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## Abstracts

## **Plenary Lecture 1**

### **Proteomics - a Tool in Personalized Medicine?**

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With cellular systems several quantitative proteomics techniques are available like metabolic or chemical labelling (SILAC, iTRAQ or ICPL, etc.). However, with plasma the situation is much different. Only chemical labelling is possible, the extremely complexity of probably millions of different protein molecules (e.g. antibodies) and the unequalled large dynamic range of over  $10^{10}$  of the most abundant to the least abundant proteins hampered a more comprehensive coverage of the plasma proteome. An easy routine identification of low abundant proteins which are expected to be diagnostically and prognostically relevant is not achieved so far. Additionally, it is well known that in plasma most of the proteins are present in multiple forms caused by various posttranslational modifications and processing events. Single protein species which may be diagnostically relevant have to be quantified which hardly can be achieved by peptide based proteomics approaches. As a consequence in most reports on plasma proteomics only a few tens to a few hundred proteins have been identified and hardly any significant quantitative data are available for human plasma. In a feasibility study first results of a proteomics based individualized biomarker discovery strategy in colon cancer patients will be presented and discussed. In collaboration with the Bavarian Blood Bank (Bayerischer Blutspendedienst) well standardized samples of single individuals covering various time points from a healthy situation until a diseased state are quantitatively analysed using protein based quadruplexed ICPL labelling strategy. The advantages, potential and current limitations of such an individualized proteomics study in human plasma is discussed.

## ***Plenary Lecture 2***

### **The Environmental Challenge for Analytical Sciences**

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In this lecture the major elements of the European Union's Policy on Environmental Protection and Sustainable Development and resulting challenges for Analytical Sciences will be presented.

The priority issues dealt with are:

- Sustainable management of natural resources: air, water, soil, forestry.
- Climate change and clean energy.
- Global development cooperation.

Analytical Sciences are required to provide policy relevant information for the development and implementation of European Community legislation and form a strong pillar for a sustainable evolution of our region and our planet.

It will be shown which information needs to be provided, how the necessary quality levels can be achieved and which new approaches, e.g. combining measurements and modelling, or earth-observation with in-situ chemical/physical measurements, need to be taken to achieve an integrated assessment of the state of the environment and to develop approaches for sustainable development.

In this context the need for building-up of a Shared Environmental Information System based on the INSPIRE spatial data infrastructure will be emphasised.

The lecture will present these issues using concrete research results obtained in the Joint Research Centre, which is providing S/T based support to the European Commission in the questions relating to the Protection of the Environment and Sustainable Development of the European Union.

## Plenary Lecture 3

### Nanoanalytics - Probing Matter at the Atomic Scale

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Nanoanalytical methods allow us to probe a variety of properties of material surfaces and interfaces even down to the atomic scale. Besides structural features which might define the functional properties of materials and devices, other parameters such as mechanical compliance, chemical composition and optical properties as depending on the local composition and distribution are of utmost interest for the development, optimization and failure analysis.

Nanoscale science is strongly driven by Scanning Probe Techniques such as scanning Tunnelling Microscopy (STM) and Atomic Force Microscopy (AFM) which allow us to investigate and manipulate individual atoms and molecules, thus complementing electron- and ion beam techniques as well as laser spectroscopy. While the imaging capabilities of techniques such as STM, SFM, and near field optics (SNOM) etc. dominated the application of these methods at their early development stages, the physics of probe-sample interactions, and the quantitative nanoanalysis of elastic, electronic and magnetic surface and transport properties became recently of increasing interest. Force spectroscopy allows us, for example, to gain information about folding and unfolding processes of individual protein molecules and other biologically relevant systems. Beyond that we can do now quantitative imaging of the potential landscape of surfaces at the atomic scale thus getting valuable data for the understanding of the atomic scale mechanisms underlying, e.g. friction- and wear processes. These techniques may also open the pathway for chemical non-destructive surface-analytical investigations at the atomic scale.

Recent development of some these techniques will be discussed.

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A. Schirmeisen, A. Taskiran, H. Fuchs, H. Bracht, S. Murugavel, B. Roling  
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For further publications and topics, see:

[www.uni-muenster.de/Physik/PI/Fuchs](http://www.uni-muenster.de/Physik/PI/Fuchs)  
[www.centech.de](http://www.centech.de)

# S01: Microfluidics

## KEYNOTE LECTURE 1

### Flow Injection Techniques for Investigating the Biogeochemistry of Dynamic Marine Environments

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Instrumental analytical techniques are critical for improving our fundamental understanding of environmental processes. In addition to the challenge of environmentally relevant detection limits is the need for high temporal and spatial resolution measurements. In this context there is a growing need for rapid and reliable but relatively low cost techniques that can be remotely deployed to provide high quality environmental data.

This presentation describes the evolution of flow injection (FI) based instrumentation for aquatic environmental monitoring [1]. FI techniques now impact on a wide cross section of analytical chemistry activities, providing imaginative and practical solutions to challenging analytical problems and contributing to the improvement of data quality. Key aspects of field based FI systems will be discussed, including reaction chemistry, detector design, analytical performance, sample presentation and data processing.

Two examples will be used to illustrate the impact of FI techniques at the interface between environmental analytical chemistry and biogeochemistry. The first is the determination and cycling of phosphorus species in catchments and estuaries. Novel solid state detection coupled with on-line photochemical oxidation of dissolved organic phosphorus in natural waters will illustrate the potential of FI techniques for gathering high temporal resolution data for catchment management. A more complex FI manifold coupled with sequential enzymatic hydrolysis will be shown to identify individual classes of organic phosphorus species in the Tamar Estuary (SW England) [2]. These results will demonstrate the potential importance of the organic phosphorus fraction (which is often overlooked) as a bioavailable source of phosphorus in aquatic systems.

The second is the determination and biogeochemistry of iron in the open ocean. An FI with chemiluminescence detection (FI-CL) will be described for the determination of iron in remote, open ocean environments. As a rate limiting nutrient, iron plays a key role in ocean productivity and climate change. Data from real time monitoring of picomolar concentrations of Fe(II) and Fe(II+III) in the Atlantic and Southern Oceans will be presented and the biogeochemical cycling of iron discussed. The Atlantic Ocean data show the power of FI-CL for global scale spatial mapping of sub-nM surface iron concentrations and the Southern Ocean results demonstrate the ability to provide high temporal resolution data from truly remote locations. The procedure used for the collection of a bulk (1 L) low level (< 1 nM Fe) Atlantic Ocean sample for the preparation of a CRM and results from shipboard and global laboratory intercomparison exercises for low level iron in seawater will also be presented to emphasise the importance of 'clean' analytical protocols [3].

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## **S01: Microfluidics**

### **The Potential of Lab-on-a-Valve Fluidic Systems for Automated Sample Processing Prior to Chromatographic Separations**

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The third generation of flow injection, the so-called Lab-on-a-Valve (LOV) [1], has been consolidated as a microfluidic platform for downscaling and automation of sample pre-treatment in both the environmental and bioanalytical field. Efforts in this direction have been primarily given so far to the hyphenation of the miniaturised module to atomic or molecular absorption/emission spectrometers for determination of trace elements and biomolecules.

The aim of this lecture is to draw the audience attention to the potential of LOV as a versatile front end platform to liquid-phase column separation systems, namely, high performance liquid chromatography [2], capillary electrophoresis [3] and miniaturised affinity chromatography [4], and most notably to large-volume injection gas chromatography [5] as well, for appropriate presentation of real-life samples to the separation system. This is nurtured on its intrinsic flexibility for either precolumn or post-column microfluidic handling and analyte derivatization by means of user-friendly programmable flow and accommodation of on-line sample pre-treatment schemes (e.g., sorbent extraction in a disposable fashion, the so-called bead-injection solid-phase extraction, BI-SPE) aimed at matrix isolation and/or analyte enrichment prior to chromatographic separations. The variety of interfaces devised so far for appropriate injection of a minute, well-defined volume of processed sample in LOV into the column set-up and for minimization of band broadening effects when coupling on-line BI-SPE with reversed-phase chromatography are to be discussed in detail and illustrated via selected representative examples in the (bio)analytical and environmental fields.

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[4] H. Erxleben, J. Ruzicka, *Analyst* 130 (2005) 469

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## S01: Microfluidics

### Simplifying the Integration of Photometric Detectors in Microfluidic Devices

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The development of integrated microfluidic systems involves the incorporation of all the necessary components in a small device [1]. The overall size of microfluidic devices with integrated optical detection is sometimes limited by the dimensions of the detection components [2]. Therefore, miniaturization of the detection system is very desirable, being one of the inevitable requirements of truly portable devices. This, in addition with the increase need for develop integrated microdevices less expensive, faster and simple than ever before, breaks through the development and integration of new type of detectors, like CMOS (complementary metal-oxide-semiconductor) and CCDs (charge-coupled devices). CMOS imagers have been used as photometric detectors [2], due to their simple use, low cost, large market availability and simple signal processing.

In this work, microfluidic devices constructed by direct-milling on poly(methyl methacrylate) (PMMA) substrates [3] were developed incorporating in to the same structure CMOS imagers as photometric detectors. One key point of this type of detectors, when compared with more conventional ones, is that the use of additional optical components can be avoided. Placing the CMOS directly on to the microfluidic device was a simple way to eliminate the need of focusing lenses and, consecutively, reduce the size and increase the simplicity of the overall microdevice.

Different CMOS detectors of different brands were individually characterized in order to determine their intrinsic characteristics namely, signal stability, quantum efficiency in the visible zone of the spectrum and dark current. Thereafter, CMOS detectors were integrated on the microfluidic devices, as chemiluminescence detectors, in order to assess their characteristics for quantitative purposes, namely sensitivity, linear range, reproducibility and base line drift, being the selection made in accordance with the obtained results. The applicability of the developed microfluidic device was assessed in the chemiluminometric determination of nitrite in ground waters, through its reaction with hydrogen peroxide, originating peroxynitrite, which reacts with luminol [4].

The proposed microfluidic device allowed the determination of nitrite with good precision as well as with good recovery values in the analysis of ground water samples, showing that the use of CMOS imagers is a truly alternative as chemiluminescence detector. The integration is easily achieved and enables the development of an extremely simple and low cost alternative to conventional detectors.

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#### Acknowledgements

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## S01: Microfluidics

### Flow-Through Chemiluminescence DNA Microarrays for Multiplexed Quantification of Pathogenic Bacteria in Water

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Pathogen detection is important for health and safety reasons. Several outbreaks all over the world have shown the need for rapid, quantitative, and particularly multianalyte detection systems. Antibody and DNA microarrays provide a powerful analytical tool for the simultaneous detection of multiple analytes in a single experiment [1]. Therefore, we have developed flow-through chemiluminescence (CL) DNA [2] and antibody [3] microarray chips. CL microarrays image the signal of each spot by a CCD camera. CL is a very sensitive read-out principle for microarrays which images the light emission by a chemical reaction with the assistance of an enzyme label.

For the quantification of multiple pathogenic bacteria especially molecular biological methods like PCR are accepted for the quantification of pathogens in food or water samples. The restriction for analytical DNA microarrays was that the usually applied end-point PCR results in a high amount of amplicons which is not dependent on the pathogen concentration. Therefore, we have developed the stopped-PCR method [2]. We terminate the amplification of the target sequence in the logarithmic phase. The result is that the amplicon concentration is strongly dependent on the applied target DNA. For analysing the amplified DNA on a flow-through microarray chip we generate single stranded DNA labelled with digoxigenin which is detected on each spot by flowing with an HRP-labelled anti-digoxigenin antibodies. This strategy has increased the sensitivity and reduced the assay time. We can simultaneously quantify *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Legionella pneumophila* and *Campylobacter jejuni* by means of a multiplexed DNA hybridisation format in less than 4 hours with a sensitivity between  $10^2$  and  $10^5$  cfu/mL. This type of analytical microarrays are adaptable to our new developed automated microarray chip reader (MCR 3) [4].

#### Literature:

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## **S01: Microfluidics**

### **Miniaturized Flow Injection System with Disposable Magnetic Detectors Based on Nickel Nanowires: a Novel Tool for Fast Detection of Total Carbohydrates**

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In this work we propose a fast and accurate carbohydrate analysis employing a miniaturized FIA system with electrochemical detection using nickel nanowires. Nickel nanowires were fabricated in our lab using Anodic Alumina Oxide (AAO) template with electroplating methods and characterized by SEM. Electrodes were simply modified just dropping a solution of previously characterized nanowires in the SPE working electrode and then, active them electrochemically in order to improve the sensitivity of the method. Due to their magnetic properties, we can avoid the leaching with a magnet under the flow cell.

Analytical performance of different carbohydrates (glucose, fructose and sucrose) were systematically studied with values of  $R^2 > 0.99$  for monosaccharide calibrations and  $R^2 > 0.98$  for sucrose. Reproducibility was excellent with RSDs values less than 4% (n=12). Up 30 measurements were sequentially performed with no fouling effect in the electrode.

In addition, we also propose a very simple and fast calibration during the analysis simplifying the overall process and decreasing dramatically systematic error (below 2% in drug and food sample analysis).

The results offered in this work, are going to open new expectative in the field of *point care* testing.

## **S01: Microfluidics**

### **Flow-Through Microdispenser for the Hyphenation of $\mu$ LC-UV and IR Microscopy: Analysis of Chlorinated Pesticides at ppb Levels**

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Problems associated with flow-cell LC-FTIR interfaces are caused by the presence of the mobile phase, the obvious solution is to eliminate the solvent prior to measurement of the infrared spectrum. A number of techniques for mobile phase elimination have been reported in the past, including thermospray, concentric flow analyzer, particle beam, ultrasonic and pneumatic nebulizers. These solvent elimination techniques generate a mist of tiny liquid droplets in an uncontrolled way, with the mist being partially dried, condensed and deposited on a given target. The diameters of these deposits measure a few hundreds of micrometers, depending of the technique, being far from the optimum value, considering a reasonable sample spot of a mid IR microscope is 50  $\mu$ m in diameter. Thus, achievement of small analyte deposits is of utmost relevance for sensitive infrared detection. In this study, a flow through microdispenser capable of producing 50 pl sized microdroplets is used to achieve small deposits. The microdispenser is a high precision tool for delivering minute amounts of liquid sample in a controlled and reproducible way [1].

The usefulness of the hyphenated  $\mu$ LC-UV-IR technique for the trace analysis was demonstrated on the determination of triazines and phenylureas in river water.

The working conditions for the microdispenser as the central element of the solvent elimination interface on the droplet formation and deposition were studied. The chromatographic system consisted of an Ultimate 3000 Dionex with a 1  $\mu$ L injection loop and a C18 Acclaim PepMap (300  $\mu$ m ID x 15 cm, 3  $\mu$ m, 100 Å) column. A linear gradient from 50:50 water:methanol to 100 % methanol for 21 min with a 3  $\mu$ L min<sup>-1</sup> at 40 °C was used, being transferred all the eluent to the microdispenser without flow splitting.

For these conditions, the identification limit of the methodology is lower than 2 ng on the column, being a really promising technique for environmental trace analysis.

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## **S01: Microfluidics**

### **In-situ Depletion and HPTLC-Supported Resonance Raman Spectroscopy of Carotenoids in Pollen**

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Understanding carotenoid composition of pollen has been of great interest to plant physiologists for decades. In our effort to develop a fast, automated pollen detection based on Raman spectroscopy, we found that carotenoids in pollen are the major source of misclassification of pollen spectral fingerprints. An examination of carotenoids contained in tree pollen was accomplished by combining High Performance Thin Layer Chromatography (HPTLC) and resonance Raman spectroscopy (RRS). Extracts containing carotenoids derived from pollen were separated with HPTLC prior to analysis by RRS. The carotenoid composition of six tree pollen species was analysed (horse chestnut, large-leaved linden, european ash, willow, prunus mahaleb, tree of heaven), and the concentrations of four ubiquitous carotenoids (beta-carotene, cryptoxanthin, lutein, zeaxanthin) in these pollen species were determined. For HPTLC separation, a new highly effective multiple development protocol was established using a gradient of methylene chloride, tetrahydrofuran and *n*-hexane as mobile phase. Raman spectra were measured directly on the HPTLC plates. The resulting data were compared to Raman spectra generated in situ during photodecomposition of carotenoid molecules. The results indicate that the Raman difference spectra obtained by in situ depletion of the carotenoid molecules represent an average of the overall carotenoid constitution. They give the first in situ evidence of inter-species variations in pollen carotenoid content, structure, and/or assembly without prior purification. Regarding the application to complex in situ situations as in the case of single pollen grains, several advantages of the in situ depletion method were identified compared to purification-based carotenoid characterization, among them higher sensitivity and immediate applicability to in situ spectroscopic characterization and detection.

## **S02: Bioanalysis I**

### **Keynote lecture 2**

#### **Single Molecule Force Spectroscopy and Recognition Imaging**

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In molecular recognition force microscopy (MRFM), ligands are covalently attached to atomic force microscopy tips for the molecular recognition of their cognitive receptors on probe surfaces. A ligand-containing tip is approached towards the receptors on the probe surface, which possibly leads to formation of a receptor-ligand bond. The tip is subsequently retracted until the bond breaks at a certain force (unbinding force). In force spectroscopy (FS), the dynamics of the experiment is varied, which reveals a characteristic dependence of the unbinding force from the loading rate. These studies give insight into the molecular dynamics of the receptor-ligand recognition process and yield information about the binding pocket, binding energy barriers, and kinetic reaction rates. Applications on isolated proteins, native membranes, viruses, and cells will be presented. We have also developed a method for the localization of specific binding sites and epitopes with nm positional accuracy. A magnetically driven AFM tip containing a ligand covalently bound via a tether molecule is oscillated at a few nm amplitude while scanning along the surface. In this way, topography and recognition images on membranes and cell surfaces are obtained simultaneously.

## S02: Bioanalysis I

### Magnetic Nanoparticles - Living Cells Interaction and Application in Immunoanalysis

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Transition from macro to micro and nanostructures cardinally changes sample interaction with biological object, particularly with the living cell, and, being spread on the electrode surface, provides unique sensor properties. In this work investigation results of magnetite nanoparticles interaction with the cells of various nature and nanoparticle routes in immunoanalysis with magnetic separation are presented. Nanoparticles were synthesized by coprecipitation of  $Fe^{2+}$  и  $Fe^{3+}$  salts by water solution of ammonium hydroxide.

Interaction of  $Fe_3O_4$  nanoparticles with the human embryonal lung cells (line WI-38), sarcoma cells, sperm cells is investigated. It is stated that during the first hours nanoparticles are being localized around membrane, and endocytosis to the cells occurs after 24 hours incubation.

Electron microscopic investigations of  $Fe_3O_4$  nanoparticles interaction with gram-positive salmonella bacteria S. Typhi strain SL 7207 and E-coli strain ATCC2592. After one hour incubation  $Fe_3O_4$  nanoparticle aggregates were fixed on bacteria surface. The results obtained were applied for the development of the immunoanalysis method with magnetic separation.

The method is based on nanocomplex “pathogen microorganism marked with nanoparticles – antibodies” formation on the surface of the sensor (thick film graphite electrode). Magnetic separation and signal forming substance concentration on the sensor surface in magnetic field are applied in the process of analysis. Linear dependence logarithm of iron reduction current amplitude time derivative from cell concentration logarithm [1].

Immunoanalysis method proposed was tested by the determination of S. typhi strain SL 7207 agent in the excrements of infected animals.

Excrements of clean animals were used for blank experiment.

Standard method of microbiological inoculation on selective medium was used for comparison of the results obtained. S. Typhi concentration, determined by the proposed method, was  $6,1 \times 10^6$  cells/ml and it was  $6,1 \times 10^6$  cells/ml as determined by inoculation method. Divergence of results was 10%.

The work is carried out with financial support of the RFFI (№07-03-96068\_ural\_a) and Government of Sverdlovsk Oblast (project “Nanotechnologies in Bio and Chemical Sensors for Environmental and Human Health Monitoring”).

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## S02: Bioanalysis I

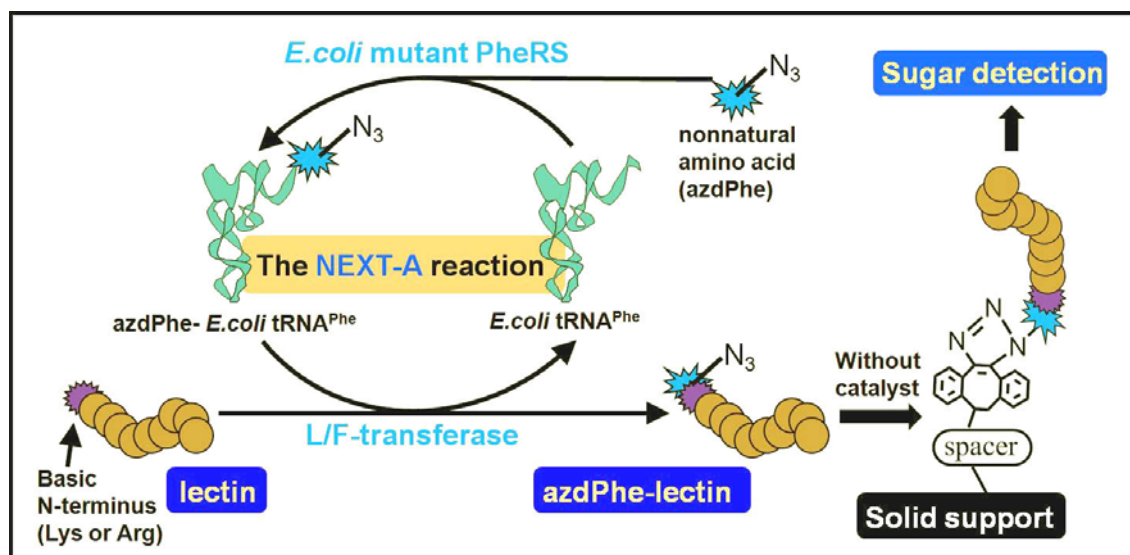
### Tag-Free N-Terminal Specific Immobilization of Lectin via the NEXT-A Reaction for Sugar Detection

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We have recently invented that nonnatural amino acids possessing functional groups can be enzymatically introduced only at the basic N-terminus of various proteins or peptides with no tags.<sup>1-3</sup> We extended this L/F-transferase-mediated functionalization of proteins in combination with aminoacyl-tRNA synthetase (ARS), namely NEXT-A (N-terminal Extension of protein by Transferase and Aminoacyl-tRNA synthetase) reaction.<sup>4</sup> Combination of the NEXT-A reaction and non-catalytic 1,3-dipolar cycloaddition enables us to immobilize any kind of active proteins onto supports with highly ordered orientation.<sup>5</sup> Lectin-immobilized gel, made by this novel system, was subjected to frontal affinity chromatography to detect specific sugars.



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## ***S02: Bioanalysis I***

### **Optical Imaging of Surface Immobilized Oligonucleotide Probes on DNA Microarrays Using Liquid Crystals**

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In this study, we report a new label-free method for the imaging of immobilized oligonucleotide probes on DNA microarrays. The imaging principle is based on the disruption of the orientations of nematic liquid crystals (LCs), 4-cyano-4'pentylbiphenyl (5CB), by the immobilized oligonucleotides on a surface. Because LCs are birefringent materials, disruption of their orientations by the immobilized oligonucleotides can manifest as optical signals visible to the naked eye. LC cells with two homeotropic boundary conditions, which align 5CB perpendicularly to both surfaces, were developed to deliver a distinct contrast between a dark background and a bright image caused by the immobilized oligonucleotides. This design also allows the quantification of immobilized oligonucleotides concentrations through the interference colors of LCs. The LC-based imaging method has a good signal-to-noise ratio, clear distinction between positive and negative results and is non-destructive to the immobilized oligonucleotides. These advantages make it a promising means of assessing the quality of DNA microarrays.

## S02: Bioanalysis I

### Holistic Analytical Technologies. Pragmatic or Utopic?

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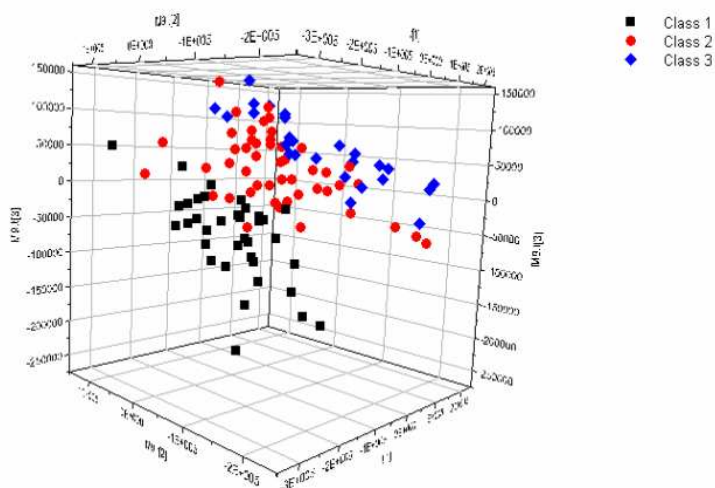
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Holistic analytical technologies represent an upcoming trend in contemporary science, especially in the case of the "Omics". Traditionally the task of Analytical Chemists was to determine a limited number of target analytes in a variety of samples. Till recently our capability restricted our results to a narrow focus of certain molecules or classes. Expected features and figures of merit of our work include: specificity, selectivity and removal of interferences. But in many cases these interferences emanate from molecules that play an important role in life sciences or in the quality of food or other products. A decade ago the determination of the whole complement of a sample couldn't appear in the thoughts of any prudent scientist. However advances in analytical technology and computing power now allow for the utopic: The holistic approach where the fingerprint of a specimen is generated and a cartographic journey is then undertaken.

LC-MS, GC-MS and NMR are the major analytical tools (to obtain the fingerprint) and multivariate statistical (Principal Component Analysis, Partial Least Squares-Discriminant Analysis) the statistical tools used in cartography.

Our group's work is in the area of biomarker discovery. We use information rich spectroscopic technologies for the discovery of biomarkers diagnostic of a disease or a certain physiology state, biomarkers of toxicity and biomarkers of drug efficacy. In the lecture technologies used for the development of robust analytical protocols will be presented along with the unavoidable problems that such non-targeted methods bear. Furthermore characteristic examples of the discovery of biomarkers will be illustrated.



## **S02: Bioanalysis I**

### **Determination of Iron in Rat Model of Secondary Iron Overload by Atomic Absorption Spectroscopy and High-Performance Liquid Chromatography**

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**Introduction:** Experimental rat model mimicking secondary cardiac and hepatic iron overload in thalassemic patients is used in non-clinical testing to assess efficacy of iron chelators. The objective of this study were to: (a) develop and optimize AAS method for iron determination in animal tissues, (b) determine level of iron in rats' heart and liver at various stages of iron overload by AAS and validated HPLC, (c) provide comparative evaluation of these two methods.

**Methods:** Male Sprague Dawley rats (N=34), body weight 250 g, were divided into treated and control groups. Treatment comprised intraperitoneal injections of iron dextran (100 mg/kg) twice weekly for 2 or 4 weeks followed by post-injection equilibration period from one to 24 weeks and euthanization. Iron content in lyophilized heart and liver tissues was determined by validated HPLC method based on iron-complex formation with desferrioxamine (J Chromatogr.B.2005, 823(2):177-83) and by AAS following microwave digestion. Certified reference materials (iron content from 184 to 16907 µg/g) were employed to test recovery and accuracy of the AAP method.

**Results:** Both types of samples exhibited normal distribution of data ( $r = 0.9490 - 0.9837$ ). However, good recovery (77-119%) was achieved, by AAS, for iron concentrations from 184-3780 µg/g, while for higher concentrations (i.e. ~ 17 mg/g) the recovery was 53% only. Calculated correlation coefficients for the data of iron content in liver and heart obtained by HPLC and AAS, were 0.835 and 0.870, respectively. A good correlation between the results obtained by HPLC and AAS was obtained for control (low iron) and moderately iron-loaded tissues.

**Discussion and Conclusions:** Iron content in experimental rat model of iron-overload can vary significantly, especially in the liver, depending on the extent and stage of iron-load. Analytical method, covering a broad concentration range, such as HPLC, provides flexibility necessary for iron determination in iron-loaded animal tissues.

## **S02: Bioanalysis I**

### **Chemical Causes of Typical Burnt Smell after Accidental Fires**

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After accidental fire there is a typical burnt smell perceptible, especially at and around the fire ground. So far, the compounds causing this long-lasting odour have not been sufficiently identified leading to problems in two aspects. First, an objective assessment, concerning the odour, is not possible. Second, the development of suitable methods of odour neutralisation has not been possible, yet. Thus, one goal of this study is to determine the compounds responsible for burnt smell present at the damaged spot.

Odourous sample materials were taken after real fire accidents. The volatile organic compounds were trapped by solid phase microextraction (SPME) fibres in the headspace of sample containing vials and analysed by means of GC/MS. Different fibre types and chromatographic columns, mainly differing in polarity, were used to cover a wide range of volatile compounds. The occurring substance classes were mainly aromatic compounds, like arenes, phenols, PAH or aromatic carbonyls. Alkanes, alkenes, aldehydes and N-heterocycles could be found as well.

Subsequently, olfactory experiments were conducted in order to identify the odourous compounds among all detected analytes. A mid-polar SPME fibre, covering a broad range of different analytes, was found to be most suitable. The loaded fibre was again transferred to the GC/MS additionally coupled with an olfactory detection port (GC/MS-O). The column flow was split: Half was led to the MS for instrumental detection and the other half to the olfactory detection port for sensory detection by five test persons. The two detection methods took place simultaneously. By this, it was possible to distinguish between the odour-active compounds and those which are volatile but odourless. Hence, the odourous compounds present at many accidental fire sites were identified. They belong to the class of substituted phenols, PAH and carbonyls.

At the moment a method to assess the degree of damage by odour based on a few indicator compounds is developed. The results of this investigation will contribute to the design of new methods for odour neutralisation and fire damage restoration in order to reduce the amount of materials and goods to be disposed.

## **S03: Process Analysis**

### **Keynote lecture 3**

#### **Novel Strategies for Environmental and Process Monitoring Using mid-IR Spectrometry**

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Advances in technology for infrared spectrometry are of key importance in the development of innovative analysis systems for use in applied environmental and process monitoring as well as in basic (bio)chemical research. These advances can include novel optical components such as light sources, detectors and fiber optics, but also supporting technologies for enabling new measurement concepts. These are needed to meet the increasing demands for reliable chemical information in our industrialized society. This presentation reports on recent developments which make use of such technological advances.

Using powerful mid-IR quantum cascade lasers, a portable analyzer for measuring oil-in-water has been developed. The new method is based on a liquid-liquid extraction step using cyclohexane followed by the determination of the extracted hydrocarbons in the cyclic extraction solvent by quantum cascade laser spectroscopy. This new method is a viable alternative to the former well established IR-method for oil in water, which used Freon 113 as the extraction solvent which, due to the ozone depleting potential of this CFC, has been banned and replaced by gas chromatography.

A standing MHz ultrasound field established between the plane ATR surface of a fiber optic probe and a piezo-ceramic element placed at a distance of a few millimeters can be used to manipulate particles in a suspension. Particles such as beads or microorganisms are captured in the nodes of the standing pressure waves. By controlling the frequency of the standing waves the particles can either be kept away from the ATR surface or pressed against it. In such a way a fiber optic in-line sensor system can be envisioned which is capable to discriminate between particles (micro-organism) and solutes (substrates and products) in a given suspension (fermentation).

## **S03: Process Analysis**

### **New Developments in FT-IR Imaging and FT-IR Miniaturization**

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*Over the last decade FT-IR imaging with focal plane array detectors has proved a powerful technique for the rapid chemical visualization of materials by a combination of spectroscopic and spatial information. Thus, selected sample regions of interest can be analyzed with reference to the identification and lateral distribution of chemical species by FT-IR spectroscopy in the transmission or attenuated total reflection (ATR) mode. The applications cover a broad spectrum ranging from medical diagnosis in human tissues to the analysis of phase separation in polymers. Depending on the field of view and the measurement technique the achievable lateral resolution varies in the range from about 3 – 60  $\mu\text{m}$ .*

In the present communication it will be demonstrated that not only the phase composition but also the phase orientation can be imaged by the novel application of polarized radiation in FT-IR transmission measurements of incompatible, anisotropic biopolymer blends. Furthermore, for the same type of samples the possibility of depth-profiling by FT-IR/ATR imaging will be addressed for the first time.

Finally, the performance of the presently smallest commercially available FT-IR/ATR spectrometer for the quantitative analysis of additives in bitumen will be discussed in some detail. The possibility of using the data of this hand-held FT-IR spectrometer in combination with chemometric evaluation routines will demonstrate that in the near future this technique may significantly contribute towards the quality improvement of road surfaces by in-situ control of bitumen before application in the process of road construction.



## **S03: Process Analysis**

### **Electronic Tongue Technology and Fourier Transform Infrared Spectroscopy for Beer Analysis – an Integrated Approach**

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The present work deals with the results of the application of an electronic tongue (ET) and Attenuated Total Reflection Fourier Transform Infrared Spectrometry (ATR-FTIR) as analytical tools for rapid assessment of beer taste and quality attributes. Fifty Belgian and Dutch beers of different types, characterized with respect to sensory properties and physicochemical parameters, were analyzed using the ET, based on potentiometric chemical sensors, and ATR-FTIR with AMTIR crystal. Both techniques have shown their ability to classify beers according to beer type and to predict taste attributes and quality parameters of beer. Using advanced chemometric techniques the output of the ET was successfully related to sensory attributes of beer such as sweetness, sourness, taste intensity, bitterness as scored by a sensory panel and to the most important instrumental parameters like for instance bitterness (concentration of iso- $\alpha$ -acids). In case of ATR-FTIR excellent correlations, typically higher than 0.9 were observed between the predicted and measured physicochemical parameters – original, apparent and real extracts, alcohol volume and energetic value. Two data analysis approaches were followed in this study: first the data were analyzed separately for each instrumental technique, subsequently, a data fusion approach was followed where data from both techniques were merged together. The latter approach resulted in improved prediction model performance. The obtained results have proven that both techniques are useful tools in the analysis of beer, since quantitative determination of important physicochemical parameters and taste attributes as well as discrimination between different beer samples is possible.

## **S03: Process Analysis**

**PAT; Flow- or Non Flow-Based, Unit Operations, Micro- or/and Nanosensors; “Microfluidics”, Real-Time; Is a Marriage with PAT Always Possible? The Reality and the Future**

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Sustainable development is a key objective of the European Union with primary concern about natural resources (waste water management, water quality and quantity), food processing and distribution, health issues, climate change, clean energy as some key issues. Microsystems technology with PAT, MPAT and NPAT become increasingly important in various operations in real-time and new innovations linking the different PAT systems successfully to microsystems technology may create fruitful solutions to the problems above. It will be shown how the establishment of PATLAB in Bucharest, Romania served as a catalyst for PAT, MPAT and NPAT. Different scenarios regarding the innovation, development, implementation and application of flow-based systems will be outlined. This will be compared with non-flow-based systems. Careful attention will be given to the reality of microfluidics as marketable applications with flow-based PAT, MPAT and NPAT, the incorporations of micro- or/and nanosensors as detection devices and if real-time is always possible. It will be discussed if there is any improvement in the configuration and construction of downscaled chemical devices and if the movement to remote sensing with innovation of high performance real-time intelligent interactive multi-point multi-species process analytical technological microsystems show that a marriage between these devices are possible. The reality of the current situation with possible future solutions will be highlighted and outlined.

## **S03: Process Analysis**

### **In-Situ Depletion and HPTLC-Supported Resonance Raman Spectroscopy of Carotenoids in Pollen**

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Understanding carotenoid composition of pollen has been of great interest to plant physiologists for decades. In our effort to develop a fast, automated pollen detection based on Raman spectroscopy, we found that carotenoids in pollen are the major source of misclassification of pollen spectral fingerprints. An examination of carotenoids contained in tree pollen was accomplished by combining High Performance Thin Layer Chromatography (HPTLC) and resonance Raman spectroscopy (RRS). Extracts containing carotenoids derived from pollen were separated with HPTLC prior to analysis by RRS. The carotenoid composition of six tree pollen species was analysed (horse chestnut, large-leaved linden, european ash, willow, prunus mahaleb, tree of heaven), and the concentrations of four ubiquitous carotenoids (beta-carotene, cryptoxanthin, lutein, zeaxanthin) in these pollen species were determined. For HPTLC separation, a new highly effective multiple development protocol was established using a gradient of methylene chloride, tetrahydrofuran and *n*-hexane as mobile phase. Raman spectra were measured directly on the HPTLC plates. The resulting data were compared to Raman spectra generated in situ during photodecomposition of carotenoid molecules. The results indicate that the Raman difference spectra obtained by in situ depletion of the carotenoid molecules represent an average of the overall carotenoid constitution. They give the first in situ evidence of inter-species variations in pollen carotenoid content, structure, and/or assembly without prior purification. Regarding the application to complex in situ situations as in the case of single pollen grains, several advantages of the in situ depletion method were identified compared to purification-based carotenoid characterization, among them higher sensitivity and immediate applicability to in situ spectroscopic characterization and detection.

## **S03: Process Analysis**

### **LIF(t) : Fluorescence Spectroscopy for Inline Process Analysis**

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The contribution gives an overview on the physical and technical bases of laser-induced and time-integrated fluorescence spectroscopy, the LIF(t) technology, and will demonstrate its efficiency in applications from the diverse areas of process analysis in chemical, pharmaceutical and polymer industry. This unique procedure offers a potential solution to measurement problems, which were considered insolvable before.

Laser induced fluorescence spectroscopy, which is one of the most sensitive techniques based on the interaction between light and matter, is particularly dedicated for inline/online process analytics. Beyond the standard spectral-resolved intensity detection, an optical excitation with short UV-laser pulses of typically  $10^{-9}$  s duration followed by an accurate single photon counting leads in most cases to significant time-resolved LIF(t) fluorescence signals. In this way, an efficient discrimination between target species (mainly of organic nature), which should be analyzed in a process matrix and the background signal is achieved.

LIF(t) is applicable in liquids process streams, on metallic and polymer surfaces as well as solids and powders. Inline analysis data will be collected continuously and processed for plant/process control, process efficiency monitoring, product quality or environmental protection reasons.

Application examples with field data will be presented shortly during the lecture:

- concentration measurement of oil, solvents or auxiliary materials in process water, cooling water and waste water (oil on water)
- concentration measurement in insulin preparations
- solvent residue determination on pesticide powder
- efficiency of lubrication, antiseize compounds and functional coatings on surfaces ( film thickness )
- surface cleanliness examination
- status of perspective application: deduction of material properties from LIF(t)-data: ( polymer viscosity determination on granules and polymer films and inline extrusion control )

## S03: Process Analysis

### Multi-Analyte *in-situ* Process Control of Industrial Biopharmaceutical Microorganism Fermentations

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An ever increasing number of highly effective pharmaceuticals are nowadays being produced by genetically modified microorganisms in advanced biotechnological fermentations. Although such fermentations are being closely controlled by a range of sensors, GMP- and FDA-compliant *in-situ* real-time process analytics for substrate and metabolite concentrations are still hard to find. Nevertheless, such systems would be of high practical interest for both process development and optimisation and production process control.

For this work, mid-IR attenuated total reflection (ATR) spectroscopy was chosen as the method with the best potential for *in-situ* real-time analysis of multiple analytes in fermentation broths. A Mettler Toledo ReactIR system equipped with a suitable ATR probe was used to monitor high cell density *E. Coli* fermentations under industrial conditions. Unlike most other probes reported by literature, this system proved to be largely immune to variations in standard process parameters, i.e. aeration, pressure, agitation and temperature. The only critical variability observed could be linked to pronounced pH changes. These have a strong influence on the vibrational spectroscopic fingerprints and hence the prediction accuracy of certain analytes.

In addition to classical multivariate calibration approaches involving reference-analysed samples, a new, fast and flexible method for generating “virtual” chemometric models was developed and tested. This method uses scaled spectra of the relevant pure substances, i.e. analytes and interferents, which are co-added mathematically. This has a number of practical advantages: First, chemometric models can be generated with minimal effort; adding additional components to even a complex model is now a matter of just a few hours. Second, cross-correlations can be effectively excluded, which greatly enhances the applicability to living biochemical systems. Finally, the – traditionally problematic - transferability of such models between different instruments is significantly improved.

With both types of models it was possible to reliably and selectively detect all the classically relevant analytes, i.e. glucose, acetate, ammonia and phosphate, with prediction errors  $\leq 0.5$  g/L at time resolutions  $< 60$  s over the full duration of industrial fermentation production processes. These advances now allow fermentations to be both monitored and actively controlled in real-time, without affecting the actual biochemical processes, stressing the microorganisms, or adding a contamination hazard.

## **S04: Education**

### **Keynote lecture 4**

#### **The Analytical Sciences Digital Library (ASDL – [www.asdlib.org](http://www.asdlib.org))**

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The utility of the Analytical Sciences Digital Library will be described. ASDL ([www.asdlib.org](http://www.asdlib.org)) is a web-based resource intended for educators, practitioners, students, and lay persons who have a need for information about analytical measurements or innovative ways to learn more about them. Funded by the National Science Foundation of the United States, ASDL is a venue that collects, catalogs, links and publishes peer reviewed web-based discovery materials pertinent to innovations in curricular development and supporting technical resources in the analytical sciences. ASDL has recently formed a partnership with the Analytical Chemistry Division of the American Chemical Society.

ASDL materials are selected for their educational value through a peer review process by experts in the field of analytical science and in the various fields of its application. Each web resource includes a detailed annotation describing the site and its useful attributes. In addition, JASDL, the online journal of ASDL, publishes peer-reviewed online articles in the categories of courseware, labware, educational practices, and undergraduate research. Because JASDL is an ungated, open source site, all publication is under the Creative Commons License (<http://creativecommons.org>) in which the author retains copyright but the licensee has the right to distribute without limit and web users can read, download, and hotlink without limit. Copies can be made, as long as the original author is cited as the creator of the material, and the copies are distributed at cost. All economic benefit, if any, from the material remains with the author.

Anyone interested in the field of Analytical Science and its applications can join the ASDL community. You can help build the collection by suggesting new sites, joining the ASDL Professionals Directory, or writing a JASDL article. Submit a poster for the ASDL poster session or volunteer to review ASDL content materials.



## **S04: Education**

### **Fostering Analytical Chemistry Education in Europe – the Measurement Science in Chemistry Consortium**

I. Leito

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[www.msc-euromaster.eu](http://www.msc-euromaster.eu)

In response to the huge need for people educated in analytical chemistry nine European universities have recently joined forces and have formed a consortium, which offers a jointly delivered Master's degree program **Measurement Science in Chemistry** (MSC). The jointly delivered study program is a fully Bologna-compliant master's level program with the volume of 120 ECTS points. The program is open to students worldwide with bachelor's degrees in chemistry (or related).

The intention of this consortium is to offer master-level education in analytical chemistry adapted to today's job market requirements for analytical chemists, especially focusing on the quality assurance of analytical measurement results. The key concepts of analytical quality – traceability, validation, measurement uncertainty, etc – are not new, but are rarely taught routinely at universities. An important part of the program is an intensive summer school course. This course covers the most advanced topics of MSC and is one of the main added value components of the JDP: no single member university of the consortium is able to cover the topics taught at the intensive course.

The consortium sets as its aim to contribute to radical improvement and harmonization of analytical chemistry higher education Europe-wide. In recognition of the academic quality of the program, ECTNA (the European Chemistry Thematic Network Association) awarded the MSC consortium the **Chemistry Euromaster**® quality label in spring 2008.

These universities were initially brought together via the EC JRC-IRMM AcadeMiC initiative, thus deploying its mission to promote a common and reliable European measurement system in support of EU policies. IRMM now acts as the mentoring organization of the consortium with the main task to ensure that the program addresses the present and emerging needs originating from the European regulatory context and to foster the accessibility of European students to this program.

Detailed information on the consortium and on the study program is available from the consortium website [www.msc-euromaster.eu](http://www.msc-euromaster.eu) and from the consortium coordinator Ivo Leito ([ivo.leito@ut.ee](mailto:ivo.leito@ut.ee)).

## **S04: Education**

### **Conditions for Euromaster Programmes in Analytical Chemistry**

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In the past symposia of this series we focused our attentions on the Bachelor level. At the Bologna Follow-up Conference in Bergen/Norway in 2005 a three-cycle system for education was adopted. As a consequence we have to progress and should start to discuss the second cycle - the master level. Only an attractive second cycle will keep potential Ph.D. students for the third cycle in analytical chemistry.

Master programs can be broad or they can be dedicated to a subdiscipline like analytical chemistry. Before we shall discuss the scope of a dedicated program, we must assess two fundamental questions: (i) is there a need for graduates from specialized master programs in analytical chemistry, and; (ii) which institutions have the human resources and the sophisticated equipment needed to run such a program?

The "Budapest Descriptors" provide a global description of the aims of a master program in chemistry. This descriptor provides the basis for planning the program, defining the aims and profile of the program, describing the various skills and competences that the graduate will have acquired by the end of the program, as well as defining the purpose of the program.

Analytical chemistry research groups at universities are usually too small to offer a master program in analytical chemistry that will fulfil all of the requirements. A well-assembled consortium could be sufficiently strong to provide the necessary human and material resources. However, common accreditation schemes may not deal with programs offered by a consortium. The European Chemistry Thematic Network Association (ECTNA) identified this problem and included consortia programs in their label system—Chemistry Eurobachelor and Chemistry Euromaster. ECTNA cooperates with four national accreditation agencies in Europe. Applications for the Euromaster label are not restricted to the European Union, as the label is an indication of quality and not region. Particularly for smaller subdisciplines like analytical chemistry, a consortium program opens up new opportunities.

## **S04: Education**

### **Model Case Study: Basic Chemometric Approach to the Determination of Metal Content in Some Environmental Samples**

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Environment is generally very dynamic system with numerous chemical equilibria occurring constantly between different compartments such as soil, air, water and vegetation. Those changes cause a high degree of environmental data variability (natural, anthropogenic, spatial and/or temporal) and logical need for extensive studies with lots of measurements. Determination of fourteen elements (variables) in thirty plant and soil samples (objects), by atomic spectroscopy resulted in large data sets. Specific elements' bioavailability is dependant on the soil geochemical properties causing a diverse mobility of elements through soil substrates and different plant tissues. We need to know if there are any relationships between variables and between the objects, to detect grouping of the objects in respect of their origin as well as of the impact potential sources of influence on significant loadings and correlations. It is rather difficult to extract any latent information by simple visual inspection of obtained results.

Starting from *a priori* knowledge and multivariate nature of conducted research, chemometrics appeared as a powerful tool in providing information needed for making conclusions and decisions concerning the reality. In that course, unsupervised learning methods, such as correlation analysis, principal component and factor analysis were applied. After the compression of large datasets and elimination of redundancies, the transformation of high-dimensional feature space to the space defined by a few factors was used for multidimensional data graphical presentation. Influences of the features (variables) were discussed through their significant loadings on extracted factors. These factors are related to the sources of the elements in the studied plants and soil samples. Additionally, as pattern cognition method, cluster analysis was also applied. It appeared as primarily useful for finding and making visible structures within the observed dataset. The selection of clustering algorithms made possible fitting the data to the problem that has to be solved.

## **S04: Education**

### **E-Learning in Applied Instrumental Analysis – The Example of Cultural Heritage Preservation**

E.A. Varella

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Videoconferencing for educational purposes is an innovative scheme using at wide range state-of-the-art equipment and supports for synchronous teaching activities. In a parallel scheme, asynchronously delivered e-learning material allows attendees to follow self-paced study in evaluated preliminary e-lectures or laboratories, and take part in assessment sessions and interactive virtual meetings through web conference technologies.

Being at the boundary of natural sciences and humanities, cultural heritage preservation – conservation science – is not easily covered in all its aspects in one institution, while harmonizing the different educational backgrounds of learners requires careful leveling of course contents. In order to permit students acquiring the necessary minimum of competences for entering the trans-national scientific community, a multidimensional e-learning system using both synchronous and asynchronous techniques has been designed and set up, by putting into operation unified virtual classrooms in dispersed areas, and establishing educational activities in both lecturing and practicing laboratory work.

The e-learning platform consists of an open-source course management system, combined to a direct dialogue tool, and an open video learning system sustaining extended self-study. Live participation ensures interacting more or less the same way, as when physically sharing the same site.

In this frame, several virtual mobility types have been interconnected, e.g. unified third cycle theoretical or practical classes, and vocational seminars; as well as “first aid line” meetings, usually founded on the shared concern about problems or the simple need for argumentation, and adopted to the requests presented by the persons involved. Laboratory training is simultaneously followed at all nodes, and is primarily focused on reviewing ambivalent results and adopting best practice examples in both physicochemical and safeguarding issues.

The educational environment is designed as a three-fold pattern – before, during and after each course unit. In the first phase learners are prepared by having available all didactic material needed. During the frontal hours they use videoconferencing, and webcasting/archiving; and afterwards they may benefit from study material, self-assessment opportunities and a meeting point with the instructor. Linguistic issues are attended to at all instances through specially designed courses on language and terminology.

## **S04: Education**

### **Platforms for E-Learning**

I.A. Kozaris

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E-learning utilizes computers and computer networks as a channel of communication additional and complementary to conventional ones, which is connecting learners with learning media, with other people (fellow learners, sources, facilitators), with data (on learning, media, people), and with processing power. Virtual Learning Environments are increasingly becoming an important part of the strategy for delivering online and flexible learning. Many institutions already have VLE's in place, though relatively few are using them with large numbers of students. Some institutions are still trying to decide what type of VLE to implement.

Learning Platform (LP) is a generic term used to describe a range of integrated web-based applications. These can include web pages, email, message boards, text and videoconference, shared diaries, online social areas, and assessment tools.

In this article we describe the different types of e-learning tools, what a "platform for e-learning" actually does, what problems is it solving, as well as the ways in which platforms for e-learning (or elements of them) are being used, or are planned to be used. Finally, we are referring to the technical specifications of the most important platform software and systems currently available; and are comparing them by listing the relevant advantages and disadvantages of each software.

# **S05: Environmental Analysis 1**

## **Keynote lecture 5**

### **What are the Analytical Needs for the REACH Regulation?**

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Over the next decade thousands of chemicals manufactured in or imported into the European Union will have to be tested, assessed and registered under the REACH regulation. Informations on health and safety of these chemicals will be provided by commercial companies to the European Chemicals Agency (ECHA) in Helsinki. In order to achieve a successful implementation of REACH, a comprehensive effort of fundamental research is imperative.

This presentation will deal with the various aspects of the assessment of chemicals related to toxicology, ecotoxicology, QSAR, and with the role of advanced analytical chemistry. If analytical chemistry is not per se mentioned in REACH, it may be part of it at every stage of the chemicals policy.

Advances of analytical chemistry particularly in

- better analytical specificities of analysed compounds including degraded and biotransformed compounds,
- low limits of detection linked to effects at low dose exposure,
- sustainable multi-component analyses to reduce human and environmental impacts and costs

will be strongly involved into the implementation of REACH as demonstrated through various examples in the presentation.



## **S05: Environmental Analysis 1**

### **Accelerated In-Needle Solid-Phase Dynamic Extraction as an Innovative Preconcentration Method for the Analysis of Organic Volatiles at ppb<sub>v</sub> Concentration Levels**

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Solid-phase dynamic extraction (SPDE) belongs to the most innovative sample preparation and enrichment techniques based on the analyte partitioning between a fluidum and a coating phase. Main advantages compared to the more traditional and widely used solid-phase microextraction (SPME) technique include the 6 times larger sorbent volume and the reduced fragility of the device.

This presentation focuses on a recent systematic research bringing forward new information on both the fundamentals and the applicability of SPDE towards VOCs analysis at ppb<sub>v</sub> levels. In contrast to SPME where the mass transfer of analytes is governed mainly by molecular diffusion, mass transfer in SPDE is enhanced by consecutive aspirating and dispensing steps creating also advective processes. However, as an important outcome of our study, it is proven that every dispensing step leads to a significant loss of retained analytes, leading to a rather low extraction yield and long sampling times (62.5 min). Both experimental and modeling results reveal that up to 48% of the analytes retained on the SPDE needle during aspiration are released again because of desorption during the dispensing step.

Therefore, we have developed a new accelerated SPDE technique (ASPDE), characterized by a one-step large volume aspiration through the needle and the elimination of the dispensing step. As a result, equilibration times were reduced by a factor of 38 compared to the aspirating-dispensing SPDE procedure. Next, equilibrium extraction with ASPDE is shown to be 5 times faster than with SPME.

Using ASPDE and subsequent GC-FID analysis, sensitivities being at least a factor of 37 and 6 times higher than those obtained with gas syringe sampling and SPME, respectively, are obtained for toluene as a model compound. LOD and LOQ values are calculated to be 56 ppb<sub>v</sub> and 173 ppb<sub>v</sub>, respectively, proving the promising potential of ASPDE for ambient/indoor VOCs monitoring.

## **S05: Environmental Analysis 1**

### **Novel Double Layer Coating System for Solid Phase Microextraction**

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Sample pretreatment is a fundamental stage of any analytical procedure. Two aims are realized during this step, namely analytes preconcentration and isolation. One of the main challenges within this area is preconcentration of polar analytes from aqueous samples where high affinity of such analytes for the sample matrix causes serious problems. Two procedures, differing in the physicochemical mechanism, are the most often utilized: partition and adsorption. Increasing the polarity of the sorbent, especially in the latter case, increases also its affinity for the sample matrix, which may lead to competitive adsorption. Although adequate sample pretreatment is possible, very detailed and time-consuming calibration procedures are needed to assess the influence of many factors upon the extraction process, which complicate the whole technique. One of the ways to avoid such problems is utilization of liquids or pseudo-liquids (e.g. polydimethylsiloxane – PDMS) as the retaining media.

Proposed solution for aforementioned problems is delivered from two, well known, techniques used for isolation of polar analytes namely membrane extraction and solid phase microextraction. The approach is an attempt to combine the advantages of the membrane techniques with those of techniques which utilize solid materials as the retaining phase. Our new system was obtained by substantial modification of the solid phase microextraction (SPME) probe. In this system, polar sorbents are separated from the sample by a hydrophobic membrane. The issue of the partial mutual solubility of the extracting agent and the sample matrix may be solved in this way, while the analytes soluble in the membrane migrate across and are retained in the suitable extracting phase. Consequently, the number of prospective extracting agents is significantly increased. Besides, chemical modifications of both phases (the sample and the extracting agent) become possible. The new membrane-SPME (M-SPME) probe was made utilizing polyethylene glycol (PEG) of 20 kDa molecular weight and polydimethylsiloxane (PDMS) as the membrane material. In this probe PEG behaves like a pseudo-liquid at the extraction stage, whereas up to date it has mainly been used as a cross-linked solid adsorbent. It has been found that such M-SPME fiber may be successfully used at the sample pretreatment stage. It is sufficiently robust and thermally stable, the latter feature permitting the usage of thermal desorption for the liberation of analytes.

## **S05: Environmental Analysis 1**

### **Microwave Induced Combustion - High End Sample Decomposition for Trace Element Analysis**

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A recently developed technique, microwave assisted combustion (MIC), breaks new grounds in solving difficult sample preparation problems. The suitability of this technique, that combines some features of microwave-assisted wet digestion and combustion in the same system, was evaluated for the subsequent determination of non metals and metals in many sample materials. Combustion is carried out in a quartz vessel pressurized with oxygen using microwave irradiation to ignite the sample. After combustion the analytes were absorbed in a suitable solution and a reflux step, if necessary, was applied in order to obtain a complete recovery of analytes. In this system the achieved temperature during the combustion was higher than 1350 °C assuring effective matrix decomposition. Up to 500 mg of organic material can be completely combusted with residual carbon content typically below 1% and eight samples can be simultaneously processed. Contrarily to combustion bomb and oxygen flask systems, the reflux step of MIC improves the washing of vessel walls and quartz holder, minimizing eventual losses due to analyte adsorption on internal parts of vessel. Not only organic samples can be decomposed by MIC, but also volatile elements like mercury can be separated from inorganic sample materials like rock and soil.

Recent applications of the new MIC technique will be discussed with main concern for tough organic materials and the analysis of volatile elements in inorganic materials.

## **S05: Environmental Analysis 1**

### **Preconcentration of Organic Substances on Polyurethane Foams: Peculiarities and Analytical Application**

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Polyurethane foams (PUF) are of great interest in analytical chemistry of organic substances. The main advantages of these sorbents are: high effectiveness, universality, chemical and mechanical stability, sustainability in organic solvents, availability and relatively low cost. The unique sorption properties of these synthetic polymeric materials are mainly due to the combination of different hydrophilic and hydrophobic active centers and reactive terminal groups.

The paper summarizes the results of investigations of sorption and chemisorption on PUF of different organic substances: polycyclic aromatic hydrocarbons, cationic and anionic surfactants, phenols, hydroxybenzoic acids and their azo-derivatives, sulfophthalein and xanthene dyes. General factors influencing on the sorption have been revealed; relations between the sorption parameters and physicochemical properties of the sorbent and sorbate have been established. A classification of the sorption systems with participation of PUF based on conception of different sorbent-sorbate intermolecular interactions has been suggested.

The ways of preconcentration of organic substances have been developed. They have given rise to the hybrid and combined analytical techniques. It has been shown that due to rational choice of volume of the sample, mass of the sorbent, using sequential sorption, one can achieve enrichment factors up to  $10^4$ . The substances can be determined directly in matrix of the sorbent by diffuse reflectance spectroscopy, luminescence and colorimetry. The examples showing advantages of such sorption-spectroscopy analytical techniques have been given for polyaromatic hydrocarbons, phenols and ionogenic surfactants.

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## **S05: Environmental Analysis 1**

### **Enrichment of Trace Constituents in Environmental Samples with Thermoresponsive Polymers**

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Enrichment techniques are often indispensable in trace analysis for obtaining sufficient precision and accuracy of analytical results. Thermoresponsive polymers are soluble in water, but become insoluble above the critical solution temperature to form gum-like aggregates. During the aggregation, different hydrophobic compounds are easily incorporated into the polymers. This process facilitates the effective enrichment and sensitive detection of trace constituents. After briefly reviewing the background of the polymer-based enrichment, up-to-date applications will be mentioned in this presentation.

Poly(*N*-isopropylacrylamide) or poly(vinyl methyl ether) was used as a thermoresponsive polymer. The polymer was added to an aqueous sample solution and heated to about 50 degrees. The turbid solution was shaken vigorously and the resulting aggregates were collected with the aid of a Teflon-coated spatula. After dissolving in a small amount of organic solvent (e.g., *N,N*-dimethylformamide or acetonitrile), the solution was analyzed by HPLC, graphite-furnace AAS or ICP-MS. Hydrophobic organic compounds in water samples were directly collected in the polymer aggregates. By this procedure, the concentration of the analytes was increased hundredfold, which allowed the highly sensitive detection by HPLC. For the enrichment of trace heavy metals, they were converted into the hydrophobic complexes with chelating agents (e.g., ammonium pyrrolidinedithiocarbamate or 8-quinolinol) and trapped in the polymer aggregates. The modified polymer having functional groups such as iminodiacetic acid was also useful for the collection of trace metals. The metals were determined by graphite-furnace AAS or ICP-MS. Because the aggregates of polymer were easily attached to bubbles, the combination with a flotation technique becomes an effective separation technique for the treatment of large volume samples. The simple and convenient polymer-based enrichment can also attract considerable attention in the field of decontamination of polluted water. The study on this area is now in progress in our laboratory.

## S06: Sensors 1

### Keynote lecture 6

#### Developing Selective Electrochemical Sensors: from Self-Assembled Monolayers to Thin Polymeric Films

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Two principal properties are essential in sensing devices, namely, sensitivity and selectivity towards the analyte. Electrochemistry is an inherently sensitive method which if coupled with high selectivity can pave the way to designing a wide spectrum of sensors. During the last years we have developed different approaches introducing selectivity elements in thin films. We have used films, ranging from a monolayer to sub-micrometer thick polymeric layers. These were made of organic and inorganic materials and used for different applications and in particular for assembling highly sensitive and selective sensors [for example: 1-4].

In this contribution, we will review our recent approaches for introducing selectivity in electrochemical sensors based on both self-assembled monolayers (SAMs) and thin polymeric films. This will include two major approaches: the formation of selective electrodes for metals, e.g.,  $\text{Fe}^{2+}$  and  $\text{UO}_2^{2+}$  based on SAMs and the development of electrochemical sensors for organic compounds based on molecularly imprinted sol-gel films. We will also present an approach which aims to benefit from both SAMs and polymers and is based on assembling mono and multilayers of polymeric films using the Langmuir-Blodgett method.

Another interesting issue is whether the binding of, for example, heavy metals by surface bound ligands is identical to that found in homogeneous solutions. Different approaches for estimating the binding of heavy metals by functionalized SAMs will be discussed [5-6].

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## S06: Sensors 1

### Glucose Oxidase Entrapment in a Sulfobutylether-Cyclodextrin-Doped-Polytyramine Film on a Platinum Nanoparticle Modified Boron-Doped Diamond Electrode

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Immobilization of a biosensing probe in an electropolymerized polymer matrix allows for precise deposition of the biomolecule on any conducting surface regardless of dimension and geometry. The amount of the immobilized probe can be easily manipulated by changing its concentration or by adjusting the film thickness. In this study, glucose oxidase (GOx) was entrapped in an electrodeposited polytyramine (PTy) film together with negatively charged sulfobutylether- $\beta$ -cyclodextrin (SBCD) on the active area of a platinum nanoparticle modified boron-doped diamond (BDD) electrode. Atomic force microscopy (AFM) imaging revealed the presence of semicircular nanofibers with a height of 40 nm and an averaged length of 795 nm throughout the electropolymerized film surface. The combined permselective film served as an excellent matrix for the GOx immobilization with high stability and reproducibility. The glucose biosensor exhibited a remarkably selective and rapid response (2s) to glucose with a detection limit of 10  $\mu$ M and linearity up to 110 mM. Glutaraldehyde cross-linking of the film with entrapped GOx completely eliminated electroactive interference caused by uric and ascorbic acids. This attractive procedure is advantageous for the fabrication of microelectrodes and microarrays.

## S06: Sensors 1

### New Electrode Materials and Arrangements for Voltammetric and Amperometric Determination of Organic Environmental Pollutants

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There is an ever increasing demand for inexpensive analytical methods for determination of submicromolar and nanomolar concentrations of various ecotoxic and genotoxic organic pollutants, modern voltammetric and amperometric methods being quite competitive in this field (1). For detection of electrochemically reducible compounds mercury is the best available electrode material (2). However, because of its toxicity, there is a tendency to substitute it by mechanically stable, environmentally friendly and easily mechanically and/or electrochemically renewable solid amalgam electrodes and paste amalgam electrodes (3) or boron doped diamond film electrodes (4). For electrochemically oxidizable substances carbon film electrodes, carbon paste electrodes (5), and metallic tubular and microcylinder electrodes are successfully applied in our laboratory. It will be demonstrated on newly developed methods for determination of micro and submicromolar concentrations of environmentally important organic pollutants, environmental carcinogens, various drugs and agrochemicals using both voltammetry in batch systems and amperometry in flowing systems (combination of HPLC and CZE with electrochemical detection).

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## S06: Sensors 1

### Voltammetric Sensors for Aromatic Nitroderivatives Based on Molecularly Imprinted Polymer Membrane on Conducting Surfaces

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Molecular Imprinted Polymers (MIP) are synthetic polymers containing recognition sites that mimic the molecular recognition properties of natural receptors with binding affinities approaching those demonstrated by natural receptors as for instance antibody [1, 2]. For these reasons they are widely used as recognition elements in sensors. In particular electrochemical transduction is convenient because of the robustness, the possibility of miniaturization, and the easy integration of MIP films or membranes with conducting surfaces [3, 4]. Here some sensors for aromatic nitroderivatives are illustrated, based on the well known reduction of these substances at potentials between -300 e -800 mV (vs Ag/AgCl). The MIP membrane gives a better selectivity and a lower detection limit, at about 10 ppb, due to the specific sorption of the substrate. The specific MIP membrane was obtained by polymerization of methacrylic acid with ethylene glycol methacrylate, in the presence of TNT. A similar membrane, but without TNT, was synthesized for comparison (NIP: non imprinted polymer membrane). The polymerization was carried out directly on the conducting surface, carbon or metal. The first one was a screen printed graphite electrode, obtained on the market, and the second one a copper electrode, prepared by photoengraving. Despite of the thickness of the membrane, tens of  $\mu\text{m}$ , an electrochemical signal was obtained by DPV. The height of the peak being directly proportional to the concentration of the template, much lower in the case of other nitroderivatives. The effect of the conditions, in particular the acidity and the preconcentration time, on the peak position and height was investigated. The detection was possible down to 10 ppb, in the case of graphite electrodes. For comparison and for elucidating the mechanism of DPV signal, a non imprinted polymer (NIP) membrane, and a membrane obtained by polymerization of the cross-linker, were examined too.

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## S06: Sensors 1

### Ion Selective Electrodes Based on PVC Membrane for Enantiomers Analysis

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Ion selective electrodes have found many applications in pharmaceutical, clinical and environmental laboratories and are being explored for numerous other applications due to low cost and short time of analysis. Most of amino acids have two optical isomers, D and L. While L-amino acids represent the majority of amino acids found in proteins, most of D-amino acids have microbial origin, not participating in protein synthesis and sometimes exhibiting toxic properties on organisms. In this paper we report pioneering work on the potentiometric detection of amino acid enantiomers, Tryptophan (Try) and Histidine (His) without to use chiral macrocycles receptors. By combining  $[[[3,3'\text{-Co}(1,2\text{-C}_2\text{B}_9\text{H}_{11})_2]]^-]$  and the protonated form of the targeted amino acid enantiomer, an ion-pair complex is obtained, which is used to prepare the ion selective PVC membrane electrodes. The chemical compositions of the compounds obtained were established by <sup>1</sup>H-, <sup>11</sup>B-, <sup>13</sup>C-NMR, FTIR and MALDI-TOF. The properties of the prepared electrodes were studied, namely slope, concentration range, detection limit, lifetime, and selectivity. The experimental results obtained for D-Try showed that the best electrode was based on di-butyl phthalate (DBP) as plasticizer (63%), displaying a linear range from  $5.00 \times 10^{-7}$  M to  $1.00 \times 10^{-1}$  M with a Nernstian slope of 60.54 mV/decade and correlation coefficient of 0.9926. The detection limit was  $2.00 \times 10^{-7}$  M and the lifetime was over one month. The selectivity of the electrodes is also reported.

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## S06: Sensors 1

### Novel Poly(Hexylmethacrylate) Composite Carbon Electrodes Modified with Keggin-Type Tungstophosphate Salts

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The demand for stable and robust chemically modified electrodes incorporating the properties of the modifier is a continuous area of interest, namely for the production of devices for diverse applications. Keggin polyoxotungstates have been widely studied for the past 20 years, due to their chemical versatility, ease of preparation, rich redox chemistry and structural properties.<sup>1</sup> As a result, they have found numerous applications in catalysis, medicine and materials chemistry.<sup>1-4</sup> Studies on the electrochemical properties of these compounds are becoming more important, due to their use as sensors and in electrocatalysis.<sup>2</sup>

We have previously prepared polyoxometalate functionalized electrodes via a one-step deposition of micrometer thick coatings by the droplet evaporation method.<sup>5,6</sup> In the present study, novel poly(hexylmethacrylate) composite carbon electrodes modified with polyoxotungstophosphates, in the form of tetra-*n*-butylammonium (TBA) hybrid salts, were prepared and characterized. The polyoxoanion salts used were  $[(C_4H_9)_4N]_4H_3[PW_{11}O_{39}]$ , and  $[(C_4H_9)_4N]_4H[PW_{11}Co^{II}(H_2O)O_{39}]\cdot H_2O$ , which have a lacunary and a metal substituted anion, respectively. Experimental results showed that the electrochemical features of the polyanion immobilized by this methodology were maintained. Also, it was demonstrated that the use of poly(hexylmethacrylate) improved the peak currents in the cyclic voltammograms. The chemically modified electrodes were stable and their preparation was easy to perform. Another remarkable advantage of these type of CPEs is its good stability due to the insolubility of hybrid salts in aqueous solutions and the affinity of the organic part toward the paste.

The scan rate and pH effects on the voltammetric features for the first tungsten reduction process of the immobilized POMs led to the conclusion that this process was diffusion controlled and accompanied by uptake of protons. Additionally, studies with  $[Fe(CN)_6]^{3-/4-}$  as electrochemical probe showed that the permeability of these molecules is significantly improved when poly(hexylmethacrylate) is used.

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## Plenary Lecture 4

### Ionics. A New Paradigm in Ion Detection in Solution

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We show an ionic diode composed of a closely spaced cation exchanger (CE) bead/membrane and an anion exchange (AE) bead /membrane with a flow channel in between through which normally high purity water is pumped. On the other side of each ion exchanger, water/electrolyte solution can be put, either in a stationary or flow-through configuration. Electrodes are put in each of these. When the CE side is held negative and the AE side is held positive, any electrolyte present in the central channel is efficiently removed: cations pass through the CE bead to the negative electrode and anions pass through the AE bead to the positive electrode. With water flowing through the central channel, the background current is very low. If an electrolyte is injected into this stream given sufficient residence time and electric field, the electrolyte is completely removed and the resulting integrated current pulse generated is directly reflective of the total charge injected, i.e., the device behaves as a "charge detector" (CD) regardless of the electrical mobility of the ions involved and unlike coulometry whether they can be oxidized or reduced in an aqueous solution. The CD thus behaves as the equivalent of a Faraday cup in solution where cations and anions are collected in separate compartments.

Conversely, when the CE side is held positive and the AE side is held negative, cations in the electrolyte behind CE and anions in the electrolyte behind AE emerge in the central channel to form an electrolyte in a manner that represents quantitative current efficiency; this thus constitutes an electrolyte generator (EG). Putting a CD electrically in series with an EG allows one to translate any electrolyte impulse injected into the CD to the impulse of the same or an altogether different electrolyte generated by the EG. This allows for example to translate an optically undetectable electrolyte to an optically detectable one. Operating the CD with a larger injection volume/flow rate compared to the central channel flow rate results in concentration amplification, as the same Faradaic equivalent of an electrolyte is generated in a much lower solution volume.

Weak electrolytes behave in another interesting manner. It is intuitive that the ionized portion will be readily transported. However, removal of ions result in further ionization and thus the overall signal becomes much more acutely dependent on the flow rate than that of a strong electrolyte, permitting ready differentiation.

As stated above these devices behave as ionic diodes, the CD and the EG respectively being comparable to a reverse biased photodiode and a forward-biased light emitting diode, respectively with the individual ion exchangers behaving as charge selective gates like *p*- and *n*-doped semiconductors.

## **S07: Food Analysis**

### **Keynote lecture 7**

#### **Community Reference Laboratories – a Measurement Infrastructure to Ensure the Harmonised Implementation of EU Food Safety Legislation**

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The free movement of safe and wholesome food is an essential aspect of the internal market and contributes significantly to the health and well-being of EU citizens and to their social and economic interests. Due to globalisation and the availability of new technological processes the European food and feed sector is becoming more and more complex. In the past several food scares have shattered the trust consumers have in their food supply. To restore confidence a complex regulatory framework has been set up in the EU. Maximum limits for a diverse array of undesirable substances in the food chain have been laid down, appropriate sampling and analysis methods for official control purposes have been described, and a rapid warning and response system has been introduced. The effectiveness of this integrated system depends to a large degree on the harmonised implementation of control, monitoring and enforcement mechanisms across the EU Member States. Community law (Regulation (EC) No 882/2004) created networks of Community and national reference laboratories that should contribute to a high quality and uniformity of analytical data produced by official food and feed control laboratories. The activities of reference laboratories should cover all the areas of feed and food law and animal health, in particular those areas where there is a need for precise analytical and diagnostic results. This objective can be achieved by activities such as the application of validated analytical methods, ensuring that appropriate reference materials and methods are available, the benchmarking of testing capabilities, and the training of laboratory staff. In the field of feed and food safety 27 laboratories were designated as Community reference laboratories (CRLs), and for animal health related matters 13 laboratories, e.g. for avian influenza, foot-and-mouth disease, bluetongue, etc. The food safety related areas include chemicals such as veterinary drug residues, pesticides, dioxins, marine biotoxins, mycotoxins, heavy metals, and biological agents such as pathogenic bacteria, parasites and viruses; it covers also issues such as food contact materials, feed additives, genetically modified organisms, and transmissible spongiform encephalopathies. Each CRL collaborates with its own network of national reference laboratories in the Member States, thus creating an EU wide system of more than 1000 laboratories highly specialised in nearly every aspect of the safety of the food supply chain. The unique set-up of this system fosters exchange of best practice among food safety professionals, has created an enormous reservoir of knowledge and experience that can be deployed rapidly in response to a food incident, and offers training to food scientists from third countries to familiarise and keep them up-to-date with analytical practices as currently applied for the implementation of the EU food policy.

## S07: Food Analysis

### Ultra High Resolution Mass Spectrometric Fingerprinting as a Powerful Tool in Food Traceability

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A lot of scientific effort has been spent to develop rapid, reliable, and cost effective analytical approaches applicable for the authentication of various food commodities. Besides of spectroscopic techniques employing nuclear magnetic resonance (NMR), Raman, or infrared spectra, a wide range of methods employing gas chromatography–mass spectrometry (GC-MS), and/or high performance liquid chromatography (HPLC) hyphenated to MS has been implemented for this purpose. Some procedures, such as matrix assisted laser desorption/ionization mass spectrometry (MALDI), direct head-space mass spectrometry (HS-MS), and/or direct infusion MS allow reduction of analysis time thanks to elimination of chromatographic separation step. Over the few recent years, a large number of novel ambient desorption ionization techniques, such as desorption electrospray ionization (DESI), atmospheric-pressure solids analysis probe (ASAP), direct analysis in real time (DART) and some others, have become available providing further improvements. Their main advantages compared to conventional techniques, involve the possibility of direct sample examination in the open atmosphere, minimal, or no sample preparation requirements, and, remarkably high sample throughput. Nowadays, screening approaches are performed using high performance ToF instruments, with mass accuracies of < 5ppm and resolutions not exceeding 15,000. However, most of the techniques and instruments currently available suffer from either poor mass accuracy and its variability in time and more significantly from resolution not sufficient to separate analytes of interest from coeluting species. Namely the mass resolution achieved plays a key role in the unambiguous identification of the most analyzed compounds in samples containing high amounts of matrix. This presentation will demonstrate the potential of ultra high resolution mass spectrometer based on the Orbitrap™ analyzer to distinguish geographical and/or species origin as well as processing practice employed for production of some popular food commodities such as olive oil, wines etc. on the basis of metabolomic profiles (MS fingerprints). Since a large volume of data is typically generated during the measurements of positive /negative mass spectra, smart chemometric tools have to be used to establish mathematical model for classification of samples. In our study, linear discriminant analysis (LDA) and artificial neural networks (ANN) have been employed for examination of selected matrices.

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## S07: Food Analysis

### Chemometric Techniques for Tracing the Origin of Foodstuff

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The increased information available to the consumers together with the competition following the institution of the Common Market (EU countries) has led safety, nutritional value, eating characteristics, ethical, environmental, economic and social aspects to be more and more important and essential topics for the food industry. In a single word, there is an increasing demand for “quality”, as a primary criterion to access the market. “Quality” is defined as “the totality of characteristics of an entity (product or service) that bear on its ability to satisfy stated and implicit needs”. It is at a glance apparent that such a generic definition results in the concept of quality encompassing a wide range of meanings. In fact, it not only refers to the characteristics or the performances of a product, but also to the consumer and to the whole manufacturing system: consumers are not merely demanding quality, they also want to know everything about the product in terms of its “life” and origin (raw materials, production methods, harvesting location).

Accordingly, a particular aspect of quality which has been gathering more and more attention during the recent years is that of the *typicalness* of the product. In fact, it has been widely reported in the literature that the geographical and botanical origin of a vegetable foodstuff are important variables regulating the overall quality of the product. This issue has been recognized by the European Union which has been introducing since 1992 a series of norms aimed at protecting these high quality productive identities, by the introduction of the Denominations of Origin (Protected Denomination of Origin, PDO, and Protected Geographical Indication, PGI). The products which are labeled by this Denomination must be produced in a well defined geographic area and manufactured using only one or few specified botanical or animal varieties. Consequently, to prevent the frauds in these field, the quality control laboratories would need an analytical method to determine the origin of the sample. Unfortunately, even if a great host of instrumental analytical techniques are at present under investigation, no one of those can be listed whose results can be directly related to the origin of the samples.

An alternative way to cope with this problem is to use mathematical and statistical methods (*chemometrics*) to process the results of a set of determination performed on the samples in order to obtain the desired classification. In this communication, the successful use of different chemometric pattern recognition methods for the discrimination of food products according to their origin will be discussed, based on several examples taken from the authors' experience (e.g. oils, wines, honey, wheat, rice).

## **S07: Food Analysis**

### **Sample Preparation Techniques for the Detection of Odour Active Compounds**

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The phenomenon of odour activity of an organic molecule is strictly related to volatility and the molecular weight of the substance. The upper limit of the molecular weight for odour activity is around 300-350 atomic mass units.

So the ideal analytical method for the determination is high resolution capillary gas chromatography. But there are several limitations and things to consider.

First the aroma fraction of a smelly product is normally the smallest part of the whole product, being normally in the milligram per kilogram range or less. Nevertheless it very often consists of up to several hundred compounds over quite a large concentration, polarity and boiling point range, so interferences on the analytical column are quite likely.

In addition the perception of odour activity by the human smell system can be very sensitive to some specific molecules, so the analytical method must be in the concentration range of the human threshold values.

Finally the odour of a product is very seldom driven by just one or a few compounds but is a complex mix of several compounds which finally contribute to the odour property of the product. Therefore the use of gas chromatography (GC-O) where the human nose is used as a sensitive and selective detector for odour activity is an essential part for the identification.

One of the most crucial points for the analysis of these target compounds is of course the sample preparation technique, where losses of the target compounds during evaporation steps or interferences with solvents due to their low molecular weight can occur. Several solvent using and solvent free methods like simultaneous distillation extraction (SDE), solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and their applicability for certain target compounds as well as their pros and cons will be discussed.



## **S07: Food Analysis**

### **Volatile Organic Sulphur and Selenium Compounds in Beers, Wines and Spirits by Solid-Phase Microextraction and Gas Chromatography with Atomic Emission Detection**

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The beverages industry considers aroma to be of a great importance as it contributes to the product quality. The effect of volatile organic sulphur compounds on beverages flavour has been well documented, whereas this effect produced by volatile organic selenium compounds has not been described. Nevertheless the use of organoselenium compounds as herbicides, fungicides and bactericides in agriculture, and the fact of dimethylselenide (DMSe) and dimethyldiselenide (DMDS<sub>Se</sub>) of being produced by phytovolatilization or by biomethylation, makes it possible to found these compounds in beverages.

A solvent-free method for the determination of several volatile organic sulphur [dimethylsulphide (DMS), diethylsulphide (DES), methylpropylsulphide (MPS), dimethyldisulphide (DMDS), dipropylsulphide (DPS), dimethyltrisulphide (DMTS), methionol and dipropyldisulphide (DPDS)] and selenium [dimethylselenide (DMSe) and dimethyldiselenide (DMDS<sub>Se</sub>)] compounds in different beverages using solid-phase microextraction and gas chromatography with atomic emission detection is presented. The Carboxen/polidimethylsiloxane fiber was the most suitable for preconcentrating the analytes from the headspace of the sample solution at 25 °C for 20 min. Volumes of 20 mL of undiluted beer were used while, in the case of wines and spirits, sample:water ratios of 5:15 and 2:18, respectively, were used. Quantitation was carried out by using synthetic matrices of beer and wine, and a spiked sample for spirits, and using two volatile organic sulphur compounds as internal standards. Detection limits ranged from 8 ppt to 40 ppb, depending on the compound and the sample analyzed. The proposed procedure was applied to a total of forty different samples. DMS and MPS were detected in all the alcoholic beer samples, while DMDS was found in three of the samples and DMTS only in one alcoholic beer. DMSe and DMDS<sub>Se</sub> were not detected in any of the samples analyzed. On the other hand, twenty of the samples analyzed appeared to be absolutely free of the studied compounds.

## **S07: Food Analysis**

### **Short Peptide Sequences as Analytical Tool in Food Analysis**

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The search for new bioreceptor has led to the production of biological recognition elements (mainly antibodies and aptamers) having high affinity and selectivity for the target analytes (i.e toxins, antibiotics). These can be implemented for multiplexing in optical or electrochemical array formats. The major drawback of this sensing approach is the effect of complex matrices as food on the biological molecule; pre-treatment of the sample for purification and pre-concentration is often required and a careful calibration for accurate quantitative data output is needed. In this work data on the exploitation of the use of short aminoacidic sequences as analytical tool will be presented. These bioreceptors possess intermediate behaviour in terms of affinity and selectivity presenting advantages in terms of cost, ease of preparation, robustness and regeneration of the bioreceptor. For example, tetrapeptides obtained mimicking the active site of the enzyme acetylcholinesterase have been studied in solution for the binding and successfully used in solid phase extraction for organophosphate and carbamate pesticides. A procedure has been optimised for the extraction of paraoxon from durum wheat and used for the detection of the organophosphate via HPLC or via a biosensing procedure based on the inhibition of acetylcholinesterase and a choline oxidase sensor.

Different procedures for the extraction of Ochratoxin A from wine, grapes, wheat and coffee have been developed using an exapeptide obtained combinatorially by the group of Giraudi (University of Turin). Analysis was carried out in HPLC with fluorimetric detector. This SPE supports can be easily regenerated for 50-80 assays and are cost effective and more robust than the conventional immunoaffinity approach..

## **S07: Food Analysis**

### **Selected Issues of Residue Analysis in Food Quality Monitoring**

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Nowadays, food safety is one of the top priorities of many countries world-wide and meanwhile deeply routed in nearly everyones mind. Food monitoring is a suitable tool that serves preventive health protection of consumers. It helps to early recognise and possibly prevent potential risks to consumers' health caused by harmful environmental contaminants, residues of plant protection products, residues of veterinary drugs or other unwanted substances detected in foodstuffs. However, the approach of targeted measures demands high standards of instrumental analytics – mostly dealing with amounts in trace analysis. As a consequence of the consistent progress in science and technology and the resultant rigorous legislation today's agri-foodstuffs branch has to face ever stricter standards and regulations. Nevertheless, the importance of food quality control cannot be overestimated and this has been emphasized by the occurrence of several mayor food alerts over the past decade, creating a certain loss of confidence among consumers. Some of the more recent affairs are presented here in more detail with the examples of streptomycin in apples as well as melamine and pesticides in foodstuff. It is not least the quick and thorough analysis of the official authorities in many countries as well as their interactive collaboration which have proven that the rapid alert system is a very important tool for health protection in the course of globalization.

## **S08: Nanoanalytics I**

### **Keynote lecture 8**

#### **Separation, Investigation, and Analysis of Nano and Microparticles in Liquid Media**

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The separation (fractionation) of particles is needed in biochemistry, macromolecular chemistry, nanotechnology, environmental studies, and other fields of science and technology. Particles bigger than about 100  $\mu\text{m}$  can be fractionated using conventional wet (or dry) sieving. Multistage membrane filtration (MMF), field-flow fractionation (FFF), and thin-cell split flow fractionation can be used to fractionate particles in a size range of 1-100  $\mu\text{m}$ . Gel permeation chromatography, MMF, FFF, and capillary electrophoresis (CE) can be used to fractionate solid and colloidal particles smaller than 1  $\mu\text{m}$  into classes based on their size, density, or charge.

MMF employing semipermeable nano, ultra and microfilters is a rather versatile method that enables the separation of particles according to their effective size to be achieved. Membrane-based fractionation is useful for determination of the distribution patterns of trace elements bound to particulate matter in natural waters and other fluids. CE is a powerful tool for separating and investigating nanoparticles. The migration of particles in the capillary is governed by their electrophoretic mobility. The main limitation of the method is the sample volume that should not exceed 10 nL. FFF is a unique chromatography-like elution method that is applicable to the separation and characterization of a broad spectrum of solid and colloidal particles, polymers, and biological macromolecules in the size range from 1 nm to 100  $\mu\text{m}$ . FFF is divided into several subtechniques according to the type of external cross-field used (gravitational, electrical, cross-flow, etc.). FFF separations occur in thin channels and the test sample weight is limited to 1 mg. A new coiled tube sedimentation FFF technique employing a long rotating column of 1.5-mm tubing bore enables this limitation to be avoided.

Investigation of particles requires the application of different instrumental techniques such as electron microscopy, photon correlation spectroscopy, laser Doppler velocimetry, etc. Chemical analysis of separated fractions of particles can be performed by various analytical techniques: atomic and mass spectrometries, chromatography, etc.

It looks to be rather perspective to develop combined fractionation methods based on the sequential separation of complex mixtures of particulate species using various approaches. Choosing the fractionation technique at each step is dependent on research tasks as well as on particles size, density, shape, and surface properties.

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## ***S08: Nanoanalytics I***

### **Utilization of Functionalized Magnetic Nanoparticles for High Throughput DNA Separation**

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The work describes an optimized process to highly efficient and convenient preparation in high throughput magnetic human DNA separation with chemically functionalized silica-coated magnetic nanoparticles. The effect of nanoparticle's size and the surface's hydrophilicity change were studied for magnetic DNA separation process, in which the optimum efficiency was explored via the function of the amino-group numbers, particle size, the amount of the nanoparticles used, and the concentration of NaCl salt. The DNA adsorption yields were high in terms of the amount of triamino-functionalized nanoparticles used, and the average particle size was 25 nm. The adsorption efficiency of aminofunctionalized nanoparticles was the 4-5 times (80-100%) higher compared to silica-coated nanoparticles only (10-20%). DNA desorption efficiency showed an optimum level of over 0.7 M of the NaCl concentration. To elucidate the agglomeration of nanoparticles after electrostatic interaction, the Guinier plots were calculated from small angle X-ray diffractions in a comparison of the results of electron diffraction TEM, and confocal laser scanning microscopy. Additionally, the direct separation of human genomic DNA was achieved from human saliva and whole blood with high efficiency.

## **S08: Nanoanalytics I**

### **Continuous Photophoretic Separation of Hydrocolloids**

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Common techniques for the analysis of particle mixtures mainly focus on the parameter particle size. The need for a precise determination of discrete particle properties arises from numerous problems in chemistry, pharmacy, life science or biology. The application of light-induced forces on particles suspended in a liquid, e. g. hydrocolloids, offers the possibility of characterization and separation due to optical properties such as refractive index or absorption [1].

At dimensions at the  $\mu\text{m}$ -scale, optical forces become capable of manipulating or trapping of microparticles. The migration induced by light is termed photophoresis (PP) and depends on the properties of the particle/fluid system. Due to the difference in refractive index of liquid and particle, photons impinging on the particle exchange momentum and induce migration. As friction force and optical force balance at equilibrium, the particle migrates with constant velocity, the photophoretic velocity. Based on the measurement of PP velocity distributions, the radius and the refractive index of single transparent spherical particles can be calculated.

For the separation of colloid mixtures, a cross-flow setup was developed. The sample flow was applied perpendicular to the laser beam, resulting in a retention distance of the particles. The retention distance is a function of size and chemical composition (refractive index) of the particles. By variation of the laser power or the cross-flow rate the resolution of the setup can be adjusted. In case of irregular or absorbing particles a stable pathway in the laser beam must be guaranteed by matching of the laser beam profile.

The gentle and contact-free manipulation of particles provides label-free discrimination of delicate samples and does not need any further sample preparation. Potential applications are the separation of irregular shaped inorganic particles or suspensions of cells and bacteria.

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## S08: Nanoanalytics I

### Carbon Nanostructures as Tools to Improve Analytical Processes

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Today, Analytical Nanoscience & Nanotechnology is an area of increasing challenge in Analytical Chemistry. From the analytical point of view, nanoparticles and nanostructured materials can be objects (analytes) or they can be used to develop new analytical tools. In this communication, an overview of the use of carbon nanostructures (fullerenes(1), carbon nanotubes(2), nanodiamonds, hybridized composites) is presented and discussed with some emphasis in the developments implemented by our research team.

To deal with the selected topic, several classifications can be considered according to complementary criteria. The most relevant of them are two, namely : A) Step of the analytical process where the carbon nanoparticles are involved : sample treatment, chromatographic and electrophoretic techniques and sensing/detection ; and B) Exceptional physico-chemical property of the nanomaterial exploited that can be : chemical, optical, electrical, thermal, mass and magnetic.

**Sample treatment** of complex matrices can be clearly improved in terms of analytical properties (i.e. recovery and selectivity) by using carbon nanoparticles. In this context, carbon nanoparticles have been used in liquid and solid phase extraction. Recent developments in carbon nanotubes based filtration membranes is also presented. Resolution of **chromatographic (GC, LC), electrophoretic (CE) and electrokinetic (EKC) techniques** can be also enhanced by using carbon nanostructures as stationary or pseudostationary phase. Finally, the promising role of carbon nanoparticles in optical and electrochemical **sensing/detection systems** is briefly outlined.

The final part of the presentation will be devoted to the prospects of the topic, by describing the most promising “niches”.

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## **S08: Nanoanalytics I**

### **Nanostructured Electrochemical Aptasensor for Ochratoxin A (OTA) Determination**

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T3: Sensor Technologies, Nano Analytics

Ochratoxin A is a naturally occurring mycotoxin produced primarily by *Aspergillus ochraceus* and *Penicillium verrucosum* usually present in a variety of foods. Previously (1) we have developed a sensor device based on Quartz Crystal Microbalance nanofunctionalized.

The interesting approach of this work is related to the using of a selective aptamer to OTA. Aptamers are nucleic acids (DNA or RNA) that selectively bind to low molecular weight organic or inorganic substrates or to macromolecules such as proteins. Anyway, this work is the first step in the realization of a sensor for OTA based on the use of the specific aptamer exploiting the known advantages of these biomimetic receptors. In literature only two papers report the development of an assay for OTA detection using this specific aptamer.

The assay developed for OTA detection was based on a direct or indirect format. All steps of the assay were carried out onto AuNPs or MBs; only the electrochemical detection was performed transferring the functionalized nanoparticles onto SPCEs. The immobilisation of the biotinylated aptamer was based on streptavidin-biotin interaction. Finally, the enzymatic product, was determined by differential pulse voltammetry (DPV).

Finally, this approach will be applied to the analysis of some OTA samples to determine the concentration of OTA and predict the risk of a possible contamination with OTA.

#### **References:**

[1] Vidal, J.C; Duato, P; Bonel, L; Castillo, JR; Anal. Bioanal. Chem (2009) 394, 575-582



## **S08: Nanoanalytics I**

### **Liquid Crystal Multiplexed Protease Assays Reporting Enzymatic Activities as Optical Bar Charts**

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We describe a highly sensitive, substrate-specific, label-free, and multiplexed protease assay which reports proteases activities as an optical bar chart, allowing test results to be easily assessed by laymen with the naked eye. First, an oligopeptide microarray having six rows of immobilized oligopeptides, with well-controlled orientations and concentration gradients, is immersed in a buffer solution containing proteases. Then, a thin layer liquid crystal is supported on the microarray to transduce the oligopeptide cleavage event into an optical bar chart of different colors and lengths. This type of optical bar chart provides very rich information such as protease concentration, incubation time, surface densities of oligopeptides, etc. Both trypsin and chymotrypsin can be detected by using this assay within 3 h. The capability of the multiplexed protease assay opens up possibilities for detecting toxins such as botulinum neurotoxins which are known to cleave proteins and affect the docking and fusing synaptic vesicles.

## S08: Nanoanalytics I

### Application of Capillary Zone Electrophoresis (CZE) and Molecular Biology Methods to the Determination of Pathogenic Bacteria for Medical Diagnosis

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The term “microbiological diagnostics” refers to a search for and identification of an etiological factor of an infectious disease. Identifying a microorganism that causes a given pathological state followed by defining this organism’s sensitivity to drugs serves as the first and necessary condition for controlling a disease and treating a patient. The identification of a pathogen that induces an infectious disease is the prerequisite of beginning a successful treatment.

The bacterial pathogen *Staphylococcus aureus* is responsible for a significant amount of human morbidity and mortality. Novel methods, based on capillary zone electrophoresis (CZE) and molecular analysis of a part of the *coag* gene were designed for the identification and the differentiation of three *S. aureus* strains. The electrophoretic measurements rely on the differential mobility of bacteria in the fused silica capillary under the direct current electric field. To perform coagulase gene typing, the repeated units encoding hypervariable regions of the *S. aureus* gene were amplified by polymerase chain reaction (PCR) technique followed by restriction enzymes digestion and analysis of restriction fragment length polymorphism (RFLP) patterns as well as sequencing. Proposed procedures, specially fast and cheap CZE, with molecular analyses as the confirmation of these results, could become an effective tool for diagnosis of certain diseases caused by different strains of *S. aureus*. Finally, the results of electrophoretic measurements with molecular analysis were compared. The results presented in this report give a sufficient and real grounds to conclude that capillary zone electrophoresis (CZE) could be a novel, fast and cheap method of identification and typing of bacterial strains. However, future investigation are necessary to improve this method and create database.

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## ***S09: Separation Technologies***

**Some People and Places Important in the History of Analytical Chemistry in Austria**

D.Burns

*The Queen's University of Belfast*

Abstract is not available

## **S09: Separation Technologies**

### **Kinetic Optimization of External Contributions to Speed and Efficiency in Fast Ultra-High Resolution HPLC – Theoretical and Experimental Considerations**

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Fast, high-resolution separations in HPLC can be achieved by the use of small particles (1.5 – 3 µm in diameter) usually packed in columns of 2.1 mm id with modern HPLC instrumentation supplying backpressures on the order of 1000 bar.

In order to fully utilize the performance of such a system, the external contributions that compromise separation efficiency have to be carefully taken into consideration.

To experimentally assess the contributions to external variance, a “zero-dispersion” system was designed that allows to individually measure the dispersion of different system components such as injector, capillaries and detection cells as a function of flow rate. Peak variances resulting from a range of system components were determined and compared to theoretical predictions.

The kinetic plot model including external contributions (1, 2) was extended to allow estimation of optimum capillary dimensions as a function of particle size and morphology, column dimensions and maximum system pressure.

Application examples in isocratic and gradient mode will be shown to demonstrate the importance of optimized system components.

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## S09: Separation Technologies

### Thermo-Responsive Stationary Phases – Synthesis, Application and Chromatographic Evaluation

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Polymers which are sensitive towards external physical, chemical and electrical stimuli are termed as “intelligent materials” and are presently widely used in medical and engineering applications. Among these factors, it seems that temperature is one of the most attractive tools as a stimulus.

One of the most commonly used thermo-responsive polymers is Poly(N-isopropylacrylamide) (PNIPAAm). PNIPAAm exhibits thermally reversible soluble-insoluble changes in aqueous solution in response to temperature changes across a lower critical solution temperature (LCST) at 32°C.

Alternative co-polymers exhibiting similar LCST behaviour are Poly(isopropylloxazoline-co-N-propylloxazoline) P(IPOX-co-NPOX) and Poly(2-(2-methoxyethoxy)ethyl methacrylate-co-oligo(ethylene glycol) methacrylate) P(MEO<sub>2</sub>MA-co-OEGMA). A huge advantage of co-polymerization is the possibility of tuning LCST in water over a wide temperature range based on varying hydrophobic-hydrophilic monomer composition via a well-defined gradient.

Here, we compare the properties of the above mentioned co-polymers to the ‘golden standard’ PNIPAAm. Thus, we report on the synthesis of thermo-responsive stationary phases by grafting these polymers onto bimodal pore size distribution silica surface and their application for the selective separation of steroids and peptides in pure aqueous environments using HPLC with temperature control. This serves as a good alternative to conventional reversed-phase chromatography, in which the denaturation of proteins in organic solvents can be avoided.

Characterization of the stationary phases with respect to grafting density, temperature and molecular weight are reported. Furthermore, chromatographic characterization regarding the separation of a mixture of steroids and peptides below and above the LCST in pure aqueous phase and under isocratic conditions is also presented.

Macromolecular engineering can also be done on the stationary phase to optimize its response towards different kinds of external stimuli. We also show possibilities of other polymeric monolithic materials synthesized for proteomics.

## S09: Separation Technologies

### Stationary Phase Design for Ion-Exchange Chromatography – From Standard Inorganic Anions and Cations to Amino Acids and Proteins

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In the more than twenty years that encompass its birth and development, ion chromatography (IC) has undergone enormous changes. While in its earliest embodiments IC was focused primarily on the analysis of inorganic anions and cations, today IC has an important role in the analysis of organic ions as well.

One of the trends in stationary phase design is the development of hydroxide-selective ion exchangers for isocratic and gradient separations. Based on ethylvinylbenzene/divinylbenzene, the resulting resins can either be surface-functionalized and agglomerated with micro-beads carrying the actual ion-exchange groups or grafted with an anion exchange polymer layer. Other trends include the use of high-capacity columns which allow the injection of more concentrated samples without overloading and peak broadening. High-capacity anion exchangers are developed for isocratic separations of inorganic anions and oxyhalides in drinking water and other sample matrices as well as for gradient separations of inorganic and organic anions improving the separation of monovalent carboxylic acids. Lately, particle diameters of the support materials and column dimensions have been scaled down resulting in significantly shorter analysis times without compromising resolution and chromatographic efficiency.

By now, the merits of hydroxide eluants in anion exchange chromatography are well understood. However, hydroxide-selective stationary phases have been prepared by using vinyl aromatic polymers. Such polymers are advantageous because they are compatible with highly alkaline eluants. While hydroxide-selective anion exchangers prepared in this way show good selectivity for common anions, the interaction of analytes such as bromate, chlorite, chlorate, and perchlorate with the  $\pi$  electrons of the aromatic backbone is an impediment to obtaining the proper selectivity in the presence of common anions. Consequently, search was undertaken for a suitable means of preparing hydroxide-selective stationary phases from entirely aliphatic components.

Ultimately, a novel electrostatic step-growth condensation polymerization method was developed using aliphatic epoxy monomers and amines. Unlike commonly employed grafting methods, where the attachment of the graft polymer is via covalent bonds, the attachment of the graft polymer in this case is the result of strong electrostatic adhesion between the cationic condensation polymer grown directly off the substrate surface and anionic surface groups introduced through sulfonation of the substrate prior to the graft step. The resulting graft composite exhibits a high degree of hydroxide selectivity, good stability under alkaline conditions, high hydrophilicity, and excellent chromatographic properties. By tailoring the reagents used in preparation of the step-growth polymer as well as the reaction conditions, a wide variety of different selectivities are obtained.

The latest trend in stationary phase design for ion chromatography is leading towards monolithic ion exchangers. Originally developed for the separation of large biomolecules such as peptides and proteins, a breakthrough is made in the development of polymer-based monoliths for the separation of small molecular weight anions, which opens the door for high throughput anion analysis.

## **S09: Separation Technologies**

### **Evaluation of Ion Pairing Chromatography versus Hydrophilic Interaction Chromatography for the Determination of Neurotransmitters in Brain Extract by LC – MS/MS**

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As neurotransmitters, catecholamines play an important role in the control and regulation of numerous brain functions. They are also believed to be involved in different neurodegenerative disorders. Due to their high interest and their low level in biological samples, many efforts have been devoted to devise simple and sensitive analytical methods for their determination.

In this communication two new complementary LC-MS/MS methods using IP-RPLC and hydrophilic interaction chromatography (HILIC) are proposed as alternatives to traditional LC methods for the analysis of catecholamines (adrenalin, noradrenalin, dopamin), indolamines (serotonin and 5 hydroxy tryptophan) and their precursors and metabolites (3,4-dihydroxy-phenylalanin, 3-methoxytyramin, tryptophan, homovanillic acid, tyrosin and 5-hydroxyindole-3-acetic acid) in brain tissues.

First, IP-RPLC method is developed by using nonafluoropentanoic acid as volatile ion-pairing reagent and C18 monolith and C18 fused core as stationary phase.

Secondly, a HILIC method is optimized using different commercially available columns, which can be classified in relation with their functional group as neutral (diol, amide, and cyano), positively charged (amino, triazole), negatively charged (bare silica as wholly porous particles or fused core particles) and zwitterionic (sulfobetaine). Our studies lead us to a better understanding of the HILIC retention mechanism and also to the selection of the most appropriate column for catecholamine analysis.

The two complementary chromatographic systems are then compared in terms of resolution, efficiency, limits of detection and quantification limits (LOD/LOQ), advantages and disadvantages. Only the HILIC system was compatible with both positive and negative ionization modes.

As the LODs obtained are in the range of 1-100 ng.mL<sup>-1</sup> for the two systems, a pre-concentration method using Oasis MCX and Oasis HLB solid phase extraction cartridges was optimized in order to enhance the LODs.

Finally the applicability of the SPE-LC-MS/MS method has been evaluated for the identification of these compounds in brain extracts.

## **S09: Separation Technologies**

### **Application of Rotating Coil Column in Analysis of Different Samples from Environmental to Crude Oil**

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Rotating coil columns (RCC) mainly used in counter-current chromatography can be successfully applied as a pretreatment technique in analysis of different samples (liquid and soil).

A particulate solid sample is retained in the column as the stationary phase under the action of centrifugal forces while organic solvents are continuously pumped through. The dynamic extraction of hydrocarbons impurities from soil samples is performed in RCC at room temperature and normal pressure. Recovery of hydrocarbons from soil samples with different organic solvents has been investigated.

Firstly application of RCC for elemental oil analysis has been suggested. Nowadays international standard methods of elemental oil analysis (ASTM D, IP, EN ISO, UOP) could be characterized by complex sample preparation procedures, insufficient detection limits and confined number of defined elements. The possibility of pre-concentration of the trace elements from oils by RCC has been investigated. Aqueous solutions of different compositions were suggested to use as a stationary phase. Analyzed oil samples were used as a mobile phase that gives a possibility to pre-concentrate trace amount of inorganic compounds in fixing volume of the stationary phase from large volume of oil. Retention volume of the stationary phase in rotating coil columns could reach to 90% of its total volume. It has been shown that the concentrating factor of elements from oils is dependent on the properties of the sample used, partition coefficients of inorganic elements to be pre-concentrated and parameters of the planetary centrifuge operation. The combination of intense extraction trace elements by RCC and high sensitivity determination method (ICP-MS) for quantitative detection of the trace elements in oil (without any additional sample preparation) has been suggested. Examples of trace elements determination in different oils using new approach have been shown.

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## **S09: Separation Technologies**

### **Organic Metabolites in Paper – an Approach for Identification of Biomarkers in Aged Books**

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In order to contribute to preserve and save portuguese paper-based cultural heritage, the ageing of paper and cellulose is being studied to better understand the mechanisms behind the degradation. The study has additional importance because, as a result of cultural tradition in portuguese families, almost everyone has paper documents (old school books and drawings, photos, old patrimony documents (deeds), old books, yellowed newspaper, bank notes, etc.) they want to keep as mementos.

Old paper used to be made from cotton rag. Early paper is made from wood pulp. Cotton, pure cellulose is comprised of a inner network of microfibers randomly organized within a mixture of waxes, pectins, proteins and other non-cellulosic materials. Wood pulp is made from cellulose (~50%) but it also contains other polymers (hemi celluloses and lignin) and a number of substances, such as rosin, starch, gelatine, alum that are added in the paper making process[1].

Paper and books undergo degradations upon their ageing. Recent investigations have shown that volatile organic compounds (VOC's) emitted upon ageing may be used to follow and evaluate the process[2].

Using SPME-GC-MS to the study of VOC's emitted from paper and books, profiles of biomarkers were identified. The methodology allows to determine the ageing of paper, its origin (cotton or wood) and its storage conditions.

In order to preserve and recognise cultural heritage, an innovative non-destructive methodology was developed using profiles of biomarkers.

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## S10: Microseparations

### Keynote lecture 10

#### Comparison of Ion Chromatography and Portable Capillary Electrophoresis for Identification of Improvised Inorganic Explosives Used in Terrorist Attacks

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Ion chromatography (IC) and capillary electrophoresis (CE) are used routinely for the determination of inorganic anions and cations. Both techniques have their inherent strengths and weaknesses. In this presentation, IC and CE are compared in terms of their stage of development, separation efficiency, separation selectivity, analytical performance parameters, method development procedures, applications, and adoption into regulatory methods.

Throughout this comparison, the techniques will be evaluated in the context of their use for the determination of inorganic anions and cations in the identification of inorganic improvised explosives. These explosives are formed from commonly available ingredients, such as fertilizers, and have been used extensively in acts of terrorism. Current counter-terrorism measures require rapid and reliable detection of these improvised explosives both prior to detonation (pre-blast) and after detonation (post-blast). The primary aim of pre-blast identification is to detect the explosive in situations such as airport screening, while the primary aim of post-blast identification is to determine the identity of the particular explosive used in order to assist in apprehension of the perpetrators. Both situations require reliable and rapid means of analysis using methods which can be operated by relatively unskilled personnel and in field-based locations.

A detailed survey of improvised explosives has been undertaken and a suite of 15 candidate inorganic anions and 13 candidate cations has been established for post-blast fingerprinting. The separations of each of these groups of ions has been developed using IC and CE, with an emphasis on the use of instrumentation which is field-deployable or portable. To this end, miniaturised columns and robust detection methods, such as indirect spectrophotometric detection using light-emitting diode detectors have been employed. These separations have been applied to traces of the explosives and also to post-blast explosive residues and have been evaluated for their capacity to unequivocally identify the particular explosive used.

## **S10: Microseparations**

### **Characterization of Recombinant Allergen Products with Capillary Electrophoresis**

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Currently more than 80 million patients are affected by allergies in Europe. Air-borne allergens are a prominent source of allergic disorders with the proteinaceous major birch pollen allergen Bet v 1 representing one of the major triggers. High-quality products of target allergens are indispensable for an unambiguous diagnosis of the disease eliciting allergen isoform as well as for a specific immunotherapy. Previously used ill-defined allergen extracts have been replaced by recombinant products meanwhile, but the latter might still contain process- and product related impurities introduced during the manufacturing. Therefore, products have to be subjected to a stringent quality assessment according to the ICH-guideline Q6B, which recommends a portfolio of complementary methods for the characterization. However, separation systems combining exceptional selectivity and minute sample consumption, such as capillary electrophoresis, have rarely been implemented in this context.

Beside several CZE and CIEF methods, a novel affinity-CIEF approach, which applied in-lab produced monoclonal antibodies (mAbs) against Bet v 1a, has been developed to tackle both physico- and immunochemical characterization of recombinant Bet v 1a products. To overcome allergen adhesion to the silica surface tetraethylenepentamine was applied in CZE for a dynamic capillary coating and a simultaneous improvement in the separation of the target isoform and coexisting impurities, this way revealing a complex constituent profile. In addition, a novel successive multiple ionic polymer layer (SMIL) coating was developed which possesses semi-permanent character and allows for exceptional separation stability. Furthermore, CIEF has been tailored to the allergen product by employing a complex mixture of carrier ampholytes. The final immunochemical characterization addressed the confirmation of the epitope integrity by affinity-CIEF. Characterization of mAbs was by MALDI-TOF-MS, SDS-PAGE and CIEF. Incubation of allergen products with mAbs was either prior to sample injection or performed directly in the separation capillary by partial filling.

## **S10: Microseparations**

### **Contactless Conductivity Detection for Microseparation Techniques**

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Capacitively coupled contactless conductivity detection (C<sup>4</sup>D) has gained considerable popularity for miniaturized analytical systems over the last few years. The method generally allows the facile detection of all charged analytes with good sensitivity, including in particular those species which cannot be quantified with optical means. The detector has been thoroughly investigated and a comprehensive understanding of the fundamental properties has been reached. Commercial devices which have been optimized in accordance to these findings are now available and can be retrofitted to existing instruments.

A range of projects based on C<sup>4</sup>D are presently carried out in our laboratory.

Applications of contactless conductivity detection in capillary electrophoresis (CE) are further explored. These include the separation of cationic and anionic enantiomers, the determination of native inorganic and organic blood electrolytes, the clinical analysis of illicit drugs as well as therapeutic drug monitoring (TDM). The monitoring of enzymatic reactions is also investigated, including the study of acetylcholinesterase inhibitors, and the electrophoretically mediated microanalysis (EMMA) approach.

A field portable CE-instrument has been designed and tested, and an automated system based on a sequential injection analysis (SIA) manifold is investigated for unattended monitoring operations in environmental or process analysis.

Contactless conductivity detection is furthermore suitable for detection in chromatographic separations. The combination with capillary electrochromatography (CEC) using miniature monolithic columns is investigated as well as the detection in miniaturized pumped HPLC and ion-chromatography.

## S10: Microseparations

### Capillary Electrophoresis: Important Tool for Clinical, Toxicological and Pharmacological Applications

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The main objective of this research is the separation of nine cholinesterase inhibitors by use of capillary electrochromatography (CEC) and micellar electrokinetic chromatography (MEKC). Cholinesterase inhibitors [rivastigmine, edrophonium chloride, pyridostigmine bromide, neostigmine bromide, galanthamine, eserine or physostigmine, methylphysostigmine, eseroline fumarate, and 1,5-Bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide] are a class of drugs approved by the U.S. Food and Drug Administration to treat Alzheimer's disease (AD) and Myasthenia Gravis (MG). In both CEC and MEKC, different polymers and polymeric surfactants are used for optimizing the separation conditions. Additionally, in this study, the open-tubular (OT) mode of CEC is used, in which fused-silica capillaries coated with thin films of physically adsorbed charged polymers are developed by use of a polyelectrolyte multilayer (PEM) coating procedure. The PEM coating is constructed *in situ* by alternating rinses of positively and negatively charged polymers. In addition, MEKC is used for the chiral separation of huperzine A. Huperzine A is an important compound used to treat AD. However, only the (-) form of this compound is biologically active, while the (+) form can inhibit the activity of the (-) form. Therefore, the separation of the (-) form from the (+) form of huperzine A is of greatest importance, particularly in the pharmaceutical industry. Finally, optimum conditions are applied to blood samples in order to establish the ability of the methods to separate the drug compound rivastigmine from all the other components that might exist in the blood sample.

## S10: Microseparations

### Scanning Contactless Conductivity Detection: Applications in the Characterisation of Modifications to Stationary Phases in Capillary Formats

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In the creation of novel stationary phases the ability to characterise these phases has generally fallen into two areas, destructive and indirect. Destructive characterisation involving techniques such as scanning electron microscopy and electron probe analysis gives detailed information about the stationary phase, however the particular column analysed is lost. Indirect characterisation, involving chromatographic evaluation of the column, leads to only information about the column as a whole.

Capacitively coupled contactless conductivity detection (C<sup>4</sup>D) affords the ability to both spatial and temporally characterise the column under evaluation. With the ability to place the detector directly on-column and vary the position along the length of the column, i.e. scanning the column, an insight into the column environment can be achieved. In the work presented here, the use of scanning C<sup>4</sup>D (SC<sup>4</sup>D) in the characterisation of surfactant modified capillary silica monoliths will be shown. This use of SC<sup>4</sup>D on-column allows the analyst to visualise the changes in stationary phase chemistry in a non-contact, non-destructive and non-invasive manner. The coating and subsequent washing of non-specifically bound surfactant can be easily profiled using SC<sup>4</sup>D, showing the equilibration process involved. In the production of polymeric capillary monolithic columns, the use of SC<sup>4</sup>D to interrogate the photografting of a series of monomers will be shown. The ability to spatially locate and evaluate the photografted zones allows for a deeper understanding of the processes involved and the effect of photografting on the chemistry of the stationary phase. The effect of varying the photografting energy can be easily interrogated by SC<sup>4</sup>D, with a direct correlation of SC<sup>4</sup>D signal to capacity of the column. The ability to scan the column repeatedly allows for the stationary phase chemistry to be profiled under changing conditions, such as pH. This process was utilised to perform on-column titrations of immobilised functional groups directly (e.g. IDA).

## **S10: Microseparations**

### **Capillary Electrophoresis Coupled with Mass Spectrometry for Melamine and Cyanuric Acid Detection in Food**

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Melamine is a toxic compound that has been found in several kinds of food. Its addition is a fraudulent way to enhance food protein content. Many cases of renal complications in infants, due to the ingestion of contaminated food have been reported in 2008. Since then, the need for new analytical methods able to detect very low amounts of melamine in foodstuff gave rise to several applications involving different techniques.

Melamine toxicity seems to increase in combination with cyanuric acid. Therefore, our work has been focused on the development of a new rapid method for the evaluation of both melamine and cyanuric acid in food products by capillary electrophoresis coupled with mass spectrometry.

Since melamine is a positively charged molecule in a wide pH range, a choice of employing an acidic buffer has been made to avoid its interaction with the inner capillary surface, while cyanuric acid at acidic pH is a neutral molecule which migrates with the electroosmotic flow. The dependence of analyte mobilities on the pH of running buffer and electroosmotic flow has also been evaluated and will be discussed. Mass spectrometry detection has been realized by Ion Trap after electrospray ionization in both the positive ion mode (for melamine) and negative ion mode (for cyanuric acid).

A calibration curve has been built for quantitative determination of the analytes in the range between 0.1 and 10 µg/g, by monitoring a selected transition for each compound. Evaluation of method performance, in terms of LOD, LOQ, linearity and recovery has been carried out. Analyses on real samples have been performed and demonstrated the suitability of the methods to evaluate little amounts of melamine added to food products, in short analysis time; therefore, our method could be proposed as a quality control test for food safety.

# **S11: Quality Assurance/Reference Materials**

## **Keynote lecture 11**

### **Certified Reference Materials (CRMs) for Environmental, Clinical, Food, and Nutritional Assessment Measurements**

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Reliable healthcare, a clean environment, and safe and nutritious foods significantly affect the quality of our lives. The quality (accuracy) of chemical measurements that underpin these three domains and ensure their integrity is critical to our health and well-being. Certified Reference Materials (CRMs) play an essential role in the validation of analytical methods, as control materials to assure measurement quality, and as tools to provide metrological traceability to national and international standards. For nearly four decades the National Institute of Standards and Technology (NIST) has provided natural matrix Standard Reference Materials (SRMs), which are CRMs produced by NIST, to assist in assessing and improving the quality of environmental, clinical diagnostic, nutritional assessment, and food composition measurements. Recent SRMs have been developed to address measurement quality needs associated with food and dietary supplement labeling and safety requirements, clinical diagnostic testing, and national human environmental exposure and nutritional assessment studies.

To address measurement needs related to food safety and nutritional labeling, NIST has developed food matrix SRMs with values assigned for nutrient and toxic elements, vitamins, cholesterol, fatty acids, and total fat content. For the expanding dietary supplement market, dietary supplement matrix SRMs have been developed, or are in progress, including ephedra, *ginkgo biloba*, saw palmetto, carrot extract, bitter orange, plant oils, multivitamin/multielement tablets, green tea, berries, soy, kudzu, and clover. In the area of clinical diagnostic testing, serum-based SRMs have been provided for the past 30 years for health status markers such as serum electrolytes, cholesterol, glucose, urea, uric acid, and creatinine. Recent SRMs for clinical diagnostic testing include troponin I, homocysteine, folate, and steroid hormones. Recent interest in clinical nutritional assessment has driven the development of SRM for vitamins D, B<sub>6</sub>, and B<sub>12</sub> in serum. SRMs to support measurements of human exposure to environmental contaminants include human serum and milk for over 100 organic contaminants, air particulate matter for organic and inorganic contaminants, and urine for trace elements including speciated arsenic. The analytical challenges in the development of these SRMs to support chemical measurements in these critical “quality of life” areas and the potential impact of these SRMs will be discussed.



## **S11: Quality Assurance/Reference Materials**

### **From Genetic Testing to Protein Quantification – Reference Materials in Clinical Analysis**

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Results of clinical analysis are providing crucial information for the diagnosis and treatment monitoring of patients. Therefore, they should be as reliable as possible. But many of the targeted analytes are complex molecules or parts thereof and the clinical relevance of them is primarily related to their functionality rather than to a total amount of molecules or their mass. Consequently the parameters of interest are large operationally defined. Therefore, international efforts driven, for instance, by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) are focused on the establishment of globally accepted Reference Measurement Systems consisting of a combination of reference methods, reference laboratories and reference materials. The latter will be the focus of this presentation. IRMM has developed together with IFCC a number of reference materials which are serving either as primary calibrants or as crucial quality control materials for the analysis of proteins, enzymes and other clinical parameters. Moreover, the needs for an appropriate level of quality assurance in the emerging area of human genetic testing require new approaches for the design and certification of reference materials. In that case the emphasis has to be on a qualitative rather than a quantitative analysis. An update on metrological principles and the state-of-the-art of reference materials for these application areas will be presented.

## **S11: Quality Assurance/Reference Materials**

### **Investigation of Out-of-Specification Results**

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Out-of-specification (OOS) test results in pharmaceutical industry are results that fall outside the specifications or established acceptance criteria. Identifying OOS test results is described in FDA Guidance for Industry "Investigating OOS Test Results for Pharmaceutical Production" (2006). Measurement/analysis/test results obtained in other industries and such fields as environmental analysis, which do not comply with regulatory or specification limits, can be named also as OOS test results. When the compliance assessment is made on the basis of a measurement result accompanied by information on the uncertainty associated with the result, the rules developed in the EURACHEM/CITAC Guide "Use of Uncertainty Information in Compliance Assessment" (2007) are applicable.

When an OOS test result is identified, important is to determine its root causes: to avoid any repetition of the situation when appearance of a next OOS test result is possible or even inevitable. Such investigation based on metrological concepts could be helpful. It should include: 1) assessment of validation data of the measurement/analysis/testing process; 2) evaluation of the measurement uncertainty components; and 3) assessment of traceability chains important for measurement parameters and environmental conditions influencing the test results. Guidelines for this investigation are under development by a CITAC and IUPAC joint project.

## ***S11: Quality Assurance/Reference Materials***

### **Analytical Chemistry and Quality Assurance for Decision Making**

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An uncomplicated method to estimate uncertainty and quality of measurement is presented. In the search for the origin of discrepancies between results of interlaboratory testing published by the IRMM of the European Commission, several series of experiments were performed including many repetitions. The search for the origin of contradictory results and incompatible uncertainties is important because results of analytical chemistry founds the basis for decision making in research and in routine tasks of health care, forensics, food safety, energy, resources and environmental monitoring.

Surprisingly large variations were observed among results on samples and methodologies that are considered uncomplicated. The experiments include measurements by ICP-MS, flame-AAS (FAAS) and graphite furnace-AAS (GF-AAS). It was thus found that expected results on certified reference materials were reproduced at appreciable frequencies. However, it was also found that significant deviations with respect to the certified values were abundant and an estimate of reliable uncertainties was obtained only after a high number of repetitions. Results of *intralaboratory* testing was evaluated as a method to estimate uncertainties that are comparable to uncertainties obtained by *interlaboratory* testing and to uncertainties predicted by the Horwitz curve. To a large extent, the uncertainty of measurement predicted by numerous results of calibrations corresponded to the uncertainties on multiple determinations of unknowns. These investigations showed that a profound deviation between the concepts of precision and accuracy prevails in instrumental analysis. It was thus proposed that a large proportion of the difference in uncertainty of measurement between laboratories may be explained by properties of the appropriate detectors. In conclusion, it is suggested that a generally higher level of uncertainty ought to be accepted in the laboratory. The importance of the law of great numbers to reliability and to the law of propagation of errors is discussed.

## **S11: Quality Assurance/Reference Materials**

### **Certified Reference Materials to Support European Air Quality Legislation – Current Status**

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The EU Air Quality Framework Directive 96/62/EC and its Daughter Directives, specifically 1999/30/EC and 2004/107/EC, regulate aspects of PM<sub>10</sub> (a particulate matter of 10 µm and less aerodynamic diameter) in ambient air. Laboratories in the EU Member States are required to monitor concentrations of arsenic, cadmium, nickel, lead and several polycyclic aromatic hydrocarbons (PAHs) in PM<sub>10</sub> to verify compliance with target and limit values set in the directives. Therefore, appropriate quality control tools are needed to ensure the quality of measurement data. Certified Reference Materials (CRMs) and proficiency testing schemes are such essential tools for analytical quality control and are required for checking of laboratory proficiency and data comparability.

Currently, no suitable CRM with certified contents of the aforementioned analytes in a matrix that would sufficiently resemble airborne particulate matter (PM<sub>10</sub>) is available. Therefore, a feasibility study on such CRM production was carried out at the Institute for Reference Materials and Measurements (IRMM) with support of the European Commission Directorate General for the Environment and the European Network of National Reference Laboratories, AQUILA in 2005 – 2007. The positive outcome from that study enabled IRMM to start the preparation and certification of two CRMs, Fine Dust (PM<sub>10</sub>-like), to be certified for the content of selected PAHs and elements, respectively.

During these projects a number of challenges had to be tackled. One difficulty was the collection of a large quantity (~ a few kilograms) of the material in a short time. Moreover, such a material should have particle size distribution close to PM<sub>10</sub>, be homogeneous and with realistic analyte content. Some of the approaches studied to solve the problems, selected results and the current status of the projects will be presented.

## **S11: Quality Assurance/Reference Materials**

### **Mass Spectrometry Screening Based Method for the Determination of Pesticide Residues in Food. Experience Gained through European Proficiency Test**

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The international trade in fresh fruits and vegetables is an important issue within the European Union. For this reason, a large number of well known and frequently applied pesticides have been banned in the European Union as a consequence of Directive 91/414/EEC ([Council Directive, 1991](#)) and ([Regulation EC, 2005](#)). However, as a consequence of the lack of international harmonization due to diverse Good Agricultural Practices (GAPs) applied by countries around the world - as well as different additional EU limitations on the use of pesticides - a great number of pesticides which are banned in Europe, are still allowed in third countries. Moreover, the misuse of pesticides under EU regulations is not only related to third countries but also to bad application of the EU GAPs established within Europe itself. Recent alerts reported by European countries have pointed out serious problems related to the presence of illegal or misused pesticides in fresh crops.

Current analytical methodologies applied to pesticide residue food control are based on the concept of “target analysis”, where liquid or gas chromatography-tandem mass spectrometry (GC-MS/MS, LC-MS/MS) in the selected reaction monitoring mode (SRM) are very powerful techniques and currently the primary choice in food control laboratories.

However, this approach, taking into consideration that there are around 800 compounds present on the market, can overload the capabilities of many food control laboratories. Therefore, other possibilities become necessary in many cases. One efficient strategy is to apply mass screening procedures based on the new mass technologies available.

In this work, we present the results obtained by the application of such screening methods as well as the results of the first European Proficiency Test to evaluate the effectiveness of that procedure.

#### **Acknowledgments**

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## **S12: Bioanalysis 2**

### **Keynote lecture 12**

#### **Antibodies as Molecular Tools for Bioanalytical Methods**

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Modern analytical chemistry is playing an increasing role in many fields, e.g., in clinical diagnostics, toxicology, pharmaceutical analysis, and environmental and food monitoring. There are continuous ongoing efforts to develop rapid, low-cost, simple and reliable tests, that could also be automated, or carried out on-site or in the field. Amongst currently emerging techniques, significant attention has been given to bioanalytics, and especially to the development of new rafts of immunological techniques.

This presentation will cover the techniques used to produce highly specific and affine antibodies against a range of differing target analytes. Whilst polyclonal (pab) and monoclonal antibodies (mab) remain the dominant binders used for many applications, new recombinant antibody (rab) techniques now provide exciting possibilities, allowing for example, more efficient manipulation of antibody binding sites. Moreover, artificial nucleic acid ligands – the aptamers – are gaining increasing interest as new recognition reagents, Immunoassays come in a diverse range of formats, and ELISAs are currently the most popular. However, as demand grows for even shorter analysis times and more user-friendly assays, other formats are also being explored. A major disadvantage of many current immunoassays (compared with separative techniques) is that they commonly have limited multianalyte capabilities. This is being addressed using multiplexed assays with immuno biochips (microarrays) or encoded microspheres (bead-based microfluidic assays). Despite historic achievements in the field of label-free assays (both optical and non-optical), labelling techniques will continue to play a leading role in bioanalytics. In addition to enzyme and fluorescence based labels, improved performance immunoassays using artificial particulate marker systems (organic and inorganic), are of increasing interest since they may permit, for example, reduced detection limits and signal amplification. Further, recent research is also increasingly looking to develop innovative and powerful novel biofunctionalized nanometer-sized silica particles, the properties of which can be tightly controlled and tailored in a very predictable manner to meet the needs of specific applications.

## **S12: Bioanalysis 2**

### **New Format of Non-Instrumental Tests: Immunoaffinity Pre-Concentration and Immunochemical Detection**

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An increasing of quantity and variety of food and environment toxicants demands development of rapid screening techniques which could be used outside the laboratory environment, at the place of sampling. The main group of non-instrumental rapid tests is immunoassay based: these methods use high specificity and affinity of antibody as the main “driving force”. The common formats of immune-tests use membranes as solid supports (e.g. dipsticks, lateral flow and flow-through tests). However membrane application has limitations for volume of extracts or samples, hence restricting sensitivity. Also in the case of high-coloured extracts visual evaluation of results could be impeded.

Gel-based immunoassay in column format was developed as an alternative approach to eliminate the above-mentioned constraints by combination of clean-up, pre-concentration and visual detection in one simple set up. The assay was performed in transparent columns with detection layer with specific antibody. The duration of the procedure was about 20 min for 6 samples. The assay was combined with a simple rapid extraction procedure and validated in accordance with EU maximum levels (for mycotoxins in food and feed, polycyclic aromatic hydrocarbons in water).

Advantages of the tests are combination of pre-concentration and detection in one column. This allowed to reach very low (for non-instrumental methods) cut-off level of assay. For example, combination of the pre-concentrating function of the IAC this competitive direct ELISA determination enables to detect benzo[a]pyrene in water samples with cut-off level at 5 ng/l. An application of the additional columns with the clean-up layer allowed us to detect mycotoxins in high-coloured samples like wine, beer, spices, coffee, etc.

## **S12: Bioanalysis 2**

### **New Stochastic Microsensors Based on Nanostructured Materials for Molecular Diagnosis**

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The material mostly used for the design of stochastic sensors is hemolysin which is a very expensive biological material. Therefore, we proposed for the design of the microsensors nanostructured porphyrins which successfully substituted hemolysin. The porphyrins were physically immobilized in either diamond or carbon paste. Chronoamperometry was used for all measurements. The behaviour of the proposed sensors proved the nanostructured quality of the porphyrins. The sensitivity and reliability of the stochastic microsensors based on diamond paste was compared with those of the stochastic microsensors based on carbon paste. The linear concentration ranges of the proposed microsensors are between  $10^{-12}$  and  $10^{-4}$  mol/L; the regression coefficients for the calibration graphs are higher than 0.9000. The new microsensors can be reliable used for qualitative and quantitative assay of different biomarkers at molecular level.



## S12: Bioanalysis 2

### Automatic Determination of Antioxidant Capacity against Biologically Relevant Species Using Flow Injection Systems

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The formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been clearly implicated in the oxidative deterioration of food products as well as in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer [1]. Considering the protective effects of antioxidants against the deleterious oxidative induced reactions involved in these pathologies, interest in antioxidant research has become a topic of increasing attention in the last few years. Hence, the existence of simple, convenient, and reliable in vitro analytical methodologies for the fast determination of antioxidant capacity of pure compounds or in complex matrices, such as foods and biological samples, is essential to this research field. In this context, flow injection techniques are an excellent tool to automate these analyses [2, 3].

Flow Injection Analysis (FIA) was proposed in 1975 by Ruzicka and Hansen as an automation tool for chemical analysis. After 34 years, this technique is well-known and accepted by the scientific community, and it also allows the performance of assays that are not feasible when carried out manually, by taking advantage of the reproducible timing attained in these systems. In the present communication, the state of the art about automatic flow-based methods for antioxidant assessment will be highlighted. Special emphasis will be given to the specific features of existing flow injection based systems for determination of scavenging capacity against biologically relevant reactive species of oxygen and nitrogen. Several features will be compared, including the analytical figures of merit, the application to real samples and the “chemistry” behind the assays. Perspectives about novel assays and current lab work will also be discussed.

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## **S12: Bioanalysis 2**

### **Characterization of Solid Nanoparticles by Capillary Electrophoresis: a Step in the Development of a New Method for Immunodiagnosics**

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The use of nanoparticles (NPs) in immunodiagnosics is a challenging task for many reasons, among which the need for miniaturization. In view of the development of a microsystem dedicated to an original, miniaturized and fully automated immunodiagnosics which aims to mimic in vivo interactions, magnetic zwitterionic bifunctional aminated / polyethyleneoxide maghemite core / silica shell NPs of adequate diameter (15 to 50 nm), functionalized with allergenic  $\alpha$ -lactoglobulin, were synthesized. Capillary electrophoresis (CE), which has been recognized as a valuable technique for the physicochemical characterization of colloidal particles, was used for their characterization. Proper analytical performances were obtained through semi-permanent capillary coating with didodecyldimethyl-ammonium bromide (DDAB) or permanent capillary wall modification by hydroxypropylcellulose (HPC). First, the bifunctional NPs (non-grafted NPs) were characterized by CE, enabling to establish a direct correlation between particle electrophoretic mobility and surface amino group density. The influence of experimental conditions (e.g. buffer component nature, pH, ionic strength, and electric field strength) on sample stability, electrophoretic mobility and dispersion, was investigated using either DDAB- or HPC-coated capillaries. Adsorption to the capillary wall and aggregation phenomena were evaluated according to the CE conditions. The proper choice of experimental conditions finally allowed the separation of the grafted and the non-grafted NPs.

The financial support for this project by Agence Nationale pour la Recherche (ANR) is gratefully acknowledged.

## S12: Bioanalysis 2

### Is there a Significant Effect of Type of Weight-Loss Strategy on Changes in Trace Mineral Serum Concentration? A Randomized Control Trial

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**Background:** Trace elements are essential to living organisms in very small quantities. Appropriate intake levels of each dietary mineral must be sustained to maintain health. We aimed to investigate the effect of weight loss on levels of Magnesium, Zinc and Copper in the serum of the participants of 3 diet types.

**Methods:** Two-year changes in the concentration of the trace elements in blood serum were followed in a dietary intervention randomized control trial (DIRECT), that was conducted in the Nuclear Research Center-Negev (NRCN) in Israel, among a sub-group of 150 participants that were randomly assigned to low-fat, Mediterranean or low-carbohydrate diet. Inductively coupled plasma mass spectrometry (ICPMS) was used for the determination of 7 trace and ultra-trace elements in normal human serum. To avoid contamination, sample preparation was minimally treated: serum samples were diluted 10-fold with 0.1N HNO<sub>3</sub> and suitable internal standard (Rh) was added to correct for matrix effects (serum) and for ion signal instability. To check the accuracy of the method aqueous standard solutions and comparison samples of biological fluids, obtained from the Centre de Toxicologie du Quebec (CTQ), (blood, serum, urine and hair) were analyzed.

**Results:** Significant changes of Copper concentrations in the serum were found after one year of dieting: from 527±117 µg/liter of Copper at baseline to 1240±370, p<0.05 at 1 year and to 1101±345 after 2 years (recommended values for Copper are 800-1750 µg/liter). All 3 diet programs brought the average concentrations of Zinc and Magnesium to the recommended levels: from 916±177 µg/liter of Zinc at baseline to 984±282 at 1 year and to 930±224 at 2 years (recommended values for Zinc are 700-1200 µg/liter). Magnesium levels were not changed throughout the intervention period: from 1.5±0.2 mg/dl at baseline to 1.5±0.6 at 1 year and to 1.4±0.5 at 2 years of intervention for all three diet participants. Magnesium values in the serum were a bit lower than the recommended values of 1.7-2.3 mg/dl.

**Conclusions:** During a two years intervention period, no advantage was noted to any type of diet over the other, regarding the serum concentrations of trace elements. It might be the result of a universal recommendation to increase vegetable intake, given to all three diet types.

## Plenary Lecture 5

### Plus Lucis: Advanced Waveguides for Enhanced Infrared Diagnostics

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Optical chemical sensors operating in the spectral range of 3-20  $\mu\text{m}$  (mid-infrared) are gaining importance in a diversity of application areas ranging from process analysis, environmental monitoring, and security/surveillance applications, to the biomedical and diagnostic field. The perspective of robust optical sensors providing inherent molecular specificity renders mid-infrared technology among the most promising sensor platforms for addressing complex samples in demanding measurement environments. Given the current advancements in light source technology (e.g., room-temperature operated broadly tunable quantum cascade lasers) and detectors, it appears of paramount importance developing mid-infrared transducers to the same level of maturity for facilitating continuous and highly sensitive direct molecular analysis in the gas and liquid phase [1-5].

Consequently, the advent of appropriate waveguide technology including mid-infrared transparent optical fibers, planar semiconductor waveguides, and novel hollow waveguide structures in combination with either protective or molecularly responsive surface coatings (e.g., diamond-like carbon, sol-gels, polymers, etc.) promises advanced infrared diagnostics applicable at extreme conditions (e.g., deep sea), for label-free biomedical analysis (e.g., in-vivo surgical monitoring), and for next-generation multi-functional analytical platforms (e.g., in combination with atomic force microscopy) enhancing cell physiological studies.

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## S13: Sensors 2

### Keynote lecture 13

#### Functional Screening Based on Optical Transduction

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Optical methods have demonstrated increasing quality in High Throughput Screening during the last decade. In the meanwhile direct optical detection techniques gained necessary technical improvement to compete with the well established methods based on fluorescence labeled assays. However, all these methods lack an essential information giving not only hits on interacting species (sequence specific) but rather identifying interaction partners within the screening run or even supply effect based information. Thus, recent approaches aim at High Content Screening. Hyphenated techniques as well as methods for functional screening will be discussed. Both approaches aim at increasing the information content of the measurements:

A combination of Reflectometric Interference Spectroscopy (RIfS) with MALDI-TOF provides information on binding events and identifies the bound species from complex mixtures at the same time. New interaction partners of virtually any molecule can be discovered and characterized with such a system. A different approach takes advantage of physiological target structures. An example is an assay for screening for kinase inhibitors, which uses antibodies to detect the reaction product.

Recent developments have led to systems that measure the effect on natural receptors directly. Estrogenic activity in river water was assessed by using fluorescence labeled estrogen receptor  $\alpha$  in a Total Internal Reflection Fluorescence (TIRF) based binding inhibition assay. This allows to quantify the total effect on the receptor without the need to quantify a large set of different compounds. The assay will consider high affinity ligands at lower concentrations and even compounds with currently unknown estrogenic activity are found. In addition a RIfS based assay that discriminates between activated and inhibited conformations of estrogen receptor  $\alpha$  has been established to complete the information.

Such a combination of assays is therefore a valuable tool in environmental analytics as well as pharmaceutical research.

## S13: Sensors 2

### Kinetic Biosensors with Intelligent Visible Motions for Chemical Analytes

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#### INTRUCTION

In living organism, protein molecule such as muscle molecule actin/myosin and ATP synthetase functioned directly as the devices for energy conversion and transfer with high efficiency. Since some biocatalyst could be catalyze chemical reaction with volume change at room temperature and pressure, mechanical force would be expected to be provided directly from chemical energy. In this paper, an active pressure system was achieved using asymmetric enzyme immobilized membrane, thus obtaining novel kinetic biosensors with intelligent analytical functions. Hydrogen peroxide and glucose were selected as model analytes.

#### EXPERIMENTAL

The active pressure system was constructed by using two funnel type glass tubes separated by an enzyme membrane as diaphragm, in which catalase was immobilized onto the single side of the dialysis membrane. By applying hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution into the non-enzyme side of the glass tube, the active pressure at the enzyme side was measured continuously, thus obtaining the characteristics of the active pressure system for hydrogen peroxide. And the intelligent kinetic biosensors were also constructed using the active pressure system.

#### RESULTS & DISCUSSION

The active pressure increased following the application of hydrogen peroxide solution into the non-enzyme side of the glass tube. The output pressure was linearly related to the concentration of hydrogen peroxide over a range of 11.8 to 123.6 mmol/l, with a correlation coefficient of 0.994 deduced by regression analysis as shown by the following equation:

$$\text{pressure (Pa)} = -15.56 + 4.35 [\text{H}_2\text{O}_2 \text{ (mmol/l)}]$$

The novel kinetic biosensors with the active pressure system also performed the intelligent and amazing chemo-mechanical sensible behaviors with not only hydrogen peroxide but also glucose solution. We will show some movies of the intelligent behaviors of the kinetic biosensors as the novel analytical devices.

## S13: Sensors 2

### Magnetic Sensor Spheres as Versatile Tools in Optical Sensing

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Sensors based on luminescence constantly gain importance in research labs and industry. Such sensors are mainly used for measuring physiological parameters such as oxygen, pH and ions. Although a variety of sensors are commercially available, there are still problems that have not been solved yet.

The wide spread fiber-optic sensors (Optrodes) for example have to be dipped into the sample and therefore they are a contamination risk. Furthermore, quartz based fibers may cause problems due to insufficient robustness.

Sensor patches enable an external readout (through glass or transparent plastics). By utilizing single sensor patches, which are immobilized on the vessel's sidewall, analytical information can only be gathered at the position of the sensor spot.

Here we present an optical sensor platform combining the advantages of existing technologies without the known drawbacks. While nowadays mainly planar substrates are used for optical sensors, this work demonstrates the benefits of using ferromagnetic spheres as substrate. Due to their properties they can easily be captured by a magnet. This enables magnetic targeting of the sensor and therefore measurements without spatial limitations. Technology transfer between sensor patches and sensor spheres for both signal readout and sensor manufacturing is possible and allows the production of a variety of sensors (e.g.: temperature; oxygen; pH,...).

Specially designed separators can be used to generate disposable sensors for fiber-optic and planar readout units. Finally, magnetic removal of the sensor sphere avoids sample contamination after the analysis.

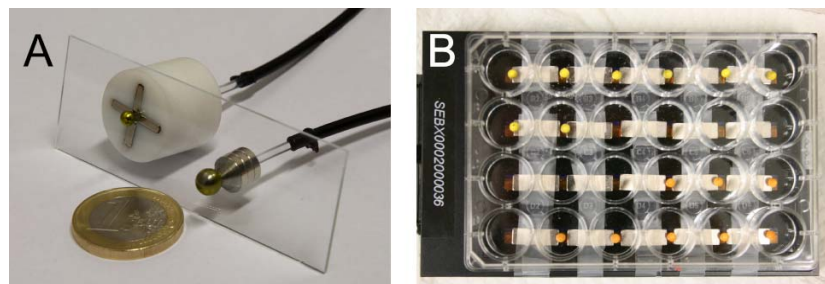


Figure 1: With the help of special separators sensor spheres can be positioned in front of an optical fiber (A) or on top of the optics of a 24-channel commercial plate reader (B).

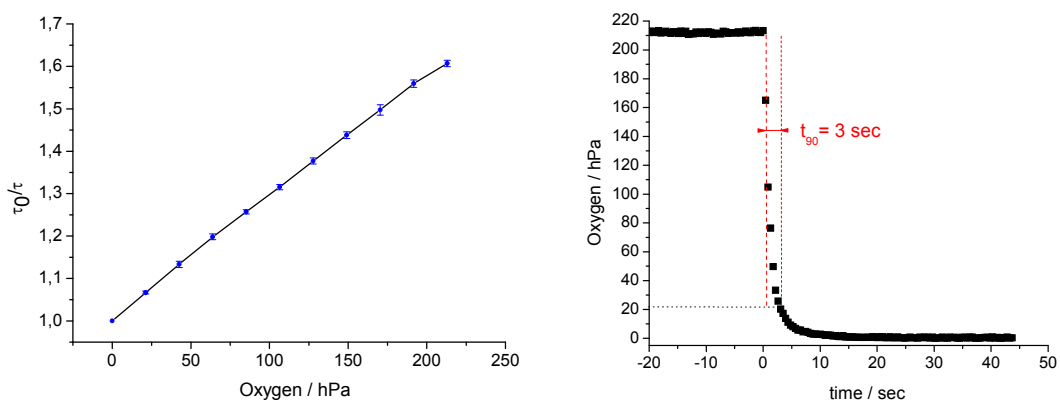


Figure 2: A: From 0% to 100% airsaturation (0 – 213 hPa) a nearly linear calibration curve (Stern Volmer plot) was obtained (left). The innovative coating technique enables the production of fast responding sensor spheres (right).



## S13: Sensors 2

### A Novel Biosensing Device Based on Modified Quartz Tuning Fork Resonator

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We present a novel approach in creating potential biosensing device using modified quartz tuning fork (QTF) resonator and sensing element made of carbon nanotubes (CNTs). QTFs have previously been proposed as cheap and simple alternatives (1) to conventional labeling-free mass-sensing quartz crystal microbalance (QCM) based biosensing devices. No method for using the tuning fork in self-excited mode for biological sensing in liquids with high dielectric permeability (like e.g. water) has been published.

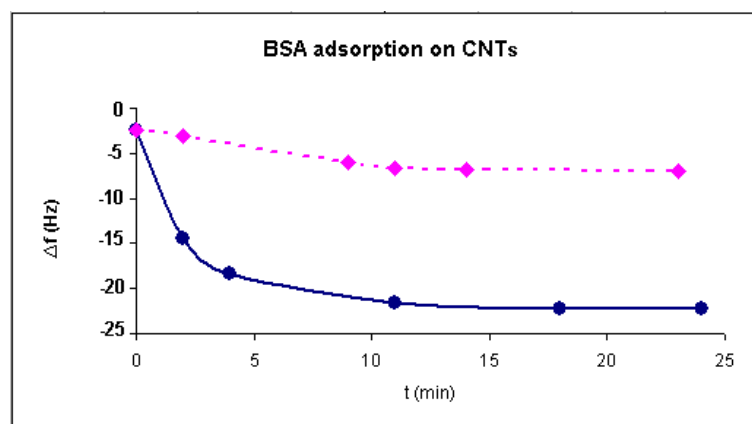
Carbon nanotubes have high biomedical potential (2) and they can easily be modified with different bio-recognition capable molecules (proteins, nucleic acids) either by using covalent bonding or non-covalent adsorption (3).

We propose a solution for the tuning fork concept by introducing the unique structure of carbon nanotubes to create a bio-recognition surface for the QTF-based sensor. Main advantage of our device is its capability of biological measurements both in gaseous and high dielectric permeability liquid environment. Performance capability of the novel method was confirmed experimentally by investigating the adsorption kinetics of BSA on CNTs in aqueous environment.

BSA adsorption rate on CNTs strongly depends on pH, having the highest rate at pH 4.8 (isoelectric point of BSA) and the lower at other pH values. In our case the modified QTF sensor was dipped into the 5mM phosphate buffer solution at pH 4.8 (blue line). BSA was introduced into solution at final concentration of 0.1 mg/ml causing the adsorption of the respective protein onto the nanotubes, resulting in rapid resonance frequency shift. In 20 minutes the resonance frequency was shifted by 19 Hz and then stabilized indicating the saturation of the surface with BSA. Experiment was repeated in pH 7 solution (pink line). In this case the same amount of BSA resulted in much slower resonance frequency shift giving the total shift of only 4.5 Hz.

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## S13: Sensors 2

### **An Optical Biosensor for on Line Heavy Metals Detection: Bioluminescent Bacteria, Technological Design and Computational Fluid Dynamics Modelling inside the Macroarray**

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In 2001, the European community published a list of priority substances in the field of water policy. In this list, heavy metals (Cd, Ni, Pb, Hg) are classified as “dangerous priority substances” the detection of which in the environment is a necessary part of European policy. In this context, we developed a biosensor with several bioluminescent bacteria for the on line detection of heavy metals.

Bacterial bioluminescence is the light produced by some bacteria mediated by the enzyme luciferase. Such biochemical reaction is coded by the complete *luxCDABE* genes (luciferase and aldehyde synthesis). Combined with appropriate promoters, the bioluminescence can be selectively targeted by several pollutants.

For heavy metals detection, three recombinant *Escherichia coli* strains were constructed with the *Vibrio fischeri luxCDABE* reporter genes cloned downstream of several metal resistance systems leading to the detection of a wide range of heavy metals after 1-hour of contact time and a detection limit under or near the pollution standards.

In a second stage, we designed a multi-channel biosensor in immobilized phase: Lumisens3. This new device, designed for the non-stop analysis of water pollution, allowed the insertion of the three above bioluminescent strains and two bioluminescent constitutive strains immobilized in a multi-well removable card (macroarray). The validation of this biosensor allowed us to demonstrate the simultaneous on-line crossed detection of one or several metals as well as the measure of the global sample toxicity.

In a third stage, we addressed the problem of the diffusion of pollutants and substrate for the bacteria since the induction time in the agarose matrix was important and the response from wells to wells was heterogeneous. We decided to use the multiphysics software COMSOL, which is capable of combining several differential equations (hydrodynamics, heat and mass transfer) in order to optimize hydrodynamics and transfer phenomena when bioluminescence occurs. Thanks to these simulations, a new card was designed lowering the induction time and increasing the homogeneity. Moreover, it was demonstrated that the inducer, Cadmium, does not restrict the bioluminescence that was limited by the oxygen at the bottom of the wells.

This new multichannel biosensor combined to a better understanding of the limiting factors influencing the detection process should contribute to propose reliable biosensor for the on line analysis of pollutants.

## S13: Sensors 2

### Porphyrins as Sensing Materials in Fiber-Optic Chemical Gas Sensors

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Optical fiber chemical sensors have attracted interest in recent years due to their remote analysis capability, high sensitivity, and almost complete immunity to magnetic interference.

Several fiber-optic humidity sensors have been reported based for example on hydration of  $\text{CoCl}_2$ , hydration of trifluoroacetophenones, and dyes which change their color upon exposure to humidity.

A new sensor which we have developed is based on a Nafion polymeric film of N-confused porphyrin applied on an optical fiber. In contrast to regular porphyrins which are macrocyclic compounds with four pyrrole groups connected by methine bridges, N-confused porphyrins (NCP) are isomers where one of the pyrrole rings is inverted. It was found that when the Nafion/NCP films were exposed to dry air ( $\text{RH} \leq 1\%$ ), they exhibited Soret bands at 406 and 459 nm (small shoulder and main peak, respectively). However, when exposed to humid air ( $\text{RH} = 77\%$ ), only one Soret band was observed and was blue shifted compared to the dry film main band. The two spectra also intercepted, indicating equilibrium between two isomers of the NCP. Tautomerism of NCP, reported in the literature for this compound when dissolved in different solvents, is suggested to be responsible for the optical changes in Nafion/NCP films. High water content decreases the acidity of the Nafion films, causing the more hydrophobic tautomer to predominate and populate the hydrophobic fluorocarbon phase in Nafion.

Upon irradiation at 470 nm, the output optical signal of the sensor was found to linearly depend on humidity over the range 0 to 4000 ppm (v/v). The response time, depending on humidity, was in the 2-6 minutes range. Good repeatability of the signals ( $\pm 2\%$ ) was obtained during 100 dry/humid cycles.

A brief description of a metalloporphyrin-based fiber-optic sensor for the detection of hydrogen which is recently being developed in our laboratory will also be presented.

## S13: Sensors 2

### Novel Double-Armed Calix[4]arene Compounds as Selective Extractants for the Determination of Heavy Metal Ions in Aqueous Environment

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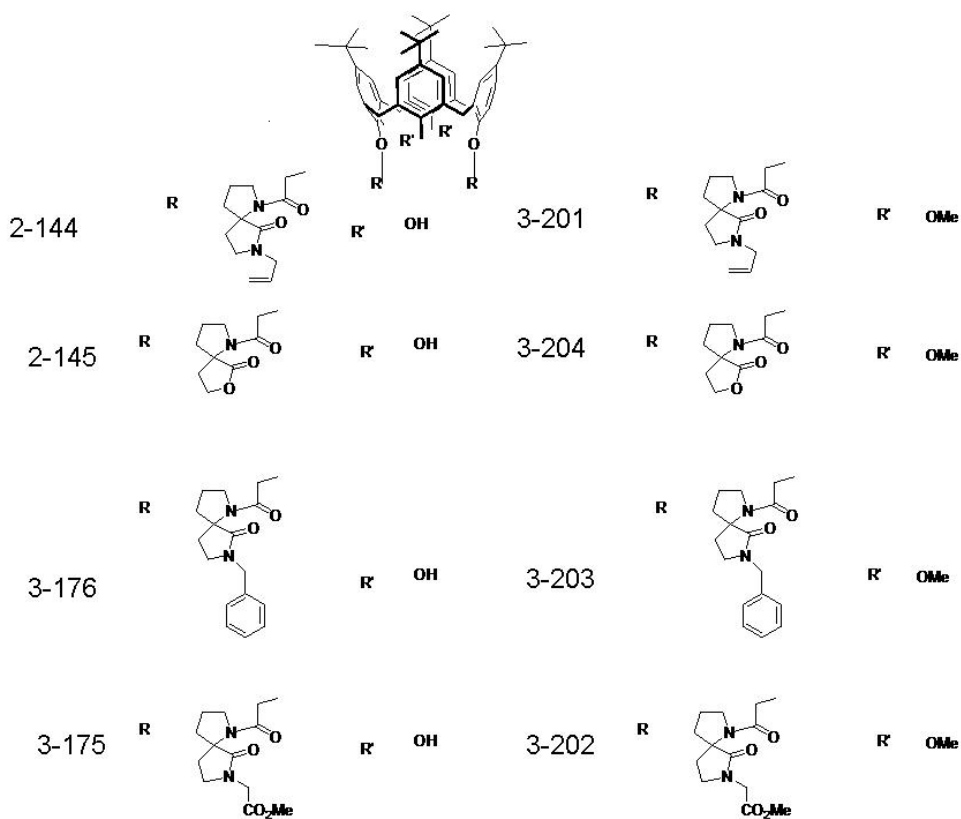
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A number of spirocyclic double-armed calix[4]arene compounds ( **Figure 1**) with amide and ester ligating groups on the lower rim have been synthesized and investigated as extractants of toxic heavy metal ions from aqueous waste water.



**Figure 1. Structure of 2-144, 3-201, 2-145, 3-204, 3-176, 3-203, 3-175 and 3-202.**

In order to study the binding ability of these double-armed calix[4]arene compounds with metal ions, such as Na I, K I, Ca II, Mg, Pb II, Ni II and Cd II, the solvent extraction of metal ions picrates from aqueous solution into dichloromethane was performed at room temperature. It was found that these ligands can be used as extractants for most of the metal ions, particularly; 2-144 obtained a good selectivity to Pb II over other alkali metal ions, alkaline and heavy metal ions. And the initial study also showed that the plasticized PVC membrane based on these lipophilic tweezers-like double-arm compounds as ionophore exhibit good optical response and significantly highly selectivity for Pb II over other tested metal ions. This has wide applications for developing selective and sensitive optical sensors for lead for water monitoring.

## **S14: Bioanalysis 3**

### **Keynote lecture 14**

#### **Advances in SPE-LC-MS/MS Analysis of Drugs and Endogenous Compounds in Biofluids**

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In bioanalytical LC-MS/MS of drugs and endogenous compounds the main drawback of many protocols either is a time-consuming and/or labor-intensive sample pretreatment step or it is an instable ionization yield due to an inadequate clean-up procedure. Matrix components co-eluting with the analyte(s) often suppress the ionization of the target compound(s) and therefore strongly diminish the accuracy of quantitation. In addition, the biological variability, i.e. the varying composition of the same biological sample matrix also affects the reproducibility of a LC-MS method. This holds especially for the abundant low MW components in a complex biofluid. Therefore, not only high MW components (e.g. proteins) should be removed prior to LC-MS but also low MW compounds which potentially interfere with the ionization process.

In this context, Solid Phase Extraction (SPE) is the clean-up technique of choice. SPE can be easily hyphenated with LC via a six-port valve, i.e. column-switching (on-line SPE-LC). For that purpose a small SPE-column is filled with tailor-made packing, i.e. Restricted Access Material (RAM) allowing a size-selective fractionation of complex (bio)fluids and a simultaneous extraction of the target analyte(s) by reversed phase, ion-exchange or affinity chromatography [1]. Such a SPE-column can be operated at flow-rates up to 4 mL/min thereby reducing the clean-up time of e.g. a 50 µL sample of human plasma to less than 60 sec. With regard to the elimination of matrix effects in LC-ESI-MS/MS, one can extend such an on-line SPE-LC platform by a second SPE-column (multidimensional SPE) which possesses orthogonal chromatographic properties [1]. Another approach is to apply the newly developed Phase Optimized Liquid Chromatography (POPLC™) kit [3]. In POPLC LC-column segments with different length and packed with different stationary phases are combined and coupled together, respectively, to create a tailor-made analytical LC-column for a given separation and MS detection problem.

Finally, for the direct injection and on-line SPE-LC-MS/MS analysis of whole blood one can apply a unique in-line processing procedure, i.e. heat-shock treatment of anticoagulated blood, which yields a novel biological fluid named cell-disintegrated blood [4].

## S14: Bioanalysis 3

### Beyond Continuous Glucose Monitoring – Detection of Bioanalytes with Boronic Acid-Appended Bipyridinium Salts

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The detection of bioanalytes, such as carbohydrates and nucleotides, is singularly important to the biomedical field, impacting areas like diagnostics, drug design, and clinical studies.

A two-component carbohydrate sensing concept based on anionic fluorescent dyes as reporters and boronic acid-appended bipyridinium salts as receptors was originally formulated.<sup>[1]</sup> A glucose sensor, based on this concept, was produced by immobilization of the sensing components.<sup>[2]</sup> This device allowed for continuous monitoring of glucose concentrations in the physiological range and is currently being commercialized for clinical use in blood glucose monitoring.<sup>[3]</sup>

To further demonstrate the analytical power of the probes, several receptors with the commercially available fluorescent reporter dye HPTS were combined in an aqueous solution-phase sensor array to differentiate between neutral saccharides and anionic phosphosugars and nucleotides.<sup>[4,5]</sup>

The “static” discrimination results were used to assay “dynamic” carbohydrate transforming enzyme reactions. Real-time fluorescent enzyme assays have been developed for sucrose phosphorylase and phosphoglucomutase.<sup>[6]</sup> These assays make use of the selective carbohydrate sensing system that detects the unlabeled enzymatic products fructose and glucose-6-phosphate. The real-time fluorescence intensity of the reporter dye was converted into product concentration, allowing initial reaction velocities and Michaelis-Menten kinetics to be calculated. The assays were also carried out in multiwell plate formats, making them suitable for high-throughput screening of enzyme inhibitors.

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## S14: Bioanalysis 3

### Novel Approaches to the Enzymatic Determination of Organic Substrates of Oxidoreductases

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The sensitivity and selectivity of the enzymatic methods for the determination of biologically active compounds and the possibility of their application to the analysis of water insoluble samples depend mainly on the catalytic activity, stability, and substrate specificity of enzymes in aqueous and water-organic media. To improve these properties of the enzymes and widen the area of the enzymatic methods application, we have proposed a number of novel approaches, such as: varying a source of an enzyme isolation and the nature of a buffer solution for carrying out an indicator reaction; forming the self-assembling systems of enzymes with amphiphilic molecules – polyelectrolytes and surfactants, and using hydrophilic ionic liquids as a component of the medium for the indicator system. Besides, the application of the effect of “substrate-substrate” activation and conjugated reactions was shown to be extremely useful to solve the problem of the selective and sensitive determination of biologically active compounds in different media.

The expediency and prospects of the application of the above mentioned approaches for the determination of organic substrates of oxidoreductases, such as plant peroxidases (isolated from horseradish, soy-beans, peanut, alfalfa, fungi), bovine serum amine oxidase, and alcohol oxidase from *Pichia pinus*, have been shown in our investigations. Numerous examples demonstrating the possibilities of the proposed approaches to improve the analytical characteristics of the enzymatic procedures for the determination of environmental toxicants, pharmaceuticals, and markers of food quality: phenolic compounds (such as hydroquinone, pyrogallol, aminophenols, chlorophenols, etc.), biogenic amines (e.g. benzylamine, histamine, and spermine), aliphatic alcohols (ethanol, methanol), phenotiazines (promazine, chlorpromazine, etc.), catecholamines (e.g. dopamine, adrenaline), and organic peroxides not only in aqueous solutions, but also in water-organic media and ionic liquids, will be discussed.

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## S14: Bioanalysis 3

### Chromatographic Determination of Water-Extractable Non-Structural Carbohydrates (NSC), Including Inulin and Starch, in Different Plant Samples

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Carbohydrates are major constituents of plants. Their composition may vary with season, light availability and vegetative stage. Additionally, composition of non-structural carbohydrates (NSC) will reflect growth and variations in photosynthesis as well as abiotic stress phenomena, like hyperosmosis. Besides the importance of studying these biological properties of carbohydrates, their exact identification and quantification in plant material is also of vital importance to establish more precise and reliable carbon balances to study biogeochemical cycles and evaluate appropriate models.

For this reason, a rugged method for the reliable determination of non-structural carbohydrates (NSC), based on 12 sugar alcohols and carbohydrates, including the trisaccharide raffinose, will be presented. Moreover, the polysaccharides inulin and starch, used for storage purposes by plants, are amenable to the procedure after appropriate hydrolysis. The method is based on High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD). Overall analysis time is 30 min. The method allows the determination of the relevant sugar alcohols, mono-, di- and oligosaccharides within a single analytical run. Inulin and starch are determined in a second run.

The method was successfully applied to a variety of plant samples: pine needles, tomato leaves and grass of the species "*Lolium perenne*". Identification of peaks is carried out by comparison of retention times with known compounds (mixture of standards) and, in case where peak assignment is obscure, by spiking the extract. Repartition and quantification of carbohydrates in the different plant samples will be reported. Besides "common" carbohydrates, like glucose and fructose, several other mono- and oligosaccharides were detected, indicating that composition of non-structural carbohydrates in plant samples is complex. Particular emphasis is given to the determination of both the polysaccharides inulin and starch which make up a considerable part of non-structural carbohydrates in plants.

## S14: Bioanalysis 3

### Determination of Human $\beta$ -defensin Antimicrobial Peptides in Saliva of Patients with Oral Mucosal Diseases

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Saliva contains several types of antimicrobial peptides that play a role in innate immunity. The Beta-defensins, human Beta-defensins (HBD)-1 and -2, have also been detected in saliva[1]. This study was designed to investigate the presence of HBD-1 and HBD-2 in the saliva of patients with various types of oral inflammation, with **oral lichen planus (OLP)**, **Behcet's disease (BH)** and **recurrent aphthous stomatitis (RAS)** in saliva and to determine whether the salivary HNP-1 concentration can be used as a marker of inflammation in patients with inflammatory diseases affecting the oral cavity and to compare with healthy volunteers. Whole saliva samples were taken from the patients and a saliva sample was similarly obtained from each control subject a single time. Each saliva sample was immediately adjusted with citric acid to a pH of 3.0 and stored at  $-20^{\circ}\text{C}$  until assay. HPLC system was used in order to isolation and determination of the Beta-defensins. The molecular weight of the HBD-1 and HBD-2 were measured by means of a triple-stage quadrupole mass spectrometer with an electrospray interface. The calibration curves for defensin HBD-1 and HBD-2 indicated a linear range at the detector with  $R^2 > 0.99$ . Our study showed a significant positive correlation the concentration of human Beta-defensins in saliva patients with especially BH and RAS as well as OLP. So, the salivary human Beta-defensins concentration can be used clinically as a marker of inflammation between these patients.

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## S14: Bioanalysis 3

### Affinity Capillary Electrophoresis Determination of the Interactions between a Lysozyme-Binding Aptamer and Mono- and Divalent Cations

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Aptamers are synthetic single-stranded oligonucleotide sequences which can have specific binding affinity to various targets ranging from small molecules to proteins, and even cells. They are isolated from random-sequence pools by an in vitro selection technique known as SELEX (systematic evolution of ligands by exponential enrichment). Due to their distinctive properties, aptamers can be employed as sensitive diagnosis agents, biomedical research tools and even therapeutics. Binding between an aptamer and its target is highly dependent on the conformation of the aptamer molecule, this latter seeming to be affected by a variety of cations. As only a few studies have reported on the interactions of monovalent or divalent cations with aptamers, we describe herein the use of affinity capillary electrophoresis (ACE) in its mobility shift format for investigating interactions between various monovalent ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cs}^+$ ) or divalent ( $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ) cations and a 30-mer DNA lysozyme-binding aptamer. This study was performed in background electrolytes of different natures (phosphate and MOPS buffers) and ionic strengths.

First, ionic strength and counterion condensation were evaluated, according to Friedl's and Manning's models, respectively, and the experimental mobilities were corrected for these two effects in view of understanding the phenomena involved in the aptamer/cation interaction. In the case of monocations, ionic strength was evidenced as the main effect modifying the effective mobility of the aptamer, but a possible interaction between the buffer components, the aptamer and the monocations was highlighted. For divalent cations, the effective mobility shift during ACE study was not only attributed to ionic strength and condensation effects, but also to a possible interaction with the buffer anions (binary or ternary complexes) and/or conformational change of the aptamer induced by the divalent cation binding. Finally, apparent binding constants were calculated for divalent cations from mathematical linearization methods, highlighting a possible conformational change.

## S14: Bioanalysis 3

### Quantification of Peptides in Biological Fluids

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Only 8 or 10 years ago, when it came to quantitation of peptides with molecular weights of between 1000 and 5000 Da from serum or plasma, there were only a very few specialists taking the first tentative steps in this field. We know from reliable sources that we were among the very first bold pioneers in this field. Since then we have developed quantitative determination methods for a large number of peptides in serum/plasma, down to 1 ng/mL and – in the case of urine – even as far as 0.1 ng/mL. Our latest preliminary trials indicate that for three peptides (5, 7 and 9 amino acids) we got down to 20 pg/mL in plasma. The following examples aim to show that flexible solutions for this difficult topic are indeed perfectly feasible.

Rough estimates suggest that as many as 5 to 20 million different peptides and proteins may be present in the human and animal organism. There follow 4 representative examples, each of which touches on a specific problem:

In the first example, small peptides with molecular weights of 217 and 199 were detected down to limits of 1 ng/mL. With such small molecular weights and such hydrophilic molecules, which thus practically rule out sample preparation, the major challenge that we addressed here was that of **selectivity**.

Another peptide, with a molecular weight of 2013, has a **recovery** rate of between 10 and 20 % following standard acetonitrile precipitation in plasma. Thanks to some bold choices of additives, we have been able to achieve both high recovery and a detection limit of 1 ng/mL in plasma and 0.1 ng/mL in urine.

The next peptide, with a molecular weight of 3039 (28 amino acids) had a serious **stability** problem in whole blood and plasma (with a half-life of approx. 10 - 15 minutes). Protease inhibitors were no help here, but a certain type of acidification solved the problem. The detection limit for this medium-sized peptide is 25 ng/mL in plasma.

The last peptide described here has a molecular weight of 3618 and is so **strongly lipophilic** that it could only be isolated from plasma by the Folch extraction procedure. These extreme lipophilic properties also entailed numerous other problems, all of which we have solved satisfactorily.

**Publications:** Naunyn-Schmiedeberg's Arch. Pharmacol., 378, 323-333 (2008)  
Crit. Care Med., 35, 1730-1735 (2007)  
LC-GC AdS, March 2008

## **S15: Safety issues**

### **Keynote lecture 15**

#### **Impact of Innovative Mass Spectrometric Instrumentation on the Analytical Determinations with Legal Implications**

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An increasing number of analytical determinations are currently executed to comply with law enforcements, regarding various aspects of both industrial production and social behavior. These issues include prevention of environmental pollution, food safety, antidoping controls, safe-driving policies, and many others. For such determinations, innovation in analytical procedures offers substantial opportunities to improve the control effectiveness, but in the same time raises questions about the reliability of results. In general, official analytical methods are rather conservative, as they should satisfy a widespread and standardized applicability.

In particular, prompt acceptance of novel mass spectrometric (MS) techniques, in the analytical methods involving legal implications, depends on the nature of the advantages that innovation will offer. New technologies that improve measurable properties (i.e. resolution, sensitivity, repeatability) are rapidly acknowledged and applied, whereas those implying a new approach to conventional inquiries (for example, new ionization or desorption methods) or providing more sophisticated information are likely to be accepted with a delay.

The presentation will provide a survey on the major instrumental advancements occurred in mass spectrometry during the last years and the effects that such innovations have produced in a variety of analytical determinations involving legal implications. Typical examples, useful to illustrate these effects, will be taken from toxicology, forensic science, antidoping activity, drug abuse in animal breeding, environmental and food-safety control. Some MS advancements have created the premises for a revolution, still in progress, within the system of accredited analytical method, while others have opened entirely new branches of forensic investigation. Recent results from the author's research activity will also be presented.

## S15: Safety issues

### Multiparallel Microchip with Fluorescent Yeast for Rapid Toxicity Screening

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Toxicity screening using yeast is widely used for different targets compounds, such as genotoxicity, endocrine disrupters chemicals (EDCs), oxidative stress factors, etc. The aim of this work was to develop a simple low cost toxicity screening test that does not require animal testing. These tests would help chemical industries screen new chemicals for environmental effects so as to comply with the new EU REACH regulations, as well as allowing the aquatic environments to be monitored for a wide range of toxic behaviour. Recombinant budding yeasts *Saccharomyces cerevisiae* containing fluorescent markers such as green or yellow fluorescent protein (GFP or YFP) are ideal candidates because they release the fluorescence without the necessity of adding any substrates.

A system has been demonstrated with multiparallel capillaries to retain different recombinant yeasts, inside the chip and expose them to potential toxic compounds. The recombinant yeast (supplied by Gentronix Ltd., Manchester, UK) contained a vector expressing GFP under genotoxic (DNA damage) conditions. After exposure of the yeast to different target compounds (dissolved in DMSO at a final volume of 1 %), they were excited at 485 nm and the fluorescence emission was detected at 520 nm under an inverted microscope. Quantification of fluorescent and comparison of the emission was performed and ANOVA analysis used to demonstrate significant differences when comparing to the control yeasts.

Retention of the small yeast particles under flow conditions was difficult in the initial design and in the most recent version magnetised yeast particles are been utilised to improve retention. Our system is demonstrated to be an excellent tool for a rapid screening test to detect different toxic effects. This method would help to avoid the production and discharge of toxic compounds by the manufacturers as well as to detect already polluted environments.

Keywords: glass-microchip, GFP, toxicity screening, recombinant yeasts

#### **Acknowledgements**

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## **S15: Safety issues**

### **Investigation into the Nature of Metal Threads on Historical Textile**

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Investigation into the nature of metal threads from historical textiles enables proper decisions about further cleaning, conservation and restoration treatment steps. Metal threads applied on historical textiles were usually strips made from metals wound around the core fiber yarn. The fiber inside the thread was made of cellulose or protein polymer (cotton, silk, wool and flax), while the metals most commonly used were gold, silver, zinc and copper. The average width of the metal strips used was in between 0.15 and 0.35 mm, and the average thickness varied between 0.005 and 0.030 mm. The most important step during textile conservation is identification of all the materials originally applied in order to understand the nature of chemical and physical degradation that may occur within the various components of the system. Methods applied should be non-destructive and sensitive enough to detect trace elements in small available sample amounts. The goal of this research was to describe the most useful procedures needed for fast and easy determination of specific metals of interest which are important for textile conservation. Therefore we propose application of the scanning electron microscopy equipped with the EDS detector and inductively coupled plasma – optical emission spectroscopy for detection of metals in solid and liquid historical samples, respectively. Examples of nine different metal threads collected from Croatian historical textile materials, in which metals were identified, will be presented.

**Keywords:** *ICP-OES, SEM-EDX, historical textile, metal threads, conservation restoration*

## S15: Safety issues

### Comparison of Different Approaches to Estimate the Limit of Detection and Limit of Quantification of the 15+1 EU Priority Polycyclic Aromatic Hydrocarbons (PAHs) in Meat Products

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European Regulation (EC) 882/2007 defines the limit of detection (LOD) and the limit of quantification (LOQ) as two mandatory performance criteria to characterize any food and feed analysis method. Commission Regulation (EC) No 333/2007 specifies that LOD and LOQ for benzo[a]pyrene must not exceed 0.3 and 0.9  $\mu\text{g}/\text{kg}$  respectively.

Despite the number of official guidelines and standards, the determination of these limits is still confusing in terms of the underlying concepts, terminology and calculations. The aim of this work is to show the effect of different approaches based on instrument calibration (ISO 11843-2 or DIN 32645), blank procedures (IUPAC recommendations and the Commission Regulation (EC) No 333/2007) or signal-to-noise ratios (European Pharmacopeia) on the level of LOD and LOQ in the analysis of the 16 EU Priority PAHs in meat samples by gas chromatography-mass spectrometry.

Different issues such as the significance of matrix, the variability of the blank signal and the dependence on time are also addressed.

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## S15: Safety issues

### Mass Spectrometry in Analysis of Precursors of Chemical Warfare Agents

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A survey focused on mass spectrometric analysis of selected tertiary amines, which may serve as precursors of chemical warfare agents and/or side products of their synthesis, is presented. Development of fast and reliable methods for their detection and identification is an important part of Chemical Weapon Convention (OPCW, 1997). Tandem mass spectrometry as a highly sophisticated technique allows identification of ions on the basis of their masses and fragmentations. Proposed structures of fragments of studied compounds (namely *N,N*-dialkylaminoethane-2-ols, *N,N*-dialkylaminoethyl-2-chlorides and *N,N*-dialkylaminoethane-2-thiols) were confirmed with deuterated standards and accurate mass measurement. Interesting minor fragmentation processes (such as elimination of ethene from propyl chain or rearrangement of sulfhydryl group) were revealed as well. A simple direct infusion of standard solutions into the ion source to perform MS<sup>n</sup> experiments have a limited potency to differentiate among positional isomers. □LC/MS/MS method was designed for the cases where the resolution based solely on differences in fragmentation is unsatisfactory. Increase in retention of the polar derivatives on reversed phase was achieved by ion-pairing chromatography. Trifluoroacetic acid, heptafluorobutyric acid and less typical 3,5-dinitrobenzoic acid were tested as ion-pairing agents to gain a sufficient selectivity. Furthermore metabolism of the precursors was studied *in vitro*. The compounds of interest were incubated with rat hepatocytes and analyzed using □LC/MS/MS. The samples and corresponding controls showed that chloroderivatives alkylate peptides on sulfhydryl group of cysteine while thioderivatives form a disulfidic bond under physiological conditions. Model experiments with *N*-acetylcysteine, glutathione and □-lactoglobulin confirmed formation of these products. This work contributes to a wider research aimed at finding of biomarkers of exposure to controlled substances.

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## **S15: Safety issues**

### **Chromatographic Method Portfolio For the Purity Control of Ionic Liquids**

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As a new generation of solvents with outstanding properties ionic liquids open a large variety of interesting applications in different “high-tech” fields.

For the analysis of these zwitterionic components a broad analytical method portfolio is used.

Beside classical reversed phase Chromatography, used for the analysis of UV active imidazolium- and pyridinium-type ionic liquids. Ion-Chromatography in different modes is used successfully for the purity control of amine- or phosphonium-type ionic liquids. The most versatile detection principle in this case is suppressed conductivity detection for the latter molecule classes without an UV-Chromophor.

On the other hand Capillary Electrophoresis and Isotachophoresis with different detection principals like contactless capacitively coupled conductivity detection or “indirect” UV detection, using buffer-electrolyte systems with high background absorption are applied for quality control of ionic liquids as well as for the reaction monitoring of organic synthetic routes.

For each of the above mentioned classes of ionic liquids several separation application examples will be presented.

Beside the analytical methods described above a set of application examples where ionic liquids might play an important role in the future in different “high tech” fields like electrochemical applications, analytical applications, Lewis acid catalysis or organic solar cells are presented also.



## S15: Safety issues

### Method Development and Validation of Sampling and Testing Methods for the Mostly Used New Generation Anesthesia Gases in Turkish Hospitals

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The anesthesia gases that are released to the hospital operating rooms are called as waste anesthesia gases and create environmental problems for the hospitals. Starting with the anesthesiologist and the surgeon all operating room staff members are affected from this low dose waste gases. In their professional routine, this exposure is continued until the weekend and the cycle is repeated, on weekly basis. The effects of exposure to anesthesia gases are reported in the literature; even cell level impact is observed in recent years. There is not much information on the long term effects for the anesthesia gases that are used most recently, such as Sevoflurane, Desflurane, Isoflurane and Nitrous Oxide in Turkey. Samples were collected between January 2005 and December 2006, from several hospitals and especially from the one which is the largest research and teaching hospitals of Turkey. A rapid and selective method for the determination of these anesthesia gases accumulated in the hospital operating rooms was developed and validated.

Samples collected from the operating rooms and their nearby halls, via handy pump and adsorbed on activated carbon then desorbed with carbon disulphide and toluene. The mean recovery was found to be 79.2 and 71.8 %, respectively. Analysis was carried out by HS-GC-FID and TCD. Methylene chloride was one of the internal standards used. Linearity was obtained ( $R^2 = 0.9968 - 0.9992$ ) and intra-day and inter-day precision studies were also realized with RSD below 5% in both cases. Besides, SPME-GC-FID results were also compared. Method we have used is easily applicable to other anesthesia gases of interest.

**KEYWORDS:** Anesthesia gases, sevoflurane/desflurane/isoflurane, HS-GC/FID, SPME

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## S16: New MS Technologies

### Keynote lecture 16

#### Hyphenated Separation Techniques in Searching of Biomarkers for Early Cancer Diagnosis

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In the recent years, much effort has been made to develop fast, high efficiency separation techniques and detection methods for determination of biologically important molecules. The rapid diagnosis of microbe-based diseases without the need of isolation of pure cultures is an obvious need and the future effective treatment can prevent the recurrence of infection and perhaps the development of cancer. There are many types of diagnostic methods used to identification of bacteria (invasive, non-invasive) but these methods usually require time-consuming and laborious procedures and therefore are not capable for fast diagnosis in case of emergency. The new approach is possible thanks to capillary zone electrophoresis (CZE), which is characterized by high efficiency of separation, high resolution, short analysis time and simplicity of automatization. Using selective modification of capillary we are able to resolve and identify different species of bacteria for example: *E. coli*, *H. pylori*, *S. aureus*, ect. These microorganisms are responsible for most common infections found in humans worldwide.

In current practice, also breath testing for volatile organic compounds (VOCs) can provides safe and non-invasive method for the investigation of human metabolism and diseases. Therefore, exhaled air gives unique possibility of fast diagnosis or evaluation of several disorders including lung cancer, heart diseases, schizophrenia, etc. Alveolar breath contains large number of VOCs analysis but most of these compounds are on trace levels (ppb or/and ppt). These concentrations are usually too low for analytical instrumentations and the sample preconcentration methods are required. The most important methods such as: thermal desorption (TD) and solid phase microextraction (SPME) are widely utilized. TD is an alternative to gas chromatograph with mass spectrometry (GC-MS) inlet system and sample injection mode. In order to increase time of sampling and reconditioning of sorbent tubes, has led to the development of SPME for the breath analysis. This technique utilized fused silica fibers coated with sorbent which extracts samples. However, progress in the breath analysis is rather limited due to lack of generally accepted and standardized methodologies of sampling, normalization and standardization of results. Probably, this is a primary reason why breath analysis could not yet been introduced into wide medical practice. Because of the high efficiencies of proposed methods, both for rapid identification of bacteria and VOCs analysis, these techniques seem to be very promising in modern clinical laboratories.

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## **S16: New MS Technologies**

### **Using Chemical Cross-Linking and LC-MS/MS for the Structural Analysis of Proteins and Protein Complexes**

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Mass spectrometry has driven many advances in the field of proteomics, making the identification and quantification of thousands of proteins increasingly routine. MS has also facilitated the analysis of protein structures, involving, for example, top-down approaches or hydrogen/deuterium exchange. Chemical cross-linking of reactive side chains in proteins and protein complexes has been used for some time, but its wider application has been compromised by low reaction yields as well as limitations in instrumentation and dedicated software. In order to improve cross-linking technology in combination with MS, we have designed an improved workflow for the study of proteins and their interaction networks.

Our analytical platform relies on three core “modules”: (i) The use of isotope-coded cross-linking reagents that impart a characteristic signature on modified peptides, (ii) the use of mass spectrometers providing high mass accuracy data (e.g. FT-ICR), and (iii) powerful software developed in house that allows to identify cross-linked peptides even from complex samples using large protein databases.

For samples of moderate complexity (several to tens of proteins), state-of-the-art instrumentation provides sufficient sequencing speed, sensitivity and dynamic range to directly identify cross-linked peptides in a single LC-MS run. This is achieved by taking advantage of charge state preselection in real time, so that the more highly charged cross-linked peptides are preferentially selected for sequencing. Advanced workflows for the analysis of more complex samples include additional chromatographic and/or electrophoretic separation steps for general or cross-link specific fractionation. For example, strong cation exchange chromatography may be employed for the enrichment of cross-linked peptides that carry more basic groups than linear peptides. A more generic, high resolution approach for fractionation is off-gel electrophoresis that separates peptides based on their isoelectric point.

We will present results that demonstrate that recent advances in separation science, mass spectrometry and bioinformatics can be combined to advance the field of protein cross-linking and make it a valuable tool for the analysis of protein structures and protein-protein interactions.

## **S16: New MS Technologies**

### **Rapid Characterization of Complex Viscous Liquids by Extractive Electrospray Ionization Mass Spectrometry**

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Liquids with complex chemical compositions and high viscosities such as honey, olive oil, ionic liquids and toothpaste etc play important roles in our life and in numerous biological, physical, chemical and industrial processes. Direct characterization of these liquid samples, without extensive sample pretreatment, remains a significant challenge to modern analytical science and technology. By initializing neutral desorption inside viscous liquids with a nitrogen gas beam, an aerosol sample containing the molecular composition of the bulk liquid is formed through a microjetting mechanism. This aerosol is directed into an extractive electrospray ionization (EESI) source via a heated Teflon tube for detection by Mass Spectrometry (MS). Unlike previously reported techniques (e.g., headspace analysis, surface desorption sampling, solvent extraction, etc.), direct characterization at the molecular level of viscous samples such as honey and olive oil samples was achieved without the need for any sample pretreatment or extraction steps. The results demonstrate that no loss of significant molecular information occurs when analyzing viscous liquid sample by EESI, in contrast to a situation where extraction is performed. Trace levels of diethylene glycol (DEG) in toothpaste was selectively detected and quantified, demonstrating the power of this methodology for rapidly analyzing heterogeneous liquid mixtures of extremely high viscosity (150,000~300,000 cP). To conclude, EESI-MS is a simple and rapid, yet powerful method to obtain molecular information of viscous liquid sample without requiring complicated sample pretreatment steps.

## **S16: New MS Technologies**

### **PTR-MS Technology for Real-Time Process Monitoring and Control in Biotechnology**

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Proton transfer reaction mass spectrometry (PTR-MS) is a well-established, chemical ionization, MS method for detection and quantification of volatile organic compounds (VOCs) down to parts per trillion by volume (pptv) levels with a time resolution of <1 s per compound. Common constituents of air (N<sub>2</sub>, O<sub>2</sub>, Ar, CO<sub>2</sub>) do not react in PTR-MS, and sample gas humidity does not interfere with VOC measurements. Hence direct analysis without the need for prior pre-concentration or sample treatment can be performed. These characteristics make the PTR-MS method ideally suited for the real-time determination of the gas-phase composition of VOCs in the exhaust gas of a fermenter for obtaining more in-depth information on the present fermentation status. The development of such real-time monitoring technologies is one of the major objectives of the process analytical technology (PAT) initiative which was initiated by the FDA for manufacturing of biopharmaceuticals. We have developed a sampling system that enables PTR-MS to be coupled to a bioreactor. This enables cultivation phases to be monitored for microbiological activity, thereby providing specific information of the status and development of a production batch. We report on measurements which have demonstrated that the PTR-MS technology is optimally suited for the real-time monitoring of bioprocesses and can contribute to the implementation of PAT compliant process development. Our long-term goal is to utilize the VOCs as control parameters for biotechnological processes such as pharmaceutical fermentations.

## S16: New MS Technologies

### Mass Spectrometry with Soft Photo Ionisation for On-Line Characterisation of Organic Products from Combustion and Pyrolysis Processes

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Resonance enhanced multi photon ionisation–time-of-flight mass spectrometry (REMPI-TOFMS) using UV-photons is a sound method for selective analysis of aromatic species in complex matrices. Recently, improved electron-beam pumped excimer lamps (EBEL) are used for the generation of VUV-light for single photon ionisation (SPI), which combined with TOFMS can additionally detect aliphatic species. These techniques allow direct monitoring of thermally evolved gases from combustion and pyrolysis without fragmentation, masking out small matrix molecules such as nitrogen. Transfer of evolved gases to the TOFMS is carried out via heated sampling lines containing filters to account for the particle load and a deactivated quartz capillary.

With these detection methods, primary organic products of biomass flash pyrolysis in a technical reactor at Forschungszentrum Karlsruhe (as part of the Biomass to Liquid process chain) were monitored in real time. The miscellaneous biomass feedstock (soft- and hardwood, wheat straw, coarse colza, miscanthus) showed distinguishable mass spectra with specific patterns. For example, with soft wood retene (degradation product of abietic acid) appears, whereas with hardwood syringol derivatives are evolving, and with coarse colza indole arises. The main detectable components consisted of furan and phenol derivatives (cellulose and lignin decomposition products).

A second application dealt with the effects of sulphur addition on the emissions of Polycyclic Aromatic Hydrocarbons (PAH) in the flue gas of a grate-fired bark boiler in Sweden. Flue gas was consistently monitored for up to eight hours. Sulphur was added either as an aqueous solution of ammonium sulphate or as pellets of elemental sulphur. Quantification of selected PAH was carried out by determining relative ionisation cross sections with respect to benzene, yielding detectable concentrations down to the ppt range. Overall, the addition of sulphur led to a significant reduction of PAH concentration and emission peaks independent of the means of sulphur dosage.

## **S16: New MS Technologies**

### **Rapid and Unambiguous Identification of Polyolefine Stabilizers and their Degradation Products with DART Mass Spectrometry**

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Over the last decades the importance of polyolefines has constantly increased. This is due to their excellent material properties and their cost-performance ratio. However, as the structure of polypropylene includes tertiary carbon atoms, stabilization is compulsory to avoid oxidation. Commonly appropriate stabilizing agents are added during the compounding procedure. Unfortunately, due to the rather harsh compounding conditions (temperature, oxygen atmosphere...) a decomposition of the stabilizers might occur during this procedure. Therefore, analytical investigations proving the level of stabilization before and after the compounding process are needed. This is also the case if a commercially available polymer granule (with an unknown degree of stabilization) of even recycled base material is employed as starting point for the fabrication of plastic products.

For this purpose a range of analytical methods exists, most affected with the need for extensive sample pretreatment. A much more straightforward approach for analyzing plastic samples is direct analysis in real time mass spectrometry (DART-MS). To demonstrate the suitability of DART-MS for the determination of a wide range of commonly employed stabilizing agents, a test set of 21 stabilizers was defined and subsequently polymer samples including these stabilizers were prepared using a lab-scale compounder. To investigate the influence of parameters such as temperature, cycling-time and speed of the extruder screw during compounding on the stability of the included stabilizers a series of experiments were performed. The produced polymer samples were subsequently analyzed using DART-MS. Ion-source parameters like discharge needle potential, potential of the grid electrode and the discharge electrode, the heater temperature and the gas flow were optimized. Both modes of ionization were tested, whereby the positive ion mode resulted in higher signal intensities for all analytes. To allow the determination of accurate masses, the instrument was calibrated with Agilent APCI Tuning Mix as mass reference. However, mass to compensate for any mass drift occurring during measurement, an internal standard (bis(ethylhexyl)phthalate) was used. The developed method allowed the unambiguous determination of all selected stabilizing agents in polymer samples. For Irgafos126 degradation of this stabilizer resulting from more harsh compounding conditions was monitored and degradation products could be identified.

# **S17: Elemental Analysis**

## **Keynote lecture 17**

### **Speciation Analysis in the Food Sciences: Current Issues Relating to Arsenic and Selenium**

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Arsenic and selenium are two elements that occur naturally in the environment, and in organisms, in a range of inorganic and organic forms. As a consequence, these two elements also appear in different forms in many human foodstuffs. Depending on the element and chemical form, these selenium and arsenic species can be essential, innocuous, or highly toxic. Speciation analysis is a relatively new field of analytical chemistry that can provide quantitative data on the various forms of an element in a sample. Such data for arsenic and selenium are essential for a realistic assessment of the relevance to human health of the presence of these two elements in foodstuffs. The importance of this analytical field in the food sciences in regard to setting maximum permissible levels for arsenic and selenium will be illustrated.



## **S17: Elemental Analysis**

### **Catalytically Active Gold Collector for the Preconcentration and Determination of Total Dissolved Mercury in Natural Waters**

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Mercury (Hg) is ubiquitous in the environment and requires analytical monitoring in all environmental compartments due to its high mobility and toxicity. In the hydrosphere mercury is bioaccumulated up to  $10^6$  times in fish and sea food. In the European Union Water Framework Directive mercury is classified as one of the 33 most dangerous pollutants. Total dissolved Hg in natural waters is normally in the pg to low ng L<sup>-1</sup> range and an extremely sensitive detection technique, e.g. cold vapor - atomic fluorescence spectrometry, is required to obtain robust analytical data. However, efficient and contamination free sampling and sample storage procedures are equally important and pose a significant challenge. Contamination from stabilizing reagents, storage containers and the ambient environment is likely due to the ubiquity of mercury. Analyte losses can also occur because of the high volatility of Hg species and adsorption to surfaces. One strategy to minimize analyte losses and contamination is to use in situ solid phase preconcentration which stabilizes mercury species at the point of collection and lowers the detection limit. A novel solid phase preconcentration method using catalytically active gold surfaces for the determination of total dissolved mercury in natural waters by atomic fluorescence spectrometry will therefore be presented. The method benefits from minimal use of reagents, since acidification is the only necessary sample pre-treatment. Preparation and characterization of the adsorbent material and preconcentration efficiency for mercury species from natural waters will be discussed. The method was automated using flow injection system coupled to atomic fluorescence spectrometry. Validation was performed by investigation of certified river and sea water reference materials. The limit of detection achieved was 80 pg Hg L<sup>-1</sup> (sample volume 2.5 mL). The experimental set-up, optimal parameters and analytical figures of merit as well as application to different water matrices (river water, lake water, seawater, wastewater) will be shown.

## **S17: Elemental Analysis**

### **Development of a Dynamic Extraction Procedure with on-Line ICP-AES Detection for Fast and Accurate Determination of Soluble Trace Metal Fractions in Airborne Particulate Matter**

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The current interest in atmospheric particulate matter (PM) is mainly due to its effect on human health. A fraction that is associated with several adverse health effects – including cancer – is the metallic portion. For this reason, a great deal of research has focused on the metal composition of airborne particulate matter. Until now in most studies total elemental concentrations were determined. However, toxic effects of trace metals in airborne PM are only expected if the metals are biologically available. Thus for risk assessment detailed knowledge about the solubility of the investigated metals is required since bioavailability depends thereon.

In the last decades, batch-wise equilibrium-based single or sequential extraction schemes have been established as analytical tools for fractionation analyses to assess the ecotoxicological significance of trace metals in ambient PM samples. Although these batch-wise liquid/solid extraction methods have gained widespread acceptance in literature, there is still a need for further improvement since these batch procedures suffer from several shortcomings, such as being tedious, time consuming and being prone to risk of contamination and to metal adsorption/re-distribution phenomena, and, more importantly, they provide no information about the kinetics of the leaching process.

In this study a procedure for the sequential extraction of airborne particulate matter with various leaching solutions and subsequent on-line ICP-AES measurement of selected trace metals in the derived extracts is presented. Analysis was performed using indigenously developed micro-cartridges, which were packed with aliquots of the investigated PM<sub>10</sub> filter samples, and continuously treated with leaching agent. Extracts were directly introduced into the nebulizer unit of the detecting ICP-AES. Evaluation of the derived elution profiles provided information about the kinetics of the extraction process and allowed differentiation between individual soluble fractions.

## S17: Elemental Analysis

### Product Analysis of the Electrochemical Reduction of the Iodinated Contrast Medium Iomeprol by LC-MS-MS and IC-ICP-MS

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Iomeprol belongs to the compound class of iodinated X-ray contrast media (XCMs), a class of diagnostics for radiographic contrast which is pharmaceutically inactive. The total amount of XCMs used worldwide is estimated to 3,500 t per year. After their application XCMs are rapidly eliminated via urine and faeces in their original – non-metabolized – form and transported via sewage to sewage treatment plants (STPs). They are not significantly removed during conventional and advanced sewage treatment processes and therefore enter receiving waters and have been found also in drinking water. According to the precautionary principle the environmental input of such persistent and hence long-living, mobile compounds like XCMs should be limited. Therefore new approaches for water treatment are of interest to eliminate XCMs before they can enter the aquatic environment. The identification of transformation products is essential to assess treatment processes, since for example the removal of organically bound iodine is considered as a key process to render the transformation products more biodegradable and less bioaccumulative.

In this work we therefore show the identification of transformation products during the potentiostatic controlled electrochemical reduction of iomeprol. The reduction process was followed by product analysis with LC-ESI-MS-MS and IC-ICP-MS. The identification is mainly based on the interpretation of the mass fragmentation and supported by a iodine mass balance. The product analysis showed a rather selective deiodination process with the successive occurrence of IMP-I, IMP-2I, IMP-3I, and a final product, respectively. The iodine mass balance based only on IMP and iodide showed a gap of about 26 % in the beginning of the electrolysis process and could be completely closed by taking the intermediates IMP-I and IMP-2I into consideration. This means that the major intermediates and final products were measured and that the reduction process is a rather selective one to remove organically bound iodine from XCM. An attractive application area would be the electrochemical deiodination of XCMs in urine of patients or hospital effluents.

## S17: Elemental Analysis

### Size Exclusion Chromatography Coupled to Electrospray Ionization Mass Spectrometry for Qualitative and Quantitative Analysis of Arsenic Interactions with Peptides and Proteins

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Arsenic-binding proteins are of toxicological importance since enzymatic activities can be blocked by arsenic interactions. In the present work, a novel methodology based on size exclusion chromatography coupled to electrospray ionization mass spectrometry (SEC-ESI-MS) was developed with special emphasis to preserve the intact proteins and their arsenic-bindings [1]. The eluent composition of 25 mM tris/HCl, pH 7.5, with the addition of 100 mM NaCl optimized for SEC with UV detection provided the highest SEC separation efficiency, but was not compatible with the ESI-MS because of the non-volatility of the buffer substance and of the salt additive. In order to find the best compromise between chromatographic separation and ionization of the arsenic-binding proteins, buffer type and concentration, pH value, portion of organic solvent in the SEC eluent as well as the flow rate were varied. Two different SEC columns providing a wide and a narrow mass fractionation range (1-300 kDa and 0.1-0.7 kDa, resp.) were involved. In the optimized procedure five different arsenic-binding peptides and proteins (glutathione, oxytocin, aprotinin, alpha-lactalbumin, thioredoxin) covering a molar mass range of 0.3 to 14 kDa could be analyzed using 75% 10 mM ammonium formate, pH 5.0 / 25% acetonitrile (v:v) as eluent and a turbo ion spray source operated at 300 °C and 5.5 kV. A complete differentiation of all peptides and proteins involved in the arsenic-binding studies as well as of their arsenic-bound forms has become feasible by means of the extracted ion chromatograms (XIC) of the mass spectrometric detection. Limits of detection in the range of 2 to 10 µM were obtained by SEC-ESI-MS for the individual proteins. The arsenic bindings of the redox-active biomolecules thioredoxin and glutathione result in a loss of their fundamental capability to regulate the cellular redox state over the dithiol-disulfide cycle. Moreover, the spatial properties of the other proteins can be altered by reduction of structure-determining disulfide bridges and subsequent prevention of reconstitution by arsenic binding.

The degree of toxicity is mainly ascribed to the quantitative extent of thiol blocking. The new method offered the possibility to estimate equilibrium constants for the reaction of phenylarsine oxide with different thiol-containing biomolecules by means of the XIC peak areas of reactants and products. The apparent constants obtained by SEC-MS were compared with ESI-MS binding studies without preceding chromatographic separation [2].

[1] A.C. Schmidt, B. Fahlbusch, M. Otto, *J. Mass Spectrom.*, in press

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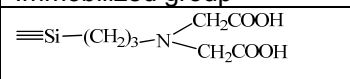
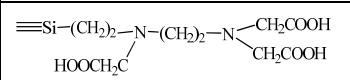
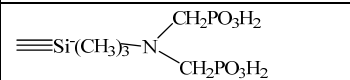
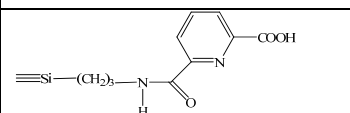
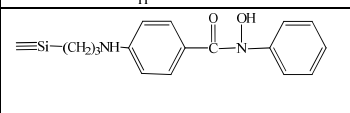
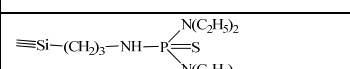
## S17: Elemental Analysis

### Organo-Silicas with Covalently Immobilized Complexing Groups for SPE of Toxic Metal Ions in Hyphenated Analytical Technique

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Eight organo-silicas with covalently immobilized derivatives of ligands having high complexing potential, namely (see table): diethylenetriamine (Dien-SiO<sub>2</sub>); 1,10-phenantroline (Phen-SiO<sub>2</sub>); amino dicarboxylic (IDC-SiO<sub>2</sub>), aminodiphosphonic (ADPA-SiO<sub>2</sub>) and ethylenediamine tricarboxylic (EDTA-SiO<sub>2</sub>) acids; pyridinedicarboxylic acid (PyDCA-SiO<sub>2</sub>), N-Benzoyl-N-phenylhydroxylamine (BPHA-SiO<sub>2</sub>) and bis(diethylamino)phosphine sulfide (DEPS-SiO<sub>2</sub>), were studied as adsorbents for dynamic pre-concentration of Zn, Cd, Hg, Pb, Ni, Cu ions from mineral and artesian water for their further determination in eluate. Optimisation of the adsorbents utilisation was performed for next parameters: capacity, selectivity, adsorption kinetics and efficiency, metal recovery and simplicity of the adsorbents preparation. It was demonstrated that all studied adsorbents have acceptable adsorption kinetics, sufficient capacity and efficiency for their application as adsorbent in dynamic SPE pre-concentration of toxic metals. EDTA-SiO<sub>2</sub> has very high capacity (up to 0.1 g of Hg per 1 g of adsorbent) but slowest adsorption kinetics and insufficient affinity to Pb ions. Dien-SiO<sub>2</sub> and ADPA-SiO<sub>2</sub> are not selective towards target group of ions. Difficulties of Phen-SiO<sub>2</sub> and BPHA-SiO<sub>2</sub> synthesis are not rewarded by their efficiency or selectivity. The best result was obtained with PyDCA-SiO<sub>2</sub> and EDTA-SiO<sub>2</sub> where recovery is reached 99% and detection limit were lowed up to 500 times.

Immobilized group	abbreviation
	IDC-SiO <sub>2</sub>
	EDTA-SiO <sub>2</sub>
	ADPA-SiO <sub>2</sub>
	PyDCA-SiO <sub>2</sub>
	BPHA-SiO <sub>2</sub>
	DEPS-SiO <sub>2</sub>

## **S18: Microchimica Acta**

### **Analytical Potential of Hybrid Nanomaterials**

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Recent years a large number of hybrid nanomaterials have been described in literature. However, they have been scarcely used in analytical sciences. In general, nanomaterials are characterized to present characteristic properties which are different from their components. Hybrid nanomaterials can be classified according its nature. In fact they can result from the combination of different carbon nanoparticles, different metallic nanoparticles or from the combination on carbon nanoparticles with metallic nanoparticles. A general overview of the synthesis of these nanoparticles and purification will be also presented.

Is this communication several hybrid nanomaterials are described with special emphasis in their properties. From this point of view two types of nanomaterials can be distinguished: i- hybrid nanomaterials which properties can be described as the contribution of the properties of individual nanoparticles, and ii- hybrid nanomaterials which present enhanced properties.

New analytical applications and uses of these hybrid nanomaterials will be presented and compared with analytical applications based on simple nanoparticles. In this context, examples such as the use of Ag-Au nanoparticles and CdSe-Ag nanoparticles in SERS, CNT-magnetita and CNT-nanodiamond nanoparticles for sample preconcentration, CdSe-polystyrene and InP-CdS in fluorescence sensing and Au-polymer nanoparticles in electrochemistry will be presented.

From the comparison will be deduced that hybrid nanomaterials present important advantages over simple nanoparticles to perform sample clean-up and preconcentration and biosensing. In general from the examples reported can be affirmed that hybrid carbon nanostructures are usefulness in sample treatment, hybrid metal-carbon nanostructures are useful in sample treatment and also (bio)sensing and hybrid metal-metal nanostructures are mainly useful in (bio)sensing.

## **S18: *Microchimica Acta***

### **Flow Field-Flow Fractionation (FI-FFF)–Multidetector: a Promising Tool for Multi-Dimensional Characterization of Nanoparticles**

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Because of their sub-micrometer size, specific surface sometimes very important and ubiquity, nanoparticles (NP) play a major role in environmental quality and elemental biogeochemical cycles. Since nanotechnologies advent and the increasing of nanomaterial-based products, NP environmental impact must be considered, all the more because few data are available about. At the same time, characterization of manufactured nanoparticle from various industrial and health sectors such as energy, electronics or biopharmacy is needed in order to better control their synthesis, properties and to increase their performances.

Accordingly, the hyphenation between Flow Field-Flow Fractionation (FI-FFF) and multi- detection including UltraViolet (UV) and Multi-Angle Laser Light Scattering (MALLS) detectors, and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) appears as a powerful analytical tool. However, relatively few studies have been dedicated to the potentiality of such coupling. The fractionation power of FI-FFF associated to the size-based MALLS detection, sensitivity and selectivity of ICP-MS are major advantages offering resolution and precision well adapted to nanometric-scale investigation. Thus, this coupling is able to reach a “virtual 3D-image” describing size, shape, chemical composition and elemental distribution on the surface/ in the volume of the nanoparticles.

Different examples will be then presented in a comparative approach, in order to illustrate the analytical potentialities of FI-FFF- ICP-MS-based coupling. Its interest for evaluating nanoparticle contribution to environmental quality and industrial control, as well as evolution perspectives will be also considered.

## **S18: *Microchimica Acta***

### **Steel Analysis by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) – a Closer Look by Electron Probe Microanalysis (EPMA)**

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LA-ICPMS is a widely used solid sampling technique especially applied in geochemistry and material sciences. The number of publications in this field has been increasing over the last decade. LA-ICPMS has many advantages compared to conventional nebulization of mineralized samples. The spatial resolution and the possibility of element profiling are outstanding advantages of the method. LA-ICPMS is more or less non-destructive, which makes it attractive when only limited amount of sample is available. Quantification however is still afflicted with several problems like inhomogeneity of the standards and a lack of appropriate matrix-matched standards.

In the present work we evaluated the applicability of LA-ICPMS (New Wave UP-213 LA system coupled to Agilent 7500ce ICPMS) for determination of major, minor and trace constituents in steel samples in order to apply the method for process and quality control. Working at laser spot sizes of 20-100 µm resulted in high standard deviations of individual elements when performing scans on polished steel samples. Possible reasons for these results could either be variations in the laser-sample interaction or inhomogeneities in the steel sample. Therefore, we had a closer look on the sample surface with EPMA (JEOL JXA-8200). The spatial resolution of EPMA is at least tenfold better than obtainable with LA. A clear segregation for most elements and zoning could be detected by means of EPMA.



## **S18: *Microchimica Acta***

### **Immuno-Pillar Chip for Rapid Point-of-Care Diagnosis**

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Immunoassay is widely used for medical diagnostics, drug discovery, biological studies, etc. and they represent some of the most vigorous activities in the field of  $\mu$ -TAS and Lab-on-a-Chip. The reduction of sample and reagents consumption and provision of rapid analyses are basically possible by miniaturizing immunoassay systems. However, multiplex detection in a microchannel is not easy. To detect multiple targets, multi-channels or multiple chips with complex procedures are usually necessary. From viewpoint of practical use, the development of multiplex detection in single microchannel is required. In this paper, we describe a rapid and easy multiplex detection of disease markers ( $\alpha$ -fetoprotein (AFP), C-reactive protein (CRP) and prostate-specific antigen (PSA)) in serum. The fabrication procedure for the immuno-pillar chip was described elsewhere. First, a mixture of anti-targets antibodies immobilized polystyrene beads (1 $\mu$ m diameter) and photopolymer (poly(ethylene glycol)-based polymer) containing a photo initiator was introduced into the inlet by capillary force. Second, UV light (365 nm) was irradiated through a photomask on the microchannel. The photopolymer was polymerized only in the exposed parts, and the exposed parts became hydrogel pillars including many anti-targets antibodies immobilized beads. We call this microchip the "immuno-pillar chip". Assay required only a pipette for the introduction of the sample and reagents except for a fluorescence microscope for fluorescence measurement. The secondary antibodies for the targets were labeled with fluorophores which emit fluorescence at different wavelength. We assayed human sera spiked with AFP, CRP and PSA by using the immuno-pillar chip. The spiked sera were introduced directly into the microchannel. The calibration curves of AFP, CRP and PSA are quite good. The total assay time was only 12 min. The limits of detection (LODs) were about 10 pg/mL for three biomarkers. From these results, we judged the immuno-pillar chip had great potential for rapid point-of-care diagnosis.

## **S18: *Microchimica Acta***

### **Quality Control with Multivariate Near Infrared Spectroscopic (NIRS) Methods in Different Production Steps of a Pharmaceutical Intermediate**

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Near infrared spectroscopy was used to develop an effective quality control and monitoring system in the manufacturing process of the  $\beta$ -lactam antibiotic intermediate 7-aminocephalosporanic acid (7-ACA). This application compasses the online control of the drying process of the respective precursor substance and the multivariate characterization of the final product as part of a process analytical technology (PAT) process control practice.

A simple functional sampling system with a NIR measuring setup was developed, which enables monitoring and control of the three relevant product parameters. The values for product assay, water content and residual solvent predicted with NIR calibrations are in good agreement with the respective reference values. Using the online monitoring system the drying process could be efficiently optimized. For the quality control of the final intermediate quantitative and qualitative NIR models can serve as alternative and extension of conventional chemical analysis methods. An over-all quality control test based on NIR spectroscopy was introduced using a multivariate model reflecting the allowed chemical quality range of the pharmaceutical substance. The composition of the calibration set had to be precisely selected and spectra acquisition, evaluation and analysis had to be extensively optimized to achieve the required sensitivity of the model. For each batch a simple chemometric summary parameter is calculated from the routinely recorded NIR spectrum according to the model to support the PAT quality control system that supervises the whole production process.

On the basis of the presented NIR applications the potential of the method could be demonstrated and the advantages of the technique could directly be capitalized on improved production and quality control.

## S18: *Microchimica Acta*

### Surface Characterization of Nanomaterials: Reinforcing Fillers and Fuel Cell Catalysts

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For an adequate characterization of highly dispersed materials which also may show a high degree of structural complexity the combination of quite different analytical techniques is essential. This includes standard methods of classical analytical chemistry and instrumental analysis as well as the development and adaptation of dedicated methods of materials research for the individual case. This shall be illustrated by presenting typical applications from the chemical industry. On focus is the surface analytical characterization of carbon blacks which are used at the large scale as reinforcing fillers for tyres or as highly efficient pigment blacks and, furthermore, the characterization of Pt/C fuel cell catalysts under realistic gas pressure. After short discussion of the techniques which are usually applied (electron microscopy, chemical analysis, surface spectroscopy) the benefits of combining such data with results on the hydrogen-related surface chemistry which is accessible by inelastic incoherent neutron scattering (IINS) will be addressed. IINS is highly complementary to Infrared-, Raman- and NMR-spectroscopy especially if materials properties such as a strong absorption of electromagnetic radiation or electrical conductivity seriously impair the use of their full potentials. IINS measurements of the proton dynamics of certain surface structures allows spectroscopical characterization down to hydrogen concentrations in the ppm-range. Furthermore, the dissociatively adsorbed, atomic hydrogen at the surface of nanometre-sized supported precious metal particles can be utilized as a selective local probe for catalytically relevant adsorption sites. Surface science measurements under hydrogen pressures such as 1000 mbar and more are possible. This allows new insights in the search for the real active sites of technical catalysts. Also the IR- and Raman-forbidden  $J=0 \rightarrow J=1$  rotational transition of the hydrogen molecule can be used as a probe for directed, selective H<sub>2</sub>/surface interactions. This will be exemplified by IINS results on activated carbons which are used for the manufacture of supported precious metal catalysts for chemical syntheses.

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## **S19: Environmental Analysis 2**

### **Investigation of 1,1-Dimethylhydrazine Behavior in Soils**

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Unsymmetrical dimethylhydrazine (UDMH) became one of the most toxic xenobiotic compounds since it happened to be used for rocket launching. Large areas of landscapes were contaminated by UDMH when its residues spilled out on soil surface from landing rocket tanks. The environmental chemistry of UDMH is a complex problem being under discussion for last several years. The identification of numerous products of UDMH decomposition is not comprehensive yet. The other problem is connected with the explanation of high stability of UDMH in soils despite of its high reduction ability. In this research such modern methods as LC-MS, GC-MS and preparative high performance liquid chromatography followed by NMR studies were applied for the investigation of the decomposition products of UDMH in model suspensions of various types of soil samples contaminated by this toxicant. Dimethylamine (1), 1-methyl-1,2,4-triazole (2), 1,1-dimethylguanidine (3), 1-formyl-2,2-dimethylhydrazine (4), methyl- and trimethyl- hydrazines, 1,5,5-trimethylformazane, 1-methyl-1,6-dihydro-1,2,4,5-tetrazine, dimethylhydrazones of acetaldehyde, formaldehyde and glyoxal were found as to be the decomposition products of UDMH. The coincidence of chromatographic and spectral properties of synthetic standards with supposed structure provided unequivocal identification of each substance. The products of decomposition (1)-(4) were found in real soil samples taken from polluted areas.

The explanation of high stability of UDMH in soils was given by analyzing of aqueous extracts before and after its alkaline distillation. The initial extract of soil after 90 days of UDMH spillage did not contain this compound, but 1-formyl-2,2-dimethylhydrazine was found in it. On the contrary, after the distillation considerable concentration of UDMH in the absence of 1-formyl-2,2-dimethylhydrazine was found. Therefore, the appearance of UDMH in final solution could be explained by hydrolysis of 1-formyl-2,2-dimethylhydrazine during samples preparation in alkaline conditions. That is why more stable 1-formyl-2,2-dimethylhydrazine erroneously identified as UDMH in soils, especially in those which are analysed after several months of UDMH spillage.

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## S19: Environmental Analysis 2

### Metal Binding Properties of Humic Like Substances of Atmospheric Aerosols

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Volatile organic components (VOC) of the atmospheric aerosols undergo various oxidation reactions and becoming more and more polar compounds. Beside the oxidation processes, however, even less well understood polymerization reactions may also take place leading to the formation of a large number of secondary organic compounds of which many are polar, water soluble (WSOC). The WSOC usually amounts to 20 – 70% of the fine (0,1-1  $\mu\text{m}$ ) fraction of the tropospheric aerosol collected by the usual sampling impactors. A further treatment of the WSOC fractions with solid phase extraction proved that 20 – 50% of these compounds are humic-like substances often referred to as HULIS in the literature.

In connection with the chemical characterization of the aerosols our current work is concerned with the ICP-MS determination of the transition metal ion content of the aerosols and with the stability constant as well as the copper(II) ion binding capacity of the WSOC components. According to our best knowledge no similar studies were reported earlier in the aerosol literature. Electroanalytical studies were performed by using the competing ligand exchange technique of the adsorption stripping voltammetry (CLE – AdSV). Competing ligand exchange is used here to avoid the problem caused by the poorly characterized complex forming ligands of the WSOC. As a competing ligand salicylaldoxime was used. Our results indicated that the WSOC components of the fine aerosol (typically the HULIS) forms stable complexes with Cu(II)-ions. Stability constants of the various aerosol samples ( $\log K = 16.0 - 16.5$ ) were calculated from the data of the CLE – AdSV experiments by using the *van den Berg – Ruzic* equation. The applied method of calculation yields also an estimate for the metal ion binding capacity of the WSOC. The obtained capacity values were in between 6.6 – 39 nM Cu(II) / mgC for the aerosols collected in various seasons.

## S19: Environmental Analysis 2

### Determination of Trace Veterinary Fluoroquinolones in Soils and Waters and Role of Photochemistry in their Degradation

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Fluoroquinolones (FQs) are highly useful antibacterial agents, largely employed for human and veterinary use. They have been detected worldwide in surface waters and soils because of their environmental persistence and photo-induced by-products formation, potentially dangerous for organisms. In this study the determination in surface waters, sandy and loamy soil samples of two veterinary FQs, Enrofloxacin (ENR) and Marbofloxacin (MAR), widely used in farming between Pavia and Milan (Italy) was investigated. Soil samples were collected from manured fields, dried at room temperature in the dark, sieved (2 mm). The FQs extracted [1] were filtered on nylon membrane (0.45 µm), preconcentrated by SPE on a hydrophilic-lipophilic and weak anion exchanger cartridges and analyzed by HPLC with fluorescent detector (FD). The great chemical stability of FQs makes these highly persistent contaminants. A possible mechanism of degradation is photochemistry [2,3]. At this purpose photo-induced degradation by-products were studied both on soils samples and spiked natural waters. The influence of matrix composition on the rate of degradation, i.e. natural organic matter but also chloride, phosphate, magnesium and calcium ions concentration, was investigated. The irradiation was carried out by using a solar light simulator and by exposing the samples under natural solar light.

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## **S19: Environmental Analysis 2**

### **Concepts and Recent Trends in Green Analytical Chemistry**

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Growing public concern over protecting our environment is obliging all chemists to modify their chemical activities in such a way that they will be conducted in an environmentally friendly manner. This can be realized within the framework of the principles of green chemistry.

The irony is that the methods used in laboratories to analyze the state of environmental pollution, as well as the analytical chemists applying them, through the uncontrolled disposal of reagents, solvents, and other chemical wastes, may themselves be the source of large amounts of pollutants entering the environment. Traditional analytical procedures require considerable quantities of chemical compounds; sampling, and especially the preparation of samples for their final determination, frequently involves the formation of large amounts of pollutants (vapors, liquid effluents—waste reagents and solvents, and solid waste). If environmental pollution by analytical reagents and so on is to be avoided, the rules of green chemistry must be introduced into chemical laboratories on a large scale. Analytical chemists strive for the traditional goals of accuracy, precision, sensitivity, and low detection limits; but by implementing green chemistry rules in laboratory practice, they are demonstrating their awareness of the impact of their work on the environment.

The main objective of green analytical chemistry is to apply analytical procedures and devices that generate less hazardous waste, are safer to use, and kinder to the environment. This objective can be achieved through the development of entirely new analytical methodologies or the modification of old ones to incorporate procedures using either fewer hazardous chemicals or at least smaller quantities of them. The general strategy toward making analytical methodologies greener involves not only changing or modifying reagents and solvents, or reducing the amounts of chemicals used, but also the miniaturization and even the elimination of sampling by measuring the analytes of interest *in situ* in real time and on-line.

The paper presents recent advances in green analytical chemistry in the context of the whole analytical process, that is, from sample collection, sample preparation, to sample analysis, as well as the characteristics of some traditional methodologies that have always been environmentally benign but were never described as “green.”

## **S19: Environmental Analysis 2**

### **Horizontal Rotating Tubular Bioreactor (HRTB) - a Promising Tool for Heavy Metal Removal from (Textile) Wastewaters**

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Heavy metals are important and frequently investigated due to their wide exposure in the environment as well as to the evidence of their specific biological and toxicological effects. Heavy metals are often found in our environment due to their frequent industrial usage and presence in industrial wastewaters which are released into streams and rivers without adequate treatment for their removal. In wastewater treatment various bioreactors with microbial biofilms are often used, but the special design of a horizontal rotating tubular bioreactor (HRTB) has shown promising removal rates in a pilot study. Thus its applicability for the removal of metals commonly present in textile wastewaters, such as chromium, manganese and cobalt, was studied. In order to optimize the removal efficiency different process parameters (inflow rate, rotation speed) were used and water samples were drawn along the bioreactor for monitoring the metal contents. The concentrations of the elements were quantified on site by UV-VIS spectrometry. Furthermore the metal uptake of the biomass was determined by ICP-MS after microwave assisted digestion of the organic matter.

The maximum removal rates obtained are 100%, 94% and 69% for chromium, manganese and cobalt, respectively. Due to these high efficiencies HRTB seems to be an appropriate tool for the treatment of heavy metal loaded textile wastewaters.



## S19: Environmental Analysis 2

### A Fast and Effective Routine Method Based on SPME and GC/ICP-MS for the Monitoring of Organotin Compounds in Surface and Sea Waters

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Among the organometallic species with relevant toxicological effects on the whole environment, organotin compounds are one of the most widespread molecules owing to their past intensive use in agriculture and paint and polymer industry. The toxicity of these persistent pollutants, namely dimethyltin (DMT), dibutyltin (DBT), and tributyltin (TBT) has been demonstrated even at the ng/l and the EU included them in the list of priority pollutants<sup>1</sup>.

In this work a rapid and very sensitive method based on solid-phase microextraction (SPME) followed by gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) was developed and validated aimed at the determination of DMT, DBT and TBT in water samples. A divinylbenzene/carboxen/polidimethylsiloxane fiber was used for the SPME sample treatment. Coupling of the ICP-MS with GC was accomplished optimizing an easy-to-fit in-house interface<sup>2</sup>. Optimal conditions were found by using the enhanced "hot plasma/protective ion extraction" introduction system.

GC-ICPMS made possible the determination of the organotin compounds at ultratrace levels, quantitation limits ranging from 0.04 to 5 ng/l. The method was also proved precise and rapid, GC-ICPMS allowing for the separation of organotin species in less than 7 minutes.

The device is technically simple, flexible, low cost and its home-made assembly is trouble free. The method was used in routine analysis of fresh and marine water samples.

<sup>1</sup>European Union, European Directive 76/464, Office of EU Commission, Bruxelles

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## **Plenary Lecture 6**

### **Microarray Technology - Current Status and Trends**

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Microarrays represent currently one of the most powerful analytical tools for the simultaneous detection of multiple parameters within one analysis step. Usually, the specific affinity reaction of nucleic acids and/or antibodies towards antigens is the most common bioanalytical method for generating multiplexed quantitative results.

Diverse fluorescence, chemiluminescence and other label-free microarray readout systems have been developed in the last decade. Especially the label-free techniques are wanted, but still need further research.

The lecture will exemplarily show the achieved development status connected with a critical evaluation of the current achievements.

Literature:

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## Plenary Lecture 7

### LC×LC: Liquid Chromatography Enters a New Dimension

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During the last few years liquid chromatography (LC) has experienced a great deal of progress. The performance of the technique is being improved by the introduction of novel types of columns, such as monoliths, and by increasing the maximum permissible pressure on high-pressure LC systems. These advances make it possible for LC users to perform a given separation more rapidly or more efficiently (*i.e.* narrower peaks).

There is a strong demand from various areas of science (*e.g.* life science, food science) to separate ever more complex samples into as many individual components as possible. For such challenging problems a better measure for the performance of a chromatographic system is the peak capacity, which can be defined as the number of peaks that may possibly be separated on a given system. The peak capacity of LC systems has been increased significantly by the advances listed above. Many hundreds of peaks can be accommodated in a single LC run. Patient researchers may even reach a peak capacity of 1000 (in about a day). While impressive, these results are still well short of the most ambitious targets. For example, the human proteome is assumed to contain some 50,000 proteins. Comprehensive two-dimensional liquid chromatography (LC×LC) constitutes a significant step in the right direction. Using this technique a peak capacity of several thousand can be achieved within a reasonable time (one or two hours).

In this lecture the potential and practical performance of one-dimensional LC and LC×LC will be compared. Different ways of performing LC×LC will be discussed and we will take a peek into the future. Is LC×LC×LC a realistic option?

## Plenary Lecture 8

### New Stationary Phases for Enrichment and Separation Technologies in the '-omics' Area – a Challenge in Analytical Separation Science

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“Omics” is a general term for a broad discipline of science and engineering for analyzing the interactions of biological molecular components in various “omes”. These include genome, proteome, metabolome, expressome, and interactome. “Ome” and “omics” are very convenient handles for describing the holistic approach for looking at complex systems. “Omics” will not only have an impact on our understanding of biological processes, but also on the prospect of more accurately diagnosing and treating disease. The development of these ‘omics’ has depended on, and has also driven advances in bioanalytical approaches including liquid chromatography, electrophoresis and mass spectrometry to permit the handling of large numbers of biological samples at high selectivity and sensitivity. Thus the design of novel stationary phases for selective enrichment and separation is one of the key points for establishing a successfully running “omics” platform. There is a need for highly efficient approaches to handle the problems associated with sample preparation, separation and identification of biological species. Many newly emerged technologies meet the basic requirements. In particular, nano-materials have a great impact on future sample preparation due to their unique physical and chemical characteristics. Novel enrichment and desalting methods based on modern SPE technologies are applied to reduce the complexity of biological samples while  $\mu$ -HPLC is used for separation, preconcentration and fractionation. Considerable progress has been made in the development of stationary phases for separation and sample preparation. Several chemistries are available for a wide range of applications which can be tailored to a specific application allowing endless possibilities in terms of selectivity tuning.

#### **Literature:**

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## **P001-A1**

### **Determination of Mitomycin C in Urinary Bladder Tissues by HPLC-MS Technique**

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Mitomycin C (MMC) is a widely used chemotherapeutic agent, applied as a single drug or in combination with other chemotherapeutic drugs. The most significant MMC toxicity in humans is myelosuppression, widely confirmed to be a delayed and dose-related effect. The determination of this antibiotic in aqueous solutions and in biological fluids, mainly plasma and urine, has been a subject of special interest in several papers, but no attention has been given to determination of mitomycin in tissues. Recently a new scheme for urinary bladder cancer was suggested. It combines mitomycin administration followed by laser exposure. Due to local character of such influence and small amount of accessible tissue, the method for the determination of mitomycin in samples of this kind should be precise and sensitive.

In current work, a new simple and sensitive technique of determination of mitomycin C in biological tissues was developed and validated. After a one-step extraction procedure the sample was analyzed on a reversed-phase column Zorbax SB-C18 using water-acetonitrile mixture as a mobile phase. The detection was performed in ESI-SIM mode (ion with  $m/z$  357 was used for quantitative analysis and  $m/z$  355 as a qualifier). The detection limit was 0.2 ng/g. The suggested method was successfully applied in clinical trials.

## **P002-A1**

### **Anti-Asthmatic Drugs in Plasma/Serum – a Major Analytical Challenge**

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#### **Introduction**

Asthma therapy is frequently effected by way of topical application to the airways. As the quantity of substance applied is in the 10 – 500 µg range, detection from plasma or serum is extremely difficult, with concentrations of between 1 – 1000 pg/mL plasma or serum. The only really viable analysing technique in this case is HPLC tandem MS. As the various drugs deployed (anti-inflammatory and bronchodilatory substances) each have very different lipophilic properties, sample preparation is also a major challenge.

#### **Experiments**

Bronchodilators:

- A: Formoterol in serum (calibration range 0.4 – 100 pg/mL)
- B: Salbutamol (=albuterol) in plasma (calibration range 10 – 3900 pg/mL)
- C: Salmeterol in plasma (calibration range 5 – 1000 pg/mL) and

Anti-inflammatory substances:

- D: Budesonide in serum (calibration range 5 – 1000 pg/mL)
- E: Fluticasone propionate in serum (calibration range 3 – 1000 pg/mL) and
- F: Ciclesonide and major metabolite M1 in serum (10 – 1000 pg/mL).

#### **Apparatus**

All work was conducted using only Sciex tandem mass spectrometers with Agilent or Perkin Elmer pumps and autosamplers. For B, E and F, an API 3000 was used; and for A, C and D, an API 4000. Methods A, B and C use ESI for ionisation, while methods D, E and F use APPI.

#### **Sample preparation**

Methods D, E and F (anti-inflammatory substances) use 0.5 – 1 mL of serum. The substances are extracted from the plasma (by liquid-liquid distribution).

In methods A, B and C (bronchodilators), offline solid-phase extraction, mostly with reversed-phase (C18) or ion exchange material, is used under very varied conditions.

#### **HPLC-MS/MS conditions**

The conditions for D, E and F are chosen so that for APPI, 10 % (API 3000) or 20 % acetone (API 4000) serves as the charge carrier (dopant) in the mobile phase.

A gradient is used from e.g. 10 mM acetic acid in 20 % acetone / 80 % water to 20 % acetone / 80 % methanol, through a C18 reversed phase column. Measurement is carried out as adduct ions in the negative mode.

Substances A, B and C are measured with ESI positive ionisation. The chromatography is with C18 columns. The mobile phases are formic acid in water or methanol.

#### **Summary**

Recent trials on the even more sensitive API 5000 have demonstrated that detection of fluticasone propionate is possible down to approx. 0.5 - 1 pg/mL of serum, and of formoterol down to 0.1 pg/mL of serum.

#### **Our publications**

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- Poster EFMC-ISMC Aug 2008, Vienna
- Poster EBF, Dec 2008, Barcelona

## **P003-A1**

### **Solid-Phase Extraction of Tetracycline from Human Urine and Control Serum**

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Tetracycline is a “broad-spectrum” antibiotic which is used to treat bacterial infections. It is often an alternative drug for people who are allergic to penicillin. Its plasma concentration is high, and excretes unchanged in the urine. For therapeutic and pharmacokinetic studies it is necessary to quantify tetracycline in physiological samples such as serum and urine.

Tetracycline is an amphoteric molecule soluble in polar and moderately polar organic solvents. It has ability to form stable complexes with divalent cations. In use is in the form of tetracycline-HCl which is very soluble in water. Because the complex urine and serum composition, it is necessary to carry out sample cleanup and appropriate extraction procedure prior to analysis.

In this study we developed SPE method for tetracycline extraction from human urine and control serum before HPLC determination.

Human control serum and urine samples were spiked with standard tetracycline solution and diluted with deionized water. The concentration range of tetracycline was from 4.0-8.0 µg/ml for serum and 3.0-7.0 µg/ml for urine. Solutions of EDTA and H<sub>3</sub>PO<sub>4</sub> were added in the samples to obtain pH 4 and to achieve precipitation of proteins and removal of metal ions.

For the solid phase extraction procedure silica phase C18 cartridges with special modification (Macherey-Nagel CHROMABOND Tetracycline, 6 ml/500 mg) were used. Sample application was performed with a flow rate of ~ 1 ml/min. After sample loading there was no need to wash column. Moreover, low recoveries were observed when the column was washed with 5% methanol solution.

As the elution solvent methanol and mobile HPLC phase (tert-butyl alcohol /phosphate buffer pH 9.0) were tested. The recovery values were poor when methanol was used. Better results are achieved when tetracycline was removed by elution with mobile HPLC phase.

The eluates were analyzed directly by HPLC-DAD on Zorbax Extend C18 analytical column without previously evaporation and reconstitution. Recovery rates of the spiked samples were 95-98% for serum and 87-94% for urine when mobile HPLC phase was used for elution.

#### **Acknowledgement**

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## P004-A1

### High Sensitivity Detection of *Streptococcus Pneumoniae* tmRNA Molecules by NASBA-Microarray

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We present a novel microbial diagnostic method that combines the sensitivity of NASBA with the high information content of microarray technology for the detection of bacterial tmRNA molecules. NASBA is a sensitive isothermal RNA amplification technology that has routinely been used in various fields of microbiology and microbial diagnostics. As DNA microarrays have recently shown great potential in investigation of microbial diversity, composition and species identification from environmental and medical samples, combination of these two powerful methods looks very promising. In our case, the NASBA protocol was modified to include aminoallyl-UTP (aaUTP) molecules that were incorporated into nascent RNA during the NASBA reaction. Post-amplification labeling with fluorescent dye was carried out subsequently and tmRNA hybridization signal intensities were measured using microarray technology.

The most favorable concentration of aaUTP was identified (1) and used in following NASBA amplifications of tmRNA from series of dilutions of *Streptococcus pneumoniae* total RNA ( $1 \times 10^2$  to  $1 \times 10^{-1}$  CFU/ml). Developed method was sensitive enough to detect and identify tmRNA molecules from common respiratory diseases causing pathogen *S. pneumoniae* at a concentration of less than one CFU per ml of culture. If we take the actual count of tmRNA in reaction into consideration, we achieved the detection limit as high as 5 molecules of RNA per NASBA amplification. Altogether, we have successfully demonstrated efficient combination of NASBA amplification technology with microarray based hybridization detection. Method is applicable for many different areas of microbial diagnostics including environmental monitoring and clinical microbiology.

#### Acknowledgement

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## **P005-A1**

### **Evaluation of Antioxidant Activity of Blood**

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Appearance and development of many diseases is accompanied by activation of free-radical processes. It relates to cardiovascular diseases, reproductive system pathology, diabetes mellitus, carcinogenesis and other. Antioxidant activity (AOA) of blood is a parameter of antioxidant system of an organism. Diagnostics of this parameter is necessary for definition of risk of occurrence of the diseases connected with deficiency of antioxidants in an organism and for their therapy.

The electrochemical method of integrated antioxidant activity of blood and blood fractions estimation, based on interaction of chosen mediator system with antioxidants of the investigated sample, was proposed. In case of whole blood and erythrocytic mass analysis the interpretation of received results is complicated with the contribution of hemoglobin to the blood total reducing activity (TRA). The calculation of cellular component of antioxidant activity in TRA of whole blood and erythrocytic mass, taking into account the stoichiometric relationships of interaction between hemoglobin standard solutions and mediator system, and also hemoglobin and hematocrit parameters.

Considering that the majority of existing methods of AOA definition is based on of radical interactions use, the phase of spontaneous thermal disintegration of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) initiator with peroxide radicals formation has been included in electrochemical method. The latent period of reaction is determined. By analogy to known methods, data about AOA of whole blood and erythrocytic mass are expressed in trolox equivalents. The obtained data correlates with the data received by electrometric method without the use of radical reaction. It confirms the results reliability of the electrometric method in a variant we offer.

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## **P006-A1**

### **Application of Ion Chromatography for the Determination of Inorganic Ions, especially Thiocyanates in Human Semen Samples as Biomarkers of Environmental Tobacco Smoke Exposure**

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Tobacco smoking constitutes a significant source of indoor air pollution in many types of closed spaces. Some research centers conduct studies on the tobacco burning process, the composition of tobacco smoke and on how it affects the bodies of active and passive smokers. The analysis of human biological fluids (human semen samples) can be a valuable source of information about the risk level for humans from environmental tobacco smoke.

One of the biomarkers of environmental tobacco smoke exposure are thiocyanate ions. Low concentrations of thiocyanate are usually present in biological fluids mainly due to the subject's diet. This may be through digestion of cassava foods (mostly in the tropical countries in Africa, Asia, Latin America), some vegetables of the genus *Brassica* containing glucosinolates (cabbage, turnip, kale) or by intake of thiocyanate-containing foods such as milk and cheese. Higher concentration of this ion, which is a metabolic product of cyanide, arises from environmental tobacco smoke. The level of thiocyanate is thus considered a good probe for distinguishing between smokers and non-smokers and its determination has been used for the evaluation of smoking behaviour.

The aim of this scientific study was to present the effectiveness of the proposed sample preparation procedure coupled with ion chromatography technique for the determination of inorganic ions, especially thiocyanates (as biomarkers of environmental tobacco smoke exposure) in human semen samples collected from passive, moderate and heavy smokers. The experimental results were put to the data handling in order to obtain maximum possible information concerning the impact of the intensity of smoking on the chemical composition of human semen samples.

## **P007-A1**

### **Investigation of Separation of Water-and-Fat Soluble Vitamins in Feeds and their Components**

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Vitamins are a broad group of organic compounds that are minor, but essential, constituents of food required for the normal growth, self-maintenance and functioning of human and animal bodies[1]. These compounds can be classified in two main groups: water-soluble and fat-soluble vitamins. Among water-soluble vitamins, the B group including B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> and B<sub>12</sub> are the most important. They play different specific and vital functions in metabolism, and their lack or excess produces specific diseases.

In the present work, a simultaneous determination of water-soluble vitamins [nicotinamide (PP), cyano cobalamine (B<sub>12</sub>), riboflavin phosphate (B<sub>2</sub>), pyridoxine hydrochloride (B<sub>6</sub>), thiamine hydrochloride (B<sub>1</sub>)], and fat-soluble vitamins [retinyl palmitate (A), a-tocopherol acetate (E), cholecalciferol (D<sub>3</sub>)] is investigated[2]. The appropriate conditions of sorbent, adsorption, washing and elution are investigated. Afterwards, high-performance liquid chromatography with solvent programming has been the appropriate tool in overcoming the overlapping of certain pairs of vitamins and also in adjusting the elution volumes of early and late elution peaks. The experimental variables studied were: application volume, elution solvents and cleaning solutions. The DAD detection of vitamins was made in real samples at different concentration levels. The accuracy of the method was tested .

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## **P008-A1**

### **Determination of Catecholamines in Mouse Plasma Using HPLC-Peroxyoxalate Chemiluminescence Detection**

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Plasma catecholamines (CAs: norepinephrine, epinephrine and dopamine) are widely used as an index of the sympathetic nerve system. However, until now, no analytical method of plasma CAs in mouse was developed. Therefore, in the work, we established the sensitive determination method of CAs in mouse plasma using high-performance liquid chromatography (HPLC)-peroxyoxalate chemiluminescence reaction detection. Automated precolumn cation-exchange extraction of diluted plasma was coupled with the separation on ODS column, fluorescence derivatization with ethylenediamine, and finally peroxyoxalate chemiluminescence reaction detection. The detection limits of the method were below 5 fmol. The methods developed needed only 10  $\mu$ l of plasma, which enabled the determination of CAs in single mouse plasma. The plasma CAs concentrations in normotensive mouse (C57BL, male) were  $6.63 \pm 1.37$ ,  $0.49 \pm 0.10$  and  $5.25 \pm 2.30$  (pmol/ml,  $n=7$ ), for norepinephrine (NE), epinephrine and dopamine, respectively. When the blood pressure was reduced in mouse, the increases of plasma CAs concentrations were observed. The developed method would be useful to investigate the roles of sympathetic nervous system in mouse.

## **P009-A1**

### **Analysis of Phytoplankton Pigments Composition in the Argentinian Continental Shelf**

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Marine phytoplankton plays important roles in CO<sub>2</sub> withdrawal from the atmosphere, primary production and biogeochemical fluxes of carbon, nitrogen, phosphorus and other micronutrients, and the types of phytoplankton present varies its influence in ecosystem and in marine biogeochemical cycles [1].

Traditionally the methodology for determining the phytoplankton biomass has been the quantification of chlorophyll a [2]. But this method is limited, since it is not possible to distinguish between different pigments or the type of phytoplankton communities present. For this reason, other methods for the determination and quantification of phytoplankton pigments are being applied nowadays.

The application of high performance liquid chromatography (HPLC) is one of the most used methods [3]. HPLC is coupled with a UV-visible detector for the determination of phytoplankton pigment individually which let determine the kind of phytoplankton communities that it is found in the area.

The studied zone, in the southwestern Atlantic and over the Argentinian continental shelf (between 36° and 50°S), represents a confluence zone located between the Malvinas and Brazil currents [4]. This zone has a wide continental shelf with shallow and euphotic waters, very rich in nutrients. For these reasons this shelf is characterized by the proliferation of phytoplanktonic blooms, giving areas with high biomass concentration [5].

In this work it has been determined the types and concentration of phytoplankton pigments. They were determined in several samples at different depths from surface down to 200 metres. Sample stations were chosen according to satellite observation at the moment of the oceanographic cruise (BLOOM 2008, March 2008).

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## **P010-A1**

### **Caffeine Immunoassay: Application for Clinical, Beverages and Environmental Samples**

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Caffeine has been in use for different purposes, from human therapeutics to insecticide in agriculture, or as a marker of the liver function in clinical diagnostics, and lately even as a marker of environmental waters contamination.

Searching for non-invasive and effortless sampling methods for human diagnostics has been an intense field of research in clinical chemistry throughout the past ten years. Salivary samples represent a major improvement vis-à-vis the classical invasive blood sampling procedure as well as the analytical challenge, mainly due to fewer matrix interferences. Caffeine body-clearance can be used to evaluate patients' liver function/dysfunction no matter what kind of matrix is selected to do it: blood, urine or saliva. Therefore salivary samples were selected and measured using the developed immunoassay and the results compared to those obtained using LC-MS/MS in MRM mode. The anti-caffeine monoclonal antibody used for this assay, the enzymatic tracer (a horseradish peroxidase conjugate) and the buffers, are also suitable for caffeine monitoring in environmental waters, several beverages (coffee, tee, soft-drinks, beer) and even shampoo and caffeine tablets. Apart from different sample preparation steps and minor changes regarding the antibody and tracer concentrations, a single analytical tool can be used for completely different applications.

The results obtained by LC-MS/MS and by ELISA are compared using different statistical tools, which were selected to assay their equivalence for being indiscriminately used in clinical, food/beverages or environmental analytical chemistry.

Acronyms used:

LC-MS/MS - Liquid Chromatography-Tandem Mass Spectrometry

ELISA - Enzyme-Linked ImmunoSorbent Assay

MRM - Multiple Reaction Monitoring mode

## P011-A1

### Genotype Determination of Canola(*Brassica Napus*) Grown in Turkey Using Molecular and Analytical Methods

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Canola with low erucic acid is also one of the [cultivars](#) of *Brassica napus*. Canola has several phytochemicals which lead to prevent cancer, cardiovascular disease, aging by scavenging free radicals in human metabolism [1]. *Brassica* species seeded in spring and winter have been bred as two different forms adapted two different cultivation environments. These two forms have distinct gene pools based on DNA markers. This distinct results in difference distribution of fatty acids, flavonoid and phenolic contents of canola genotypes. Production of improved novel seed components by combining genetic engineering and analytical methods with classical plant breeding new innovations as modern biotechnology will result in functional seed with high quality.

In this study, the difference between winter and summer species of canola was studied by molecular markers and analytical methods. First of all, genomic DNA from canola genotypes was extracted with EZ1 automatic nucleic acid isolation system. DNA from each seed was amplified with OPA primer series (OPA1-10) in thermal cycler by randomly amplified polymorphism DNA (RAPD-PCR) technique. The difference between two genotypes of canola in molecular level was identified by polymorphic or nonpolymorphic bands [2].

In analytical methods, fatty acid content of the methanolic extracts of both species was identified by gas chromatography. Antioxidant activity of the extracts was determined by several spectroscopic methods including DPPH, reducing power, metal chelating,  $\beta$ -caroten-linoleic acid system and CUPRAC methods [3]. Several phenolic compounds of the extracts were analysed by using high performance liquid chromatography. Obtained results from two genotypes of canola in molecular and analytical level were compared.

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## **P012-A1**

### **Impact of Xanthohumol on the Yeast Vitality and Viability: Micro-Scale Approach**

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The health-promoting properties of xanthohumol (XN) have attracted attention in the brewing community concerning the production of XN-enriched beers. However, in several studies it was found that the fermentation process is a factor responsible for a large reduction in the level of XN during the beer production [1].

It is well known that the yeast physiological status is a key factor affecting not only fermentation consistency and performance but also key quality properties of the final beer [2]. In order to understand how XN affects the metabolism of the yeast cells, in the present work the yeast viability (live/dead status of cells) and vitality (activity of the live cells), were studied and evaluated during the production of a XN-enriched beer. Three different brewing trials were performed: (A) control – wort without XN, (B) wort with 1 mg/L of XN, and (C) wort with 10 mg/L of XN. Viability by citrate methylene blue staining was performed according to the EBC Analytica method [3] whereas vitality was determined by the “vitaltitration” method specifically developed by this research team [4]. The XN content was determined by HPLC-ESI-MS/MS with a previously step of purification and concentration of the sample by solid-phase extraction (C<sub>18</sub>).

The results showed that the viability of the yeast was greater than 85% upon completion of fermentation for all brewing trials. In respect to the yeast vitality, the XN-enriched beer (10 mg/L) showed higher yeast vitality (*good* vitality) in the end of the fermentation. In opposite, the control trial (A) and trial with 1 mg/L of XN (B) showed lower yeast vitality (*bad* vitality) compared to the beginning of fermentation. These results suggest that XN has a positive effect on the physiological condition of the yeast, by a mechanism that is now under investigation.

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## **P013-A1**

### **High Efficient DNA Separation with Aminofunctionalized Mesoporous Silica Fine Particles**

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In this work, we reported the easy preparation of highly ordered mesoporous silica fine particles (MSFPs) by the addition of transition metal salts such as  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  in the surfactant micellar solution. As far as a simple synthetic process is concerned without employing any organic co-solvents, the addition of metal salts plays a key role in the self-assembly of surfactant micelles and complexes of surfactant micelles and silicate species. Moreover, the obtained MSFPs were demonstrated by high efficient DNA separation through the molecular assembly as a function of the number of amino group tailored such as mono-, di-, and tri-amino-functionality. The DNA separation efficiency was explored via the function of the amino-group number, particles size, used amounts, and the NaCl concentration. The DNA adsorption yields were high in terms of the use of triaminofunctionalized MSFPs and DNA desorption efficiency showed the optimum level at over 3.0 M of NaCl concentration. Comparing with conventional micron-sized mesoporous silica based processes for filters or membranes, this approach of the utilization of nano-sized materials to DNA separation provides many immediate advantages, including higher surface-to-volume areas, enhanced binding rates, higher efficiency, and higher specificity.

This preliminary study could enable the design and construction of an automatic system with high-throughput biomolecular purification with functionalized MSFPs, and would be applied for clinical diagnoses and proteins/enzymes recognition processes using the appropriate surface modification technique.

## **P014-A1**

### **Simultaneous Determination of Pregnenolone and 17- Hydroxypregnenolone Secreted by Bovine Adrenal Cortex Cells Using Immobilized Cholesterol Oxidase as a Pre-Column Reactor**

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A specific ultraviolet detection system using an immobilized cholesterol oxidation enzyme reactor/ semi-micro high-performance liquid chromatography has been developed for the simultaneous determination of pregnenolone (Pre) and 17-hydroxypregnenolone (17OHPre). Pre and 17OHPre were converted to progesterone and 17-hydroxyprogesterone by the immobilized enzyme packed into the reactor column, thus they were able to monitor by UV absorption at 240 nm. Pre is initially synthesized from cholesterol by cytochrome P450<sub>sc</sub> cleavage. The simultaneous determination of Pre and 17OHPre, which are compounds of the early steps in steroid synthesis, is useful to investigate steroidogenesis in the cells. The analytical system constructed here was good enough for the simultaneous determination of Pre and 17OHPre in the medium of bovine adrenal cortical cells. The calibration curves for Pre and 17OHPre had a good linearity at the range of 0.3-10 and 0.4-10 microg/ml with a correlation coefficient of 0.9996 and 0.9989, respectively. The detection limit at a signal-to-noise ratio of 3 was 0.12 and 0.08 microg/ml. The conversion rate of Pre to Pro and 17OHPre to 17OHPro was 90.3 and 99.6%, respectively. Addition of trilostane, an inhibitor of 3-beta-hydroxysteroid dehydrogenase, permitted to determine Pre and 17OHPre as a result of stopping the cascade of steroidogenesis. The contents in the medium of the bovine cells stimulated with ACTH under the presence of trilostane for 1.5hr-cultivation, were  $0.459 \pm 0.016$  and  $0.333 \pm 0.009$  microg/ml, respectively. This method is convenient for the simultaneous determination of Pre and 17OHPre secreted from the cells.

## **P015-A1**

### **Simultaneous Determination of Ten Steroids Using Sweeping-Micellar Electrokinetic Capillary Chromatography and its Application to Bovine Adrenal Cortex Cells**

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Adrenal hormones are related to a variety of physiological responses. For example, cortisol is a key stress-induced hormone secreted from adrenal cortex. To investigate the regulatory mechanism of steroidogenesis, it is very important to isolate the steroid hormones and determine the amounts. Shen et al. reported that sweeping-micellar electrokinetic capillary chromatography (MEKC) /UV-absorption method was useful for the determination of six steroids (progesterone, 17-hydroxyprogesterone, 11-deoxycortisol, corticosterone, cortisone and cortisol) using tetradecyltrimethylammonium bromide (TTAB) as a surfactant [Electrophoresis, 27: 1255-1262, 2006]. In this study, we have tried to develop the MEKC/UV-absorption method for determining four additional steroid hormones (aldosterone, androstendione, estradiol and testosterone) simultaneously, and by using (R)-(+)-1, 1'-bi (2-naphthol) as an internal standard. And then, we have applied to determine the contents of steroid hormones in the medium secreted by bovine adrenal cortical cells. A sweeping-MEKC buffer consisted of tris-HCl buffer (pH8.0) containing 10 mM TTAB. Steroid hormones in the medium secreted by bovine adrenal cortical cells were extracted with ethyl acetate. Sample was injected 50 mbar at 50 sec. The linearity for all analytes was ranged from 0.195 to 12.5 microg/mL with the correlation coefficients over 0.99. When bovine adrenal cortical cells were stimulated with ACTH, the contents of cortisol and cortisone in the medium were 0.869 and 0.239 microg/mL, respectively. A protopanaxatriol-type saponin, metabolite of ginsenosides, significantly inhibited the production of cortisol and cortisone in bovine cells stimulated by ACTH. The improved MEKC/UV-absorption method will be adapted for the evaluation of substances responsible for steroidogenesis.

## **P016-A1**

### **Development and Validation of a Method for the Detection and Confirmation of Biomarkers of Exposure in Human Urine by Means of Restricted Access Material - Liquid Chromatography - Tandem Mass Spectrometry**

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The present paper describes the development and validation of a LC–MS/MS method for the determination and confirmation of biomarkers of exposure to different types of xenobiotics in human urine. The selected target compounds were pesticides or their metabolites (1-Naphthol, 2,4,5-T, 2,4,5-TP, Chlortoluron, Diuron and 3-Chloro-4-tolylurea) and several compounds of industrial origin (2,4,5-Trichlorophenol, Bisphenol-A and Bisphenol-F). The method combines the use of a restricted access material (RAM) coupled On-Line to a LC-IT-MS system. In this way, a rapid and efficient matrix cleanup was achieved, reducing the manual sample preparation to sample freezing and filtration. The ion trap (IT) mass-spectrometry detector provided the selectivity, sensitivity and robustness needed for confirmatory purposes. The on-line RAM-LC-IT-MS method developed here has been validated as a quantitative confirmatory method according to the European Union (EU) Directive 2002/657/EC. The validation steps included the verification of linearity, repeatability, specificity, trueness/recovery, reproducibility, stability and ruggedness. Decision limits (CC $\alpha$ ) and detection capabilities (CC $\beta$ ) were also calculated, and ranged from 3.6 to 16.5 ng mL<sup>-1</sup> and from 6.0 to 28.1 ng mL<sup>-1</sup>, respectively, in fortified urine samples. Repeatability and reproducibility (intra- and inter-day) were evaluated at two concentration levels, being 12.7 % or below at the concentration corresponding to the quantification limits. Matrix effects and non-targeted qualitative analyses were also evaluated in fortified urine samples. The results of the validation process revealed that the proposed method is suitable for reliable quantification and confirmation of biomarkers of exposure to xenobiotics in human urine at low ng mL<sup>-1</sup> levels. The method is therefore appropriate for application within the context of the biological monitoring of these biomarkers and as a general screening method.

## P017-A1

### A Validated Gas Chromatography-Mass Spectrometry Method for Analysis of Ezetimibe in Human Plasma

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A new, specific, sensitive and validated gas chromatography-mass spectrometry method was developed for analysis of Ezetimibe (EZE), a novel, specific and synthetic hydroxymethylglutaryl coenzyme A reductase inhibitor and used as antihypercholesterolemic drug. Ezetimibe was derivatized using silylating agent, *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) for analysis by GC-MS. The derivatization reaction was optimized. The separation was carried out on HP-5ms (5%–phenyl methylpolysiloxane, 30 m x 0.25 mm i.d. with 0.25 µm film thickness) capillary column. Injection was performed in the splitless mode. EZE was extracted from human plasma with methyl tert-butyl ether. Trimethylsilyl ether derivative of EZE was determined in selected ion monitoring mode. Methyltestosterone used as internal standard. The method was validated with respect to limits of detection and quantitation, precision, accuracy, linearity, specificity, stability, and recovery. The limits of quantitation and detection were found as 15 and 10 ng mL<sup>-1</sup>, respectively. The method was found to be linear in the range from 15 to 250 ng mL<sup>-1</sup>. The intra- and inter-day precisions (RSD) were less than 6% and accuracies (bias) for intra- and inter-day accuracy were found between –4.04 and 9.71% at four different concentration levels (15, 40, 100, 250 ng mL<sup>-1</sup>). The proposed method was successfully applied to real human plasma samples collected from hypercholesterolemic patients for determination of total EZE.

**Keywords:** Ezetimibe, gas chromatography-mass spectrometry (GC-MS), silylation, human plasma, validation

## P018-A1

### Development of a Simple, Cheap Routine Method of Determination of Kynurenine in Plasma Blood by HPLC

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A new simple, fast, method of determination of kynurenine in plasma blood by HPLC, ultraviolet light filter detection have elaborated on.

Kynurenine is the main metabolite in tryptophan metabolism. It takes part in the pathogenesis of many disorders. The determination of kynurenine is important for diagnosis of the remission of epilepsy.

We used a chromatograph made by Laboratorni Pstroje Praha which consists of isocratic pump HPP 4001, photometric detector LCD 2563, line recorder TZ 4601, Rheodyne Model 7125 injection valve with sample loop 20  $\mu$ l (Rheodyne, Cotati, California, USA), glass columns (150 mm $\times$ 3,3 mm i.d. packed with Separon SGX CN 7  $\mu$ m) in a steel cartridge, Analog to Digital Converter (NeoChrom, Research on Demand Lab Company, Ukraine, Zaporozhye). All previous scientists had used C<sub>18</sub> or C<sub>8</sub> phases[1-3].

An ultraviolet light filter at 365 nm was used. The mobile phase consists of 10 mmol/L ammonium acetate and acetic acid with a pH value 3.1. We studied dependence of retention time from pH, the content of salt. During raise of the pH level, retention time increases. We tried to describe the mechanism of separation. It was obtained satisfactory separation of kynurenine from matrix (Fig. 1).

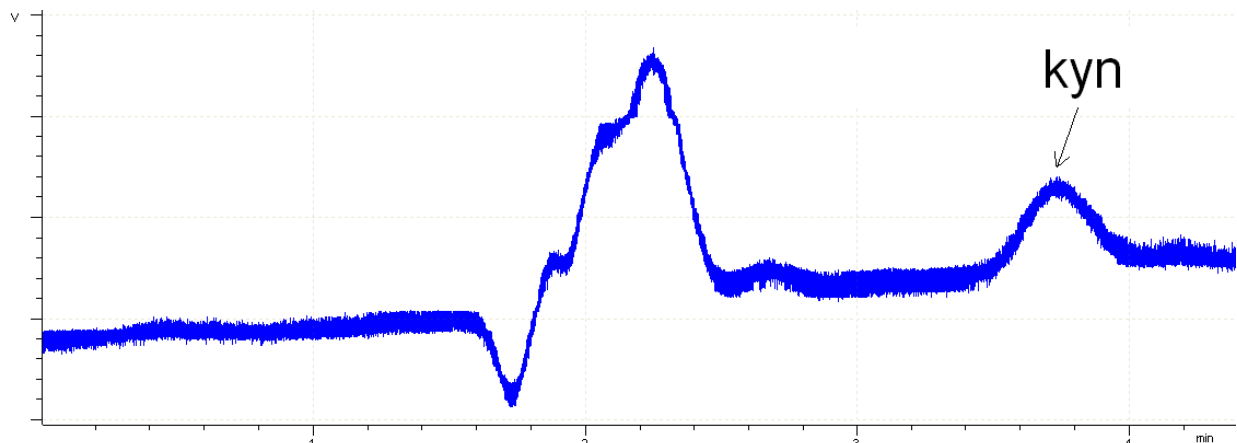


Fig. 1. Chromatogram of kynurenine in plasma blood of patient with epilepsy.

Acknowledgement. The authors are thankful to company "Research on Demand Lab" <http://neochrom.biz> for providing of equipment of NeoChrom.

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## **P019-A1**

### **Automated Versus Manual Solid-Phase Extraction for the Simultaneous Recovery of Selected Contaminants from Food (Coffee) and Biological Matrices (Urine, Faeces)**

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Performing a solid-phase extraction (SPE) manually can be a time consuming process, especially when large numbers of samples must be processed. Hence, automating manual SPE methods can afford various benefits such as high sample throughputs and reproducible results. Yet, performances of the automated procedure need to be validated. In this study, we compare the performances obtained under similar conditions with both a manual SPE system (Visprep, Supelco) and an automated SPE apparatus (ASPEC, Gilson). The automated extraction system uses positive pressure to process samples instead of vacuum for the classical manual SPE. Both SPE systems have been applied to the extraction of selected coffee contaminants, including two polycyclic aromatic hydrocarbons (benz[a]anthracene and benzo[a]pyrene) and two mycotoxins produced by some species of fungi belonging to the genera *Aspergillus* and *Penicillium* (ochratoxins A and B). Hydroxylated derivatives of benz[a]anthracene and benzo[a]pyrene have been also included in this study, as they may be formed *in vivo* after coffee ingestion as possible metabolites. All the final extracts were further analyzed by high-performance liquid chromatography coupled to a fluorescence detector. The influence of several parameters has been investigated on both systems, and some differences can be noted. Our results show that the SPE procedure optimised with a manual system needs further optimisation of selected parameters for achieving quantitative recoveries for all the targeted contaminants with an automated system. The advantages of the automated SPE procedure over the traditional manual SPE method will be discussed in terms of overall throughput and performance.

## P020-A1

### HPLC-ESI-MS/MS Method for Multimarker Monitoring of Oxidative Stress in Body Fluids

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Oxidative stress can be defined as the body imbalance between production of reactive oxygen species (ROS) and biological system ability to readily detoxify the reactive intermediates or easily repair the resulting damage. ROS generated close to cell membranes oxidize membrane phospholipids (membrane lipid peroxidation), which can be further transformed in a chain reaction. Endogenously generated aldehydic lipid peroxidation products are malondialdehyde,  $\alpha,\beta$ -unsaturated aldehydes (mainly 4-hydroxynonenal and 4-hydroxyhexenal) and saturated aldehydes (C6, C7 and C9). Aldehydes are formed by lipid peroxidation of  $\omega$ -6 (arachidonic acid, linoleic acid) and  $\omega$ -3 (oleic acid) polyunsaturated fatty acids. The aldehyde biomarkers quantification in various body fluids (exhaled breath condensate, plasma and urine) represent an interesting tool for oxidative stress induced diseases diagnostics. The work presents a new method for the determination of aldehyde-biomarkers in body fluids based on the LC-ESI/MS/MS. Malondialdehyde, 4-hydroxynonenal, and saturated aldehydes (*n*-C6 to C13 aldehydes) were quantified after derivatisation with Girard's reagent T. LC-ESI-MS/MS operated in neutral loss (NL) mode was used for its exceptionally high degree of selectivity and sensitivity. The developed method enabled unequivocal parallel determination of several oxidative-stress biomarkers at the only one analysis run. The method was optimized and validated. Finally, the method was tested on real clinical samples collected from patients with different oxidative stress induced disorders (silicosis, asbestosis, pleural hyalinoses) and compared to the control group of healthy subjects.

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## **P021-A1**

### **Ultrasensitive Nanoparticle-Enhanced Surface Plasmon Resonance Imaging Detection of Specific DNA Sequences by Peptide Nucleic Acids**

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A major challenge in the area of DNA detection is the development of rapid and multiplexed methods that do not require the labeling and the polymerase chain reaction (PCR) amplification of the DNA sample. Both the above procedures entail additional steps in sample pre-treatment, are time consuming and require expensive reagents. In this perspective, several label-free and PCR-free approaches able to perform DNA analyses with high-throughput, low sample consumption and ultrasensitivity (1 pM sensitivity must be reached for the detection of unamplified DNA) have been proposed.

In this communication the results we obtained in the ultrasensitive detection of non-amplified DNA will be presented. The method is based on the use of surface-immobilized peptide nucleic acids (PNA) probes in combination with continuous-flow microfluidics and nanoparticle-enhanced surface plasmon resonance imaging (SPRI) biosensing.

The sandwich hybridization strategy allowed the discrimination between fully matched and single base mismatched sequences even at the 1 fM concentration. In addition, the microfluidic management of the fluids allowed a multiplexed determination of the SPRI responses. The discrimination was obtained by using 150 zeptomoles of the DNA target.

## **P022-A1**

### **Open Bridge-Structured Gold Nanoparticle Film for Electrical DNA Detection**

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Fluorescent-based DNA chips have been well-known as conventional large-scale gene analysis tools. However, these chips are currently expensive since their manufacturing requires sophisticated instruments such as hybridization equipments and fluorescent scanners, which has prevented their clinical use and has confined their utilization to research fields. In this regard, we have developed a novel label-free technique based on the change in the resistance of a gold nanoparticle (AuNP) array due to a change in an open bridge structured by hybridization.

The resistance of the open bridge structured AuNP array immediately decreased and became constant in 60 s. The  $\Delta R$  value, defined as the difference in the resistance before and after the hybridization, was 100 m $\Omega$  with an S/N ratio of over 30. The sensor showed response over a wide concentration range (1 nM-100  $\mu$ M) with a detection limit of 5.0 fmol. To verify the effectiveness of this system for the identification of DNA mismatches, we carried out experiments using the targeted DNA along with complementary, 1-bp, 2-bp, and 24-bp mismatched (fully mismatched) DNA sequences. The response was the highest for the cDNA and decreased with an increase in the number of mismatched bases. Finally,  $\Delta R$  hardly changed at the fully mis-matched sequence ( $\Delta R < 10$  m $\Omega$ ). It implies that resistance change by hybridization can be directly detected with a resolution that is sufficiently high for the detection of SNPs.

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## P023-A1

### Voltammetric Detection of the Damage Caused to DNA by the Nitro Derivatives of Fluorene Using an Electrochemical DNA Biosensor

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The emissions of gasoline and diesel engines contribute significantly to ever increasing pollution of living environment. A specific part of exhaust gases is composed of nitrated polycyclic aromatic hydrocarbons (NPAHs). In the eighties and the nineties of the last century, L. Möller studied influences of NPAHs and their *in vivo* metabolites on living organisms and their DNA [1]. In our work *in vitro*, an electrochemical DNA biosensor based on the screen printed carbon paste electrode (SPCPE) has been used for investigation of the interaction between nitro derivatives of fluorene (2-nitrofluorene and 2,7-dinitrofluorene were chosen as model representatives of NPAHs) and calf thymus DNA. Two types of DNA damage have been investigated and electrochemically detected at the DNA/SPCPE biosensor: the DNA damage caused by short-living radicals generated by the electrochemical reduction of nitro group, previously described by Abreu [2], and the damage caused by the direct interaction with the studied compounds [3]. The type of direct interaction between 2-nitrofluorene and DNA has been characterized using previously tested DNA intercalators (metal complexes of 1,10-phenanthroline) [4].

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## **P024-A1**

### **Headspace Single-Drop Microextraction for the Determination of a Group of Volatile Compounds Involved in the Toxicity and Flavor Characteristics of Smoked Foods**

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Food smoking is one of the oldest food technologies and has been in use for thousands of years not only to give foods a particular organoleptic profile but also to inactivate the actions of enzymes and microorganisms. Several studies have reported an extensive list of organic compounds found in biomass smoke including acids, alcohols, carbonyls, esters, furans, lactones, polycyclic aromatic hydrocarbons, phenols, and other miscellaneous compounds. Among these compounds, phenols and methoxyphenols are components of great importance for the smoke flavor, preservation of foods, and antioxidant effects. Phenols derivatives are characteristics for spicy odors, whereas the derivatives of guaiacol (2-metoxifenol) present woods and medicines odors, and the derivatives of syringol (2,6-dimethoxyphenol) present toasted flavors. Other volatile compounds, such as cresols, are toxic and their presence is therefore non-desirable in smoked foods.

Many conventional methods have been used to sample and analyze volatile compounds present in biomass smoke. Recent trends to minimize the organic solvent consumption in the extraction procedures have lead to the development of several microextraction techniques.

Headspace single-drop microextraction (HSDME) is a relatively novel extraction technique which presents many advantages such as low cost, speed, and little sample and solvent consumption. Briefly, in the technique a drop of few micro-liters (typically 1–5) of a solvent (normally an organic solvent) is suspended at the tip of a micro-syringe. The drop is exposed to the headspace of the sample for a given time at a given temperature, then retracted into the syringe and directly transferred to an appropriate chromatographic analytical system.

This work focuses on the utilization of HSDME combined with HPLC-UV for the quantitative determination of a group of volatile compounds involved in flavor properties (vanillin, 3-metoxyphenol, 2,6-dimetoxifenol, 2-ethylphenol and 3-ethylphenol) and toxicity (phenol, o-cresol, m-cresol and p-cresol) in smoked foods. Several solvents have been tested in the extraction process, being the organic solvent 1-decanol and the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate the most adequate ones to work with. The extraction variables (drop size, extraction time, temperature, pH, among others) have been optimized using an experimental design.

## **P025-A1**

### **Geographical Differentiation of Tequila According to their Mineral Content**

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Tequila is an alcoholic beverage made from the fermentation and distillation of agave juice. The elaboration of tequila is restricted by Mexican law to the blue agave (*Agave tequilana* Weber var. azul) produced in some geographic areas, namely, Jalisco, Nayarit, Michoacan, Guanajuato and Tamaulipas. There are four different kinds of tequila according to the characteristics acquired during the elaboration process of the distillate. These types are silver, gold, aged and extra-aged. Tequila production reached in 2007 well over 162 million liters, and almost half of this production was for exports. In food control, efficient methods are required for the identification of the geographical origin and authenticity of the products. The metal content in these four varieties depends on the elaboration process and the raw materials. The dilution water can be the main source of elements like Ca, Mg and Na. The presence of S may be due to the agave plant used in the elaboration or SO<sub>2</sub> used to prepare the oak cask for ageing the product. Cu and Zn may come from the still used in distillation. In this work, the content in Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, S, Sr and Zn were determined by inductively coupled plasma atomic emission spectroscopy and used to perform the geographical differentiation of silver tequila from the state of Jalisco. Different production areas were considered, Guadalajara, Tequila and Amatitlan. Linear discriminant analysis was applied. Two discriminant functions were extracted and the variables Ba, Cu, Mn, S, Sr and Zn were the most discriminant. This model allowed the geographical differentiation of silver tequilas.

## **P026-A1**

### **Challenges in the Application of Rabbit-IgG for the Development of Lateral Flow Devices**

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Lateral flow devices (LFDs) are user friendly chromatographic strip tests for the rapid detection of various analytes such as microbial analytes, different toxins, antibiotics and food allergens. The most famous or known application is the pregnancy test.

The principle of LFDs is – similar to ELISA – based on an immunological antibody-antigen reaction. For immediate read-out of the test result, the applied antibodies have to be labelled with coloured particles such as carbon (Van Amerongen et al., 1994; Seydack, 2005), latex (Dávalos-Pantoja et al., 2000; Dávalos-Pantoja et al., 2001) or colloidal gold (De Roe et al., 1987; Geoghegan, 1988; Gasparyan, 2005).

In the presented work rabbit-IgGs were used, which were produced against protein extracts of potentially allergenic food such as peanut, hazelnut, and egg white. Colloidal gold was chosen for labelling the antibodies. The application of polyclonal rabbit antibodies in immunodiagnostic tests offers the advantages of rapid availability of antiserum, high yield, and usually high specificity and sensitivity for the antigen. Nevertheless, the production of stable antibody-gold conjugates is critical and dependent on various factors such as IgG purification from serum, determination of the coupling ratio, and finally conjugation conditions including pH-value, time and buffer system. An overview about drawbacks and their successful overcoming during our work on the development of lateral flow devices for the detection of potentially allergenic food proteins is given.

This work is part of the Christian Doppler Pilotlaboratory for Rapid Test Systems for Allergenic Food Contaminants.

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## **P027-A1**

### **Specific Analytical Approach, Using Spiked Preparations, for the Determination of the 24 Volatile Allergenic Fragrance Substances, Restricted under Council Directives, in Perfumes Using Mass Spectrometry Detection**

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The ingredient content of cosmetic products is variable and complex mainly due to the type of formulations and substances involved. One of the consequences of this complexity is an important problem of specificity for the quantification of low concentration analytes in the product even using selective analytical methods.

Mass spectrometry is often used to obtain a better specificity on the assay results. Nevertheless, for complex matrices, such as cosmetics, interferential compounds could subsist and prevent a correct quantification using a standard quantification approach (direct quantification of the analyte in the sample using a standard calibration curve).

According to the number of substances involved in their manufacture and the diversity of the volatile allergenic fragrances substances, perfumes could be considered as examples of complex matrices (presence of many interferential compounds).

In order to quantify each allergenic fragrance substances that could be present at very different amounts in the analyzed sample (from 0 to 10,000ppm depending on the allergen), and check the specificity of the detection for each compound, an analytical approach using spiked and un-spiked preparations for each sample assay is proposed.

The analytical method used for the separation of all the 24 volatile allergenic fragrances using mass spectrometry detection is given. The identification of allergenic compounds is carry out in full scan, whereas the quantification is performed in SIM mode using when needed a specific quantification approach to overcome any possible interferences.

For each assay, 3 un-spiked preparations at 3 different dilutions levels (1/10, 1/100 and 1/1000) and 3 spiked preparations at 3 different spiked levels (10ppm, 100ppm and 1000ppm) are systematically carried out.

For each allergenic fragrance, a recovery is determined to check the specificity of the allergen detection and evaluate the accuracy of the quantitative result given.

## **P028-A1**

### **The Importance of Subsample Preparation Practices in the Analysis of Crude Oils**

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There have been many subsample preparation practices proposed to reduce sources of error in analysis of crude oils and petroleum products. Optimal subsample preparation allows keeping the degree of representativity of the final aliquot going into the laboratory analyzer. The objective of this work was to discuss some experiences in order to understand the importance of sampling practices to obtain representative subsamples of crude oils and petroleum products, and measurements with minimized uncertainty whenever possible.

The impact of the type of recipient and the storage time on physical and chemical quality of crude oil (27 °API) stored was studied. Two-way analysis of variance was a good tool to separate and estimate the causes of variation when comparing the results of physical and chemical parameters for crude oil samples under different storage conditions along the nine weeks of storage.

The analysis of variance showed that the subsamples should not be used to vapor pressure test unless in case of interlaboratory crosscheck, and may be considered as the first test from the bottle subsample. This conclusion is supported by the fact that excellent results was obtained four and seven weeks after the sample collecting provided that it is kept in metallic recipient under shadow.

Another approach is in relation to the storage time. Sometimes long periods of crude oils samples storing, in a complete evaluation, are necessary. But there is a trend to reduce some concentrations, for instance, acidity and sulfur content. It is known that the good practice to these determinations should be testing them within one hour after sampling. So the reanalysis of this kind of storage sample requires criteria for comparisons.

The standardized practices provided quality improvement to our laboratory.



## **P029-A1**

### **Multi-Walled Carbon Nanotubes as Efficient Solid-Phase Extraction Materials of Organophosphorus Pesticides from Fruit Juices**

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Beverages based on fruits are currently receiving considerable attention. Their market potential is growing since they are highly nutritious and they contain various bioactive compounds with antioxidant activity as well as phenolic compounds. Among them, fruit juices constitute the most important part of fruit based beverages commercially available. They are daily consumed in high quantities (more than alcoholic beverages), especially by children, that is why the analysis of pesticides residues in this type of samples is of high importance.

In this work, multi-walled carbon nanotubes (MWCNTs) have been used for the first time as solid-phase extraction (SPE) sorbents for the extraction of eight organophosphorus pesticides (i.e. ethoprophos, diazinon, chlorpyrifos-methyl, fenitrothion, malathion, chlorpyrifos, fenamiphos and buprofezin) from different commercial fruit juices (i.e. apple, grape, orange and pineapple). The developed method, which involves SPE and direct gas chromatography with nitrogen phosphorus detection analysis, is very fast, simple and cheap: only 1:1 dilution with Milli-Q water and pH adjustment to 6.0 of 10 mL of juice is necessary prior to a quick MWCNTs-SPE procedure that used only 40 mg of stationary phase. Mean recovery values were above 73% for all the pesticides. Matrix matched calibration was carried out for each sample matrix since statistical differences between the calibration curves constructed in pure solvent and in the reconstructed juice extracts were found. Limits of detection ranged between 1.85 and 7.34 µg/kg. The proposed method was also applied to the analysis of this group of pesticides in several commercial juices.

## P030-A1

### Solid-Phase Extraction (SPE) in Patulin Analysis: Comparison with Small Scale Microextraction by Packed Sorbent (MEPS) Methodology

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Patulin is a mycotoxin that can be found in apples, pears, grapes, vegetables and cereals and their food products [1]. Studies have shown that patulin has immunotoxicity, neurotoxicity, immunosuppressive and teratogenic effects [2]. Its occurrence led the EU to establish 50µg/Kg as a maximum of patulin in apple juices and ingredients, 25µg/Kg for solid apple products and 10µg/Kg in food for children [3].

Due to its occurrence as a natural contaminant of apple juice, several methods have been developed for measuring patulin. Using a derivative, GC/MS was used to detect its presence in apple juice [4]; underivatized patulin demonstrated to be susceptible to negative ion chemical ionization (CI) GC/MS technique but its quantification is based on LC [4].

Nevertheless the more extensively method used to analyse patulin is so far the reversed phase HPLC due to its [5, 6]. Some studies have also been made to separate the patulin from other biologically important compounds; conditions like the composition of the mobile phase were established [7, 8].

Sample preparation acquires importance since simple solvent solvent extraction and liquid-liquid extraction are time consuming, expensive, use considerable amounts of organic solvents and, in some circumstances, causes patulin degradation. A recent paper reported the SPE for the patulin analysis [9].

This study shows an improved methodology using SPE as sample preparation. Comparison between the use of normal phase and reversed phase SPE was done. A small scale methodology using MEPS was also developed, for the first time.

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## P031-A1

### Analyses of Maillard Reaction Products in Raw and Cooked Sweet Potato (*Ipomoea Batatas*)

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Sweet potato, *Ipomoea batatas* (L.) is a perennial morning-glory (Convolvulaceae) vine that has been cultivated worldwide for over 5000 years for its edible tubers. Today, sweet potato is cultivated in developing countries, because, among other factors, it is easy to propagate, tolerates low temperatures, and requires low level inputs of water and fertilizer (1; 2).

Monosaccharides, disaccharides and fatty acyl glycosides have been described in the tubers of the *Ipomoea batatas* (3; 4). Due to its chemical composition medicinal properties have been attributed to this plant (1).

Maillard reaction (MR) may occur during food processing and/or storage, particularly at high temperatures, in carbohydrates and proteins containing foods. The reductor sugars and proteins are the main compounds involved in the initial states of the MR. In advanced stages of MR, undesirable compounds such as furfurals can be found. This chemical conversion has been reported to occur, for example, during the storage of fruit juices and balsamic vinegars [2,5] and EU have legislated about the maximum levels allowed (5).

In order to understand what happens during cooking process and to evaluate the implication of Maillard products in *Ipomoea batatas* consumption we began the study of furfurals in the raw and cooked sweet potato. The organic compounds of uncooked and cooked by baking were studied by solid-phase microextraction (SPME) and GC-MS. Different SPME fibers and analytical conditions were studied. The contribution of these critical constituents to the aroma and to the chemical composition of sweet potatoes has been evaluated.

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## **P032-A1**

### **A New Rapid Analysis Method for the Determination of Pesticide Residues in Vegetables by GC-MS**

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An analytical method for the determination of pesticide residues (including Organophosphorus, Organochlorine, Carbamate, and Pyrethrins) in various vegetables was developed. Extraction of pesticides from vegetable samples was done by Dichloromethane with the addition of Ultrasonic Assisted Extraction, and final analysis was made by GC-MS using the optimum ionization mode (Electron ionization (EI) for each pesticide) with selected ion monitoring (SIM) mode. The method has been validated in order to be applied on real samples. Recovery studies have been done at 0.05, 0.1, and 0.2 mg/kg fortification levels of each pesticide and the range obtained was >90% with the relative standard deviation of < 4% for each pesticide.

Keywords: Pesticide residues, Vegetables, GC-MS /SIM.

## **P033-A1**

### **Analysis of Priority Polycyclic Aromatic Hydrocarbons in Marine Fish**

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The consumption of fish is recommended as a mean of preventing cardiovascular and other diseases and has greatly increased over recent decades in many European countries, like Portugal in which, the annual per capita consumption of fish is 59 Kg against the European consumption of 22 Kg. Polynuclear aromatic hydrocarbons (PAHs) are well-known environmental pollutants at low concentrations and are included in the European Union and US Environmental Protection Agency priority pollutant list due to their mutagenic and carcinogenic properties [1]. Most individuals are exposed to PAHs predominantly from dietary sources, and when making food choices, consumers are faced with the dilemma of reconciling differences between health benefits and exposure to chemical contaminants.

From the analytical point of view, isolation of PAHs from biological matrices most often involves complicated extraction and clean up procedures to provide extracts ready for the accurate analytical determination. QuEChERS method is a simple, rapid, and inexpensive procedure requiring little labor and few materials, space, and solvents. One QuEChERS method has achieved the status of Official Method of AOAC International [2] and another one is already a draft from a technical commission of the European Committee for Standardization.

The aim of this work was to test and adapt the QuEChERS methodology to the extraction of 16 PAHs from chub mackerel (*Scomber japonicus*), in order to assess the safety of this fish specie. Individuals were also characterized relatively to their weigh, length, gender, water and fat content. The average concentrations of the sum of the detected PAHs in the analysed samples ranged from 3.34± 0.96 ng/g to 5.03± 0.98 ng/g.

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## **P034-A1**

### **Vitamin B12 Speciation – Water and Enzymatic Extraction**

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Small amounts of vitamins and minerals are essential for the human for normal, healthy growth and development. Human organisms cannot be stimulated to produce such essential micronutrients. They must be regularly provided with the food. The best way to increase the intake of natural microminerals is consumption of specially designed functional food. This supplemented food (e.g. eggs, milk or meat) is prepared by special feeding of farm animals, which results in enriching the products with substances usually missing in typical daily diet.

Vitamin B12 (containing cobalt) is well known as cobalamin. In fact it is a family of compounds including major forms: cyanocobalamin, hydroxycobalamin, adenosylcobalamin and methylcobalamin. These natural species of vitamin B12 occur in meat, fish and eggs, but the only one form – cyanocobalamin - is used to enrich food products. B12 is important for human metabolism, in red blood cells production and in the maintenance of the central nervous system. These are main reasons, why it is supplied to chicken eggs (1).

The goal of this study was the identification of vitamin B12 forms in chicken eggs. The idea was to develop a new analytical method to perform quality control of food products based on coupling size exclusion chromatography (SEC) with inductively coupled plasma mass spectrometry (ICP MS). It is robust, element specific technique which allows to distinguish various specific biomolecules in food.

Extraction of water soluble compounds from chicken eggs has been optimized. The obtained results showed that from both yolk and white intact vitamin B12, its adducts with proteins as well as decomposition products were isolated. The proteins involved in adducts were identified as albumin and phosvitin, the most abundant proteinaceous species in chicken eggs.

The experiments were also performed in the system simulating human digestion conditions (enzymatic extraction). Among products of such designed process two species were found – probably adducts with proteins and intact form of vitamin B12; the last one seemed to be adenosylcobalamin or methylcobalamin. These forms have been proposed as the only vitamin B12 forms present in chicken eggs.

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## **P035-A1**

### **Characterization of Interactions between Natural Organic Matter and Metals by Tangential-Flow Ultrafiltration and ICP-OES**

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Molecular size fractionation of the natural organic matter (NOM) in water samples from the Serra de Itabaiana National Park (Brazil) was used to characterize the ageing process after metal ions complexation. A special five-stage tangential-flow ultrafiltration device was used to separate NOM into six size fractions (F1: >100; F2: 100-50; F3: 50-30; F4: 30-10; F5: 10-5; F6: <5 kDa). The distribution patterns obtained showed that metals (Al, Ba, Cr, Cu, Fe, Mn, Ni, Pb and Sr), that were analyzed by ICP-OES, were preferentially complexed by the F1 fraction, which contained a higher concentration of dissolved organic carbon (DOC). The following decreasing order of carbon distribution in the different fractions was obtained: F1>>F3>F2=F4>F6>F5. Fractions F2 and F5 presented the highest levels of humification, and F6 the lowest. After 30 days, new distributions of DOC and metals were observed. Highest DOC was still found in F1, while the following complexation order was obtained: F1>F2>F3>F4>F5>F6. The metals presented similar and homogeneous distributions in all fractions. The species formed between NOM and spiked metals showed distribution patterns which changed as a function of complexation time, indicating the existence of a transformation process and inner rearrangements of the binding sites within the NOM, with a homogeneous trend of metals distributions in all size fractions. The more humified fractions exert a large influence on the complexation of metals in the aquatic environment, and this makes possible a better understanding of the effect of transport processes, accumulation and bioavailability on metal balances in natural waters.

## **P036-A1**

### **Heavy Metal Levels in Muscle Tissue of Horse Mackerel (*Trachurus Trachurus*)**

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Much attention has been focused on potential human exposures to metals like cadmium, lead and arsenic, via the consumption of fish species. The adverse human health effects associated with exposures to heavy metals are diverse and include, but are not limited to, neurotoxic and carcinogenic effects [1].

The Portuguese annual consumption of fish is 56.9 kg per capita year<sup>-1</sup>, proving that Portugal is the biggest consumer among all the EU countries.

In the present study, fresh samples of Horse mackerel (*Trachurus trachurus*), a highly appreciated fish in Portugal, were purchased from different local markets in Oporto region (NW Portugal) during 2007 to 2008 and were characterized concerning their heavy metal contents. Sample collection and biometric characterization were performed in accordance to the EPA Guide No 823-B-00-07 [2]. Specimens were separated in two groups: males and females. 67 individuals were manually headed, eviscerated and filleted. Each sample used for further analysis was constituted by the edible parts of, at least, 4 individuals and a minimum mass of 200 g. Homogenised samples of edible fish tissues were digested in a Microwave Accelerated Reaction System for Extraction and Digestion (CEM, Mathews, NC, USA) and quantitative determination of metals were performed by graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman effect background correction. Influence of the biometric parameters was statistically studied. The results revealed that heavy metal levels in muscle tissue of Horse mackerel were below the total provisional tolerable daily intake and maximum level established by the European Commission Regulation.

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## **P037-A1**

### **Analysis of Auramine O in Food Products Using Liquid Chromatography with Tandem Mass Spectrometry Detection Method**

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Auramine O (also known as Basic Yellow 2) is a diarylmethane dye used as a fluorescent stain and it is hazardous when ingested. In its pure form, Auramine O appears as yellow needle crystals. The use of Auramine O is not permitted for use in food products and some countries have reported the presence of Auramine O in some soy-based food products and tea leaves. A novel HPLC tandem mass spectrometry method for the detection and quantification of Auramine O in food products was developed to address this need to detect such colour contaminant in food. Due to the lack of availability of an isotopic standard, the quantitation of Auramine O was done using the standard addition approach. The limit of detection achieved for this method is 10 µg/kg.

## **P038-A1**

### **Aptamer Design for Food Allergen Biosensors: a Case Study for Lysozyme**

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Food allergy is a major health concern in both children and adults. Hen egg is one of the food components mostly reported to cause adverse reactions in infants and young children. Lysozyme is one of major allergens in egg – white protein appearing not only in foods but also in many health care products. It may cause mild to severe allergic reactions in small amounts. In this study we present a novel approach for selecting aptamers with high affinity and selectivity using Capillary Electrophoresis - Systematic Evolution of Ligands by Exponential enrichment (CE-SELEX). To perform CE-SELEX, the random DNA library was incubated with lysozyme and the bound sequences were isolated from unbound sequences using capillary electrophoresis and detected with laser-induced fluorescence (LIF) detection. Subsequently, the bound sequences were PCR amplified and purified to obtain an enriched ssDNA pool for further selection rounds. During the selection process the dissociation constant of the ssDNA pool decreased from the micromolar to the low nanomolar range within five rounds of selection. The affinity and the specificity of the selected ssDNA aptamers to lysozyme were evaluated using fluorescence anisotropy, surface plasmon resonance and affinity capillary electrophoresis. The selected aptamer had a dissociation constant of 3 nM as determined by fluorescence anisotropy. The aptamers were successfully challenged for specificity against other egg white proteins. The high affinity aptamer opens up possibilities for the development of an aptamer based bio-assay for the detection and quantification of lysozyme in food and pharmaceutical products.

## **P039-A1**

### **Comparison of Possibilities of Solid-Phase Microextraction and Stir Bar Sorptive Extraction for Determination of Some Beer Flavors**

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Beer flavour, a combination of odour and taste, is a very important factor in the consumer's perception of the quality of beer. The total sensory profile of beer is the main contributor to the success of this product in the market. Thus, the ability to be able to analytically determine the most important flavour groups in beer is important. Esters are one of these groups. A typical flavour characteristic contributed to beer by most esters is a pleasant fruit flavour.

The aim of this work was comparison of optimised solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) methodologies for isolation and quantification of ethyl esters in Czech beers. Due to the great efficiency of SBSE, the solvent back extraction of compounds sorptived in stir bar was used instead of the thermal desorption. Gas chromatographic analysis with flame ionisation detection was used for the determination of these compounds. Several experimental parameters that influence in sorption processes were studied, namely extraction time and temperature, salting-out effect and solvents using for solvent back extraction of the stir bar. The performance of these two methodologies was evaluated in terms of repeatability, precision, linearity and recoveries.

## P040-A1

### Chlorination of Acidic Pharmaceuticals: Investigation by Experimental Design and Liquid Chromatography-Electrospray-Mass Spectrometry

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Acidic pharmaceuticals reach surface water via wastewater treatment plant discharges. Then, this surface water is often used as a source of drinking water. At this point, some published works have already reported that some of these contaminants can be degraded upon chlorination [1,2], but possible transformation pathways still remained unclear. Thus, this work has investigated the degradation of seven acidic drugs and two metabolites during chlorination in detail. A liquid chromatography-mass spectrometry triple-quadrupole system (QqQ) was used to follow the time course of the pharmaceuticals and by-products, while a hybrid quadrupole time-of-flight system (Q-TOF) was also employed in the identification of the by-products. Under strong chlorination conditions (10 mg/L chlorine, 24 h), only four of the target drugs were significantly degraded (salicylic acid, naproxen, diclofenac and indomethacin), which were then subjected to further evaluation. The degradation kinetics of these four compounds was investigated at different concentrations of chlorine, bromide and pH of sample by means of a Box-Behnken experimental design. Depending on these factors, half-lives varied in the ranges: 23-573 h for salicylic acid, 13-446 min for naproxen, 5-328 min for diclofenac and 0.4-13.4 min for indomethacin. Also, it was observed that chlorine concentration was the overall most significant factor, followed by the bromide concentration (except for indomethacin), resulting both factors in faster degradation kinetics as they are also increased. The degradation of salicylic acid, naproxen and diclofenac consisted on the electrophilic aromatic substitution of one or two hydrogens by chlorine and/or bromide. Moreover, for diclofenac, two other by-products corresponding to a decarboxylation-hydroxylation pathway from the monohalogenated products were also identified. Indomethacin degradation did not lead to halogenation products but to oxidation ones and the cleavage of the molecule to yield 4-chlorobenzoic acid. Finally, the investigation of these by-products in real samples by LC-MS/MS (QqQ) in multiple-reaction monitoring showed that the halogenated derivatives of salicylic acid occurred in all the drinking water and wastewater samples analysed.

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## **P041-A1**

### **Micro-Focus X-Ray Fluorescence ( $\mu$ -XRF) Analysis with a Scanning Electron Microscope (SEM)**

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Modern SEM's have different analytical equipments for determination of the specimen element composition. Electron probe microanalysis (EPMA) has become in the recent decades a standard analytical method at SEM. Based on the technological development of low-power X-ray tubes and X-ray optics in the last few years a new analytical tool for SEM has established:  *$\mu$ -XRF with SEM*. The excitation with polychromatic photons is a valuable completion to the analytical capabilities at a SEM, this being enabled by attaching an X-ray source to the SEM.

The analysis with  $\mu$ -XRF takes advantage of the low signal-to-background ratio in comparison to EPMA. Typical detection limits of  $\mu$ -XRF for most elements are one order of magnitude lower than those of EPMA, *i.e.* below 100 ppm. The FWHM of the excitation X-ray beam ranges between 50 and 100  $\mu$ m. The analysed volume is determined mainly by the absorption of the fluorescence radiation and not only by the penetration depth of the excitation X-ray beam.

The coupling of EPMA and  $\mu$ -XRF takes into account the advantages of both individual analytical methods. This is illustrated through representative applications:

- analysis of the light main elements with EPMA and of the trace elements with  $\mu$ -XRF, respectively
- characterisation of specimens with inhomogeneous element concentration (bulk analysis and surface sensitive analysis).

## **P042-A1**

### **Anion Exchange Liquid Chromatography for the Determination of Nucleotides in Baby and/or Functional Foods**

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Baby foods combine a wide range of different matrices: non-fatty baby foods based on fruits and vegetables, fat foods based on meat/egg/cheese and cereal-based foods with different fat contents. Moreover, breast milk and infant formulas are also included. Functional foods satisfy the basic nutritional needs and, moreover, provide health benefits; dairy functional foods and functional ingredients containing milk represent a growing market. Nucleotides are monomers which constitute the nucleic acids, having immune-, lipidic- and digestive-related functions. Their addition to infant formulas is authorized at a maximum concentration of 5 mg/100 kcal, which is equivalent to the amount in maternal milk. In this study, a sensitive, selective and solvent-free procedure is proposed for the rapid determination of monophosphate nucleotides (cytidine 5'-monophosphate, uridine 5'-monophosphate, adenosine 5'-monophosphate and guanosine 5'-monophosphate) in baby foods. The method is based on the deproteinisation of foods and direct analysis by anion exchange liquid chromatography (LC). Nucleotides are ionic and very polar organic compounds and were separated on a Tracer Extrasil SAX strong anion exchange column with isocratic elution using 0.01 M dihydrogenphosphate buffer (pH 3.5) as mobile phase at a flow-rate of 1 mL/min and detected by diode-array detection. The sequence of the analytes as a function of the eluting time was: 5'-CMP (2.75 min), 5'-AMP (4.75 min), 5'-UMP (6.48 min) and 5'-GMP (8.10 min). The LC method was validated for linearity, detection and quantitation limits, selectivity, accuracy and precision. The recoveries obtained for spiked samples were satisfactory for all the analytes. Twelve different baby food samples were analyzed corresponding to five infant formulas (starting, follow-on, prebiotic and supplemented), two fermented milk, breakfast cereal (multicereals with honey), two puree samples (vegetables and chicken with rice) and two lyophilized puree samples (peach with banana and fruit salad). The highest levels of nucleotides were found in supplemented infant formulas and the lowest levels in the puree samples.

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## **P043-A1**

### **Monitoring of Copper in Clarified Apple Juices**

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The increased awareness of healthy lifestyle leads to a higher consumption and relevance of apple juice in human nutrition. Furthermore products obtained by organic farming, i.e. growing of plants without using synthetic inputs, such as synthetic fertilizers and pesticides or genetically modified organisms are preferred. Inorganic copper compounds being traditional fertilizers for apple trees are not considered as synthetic fertilizers, thus they are used also in organic farming for soil or foliar application. Since the fertilizer application rate affects the nutrition of apples, the applied copper might be also reflected in the copper concentration of apple juices.

The diet is the main source of trace elements and exposure to dietary trace elements has a direct impact on human health. Copper is an essential element for humans, the average content ranges from 50 to 250 mg per person. Copper being part of at least 13 enzymes plays a vital role for many organisms. Furthermore copper is necessary for the proper metabolism of iron, maintenance of connective tissue, pigmentation of skin and hair.

Thus the determination of copper in commonly consumed food stuff and beverages is of concern. The limit concentration for copper in apple juices given in the Codex Alimentarius Austriacus is 5 mg/kg. Twenty clarified apple juice samples commercially available in Croatia and Austria were analyzed for their copper content. Prior to quantification by inductively coupled plasma – optical emission spectrometry (ICP-OES) the juices were digested by a microwave assisted digestion system using HNO<sub>3</sub>. The copper concentrations were all below the limit of detection (17 µg/kg).

## **P044-A1**

### **Control of the Probabilities of False Non-Compliance and False Compliance in the Colour Qualification of Spanish Young Red Rioja Wines**

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The product characterization is becoming increasingly important nowadays. To properly typify products it is needed, not only to separate them from others, but to guarantee a suitable protection of the specificity of the product (producer side) and the protection against frauds (consumer side). Conceptually, this is inside class-modelling techniques, whose distinctive characteristic is the possibility of estimating sensitivity and specificity for each model. Sensitivity measures the capacity of the computed model to recognize its own objects whereas specificity measures the capacity of the model to reject foreign objects. Thus, both parameters jointly describe the computed models and allow evaluation of the confusion among categories or detection of outlier data.

We need class-models with high sensitivity and specificity but, in general, these parameters show opposite behaviour: when one increases, the other decreases. In this work we look for optimal class-models for a given problem, that is, the pairs of optimal values of attainable sensitivity and specificity, so that the user may decide considering existing requirements and can change its decision if the requirements change.

The procedure is shown using 129 samples of young red wine. In each sample, the CIELab parameters and eleven routine oenological measurements were determined. These samples are divided in two categories depending on whether they comply or do not comply the quality criteria related to colour (sensory property) established by the Denomination of Origin Rioja as assessed by the official committee of wine-tasters.

Then, PLS-CM [<sup>1</sup>,<sup>2</sup>] is used to model the qualitative response as a hypothesis test. Note that the probabilities of false non-compliance and false compliance associated to the hypothesis test are in fact the complement of sensitivity and specificity, respectively.

The previous approach is compared to the results obtained by direct estimation of the Pareto-optimal front in the two probabilities [<sup>3</sup>].

Both cases allow estimation of the risk curve that relates the simultaneous expected behaviour of the probabilities of false non-compliance and false compliance.

#### **Acknowledgments**

The work was partially supported by Caja de Burgos and University of Burgos.

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## P045-A1

### Usefulness of Front Face Fluorescence and Partial Least Squares Class-Modelling for Screening of Sulfonamides in Milk Evaluating the Probability of False Compliance

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Screening methods are used to detect the presence of a substance or class of substances at the level of interest and they are specifically designed to avoid false compliant results [1]. In the case of sulfonamide group, European Union establishes that the combined total residues of all substances within it in milk should not exceed  $100 \mu\text{g kg}^{-1}$ . What's more, the percentage of non-compliant results for antibacterial agents has recently increased [2]. So, the availability of suitable screening test is an element key in these residues detection.

In some screening methods, such as STAR protocol [3], detection level ranges for sulfonamides are between  $100\text{--}200 \mu\text{g L}^{-1}$  for sulfamethoxazole, sulfadiazine, sulfamerazine and sulfachloropyridazine, and  $600\text{--}1000 \mu\text{g L}^{-1}$  for sulfamethazine and sulfathiazole. This method is unable to fulfil the criterion,  $\beta$  (probability of false compliance) less than 0.05 demanded by Decision 2002/657/EC [2]. In other microbial assays published, no evaluation of  $\beta$  is provided.

In this work, a rapid, simple and cheap procedure for the determination of these six sulfonamides in milk is proposed based on the use of front face fluorescence spectroscopy and Partial Least Squares (PLS) calibration. Milk samples are pre-treated with a unique easy step of derivatization with fluorescamine. It has been established with previous studies that for a six sulfonamides mixture, the multivariate analytical sensitivity at  $100 \mu\text{g L}^{-1}$  for PLS calibration with emission fluorescence spectra is  $137.5 \mu\text{g L}^{-1}$ .

To use this procedure as a screening method, 35 samples from 11 commercial brands of milk spiked with a six-sulfonamides mixture at 8 levels of concentration (0, 30, 60, 100, 140, 170 and  $200 \mu\text{g L}^{-1}$ ) are analysed by PLS class-modelling [4]. For  $\beta = 0.05$  (threshold value established by European Union), the probability of false non-compliance,  $\alpha$  is 0.16 allowing the suitable screening of these six sulfonamides.

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## **P046-A1**

### **Determination of FFA Content in Corn Oil by Using a Modified HPLC System II by Automatic Injection**

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The purpose of this work was to develop a simple, fast, easy to use, solvent saving automated determination of corn oil by FIA handling of oil samples avoiding pre-treatments outside the flow system. So, an HPLC system was modified displacing analytical column by reaction coil and used for determination of FFA content in corn oil. An n-propanol solution was used as carrier phase and potassium hydroxide and two indicators (phenolphthalein, PHP and bromothymol blue, BTB) were used as reagent at micro-liter level.

Firstly, it was performed to optimize some parameters such as flow rate of carrier phase, reaction coils (length, geometry and internal diameter), sample volume, reagent volume and concentration (both bases and indicator concentrations), temperature. Corn oil samples prepared at a definite FFA value (according to AOCS method, FFA;  $0,09\pm 0,02$  and  $2,42\pm 0,02$  mg KOH/g oil) were used for exhibiting of ascendency of flow injection. The proposed method was based on the linear relation between the FFA content (%) and the area of the FIA peak. The decrease in absorbance at 580 nm was measured, and results were given as % oleic acid (9-octadecenoic acid).

In summary, a novel FIA method was developed to determine the free fatty acid (FFA) content of corn oil samples [FFA  $>0,09$  and  $<2,42$ ] with good accuracy, high sensitivity, simplicity of operation and in less time than conventional titration.

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## **P047-A1**

### **Automated Dynamic Headspace Coupled with TD-GC-MS: A Novel Method for the Analysis of Volatile Compounds in Wine**

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The study of wine aromas has always been present in the world of Enology. In this sense, an improved knowledge of wine composition may help the prediction of shelf-life and allow an assessment of the influence of technological steps and storage conditions on quality [1].

Static equilibrium headspace sampling is commonly used for GC determination of volatiles in solid and liquid samples. Since this technique relies on the analyte partitioning between the sample and headspace and uses a fixed injection volume, it may not provide adequate detection limits, particularly for higher molecular weight compounds and for polar analytes in aqueous samples [2]. Dynamic headspace can solve some of these drawbacks.

In this study, we describe the use of an automated dynamic headspace sampler for the determination of a large variety of polar aroma compounds in wine samples. Sample preparation was performed by Automated Dynamic Headspace (DHS) based on adsorbent traps (Tenax) and automatically dry purged to further eliminate trace water before its introduction into the integrated thermal desorption unit. Finally, the analysis was carried out with GC-MS detection. To achieve the optimum extraction performance, several main extraction (pH, NaCl and ethanol addition) and trapping parameters (volume purge, dry flow, dry volume, flow purge and trapping and drying temperatures) were investigated. Finally, thermal desorption conditions were also optimised including cryo-focusing temperature, vent flow and vent pressure. Under the optimised experimental conditions, the method showed good linearity and repeatability, as well as advantages such as sensitivity, simplicity and high feasibility. Then, the extraction yield of these analytes from several wine samples was determined and compared to stir bar sorptive extraction (SBSE) optimised in a previous work [3].

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## **P048-A1**

### **The Evaluation of Cottonseed Oil Quality in Refining Process by Using HPSEC Technique**

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Cottonseed oil, which has an important portion in vegetable oil sector and a primary location in view of production. It is stressed that cottonseed oil's refinery isn't realized at the desired level since food products prepared by using cottonseed oil have short shelflife and bad quality. To guarantee an effective quality control for a high quality cottonseed oil refining, there is a need of good methods to investigate the effect of refining parameters.

The aim of this study was to determine nonpolar and polar parts proved to be very suitable for the measurement of deterioration during refining process. With the proposed method, the polar and nonpolar compounds were separated according to the IUPAC method. After elution, samples were dissolved in THF for HPSEC analysis. The system consisted of a 20 µL loop, a series of PLgel columns, each of 300 x 7.6 mm i.d., 5 µm, 500, and 100A°, respectively. 0.2 g oil were dissolved in THF. In experiments it was tried to optimize several parameters.

Analyses of polar compounds in samples taken at different steps of the refining process indicated that the quality of crude oil could be deduced from the resulting refined oil by virtue of certain markers of oxidative and hydrolytic alterations. MW than that of oxidized triglyceride monomers are formed, mainly as a consequence of the high temperature in the bleaching and deodorization. On the other hand, oxidized triglycerides and diglycerides remain after refining, so their level is a measure of oxidation and hydrolysis, regardless of whether the oil is crude or refined.

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## **P049-A1**

### **Rancimat Method to Determine Cottonseed Oil Stability in Refining Process Steps**

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The oil-processing methods affects the oxidative stability of an oil, and the production of commercial plant oils involves a number of potentially destructive steps. So, crude oil has higher oxidative stability compared to refined oil, and this is mainly due to high tocopherol concentration in crude oil.

In this study, the effect of refining process on cottonseed oil production quality was investigated by using the Rancimat method. This method yields standard quality control parameters for the production of oils and fats in the food industry or for incoming goods checks in further processing.

The Oxidative Stability Index (OSI) of cottonseed oil samples was determined by the AOCS Cd 12b-92 method. All experiments were carried out using a 743 Rancimat, 3,0 g of oil was weighed into the reaction vessel placed into the heating block kept at 120°C, air flow was set at 20 L/h. The conductivity of this solution was measured and recorded.

Samples taken from different refining process steps indicated that crude cottonseed oil and washing step of oil samples were more stable to the oxidation than other step's samples due to higher concentrations of tocopherols. Especially, it was observed that oxidation stability was decreased markedly in bleaching and deodorization steps applied high temperature compare to other steps. Induction times for crude oil, washing, bleaching input, bleaching output and deodorization steps were found to be that 3.41 h, 3.58 h, 3.14 h, 3.10 h and 2.73 h respectively.

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## **P050-A1**

### **Investigations of the Effects of the Application of Neonicotinoid Insecticides on Apiculture by LC-MS/MS**

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The neonicotinoids such as clothianidin, imidacloprid and thiamethoxam, constitute a class of potent and widely used insecticides. They are used inter alia as seed treatment agents in maize crop as a measure of pest control against the corn rootworm. However, the neonicotinoid insecticides are highly toxic towards bees. Bees might come into contact with the neonicotinoids upon the sowing of treated seeds, if a substantial abrasion of the insecticide occurs. Another route might consist of plant exudates, such as guttation droplets and nectar. Consequently, the use of neonicotinoids may have negative apicultural effects in a direct way in terms of beekeeping (loss of bees) as well as in an indirect way as honey may become contaminated with residues of these pesticides. In this context investigations using LC-MS/MS were carried out. A method for the determination of neonicotinoid residues in honey and other matrices was developed. Details regarding sample preparation, measurement and data evaluation are presented and the obtained results discussed.

## **P051-A1**

### **Determination of Copper in Organic Vegetable Samples by GFAAS after Ultrasound-Assisted Extraction**

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The consumption of organically grown vegetables has shown a significant increase. These foods are considered healthy because, according to the literature, they have a higher nutrient content and are totally devoid of toxic agrochemicals [1]. Few studies have focused on the micromineral content in organic foods, particularly of copper – trace amounts of which are known to be essential to health human but which is toxic in excessive quantities [2]. Copper toxicity and nutritional content in human and animal food are concentration-dependent. The deficiency, as much as the excess of this metallic micronutrient, can cause alterations in the physiological functions increasing the susceptibilities of the human and animal to infections [3]. In view of the foregoing, the present study aimed to determine copper in vegetable samples using ultrasound in the analyte extraction process, and subsequent quantification by GFAAS. Using 0.10 mol/L of HCl as extraction solution, the optimal conditions of extraction were established as follows: 100 mg of sample mass; sample granulometry of less than 60 µm; sonication time of three cycles of 10 s and sonication power of 102 W. The proposed method was applied to determine selenium in samples of organically grown vegetables and its results proved compatible with those obtained from samples mineralized by acid digestion in a microwave oven.

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## **P052-A1**

### **Using Spectroscopy FTIR Analysis in Honey Characterization and Official Parameters Determination**

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Honey is very important in the modern diet due to its nutritional features, its genuineness and healthiness. Honey composition is quite simple but its analysis is complicated by the different techniques implicated, e.g. refractometry and conductivity for moisture and ash determinations, liquid chromatography for hydroxymethylfurfural and sugars determinations, enzymatic reaction for diastase content and titration for acidity content determination, whereas FTIR spectroscopy allows to have many parameters with a single determination and only dilution as sample preparation. Aim of this work was to evaluate the effectiveness of FTIR analysis in honey characterization and official parameters determination.

An IR Fourier transform-spectrometer for liquid was used (Milkoscan FT2, Foss Electric) with three waves lengths between 240 and 1299 nm (250-405 nm, 445-460 nm e 735-770 nm), with WINISI II 1.50 software for data elaboration. Honey samples, 2470 at all, were from national competition selections.

For parameters calibration sets from 754 (moisture) to 364 samples (minor sugars) were used.

Honey samples were diluted with water and analyzed for the following parameters: moisture, fructose, glucose, sucrose, HMF, diastase, Pfund color, minor sugars (erlose, maltose, isomaltose, maltulose, melezitose, rhamnose, raffinose, threalose, turanose), proline, conductivity and polarimetry.

The principal constituents curves showed good correlations whereas the minor carbohydrates ones looked promising. With IR technology it was possible to verify the principal official requirements and a qualitative valuation of honey quality in a quick and cheap mode. It was even successfully possible, for some monofloreal honeys to compare FTIR data with European monofloreal honey profiles (L. Persano Oddo, *Apidologie* 35, 2004).

Methylantranilate is a marker for citrus honey and promising correlations were obtained between FTIR and chemical analysis, showing, also in this case, the possibility to obtain, in a cheap and quick manner, the most part of official parameters and monofloreal features determination.



## **P053-A1**

### **Assessing Olive Oil Quality Using FTNIR Spectroscopy**

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Quality assessment of final foodstuffs is a fundamental parameter in food production in order to maintain high quality products. Olive oil, especially extra virgin olive oil, is one of the well recognized important component of a modern diet, due to its healthy and nutritional quality. Several different analysis are required by official methods, like COI methods and EC Regulations 2568/1991 methods to distinguish among the different olive oil categories and to verify oil quality; they appear to be very complicated, time spending and expensive. More attractive are alternative and non destructive techniques such as spectroscopy.

Aim of this work was to verify the possibility of NIR spectroscopy in assessing oil quality.

NIR analysis were performed using a Fourier transform-NIR spectrometer (NIRFlex N-500, Büchi Labortechnik AG, Flawill, Switzerland) in the 4,000-10,000  $\text{cm}^{-1}$  range at 8  $\text{cm}^{-1}$  resolution with NIRCAl 5.2 software for calibration. Samples were analysed using quartz cells (5 mm pathlength) in transmittance mode. Every spectrum was a 8 scans average. 160 oil samples coming from different italian regions were analyzed. Good correlations with chemical analysis were obtained for lipid composition (miristic, palmitic, heptadecanoic, stearic, arachidic, behenic, lignoceric, palmitoleic, heptadecenoic, oleic, eicosenoic, linoleic and linolenic acids), K 232, K270,  $\Delta$ K, peroxides and acidity; that means the possibility to have a rapid "screening" for virgin olive oil because these analysis are required, along with organoleptic analysis, to establish the trueness of a virgin olive oil. Further attempts were made to have good correlations for other, more specific, features, like tocopherols, poliphenols and antioxidants moieties. According to our results, NIR spectroscopy seems to be a promising technique for oil quality index.

## **P054-A1**

### **Use of Gas Chromatography-Quadrupole Mass Spectrometry Methods as a Strategy for Detection and Determination of Suspected Allergens**

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In the present work a strategy for the qualitative and quantitative analysis of 24 fragrance containing products suspected to cause skin reactions is reported. The analytes belong to different classes of compounds with different polarities: alcohols, carbonyl compounds, esters and lactones, cyclic hydrocarbons and phenols.

The applicability of a headspace (HS) autosampler in combination with a gas chromatograph (GC) equipped with a programmable temperature vaporizer (PTV) and a quadrupole mass spectrometric (qMS) detector is explored. By using headspace, sample preparation is reduced to placing the sample in the vial. This reduces the analysis time and the experimental errors associated with this step of the analytical process. No filtration or preconcentration steps are required. In addition, the proposed methodology does not require a bidimensional technique such as GC x GC as in most methods described to date. Monodimensional gas chromatography coupled to conventional quadrupole mass spectrometric detector is used and the 24 analytes were appropriately separated over a running time of 11.92 min.

Two different injection techniques were used and compared in this work: solvent vent injection and classical split hot-injection. The first one is an alternative to improve sensitivity maintaining the simple headspace instrumentation and it is only used for those compounds in trace levels. The signals obtained when split injection is used allow quantification of all the compounds in most cases. Detection and quantification methods were sufficiently low to enable the estimation of allergen compounds according to European Union. It should be emphasized that the method showed good precision and accuracy.

## P055-A1

### Comparison of Derivatization Methods on Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry Hybridation for Determination of Bisphenol A and Bisphenol S in Canned Foods

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Bisphenol-type compounds are raw materials used in the production of epoxy resins and polycarbonate plastics which are widely used in dentistry and food and drink packaging. At higher temperatures, the resin can decompose and the migration of bisphenols from packaging to food can be more intensive. Moreover, bisphenols have been proved to be estrogenically active and to have carcinogenic activities. This study describes the determination of bisphenol A, bisphenol S and biphenol by coupling gas chromatography with mass spectrometry (GC-MS) and using a new environmentally friendly sample pretreatment system based on solid-phase microextraction (SPME). A derivatization process was necessary to convert the non-volatile compounds into volatile derivatives and two reactions were compared. The derivatization with acetic anhydride (AA) was performed *in situ* in a 5 mM Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> buffer solution and analytes were extracted by direct immersion (DI) using a PA fiber (85 μm) at 90 °C for 40 min with stirring at 1500 rpm. However, for the derivatization with bis-(trimethylsilyl)trifluoroacetamide (BSTFA), the analytes were first extracted by DI using the PA fiber at 70 °C for 40 min with stirring at 500 rpm and then, the fiber was removed, dried using a nitrogen stream for 2 min and introduced into the headspace of BSTFA at 50 °C for 30 s. After derivatization, the analytes were desorbed in the injection port of the GC in the splitless mode at 280 °C for 4 min. The separation was carried out by coupling GC-MS in the selected ion monitoring mode (SIM), and using a SLB<sup>TM</sup>-5MS column. The GC temperature programmed was start temperature of 80 °C and increase to 320 °C at 20 °C min<sup>-1</sup>. The sequence of the ions selected as a function of the eluting time was: biphenol (186, 7.55 min), bisphenol S (165, 9.26) and bisphenol A (213, 10.09) when using BSTFA. Retention times were slightly retarded when using AA. The method allowed the determination of bisphenol-type compounds in canned foods, and it was validated for linearity, detection and quantitation limits, selectivity, accuracy and precision. Recoveries obtained for spiked samples were satisfactory for all compounds. Samples of different type of foods stored in cans internally protected with epoxy laques were obtained commercially from different manufactures and analyzed immediately being opened. Samples include peans, peans with carrots, sweet-corn, artichoke, mushroom and been shoot. Found levels were higher for bisphenol A in most samples.

## **P056-A1**

### **Comparative Carotenoid Content of Sea Buckthorn (*Hippophae Rhamnoides L.*) Berries, Seeds and Leaves Using HPLC Coupled with UV-VIS Spectrometry**

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Berries (SBB), seeds (SBS) and leaves (SBL) of sea buckthorn (*Hippophae rhamnoides L.*, ssp. *Carpatica*) cultivated at the Fruit Research Station from Bacau, Romania were analyzed in order to compare their pigments (carotenoids and chlorophylls) fingerprint and content. The comparative fingerprint of carotenoids and chlorophylls were done using High Performance Liquid Chromatography (HPLC) coupled with Photo Diode Array detector (PDA) on a LiChrosorb RP 18 column (5  $\mu$ m) while the total carotenoids and chlorophylls content were determined by UV-VIS spectrometry. The HPLC chromatograms showed that lutein was the major compound ( $t_R$ = 11.09 min) followed by  $\beta$  carotene ( $t_R$ = 25.04 min), zeaxanthin ( $t_R$ = 11.52 min) and  $\beta$  cryptoxanthin ( $t_R$ = 18.14 min), chlorophyll b ( $t_R$ = 17.17 min) and chlorophyll a ( $t_R$ =23.82 min). In accordance with the HPLC fingerprint, the chlorophylls content was: 16.36% in SBS, 0% in SBB and 41.16 % in SBL. Concomitantly, the spectrophotometric results regarding the total carotenoids concentration was 4.34, 293.59 and 450.04 mg /kg fresh sample, for SBS, SBB and SBL respectively. Considering the overlapping of carotenoids and chlorophylls (in SBS and SBL) absorbance at  $\lambda_{max}$ =450 nm, we noticed that quantitative determination of carotenoids was overestimated. Furthermore, we corrected these values by subtracting the chlorophylls concentration, so the final corrected total carotenoid values became 3.63 and 264.80 mg /kg fresh SBS and SBL, respectively.

Further investigations will be focused on other sea buckthorn Romanian species, in order to obtain their metabolomic profile.

## **P057-A1**

### **Determination of Nitrite and Nitrate in Meats Using Capillary Electrophoresis**

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In this work, a capillary electrophoresis (CE) method using direct UV detection (210 nm) for the separation and simultaneous determination of the nitrites and nitrates in foods is described. The analyses were performed in a 75  $\mu\text{m}$  i.d. uncoated fused-silica capillary with 48.5 cm length (effective length of 40 cm) using a running buffer consisting of 60  $\text{mmolL}^{-1}$  borate and 0,2  $\text{mmolL}^{-1}$  cetyltrimethylammonium bromide (CTAB) as electroosmotic flow modifier. Samples were injected hydrodynamically by applying 30 mbar pressure during 12 s. Analytes were separated within 6 min with a voltage of -10 kV. Meats samples were weighed (5 g) in a tube and deionized water (50 mL) was added and shaken for two minutes. The suspension was incubated for one hour in a warm water bath at 80°C. After cooling it was filtered and diluted to 50 mL. An aliquot of 1mL was diluted to 10 mL and this solution was filtered through from a 0.45-mm cellulose acetate filter disc and injected in CE. For quantitative purposes, sulfite was chosen as the internal standard. Method validation parameters were determined revealing good migration time repeatability (< 0.8% RSD) and peak area repeatability (< 1.1% RSD). Analytical curves for nitrite and nitrate were linear in the 0.2 – 2.5 ppm and 0.5 – 5 ppm interval ( $r > 0.998$ ), respectively. Limits of detection were in the 0.148 for nitrite and 0.169 ppm for nitrate. The extraction recoveries were higher than 98%. The developed method was applied to the determination of different kinds of meats (sausage, ham, salame, bacon and others) from Brazil. The range of preservatives found were from 17,33 – 46,39 ppm for nitrite and 69,91 – 198,06 ppm from nitrate. The samples presented content of nitrate and nitrite below Brazilian legislation tolerate limit (150 ppm to nitrite and 300 ppm to nitrate).

## **P058-A1**

### **The Application of Near Infrared Spectroscopy Technology for the Determination of Moisture, Fat and Oleic Acid Composition in Sunflower Seeds**

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Sunflower oil has a low proportion of saturated fatty acids (10%) and a high proportion of unsaturated fatty acids (oleic acid (15-30%), linoleic acid (55-75%)). The relative proportions of fatty acids in sunflower oil are appreciated by oil companies, especially the high levels of oleic acid. Other important parameters in the sunflower seeds quality control are moisture and fat.

In the present work we set up and validated a method for the analysis of moisture, fat and oleic acid in sunflower seeds by means of near infrared spectroscopy technology. The results show that the analytical method discussed, employing the near infrared spectroscopy technique can be used in the determination of moisture, fat, and high-low oleic acid in samples of sunflower seeds in a range between 4.61-21.41% for moisture, 38.36-49.61% for fat, and 60.0-93.1% for oleic acid, with results comparable to those obtained with classic chemical methods.

Moreover, a stepwise discriminant analyses was performed to determine those wavelengths most useful in classifying the sunflower seeds by high-low oleic acid composition, using Wilk's lambda as statistical selection criterion for the variables. The model of discrimination allows the classification and prediction of sunflower seeds with high or low oleic acid with a prediction rate of 90.5% in internal validation and of 89.4% in cross-validation.

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## **P059-A1**

### **Comparative Analysis of Ethanol Kinds with Physico-Chemical and Biological Approaches**

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In the given research two high quality ethanol samples obtained from an edible raw material were compared. The aim of the investigation was to show to what extent the physico-chemical parameters of the initial ethanol influence on the physiological activity of the ultimate food product (vodka). The term "physiological activity" means the reaction of different biological systems as a response to the alcohol action. There are different levels of such activity: a mortal toxicity (lethal doses), an ethanol narcosis (sublethal doses), changes of behavior because of intoxication (middle doses) or the taste and olfactory reception during degustation (small doses).

Biological researches were carried out on laboratory mouse males. Approved test on ethanol acute toxicity and analysis of conditioned reflex activity during alcohol intoxication were applied. Acute toxicity was induced by intraperitoneal injection of 40 % alcohol. Fuddle state was modulated by alcohol entering *per os*. Analytical expertise of ethanol samples was performed with gas chromatography and chrommass analyses.

Our data showed that admixture concentrations contained in tested ethanol samples were much less than that approved by quality standards of Russian Federation. But investigated ethanol kinds demonstrated a different physiological activity. A sample containing a less concentration of additives caused a more toxic effect. A less toxic sort of alcohol had a higher level of the degustation expertise and consumer demand. The physiological and consumer characteristics of alcohol were likely to be dependent on the presence of natural ingredients in ethanol employed for vodka production. Biological approaches were more sensitive in estimation of the toxicity of alcohol than physico-chemical techniques.

## **P060-A1**

### **Analytical Determination of Tocopherols in Vegetables Oils by Non-Aqueous Capillary Electrophoresis with Fluorescent Detection**

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Tocopherols are compounds well known by their antioxidant properties and their analysis in vegetables oils is interesting and there are numerous papers dealing with their determination, using diverse analytical techniques as HPLC, GC and others. Capillary electrophoresis offers useful modes as MEKC, CEC or NACE [1] for the separation of compounds with poor water solubility, however this technique has been poorly explored in the analysis of tocopherols [2-3]. NACE uses organic solvents with electrolytes as BGE and it can simplify the step of the pretreatment of the oil sample. For this reason, we have chosen NACE to carry out the separation of the tocopherols. Additionally, in order to achieve greater sensibility and selectivity, a fluorescence detection on-column has been used in this study. The excitation and emission wavelengths were 297 and 320 nm, respectively. For the separation of tocopherols, a BGE consisted in 12 mM sodium borate, 60 mM sodium cholate and 12 mM sodium hydroxide in MeOH is used. The optimum composition of the BGE has been obtained by experimental design with response surface methodology. Samples are injected hydrodinamically at 30mbar during 3 sec, the temperature of separation was chosen at 50 °C and the voltage of separation at 20kV the first 13 min and 22 kV later. In these conditions, the peaks of  $\alpha$ ,  $\beta+\gamma$ , and  $\delta$ -tocopherol are separated in less than 20 minutes. Calibration curves are linear in the range of 1.0 and 50.0 ppm of tocoferol and DL values of 0.4 ppm for  $\alpha$ , 0.2 ppm for  $\beta+\gamma$  and 0.2 ppm for  $\delta$ -tocopherol are obtained. The developed method was applied to the determination of the tocopherols in vegetables oil samples using a solid phase extraction. For that, the oil sample was diluted in hexane, passed through to a silica cartridge, eluted with diethyl ether and evaporated under nitrogen. Finally the residue was reconstituted in a mixture of 60:20:20, BGE:ethanol:methanol and it was injected in the CE. Recoveries next to 100% were obtained to the sunflower and corn oils but in olive oil these are lower for  $\alpha$ -tocoferol.

#### **Acknowledgements**

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## **P061-A1**

### **Optimisation of a Solid-Phase Extraction Method for the Simultaneous Determination of Selected Coffee Contaminants from Aqueous Samples (Water, Coffee Brew, Urine and Faecal Extracts)**

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Chemical contaminants may be present in the food chain and, in recent years, great concern has emerged on the development and validation of rapid analytical procedures for food analysis, for further risk assessment. However, food analysis results in an assessment of external exposure, without giving any idea of contaminants bioavailability in the human organism after food ingestion. Yet, the physical and chemical nature of the food matrix is known to affect the bioaccessibility of contaminants upon ingestion. In addition, the role of the human gut microbiota has faced a growing interest in recent years, as it may contribute to the metabolism of these contaminants, resulting in either an activation or deactivation of their toxicity. With a view to study the biotransformation processes of coffee contaminants by the human gut microbiota, we have developed a reliable analytical procedure for an accurate determination of these contaminants (and possible metabolites) in a broad range of aqueous matrices (coffee brew, urine and aqueous faecal extracts). We present the development of a sensitive and selective solid-phase extraction method for the quantitative determination of selected contaminants of coffee brew, namely two polycyclic aromatic hydrocarbons (PAHs) (benz[a]anthracene and benzo[a]pyrene), three hydroxy-PAHs (considered as possible metabolites) and two mycotoxins produced by fungi belonging to the genera *Aspergillus* and *Penicillium* (ochratoxins A and B). The influence of experimental extraction parameters were tested: sorbent nature and weight, sample pH and volume, contaminant level in the sample, washing solvent nature, eluting solvent nature and volume. Final extracts were analysed by high-performance liquid chromatography coupled to a fluorescence detector. Optimised extraction conditions were found for these selected contaminants from spiked water samples, and further applied to their determination in coffee brew as well as urine and aqueous faecal extracts.

## P062-A1

### Determination of Phenolic Composition and Antioxidant Activity of Selected Serbian Fruits

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*Natural antioxidants*, particularly in fruits have gained increasing interest among consumers and the scientific community. Epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Temple, 2000). This beneficial effect is primarily attributed to the occurrence of phenolic composition, carotenoids, vitamins and minerals. Over 8000 phenolic compounds have been identified from plant materials. These compounds have been categorized into different groups such as phenolics, flavonoids, flavonols, anthocyanins, tannins etc. The quantity and the composition of phenolic compounds in fruits are influenced by genotype, storage conditions, extraction procedure, and environmental conditions (Robbins, 2003).

The objectives of this study were to determine the content of total phenolics, flavonoids, flavonols, tartaric esters, anthocyanins, polymeric color (tannins) and antioxidant activity in originally Southern Serbia's fruit extracts of strawberry (*Fragaria vesca*), blackberry (*Rubus fruticosus*), raspberry (*Rubus idaeus*) and sour cherry (*Prunus cerasus*). The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging assays and was ranged from 90.87% in strawberry to 94.13% in cherry extract. The contents of investigated phenolic compounds showed highly correlation with the total antioxidant capacity of investigated fruits. The highest phenolic compounds contents were observed in a raspberry and the lowest were observed in a strawberry among selected fruit extract. The polymeric colour (tannins) ranged from 20.81% in cherry to 50.73% in raspberry fruit extract. Evaluation of antioxidant compounds showed its considerable levels in frozen fruits and suggested their health-promoting properties. The effects of light and temperature on total phenol content in methanolic extracts of selected fruits were examined in an experimental setting designed to mimic storage conditions. Total phenolics in control samples kept in the dark at 8°C were not significantly different from those exposed to the light and kept at 20°C during 60 days. In conclusion, all of these results show that the investigated fruit extracts can be used as an easily accessible source of natural antioxidants and as a possible food supplement.

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## P063-A1

### Comparison of New Spectrophotometric and Radical Scavenging Assays for Estimating Antioxidant Activity of Wine

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Nowadays, there is considerable interest in finding out about antioxidants that are consumed in the habitual diet. It is known that antioxidant compounds are involved in reducing the risk of many diseases, e.g. atherosclerosis, cardiovascular and neurological diseases and cancer, associated with oxidative stress (Johnson, 2002). Antioxidant activity is the common assay used and widely accepted by researchers as an anticancer indicator. It has been proposed that regular consumption of red wine in moderate amounts reduces the risk of coronary heart disease via protection of LDL against oxidative damage. Polyphenols have shown strong antioxidant effects and so it has been suggested that they are responsible for neutralizing the excess of reactive nitrogen and oxygen species (Sanches-Moreno et al., 2000). There are different spectrophotometric methods to evaluate the *in vitro* antioxidant capacity of phenolic compounds, biological fluids and tissues which involve different mechanisms. The antioxidant activity of wine has been thoroughly studied and a wide variety of methods have been developed to evaluate it. The most frequently used are those that employ a chromogen of a radical nature. The presence of antioxidants leads to the disappearance of the radical, and is followed by absorbance measurement.

The purpose of this work was to present a new rapid, relatively sensitive, and low-cost spectrophotometric method enabling the *in vitro* determination of the antioxidant activity of wine. The method is based on its inhibition effect on the reaction between hydrochloric acid and bromate. This assay involves addition of a known excess of bromate to appropriately diluted wine sample in an acid medium, followed by determination of residual bromine and chlorine reacting with methyl orange and measurement of absorbance at 505 nm 5 min after addition of the last drop of the bromate solution. The absorbance increases linearly with antioxidant activity of tested wine (correlation coefficient is 0.9994). The antioxidant activity of tested wines was compared with the antioxidant activity of same wines evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging assays. In general, it could be concluded that the antioxidant capacity depends of the used method.

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## P064-A1

### Application of Copper Amalgam Electrode for Voltammetric Determination of Selenium Content in Fruiting Bodies of Selected Edible Mushrooms

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The chemical composition of edible mushroom species from ecologically safe areas varies and is already known. Dried mushrooms consist, proteins, carbohydrates, vitamins and microelements. One of the microelements appearing in popular mushrooms is selenium.

The objective of this work was to determine selenium level in twelve selected fruiting body caps of macrofungi species from *Basidiomycetes*, most popular and appreciated edible mushrooms. The method based on cathodic stripping voltammetry with differential pulse step performed at HCADE (*Hanging Copper Amalgam Drop Electrode*) has been applied. Applied electrode is characterized by simple handling, regeneration facility with good repeatability and reproducibility. Parameters, such as LOD=0.25nM (0.02 $\mu\text{gkg}^{-1}$ ) and LOQ=0.75nM (0.06 $\mu\text{gkg}^{-1}$ ) at pre-electrolysis time (120s) could be estimated.

Selenium content of investigated samples were determined in the range of 0.49–11.7mgkg<sup>-1</sup> of dry matter. The highest value of selenium content was found in *Boletus edulis* and lowest in *Letiporus sulphureus*. Results shows significantly differences between selenium assimilation in mushrooms species.

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## P065-A1

### Method Development for Daidzein, Genistein and Formononetin Quantification in Coffee

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Isoflavones have been associated with several health protective effects, namely hypocholesterolemic, anticarcinogenic, antiosteoporotic, and menopausal symptoms relief. They are structurally related to 17 $\beta$ -estradiol, being categorized as weak estrogens [1]. Soy and derivatives are the main sources of these compounds [2]. However, it is also important to evaluate the contribution from other foods to the total isoflavones intake by populations.

The aim of this work was to develop and validate a simple analytical method for accurate quantification of isoflavones aglycones (daidzein, genistein and formononetin) in coffee, by HPLC/DAD. Therefore, several extraction procedures were tested [3, 4, 5]. Method efficiencies were compared in terms of standard recoveries, chromatographic resolution, and relative peak areas. Several modifications were also tested in order to improve method performance.

In the proposed methodology, the aglycones were released by a hot acid hydrolysis, in the presence of methanol, BHT (as antioxidant) and 2'-methoxyflavone (as internal standard). Chromatographic analysis was performed by HPLC/DAD after neutralization. The method showed high correlation coefficients ( $r > 0.999$ ) for standards subjected to the entire procedure. For samples, good intra- and interday precisions (<7%), good accuracies (recoveries of 95 $\pm$ 1%) and quantification limits between 14 and 25 ng/mL were achieved.

Levels of 0.62-1.08, 0.05-0.71 and 1.20-5.80 mg/100 g (dwb) were found for daidzein, genistein and formononetin, respectively, in several roasted coffee samples ( $n=6$ ), by applying this methodology.

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## **P066-A1**

### **Changes in the Ergosterol and Deoxynivalenol Contents in Barley and Malt during Malting Process**

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Deoxynivalenol and ergosterol content in barley and in malt that was produced from this barley was determined. There were used different varieties of barley, which were grown after different crops in different parts of the Czech Republic. The malting was carried out in the VÚPS, a.s. laboratory in Brno. Totally, 20 samples of barley and 20 samples of malt were analysed. The alkaline hydrolysis with extraction into the hexane was applied for the gain of the ergosterol from cereals. Extraction with acetonitril/water and subsequent solid phase extraction (SPE) for deoxynivalenol extraction from samples was used. The analysis was achieved by high performance liquid chromatography with UV detection (ergosterol) and mass spectrometry detection (deoxynivalenol). The calibration was carried out by standard addition method.

We assessed the influence of malting process to the amount of these two compounds from received results. Ergosterol concentration ranged between 0.88 and 15.87 mg/kg in barley and between 2.63 and 34.96 mg/kg in malt and it's content increased in 95 % of the samples after malting. The malting process was observed to have a significant effect on ergosterol concentration ( $P = 0.07$ ). The deoxynivalenol contamination of samples was low and the legislation limit 1250  $\mu\text{g}$  deoxynivalenol/kg for unprocessed cereals were not exceeded. The maximum concentration of deoxynivalenol reached 641  $\mu\text{g}/\text{kg}$  in barley and 499  $\mu\text{g}/\text{kg}$  in malt respectively. Any statistically effect of malting to deoxynivalenol content was not found out.

The relationship between ergosterol and deoxynivalenol content was evaluated too. Linear correlation provided very low coefficient (barley  $R = 0.02$ , malt  $R = 0.01$ ). As emerged from results we can not consider the ergosterol content as deoxynivalenol contamination indicator in naturally moulded samples.

## **P067-A1**

### **Carbohydrate Chromatography – Ion Chromatography: a Combined Approach in Quality Assessment of Apple Juice**

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Apple juice has a strong position in the world consumption ranking, being appreciated not only for its taste and nutritional properties, but also for its health effects, being known to help protect from many diseases associated with aging such as heart disease and cancer. Apple juice is also valued because it's lower in sugar than just about any other type of juice and it's not as acidic as citrus juices.

However, all these features are related with a proper juice quality.

High performance liquid chromatography (HPLC) is commonly used to check the quality of fruit juices by analyzing their oligosaccharide fingerprint profile, as a tool for quality control and for checking adulterations.

This study investigates the use carbohydrate chromatography and ion chromatography for obtaining the profiles of fructose, glucose, sucrose, sodium, potassium, magnesium and calcium as indicators of apple juice quality. Commercial apple juices samples obtained from the Romanian market were analyzed during 2008 – 2009, fourteen brand being investigated. Carbohydrates analysis was accomplished using a HPLC Shimadzu system with differential refractive index detection, isocratic separations being achieved on a EC 250/ 4 Nucleodur 100 – 5 NH<sub>2</sub> RP column, using as mobile phase a mixture of acetonitrile : water (23 : 77), at 1 ml/ min and 35<sup>o</sup>C. Cation analysis were performed on Shimadzu system with conductivity detection, equipped with an Universal Cation 7u column maintained at 40<sup>o</sup>C; the mobile phase was a 3 mM HNO<sub>3</sub> solution, the flow rate being 0.5 ml/ min.

The proposed approach has the advantage of a minimum sample workup (consisting in dilution followed by filtration), being low time-consuming, enabling also the detection of common fraudulent practices. A final cluster analysis of the obtained data lead to the classification of the analyzed juices according to their established carbohydrates and cations composition.

## **P068-A1**

### **Effect of Processing on Carotenoids in Fortified Bread**

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Vitamin A deficiency is one of the most severe public health problems of people from developing countries, becoming also a serious problem in some cases of unbalanced diets. To avoid it, bread fortifications with provitamin A carotenoids can be an important alternative, as bread is a main vector in many populations' daily nourishment.

This study deals with bread fortification using a natural source of provitamin A carotenoids: *Cucurbita maxima* fruits (pumpkins). Fortified bread samples were obtained through the direct procedure, in which a puree obtained from baked pumpkin fruits was added to a classical yeast – based dough. Carotenoid analysis was accomplished using high performance liquid chromatography with photodiode-array detection; separations were achieved on a Nucleosil 120 - 5 C18 column, with a gradient using acetonitrile : water (9 : 1) and ethyl acetate. Twelve carotenoids were identified in fortified bread matrix, from which four carotenes ( $\alpha$ -carotene,  $\beta$ -carotene, 9Z- $\beta$ -carotene and 15Z- $\beta$ -carotene) and eight xanthophylls (neoxanthin, violaxanthin, cucurbitaxanthin A, lutein, zeaxanthin,  $\alpha$ -cryptoxanthin,  $\beta$ -cryptoxanthin and 5,6-epoxy- $\beta$ -carotene). Unfortified bread contains only traces of two carotenoids, lutein and  $\beta$ -carotene, both originating from flour. Starting from an total mean carotenoid concentration of 92.87  $\mu\text{g/g}$  dough, the final total mean carotenoid concentration recorded in bread samples was 69.65  $\mu\text{g/g}$  bread, while the provitamin A activity decreased from 3.61 to 2.38  $\mu\text{g R.E. / g}$  dry weight. The major carotenoids in fortified bread are lutein, cucurbitaxanthin A and  $\beta$ , $\beta$ -carotene; the most stable carotenoids during processing proved to be lutein and cucurbitaxanthin A, thermal processing being mainly responsible for carotenoid degradation. The proposed bread fortification leads to a product with improved appearance and taste, with higher porosity and volume, containing also a significative amount of carotenoids, which can create new markets for producers.



## **P069-A1**

### **Simultaneous Determination of Uric Acid and Purine Profile in Milk Using RP-HPLC and Diode Array Detection**

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Uric acid is an enzymatic end product of dietary purine nucleotide metabolism and a potent antioxidant. The content of purine compounds in milk varies with the associated mammal species. Hence, the aim of this work was to develop HPLC method for the determination of uric acid and purine profile (xanthine, hypoxanthine, adenosine, guanosine, adenosine 5'-monophosphate and guanosine 5'-monophosphate) in bovine and goat milks. Method was optimized and its performance evaluated with application of new internal standard N<sup>6</sup>,O<sup>2</sup>-dibutyryl-adenosine 3',5'-cyclic monophosphate which expressed satisfactory chromatographic behaviour, high stability and characteristic UV spectra. Milk samples were pretreated by dilution, protein precipitation with perchloric acid, centrifugation and filtration followed by HPLC separations using an Eclipse-XDB C18 RR column. During method set up, several factors (amount of sample, dilution factor, pH of mobile phase, time standing, centrifuge speed, volume of perchloric acid) were tested. Chromatographic separation was achieved on column by linear gradient of two mobile phases, phosphate buffer (pH-6) and methanol. The effluent was monitored using a Diode Array detector set at 280nm for uric acid and 254nm for purines. Validation was carried out by standard additions method, with recoveries of 88.2-96.7%. The precision of the method was also evaluated and reported coefficients of variation (CV) as less than 6.1. Upon development, the technique was applied on bovine and goat milks in order to study the distribution of purines therein. Goat milk revealed the lower uric acid ( $42 \pm 6.7\mu\text{M}$ , n=16) than values found in bovine milk ( $78 \pm 29\mu\text{M}$ , n=16). Moreover, bovine milk contains considerably lower amounts of purines and their exact chemical composition differs from those in goat milk. Taken together, our results indicate that these two mammal milks possess different patterns in purines metabolism.

## **P070-A1**

### **Simultaneous Determination of Nitrate, Nitrite and Polyphosphate Ions in Meat and Fish Products by cITP Method**

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Salt, nitrate and/or nitrite and phosphate ions are widely used as preservatives in meat and fish production. The use of phosphates and salt in meat products improves juiciness and tenderness and increases the weight of saleable product, due to the retention of added water. Nitrates and nitrites, used in combination with salt, serve as important antimicrobial agents in meat to inhibit the growth of bacterial spores that cause botulism, a deadly food-borne illness.

The many methods already exist to determination of phosphate, nitrate and nitrite ions but they are often complicated and difficult to use in-line. In this work we proposed simply and rapid capillary isotachophoretic method (cITP) for the simultaneous determination of nitrates, nitrites and phosphates ions (tripolyphosphates, pyrophosphates and orthophosphates) in meat and fish products.

15 meat products samples and 10 seafood samples were purchased from local markets. The products acquired compounds of phosphorus(V), nitrites and sodium chloride in composition (declaration of producer). The food were minced, homogenized and extracted with redistilled water using an orbital shaker for 30 min. The extracts were separated using centrifuge at 9000 rpm for 30 min, followed by double filtration. All extracts were transferred into a 50 mL volumetric flask, made up to the mark and analysed with one-dimensional cITP. Furthermore, the content of chlorides in meat samples were determined by Mohr method.

Analysis of nitrates, nitrites, orthophosphates and polyphosphates were performed with LE: 10 mM HCl + 0.2% hydroxyethylcellulose, HEC + 3 mM bis-tris-propane, BTP +  $\beta$ -alanine, BALA (pH = 3.6) and TE: 5 mM citric acid (pH = 3.5). A driving current of the pre-separation capillary was 200  $\mu$ A, which during detection decreased to 150  $\mu$ A.

Principal component analysis was performed for the results of nitrates, nitrites, phosphates and chlorides of the studied food samples using the Statistica (Windows software package) (version 8.0, 2007). PCA score plot was used to determine, whether various food samples could be grouped into different classes.

#### **Acknowledgement**

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## P071-A1

### Spectroscopic Methods for the Analysis of Aflatoxins in Wine

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The *Aflatoxins* are a group of mycotoxins produced as secondary metabolites by certain strains of the fungi *Aspergillus flavus* and *A. parasiticus*. Among 18 different types of *Aflatoxins* identified, major members are *Aflatoxin B1*, *B2*, *G1*, *G2*, *M1* and *M2*. These types of mycotoxins structurally refer to the group of difuranocoumarins and are classified in two broad groups according to the chemical structure: the difurocoumarocyclopentenone (e.g. *AFM1*) and the difurocoumarolactone (e.g. *AFG1*) series, respectively.

*Aflatoxin*-producing members of *Aspergillus* are common and widespread in nature. They can colonize and contaminate grain before harvest or during storage. Crops which are frequently affected include cereals, oilseeds, spices, and fruits.

*Aflatoxin B1* (*AFB1*, figure 1) is normally predominant in amount in cultures as well as in food products and is the most toxic. Pure *AFB1* is pale-white to yellow crystalline, odourless solid. *AFB1* is soluble in methanol, chloroform, acetone, acetonitrile and water.

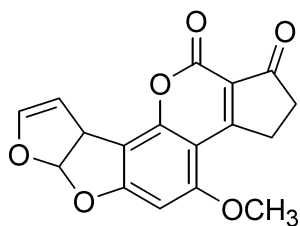


Figure 1: Chemical structure of *Aflatoxin B1*

*Aflatoxins* are potentially toxic, carcinogenic, mutagenic, and immunosuppressive agents. The ability of *Aflatoxins* to cause cancer and related diseases in humans given their seemingly unavoidable occurrence in food and feed make the prevention and detoxification of these mycotoxins one of the most challenging toxicology issues of today.

Within the joint research project “*ProSenso.net2*” non-invasive and non-destructive spectroscopic methods (such as absorption and luminescence spectroscopy in the UV/Vis/NIR-range as well as Raman spectroscopy) are reviewed for the identification of mycotoxins in real-world samples. In a first step, the basic photophysical parameters (such as excitation and emission wavelength, fluorescence decay time, and fluorescence quantum efficiency) of *AFB1* are determined. Preliminary results of the analysis of alcoholic beverages indicate that for qualitative and quantitative determinations of *AFB1* and related compounds in such complex matrices, 2-Photon-Absorption-based fluorescence techniques are promising tools for in-situ detection.

The spectroscopic techniques are complemented by additional chemometric tools (*Principle Component Analysis*, *Partial Least Squares Regression*) to extract the desired chemical information, e.g. with respect to presence of *Aflatoxins*.

Acknowledgement: This study is financially supported within the joint research project „*ProSenso.net2*“ (*PSn2*) which is funded by the German Federal Ministry of Education and Research (BMBF).

## **P072-A1**

### **Determination of Dioxin-Like PCBs in Fish Oil Pills by GC-MS: Levels and Improvements in Sample Preparation**

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Polychlorinated biphenyls (PCBs) are classified as persistent organic pollutants (POPs) because of their persistence and ubiquity in the environment. They have been widely used for many applications, especially as dielectric fluids in transformers and capacitors and coolants. Although its use is no longer permitted, PCBs are still found in environmental media, and in biological tissues.

Theoretically 209 different PCB congeners are possible but only 4 non-ortho-substituted PCBs and their 8 mono-ortho-derived are structurally among planar and are so-called coplanar. According to combined health effects considerations and different biological tests, coplanar PCBs are considered to be the most toxic. Due to the close toxicological similarity to dioxins, these compounds are considered dioxin-like compounds. Toxic Equivalency Factors (TEFs) have been established for these twelve compounds by WHO. For this reason, it is desirable to identify and quantify individual coplanar congeners present in environmental samples.

PCBs tend to be accumulated in the lipid compartment. Therefore, it is of special interest to investigate their levels in fatty samples such as fish oil, due to the biomagnification occurred in aquatic ecosystems through the trophic chain. Because of the complexity of this sample matrix, the analysis with gas chromatography usually requires preliminary purification consisting of combination of various procedures. However this leads to a laborious clean-up procedure with a large consumption of time and reagents.

This work aims to develop a full methodology for the determination of non-ortho substituted PCBs (IUPAC Nos. 77, 81, 126 and 169) in fish oil with a sample preparation consisting of a single step in order to improve the existing ones in economy, speed and respect for the environment without prejudice to their physical and chemical properties. The reason why we have focused on these four congeners is that they have been assigned higher TEF than mono-ortho congeners. The developed analytical pre-treatment is based on the use of multilayer columns of silica activated neutral and amended with H<sub>2</sub>SO<sub>4</sub> at different concentrations. Columns were washed with 20 mL of hexane and the analytes were eluted by vacuum with 15 mL of hexane. Extracts were evaporated to dryness and reconstituted with 50 µL of hexane prior to analysis. Gas chromatography coupled to mass spectrometry (GC-MS) was used for the separation and determination of the analytes. The GC-MS conditions have been optimized. The MS quadrupole was set to 280°C and the analytes were ionised by electronic ionisation at 70 eV. The SIM mode was selected for the analysis and quantification ions were selected for each analyte.

Under these conditions, the instrumental limits of detection of the analytes ranged from 0.40 to 1.67 ng mL<sup>-1</sup> and the response of the detector was linear up to 170 ng mL<sup>-1</sup>. Relative standard deviation of the areas for each and every compound was below 3.50 % in all cases ( $n = 10$ ). The methodology was validated by spiking two samples of cod liver oil with 1.0 and 1.5 ng of the analytes, respectively. The recoveries were between 77 and 125 %.

The methodology was applied to the determination of the analytes in three kinds of commercial fish oil pills with concentrations ranging from not detected to 0.86 ng g<sup>-1</sup>.

## **P073-A1**

### **Application of Commercial Biosensors for L-Lactate Assay in Fruit Juices and Traditional Fermented Food**

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During realization of Collective Research Project QUALI-JUICE (COLL-CT-2005, Co Nr 012461) the research regarding application of biosensors in early detection of contamination with lactic acid bacteria during production process by measuring concentration of lactic acid. The assay of L-lactate with enzyme kits is time and work consuming and expensive. Because of that one of the aims of QUALI-JUICE project is application of commercial biosensor for L-lactate for routine assay during the production process.

Two commercial biosensors were tested : BIOSEN\_C Line sport (EKF, Germany) and LactatProfi 3000 (ABT, Germany). Both biosensors are dedicated for analysis of L-lactate in blood and because of that their use for analysis in apple juice must be validated. To validate the biosensors the stability, accuracy and reproducibility were estimated. For L-lactate contents lower than limit of measurement by biosensor the methods of measurements were proposed by using the method of internal standard. The biosensors were tested for interfering substances like polyphenols and ascorbic acid. It was found that ascorbic acid and gallic acid were causing significant increase of the results of measurements. To obtain more accurate results with biosensors different methods of preparation of samples were tested : adsorption of interfering substances by polivinylpyrrolidone (PVPP), nylon 6 and activated charcoal and oxidation of polyphenols with tyrosinase. The best results were obtained for the samples treated with nylon 6 and PVPP. Concentration of L-lactate was measured in commercial apple juices, samples of apple must and concentrate obtained from the production line of VINKON company (Poland), other juices and traditional fermented food. For comparison L-lactate was assayed using enzyme kits (MEGAZYME, Ireland). It was found a good correlation between results obtained with biosensors and enzyme kits although the values obtained with biosensors were overestimated. The obtained results showed that both biosensors could be used for routine L-lactate assay during apple juice production and to control L-lactate content in fruit juices and fermented food. The EKF device gives more accurate results but is more expensive and is typical laboratory stand. The ABT one is less accurate but because it is a handy one and inexpensive thus it could be at line.

## **P074-A1**

### **Chemical and Microbiological Characterization of Typical East Piedmont (Italy) Salami**

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The general term “salami” indicates encased meat products, very diffused and largely consumed because of their textural, sensorial and nutritional properties. Different kinds of salami can be distinguished as a function of several factors, as fineness of the meat, formulation, consistency, storage conditions. To protect the peculiarity of a typical product, it is necessary to identify and quantify those variables that better describe its characteristics. Variables and characteristics permit to promote the product through the development of a certificate of origin that also reports the production process and the geographical provenance.

The present paper presents a wide characterisation study of typical handmade salami produced in the Alessandria province (North-West of Italy), with the scope of screening the characteristics of six products (*Muletta Monferrina*, *Salame Nobile del Giarolo*, *Filetto Baciato*, *Tipico Tortonese*, *Filzetta* and *Salamini di Mandrogne*) and follow their evolution along with ripening. Microbiological and chemical analyses were carried out regarding both non-volatile and volatile fractions.

The overall results obtained point out that the products investigated do not deviate from analogous European products and some considerations can also be drawn with respect to the nutritional characterization of the samples. To this purpose, the attention has to be focussed on BA content, as their profile can be related to the ripening working out conditions.

All the data collected were treated by multivariate statistical analysis techniques as Principal Component Analysis (PCA) and Cluster Analysis. PCA was firstly applied to the overall set of data collected at all the ripening stages considered, to provide a general description of the relationships existing between samples and variables. Then, the analysis was focussed on the samples collected at the selling stage only, to provide a description of the products as they reach the consumer table.

The application of PCA and cluster analysis show the existence of three main groups of samples and namely *Salamini di Mandrogne*, *Muletta* and *Nobile Giarolo*, that can be characterised and discriminated by different variables.

## P075-A1

### Research on New Spectroscopic Methods in Determination of Polyphosphates in Meat Products

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Phosphates are currently very important group of additives in modern food industry. Phosphate food additives serve many functions including acidification, buffering, anticaking, leavening, stabilizing, emulsifying, water binding, and protection against oxidation. Phosphates are added to a variety of processed foods, including some baking mixes, colas, meat and poultry products, cheeses, canned tuna, puddings, toothpastes, and other products, according to background information on the web site of the International Food Additives Council (IFAC). The chemistry responsible for the wide array of functional properties of phosphates is not fully understood but undoubtedly is related to the acidity of protons associated with phosphates and the charge on phosphate ions. It should be mentioned, however, that there is considerable controversy about mechanisms of phosphate functionality, particularly as it relates to enhanced water-holding capacity in meats and fish.

New discoveries about lung cancer suggested possible correlation between lung cancer risk and high level of phosphates<sup>1</sup>. Increasing consumption of condensed (pyrophosphate and tripolyphosphate) phosphates form probably will be a big medical problem in near future. Improving current analytic methods in determination of phosphates and elaborating new method would allow minimizing of risk cancer.

Popular spectroscopic methods such as UV-Vis spectroscopy and spectrofluorimetry have a lot of advantages including simplicity and precision of analysis, low price of instrument with satisfactory LOD. Fluorescent anion sensors are very appealing because they allow for low detection limit analysis. A fluorescent dinuclear zinc complex has been prepared by an easy "one pot" method in which the lysine based Schiff base ligand is generated *in situ*<sup>2</sup>. According to previous presented references we try to apply this complex in a quantitative analysis of phosphate ions by decrease of fluorimetric intensity<sup>2</sup>. Also we observed changes of wavelength observed in UV-Vis spectroscopy which would cause differentiate between pyrophosphates and orthophosphates ions.

New simple spectroscopic methods in phosphates analysis will contribute a better control of food additives.

#### Acknowledgement

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## **P076-A1**

### **Quantitative Analysis of Lincomycin and Narasin in Livestock Products by Liquid Chromatography-tandem Mass Spectrometry**

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Veterinary drugs might be used to treat and prevent the livestock diseases. However, misused and overused veterinary drugs in livestock products could affect the health of human. Therefore, Korea maximum residues limits (MRLs) for lincomycin and narasin in livestock products were set in 2008 in order to intensively control their use. In this study, the residues of lincomycin and narasin for livestock products were monitored. Livestock products (n=159) were collected from Korean markets. The analytical procedure is based on C18 solid-phase extraction cartridges and extraction with acetonitrile. Analytes were detected using liquid chromatography-tandem mass spectrometry with positive ion mode. The recoveries of their analytical method in different matrices were found ranging from 89.5 to 98.2% and 80.8 to 99.0% for lincomycin and narasin, respectively. The corresponding limits of detection were <0.01 ug/kg and <0.6 ug/kg for lincomycin and narasin, respectively. Narasin was not detected in any samples. Lincomycin was detected in cow milk with 0.1 ug/kg, in swine meat with 0.3 ug/kg and in egg with 25.2 ug/kg and its concentration was under MRL(150 ug/kg in milk, 200 ug/kg in swine meat and 50 ug/kg in egg) of lincomycin. Based on the result of the monitoring, there was no violation for Korea MRLs of veterinary drugs in livestock products.



## **P077-A1**

### **Analysis of Sweet Cherries Antioxidant Content**

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Nowadays, there is an increasing interest in the consumption of fruits in the human diet. Many studies show that their large variety and their antioxidant content have an important role in the protection against degenerative diseases such as cancer, heart diseases, brain dysfunctions, cataracts and others.

Rich in water, sugars, vitamins, mineral salts, sweet cherries are counted among the most appreciated fruits and among the richest ones in phenolic compounds. These compounds are secondary metabolites possessing an aromatic ring bearing one or more hydroxyl groups. They have an important antioxidant activity depending on their structure. Major phenolics in sweet cherries are anthocyanins followed by phenolic acids.

The aim of our study was to assess the feasibility of extracting antioxidant compounds from sweet cherries by a "green", rapid and not expensive technique: solvent free microwave assisted extraction. First of all experiments were conducted in order to determine the optimum microwave extraction conditions. The analysis of the crude extracts was achieved in reversed phase liquid chromatography. The richest extract was submitted to a fractionation by centrifugal partition chromatography in order to simplify it and make the LC-MS analysis of each fraction easier to interpretate. Results are according with literature studies and show a large amount of cyanidine-3-glucoside and cyanidine-3-rutinoside and derivatives content.

## **P078-A1**

### **Optimisation of a Solvent-Free Microwave Assisted Extraction of Antioxidants from Sea Buckthorn (*Hippophaë Rhamnoides*) Berries**

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Sea buckthorn, *Hippophaë rhamnoides* (Elaeagnaceae), is a plant naturally present in Asia and Europe which contains various chemical compounds that makes *H. rhamnoides* an ideal plant model to develop new techniques and find bioactivity. A simple and rapid solvent-free microwave assisted extraction (SFMAE) procedure was developed and optimized for antioxidants from *H. rhamnoides* berries. Whole fresh berries were irradiated with microwaves in closed system vessel without adding any solvent. Optimization of the extraction method was achieved by using a two-level full factorial design on parameters such as microwave power, extraction time and number of cycles. The effect of these factors was evaluated from antioxidant activity of extracts, and tests used were the 2,2'-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method, the determination of reducing power with the Ferric Reducing Ability of Plasma (FRAP) assay, and the estimation of total phenolic contents using the Folin-Ciocalteu method. Results showed that the effect of each factors is significant at 5% and the interaction between time and power in case of the Folin method. The richest and most active extract was obtained with 1000 W, 50 sec and 5 cycles. In order to promote SFMAE, results obtained for the optimum were compared with those obtained by water and ethanol MAE, and also by other extraction techniques using water as solvent such as maceration, pressing and accelerated solvent extraction (ASE) at different temperatures. It is appeared that SFMAE extract was found to be the best. Furthermore SFMAE respect green chemistry approach and it is a rapid and cheap extraction technique which doesn't need any pre-concentration step of the sample.

## **P079-A1**

### **Flavour&Fragrance Analysis: Easy Heart Cut MDGC with Mass Spectrometric Detection in 1<sup>st</sup> and 2<sup>nd</sup> Dimension**

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In flavour and fragrance analysis co-elutions are often observed in one-dimensional gas chromatography. In order to separate those regions are transferred into a second column using heart cut multidimensional gas chromatography GC/GCMS [1]. A Carbowax column with 30 m, 0.25 mm i.D. and 0.25 µm film was coupled to a chiral RTX-5 30m, 0.25 mm, 0.25 µm in the second dimension in order to separate chiral compounds.

To have also identification of the peaks in the first dimension an FID/MS splitting was realised. This was created by a capillary split connection to feed the effluent at the FID (1<sup>st</sup> dimension) partly into MS simultaneously (1/15 relativ to FID). For this a deactivated fused silica tube (1m, 0.175 mm ID) was lead via the interface from the GC of the first dimension into the second dimensional GCMS. Both columns the split connection and the second dimensional column are mounted into the MS detector by using a special connector [3]. While the FID chromatogram can be used for an area normalisation report the MS full scan data can be used for identification. Chiral compounds can be then transferred to the second column in a subsequent run. In cut runs the FID/MS splitting transfer line has to be blocked to prevent co-elutions of cut peaks from the second dimension with first dimensional analytes. This is achieved by a pressure increase of an auxiliary pressure unit which reverses the flow in the splitting line. Several cuts were done on commercial flavours. Below major peaks of for example linalool and terpineol the two enantiomers of each are resolved after transfer to the second dimension. The identification was done using the library FFNSC1.3 dedicated to Flavour and Fragrance compounds with linear retention indices.

As a conclusion this multidimensional GC/GCMS configuration offers easy and reliable analysis of flavour and fragrance samples.

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## **P080-A1**

### **A Bioassay Guided Approach to Identify Metabolites in Culture Filtrates of Microorganisms that Exhibit Activity against *Fusarium Graminearum***

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*Fusarium graminearum* (teleomorph *Giberella zeae*) is a plant pathogen that can cause Fusarium head blight disease (FHB) in wheat. This disease leads to mycotoxin contamination; the most prevalent toxins include trichothecenes, and zearalone, which are harmful for both humans and animals. Therefore, FHB causes enormous economic losses each year.

FHB cannot be controlled easily by the application of conventional chemical fungicides; they are able to reduce the affection of *F. graminearum*, but the mycotoxin contamination cannot be diminished reliably in this way. Therefore it is of special interest to discover natural compounds with specific effects to protect wheat plants against this pathogen.

A major goal of our project is to identify microorganisms and their bioactive natural metabolites which exhibit activity against FHB in wheat.

Microorganisms that were isolated from natural habitats were cultivated and tested for their growth-inhibiting activity against *F. graminearum*. Active microbial culture filtrates were analyzed in a bioassay guided approach using SPE and HPLC in respect to confine fractions that exhibit activity. The active fractions obtained were analyzed via LC-MS/MS using an LTQ OrbiTrap XL. The full scan raw data were interpreted using XCMS with the method CentWave, peak annotation was done using the XCMS package CAMERA. Additional LC-MS/MS experiments were done considering the candidate *m/z* values obtained by XCMS analysis. Subsequently, the Seven Golden Rules Software was used to receive candidate structures for the elemental composition corresponding to the neutralized accurate masses of interest. First results of the identification experiments will be presented.

## **P081-A1**

### **The Influence of the Barbecue Process on the Concentration of Polycyclic Aromatic Hydrocarbons (PAH) in Food**

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We have investigated the influence of different methods of barbecuing on the formation of polycyclic aromatic hydrocarbons (PAHs) in meat. Thereto, three pieces of meat with a varying fat content (steak, sausage and spare ribs) have been prepared by a chef in five different ways: on a classical gas barbecue, a charcoal barbecue, a charcoal barbecue with use of a perforated aluminium dripping pan, a charcoal barbecue with coconut charcoal briquettes and a gas barbecue with roofed burners. The preparation of each kind of meat was repeated for the five different barbecuing methods.

After preparation, the meat has been analyzed for the presence of PAHs. The samples were saponified by refluxing with an ethanolic KOH-solution. After addition of water, the PAHs were extracted with cyclohexane. Clean-up was performed on a combined silica-alumina column. The 16 PAHs prioritized by the Scientific Committee on Food and the joint FAO/WHO Expert Committee on Food Additives were determined by gas chromatography-high resolution mass spectrometry (GC-HRMS).

The concentration of the individual PAHs in the samples varied between < 0.5 µg/kg and 84 µg/kg. Total amounts of the selection of sixteen PAHs were calculated and varied between 4 µg/kg and 197 µg/kg.

The results indicate that barbecuing with roofed burners results in lower PAH concentrations compared to a classical charcoal barbecue and a classical gas barbecue. Indeed, by horizontal grilling fat can drip into the open flame giving rise to the formation of PAHs. In case of horizontal grilling the concentration of PAHs can be significantly reduced by using an aluminium dripping pan. This dripping pan prevents the contact between fat and the open flame. Furthermore, the PAH formation was shown to be dependent on the fat content of the meat. Meat with a higher fat content resulted in a higher concentration of PAHs after preparation.

## P082-A1

### Comparison of the Antioxidant Activity in Typical Fruits from Madeira Islands

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Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer. The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids.

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including 2,2- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); 2,2- diphenyl-1-picrylhydrazyl (DPPH) ferric reducing antioxidant power (FRAP), and the oxygen radical absorption capacity (ORAC) (

The aim of this research was to compare the efficiency of ABTS, DPPH, FRAP, and ORAC assays to estimate antioxidant activities and their correlations with total phenolics, and total flavonoid contents in different kind of fruits extracts typical from Madeira Archipelago. Fruit extracts for total phenolics and antioxidant activity measured in methanol extract (AOAM) analysis were prepared using the methodology developed by Paixão *et al.* with some modifications. The antioxidant potential was estimated by the following methods: DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazil radical) and ABTS<sup>•+</sup> (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid). The antiradical activity ( $A_{AR}$ ) was determined as follows:  $A_{AR}(\text{mg(GAE)/L}) = 0.9845 \times \ln(\% \Delta A_{515}) + 2.8351$  ( $r^2 = 0.9886$ ), was determined from logarithmic regression, after plotting  $\ln(\% \Delta A_{515})$  of known solutions of gallic acid against concentration (mg/L), where  $\% \Delta A_{515} = [(A_{515(0)} - A_{515(30)}) / A_{515(0)}] \times 100$ . Reducing power ( $P_R$ ) was expressed as quercetin equivalents (mg(QE)/L) from the following equation:  $P_R = (0.0015 \times A_{525} + 0.1094) \times F_D$  ( $r^2 = 0.9969$ ) was determined from linear regression, where,  $F_D$  is the dilution factor. All assays were made in triplicate.

## **P083-A1**

### **Dispersive Liquid-Liquid Microextraction for the Determination of Several Endocrine Disrupting Phenols in Seawater Samples**

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The term endocrine disrupting chemicals (EDCs) is broadly used to define a group of chemicals that may interfere with the function of the endocrine system in living organisms. Chemical exposure to EDCs may also affect human fertility. Relatively large amounts of EDCs enter the environment via sewage treatment plants (STP) effluent outfalls. Therefore, EDCs can be released into surface water, discharged into the sea, or adsorbed onto sewage sludges or sediments. In addition to this, EDCs can be accumulated by marine organisms. Therefore, it results necessary to develop analytical methods to determine EDCs in environmental samples. Among the wide list of possible EDCs, endocrine-disrupting phenols (EDPs), alkylphenols (APs) including nonylphenol (NP) and bisphenol A (BPA), are widely present in household and industrial processes.

A large number of methods have been developed to determine EDPs in the aquatic environment, including in drinking water, river water and wastewater. Limited data are present in the literature about EDP determinations in marine water. Such analyses are particularly difficult, mainly due to the high saline content of the matrix. The conventional extraction methods are in general labor-intensive if employing derivatization and/or solvent-exchange steps, as well as time-consuming. Many traditional organic solvents are often used for these steps and exhibit moderate-to-high toxicity. In addition, they are hazardous, flammable, and damaging to the environment. In recent years, several methods have been developed to eliminate or at least reduce the amounts of organic solvents used in analytical processes, such as solid-phase extraction (SPE) or solid-phase microextraction (SPME). Dispersive liquid-liquid microextraction (DLLME) is a quite recent extraction technique being characteristic for its simplicity of operation, rapidity, low sample volume, low cost and high enrichment factor. This method employs a mixture of a high-density extraction solvent, and a water miscible and polar disperser solvent. In DLLME, the extraction efficiency depends not only on the extractant, but also on the type and volume of the dispersive solvent.

The present work describes the utilization of DLLME for the determination of a group of EDPs from seawater samples. The selected extraction solvent was 1-decanol, and the disperser solvent was acetonitrile. The overall extraction procedure has been optimized by means of an experimental design. Average extraction efficiencies of 95.4% and relative standard deviation lower than 6.1% have been obtained. The method has been applied to real seawater samples from contaminated locations.

## **P084-A1**

### **Utilization of Bis[(Trifluoromethyl)Sulfonyl]imide-Based Polymeric Ionic Liquid as Selective Coating in Solid-Phase Microextraction for the Determination of a Group of Endocrine Disrupting Chemicals**

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Solid-phase microextraction (SPME) is a popular solvent-free sampling-extraction technique which has gained a lot of attention in recent years. SPME consists of a fiber that is coated with a stationary phase typically composed of a liquid polymer, solid sorbent, or a mixture of both. Equilibrium is established between the analyte and the coating material when the fiber is exposed to a solution. SPME can be conducted in both headspace and direct-immersion sampling mode. Several SPME coatings are commercially available, being the most popular polydimethylsiloxane and polyacrylate. The need for new coating materials is underscored by the fact that SPME methods must achieve high sensitivity and selectivity. The coating material must be designed to be resistant to extreme chemical conditions, such as pH, salts, organic solvents, and modifiers. To achieve long fiber lifetimes, the coating should be thermally stable to avoid excessive losses during the high temperature desorption step while also maintaining physical integrity of the film.

Ionic liquids (ILs) and their polymerized analogs constitute a class of non-molecular, ionic solvents with low melting points. ILs are in many cases comprised of bulky, asymmetric N-containing organic cations (e.g., imidazole, pyrrolidine, pyridine) in combination with a wide variety of anions, ranging from simple inorganic ions (e.g., halides) to more complex organic species (e.g., triflate). ILs have negligible vapor pressures at room temperature, possess a wide range of viscosities, can be custom-synthesized to be miscible or immiscible with water and organic solvents, often have high thermal stability, and are capable of undergoing multiple solvation interactions with many types of molecules. In this sense, ionic liquids constitute an interesting alternative to regular SPME coatings.

The purpose of this work was to evaluate the performance of an absorbent SPME coating based on the bis[(trifluoromethyl)sulfonyl]imide-based polymeric ionic liquid. This kind of coating does not need to be re-coated after every extraction, possesses exceptional thermal stability, highly reproducible extraction efficiencies, and long lifetimes. The analytes evaluated were a group of six endocrine disrupting phenols, five polycyclic aromatic hydrocarbons, five chlorophenols and three alkylphenols in different water matrices. The direct immersion mode was used in all experiments. Furthermore, a comparative study with a PDMS fiber has been carried out.



## **P085-A1**

### **Optimization of Pressurized Fluid Extraction of Nitrated and Oxygenated Polycyclic Aromatic Hydrocarbons from Atmospheric Particles**

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Nitrated (NPAHs) and oxygenated (OPAHs) polycyclic aromatic hydrocarbons, either directly emitted by combustion processes or formed in the atmosphere by both gas and heterogeneous reactions of PAHs induced by atmospheric oxidants (Allen et al., 1997 ; Perraudin, 2005), can be part of atmospheric particles . Little is known about their behavior and typical concentrations in airborne particulate matter. The objective of this study was to optimize the pressurized fluid extraction step using experimental design methodology.

Blank filters spiked with a known amount of each compound were extracted using an ASE200 apparatus (Dionex). Five extraction parameters, potentially affecting the extraction efficiency, have been optimized: temperature; pressure; static extraction time; flush volume; purge time. The samples (concentrated if necessary) were analyzed by ultra performance liquid chromatography/mass spectrometry.

The mean recovery value of all studied compounds was selected as the experimental response. A fractional factorial design involving 16 experiments was chosen as the screening method. The upper and lower values tested for each factor corresponded to the ASE technical constraints. This design was applied to identify the significant factors and 2<sup>nd</sup>-order interactions which were further optimized with a response surface design. The method was successfully applied to the analysis of real samples using optimal conditions.

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## **P086-A1**

### **Concentration of Benzene Carboxylic, p-toluic and Terephthalic Acids on Polymeric Sorbents and their Determination in Waters through High-Performance Liquid Chromatography**

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Carbon acids are among the most widespread components of natural mediums of different origin. It is not seldom that they constitute a considerable part of an organic substance in natural waters.

Chromatographic methods of analysis are most frequently used for separate-component determination of carbon acids. Notwithstanding high sensitivity of such methods, preliminary samples preparation is required, including concentration of the substances contained in trace quantities.

Solid sorbents use is quite perspective at concentrating organic compounds out of natural waters. Sorptive concentration proves to be increasingly widespread due to its simplicity, minimum quantity of toxic organic solvents.

This research is aimed at selecting the most optimum parameters for separation of benzene carboxylic, terephthalic and p-toluic acids on chromatographic column and determining their best extraction conditions out of water mediums. The content of the acids in question has been detected through high-performance liquid chromatography, its reversed phase variant. Quantitative estimation has been performed by absolute calibration method.

Solid phase concentration of carbon acids on Oasis MAX sorbent has been tested under the research in question. The eluent volume and origin effect on the degree of acids extraction out of pH water solution has been studied. Isotherms for benzene carboxylic, terephthalic, p-toluic acids sorption out of water solution at their combined presence have been determined.

Oasis MAX polymeric sorbent has proved to be relevant for benzene carboxylic, terephthalic, p-toluic acids concentration.

## **P087-A1**

### **Contribution Role of Some Substituted Phenols to Phenol Index Formation at Waste Waters Analysis**

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Phenols are brought into the environment by waste waters from petroleum chemical and refinery plants, pharmaceutical and construction materials plants, housing and communal services, as well as by storm flows from mainline railroads and petrol stations.

The most widespread phenol test method (spectrophotometric method) enables to obtain data on total amount of volatile phenols, i.e. phenol index.

Phenol index determination is undoubtedly rational and accessible method of obtaining data on the content of similar pollutants in water, in this very case of those substances that are able to form colored compounds with 4-aminoantipyrin. However, practice shows that substances, not relating to the class of phenols can enter this compound group too. Thus, in some cases phenol index value is not identical to the total phenol content and does not reflect real state of water pollution with these compounds.

In this research aimed at the assessment of contribution of some substituted phenols to the formation of «phenol index» the effects of the following compounds have been analyzed: phenol, 2-methylphenol, 4-methylphenol, 2,6-dimethylphenol, 2,4-dimethylphenol, 2-chlorophenol, 4-chlorophenol, 2,4-dinitrophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 4-nitrophenol, 4,6-dinitrophenol, 3-methoxyphenol, 4-methoxyphenol, guaiacol, 2,6-dimethoxyphenol, hydroquinone, resorcin, pyrocatechol, pyrogallol, phloroglucinol, 2-aminophenol, 3-aminophenol, 4-aminophenol, 2-tertbutylphenol, 4-tertbutylphenol, 2,6-ditertbutylphenol, as well as non-phenol substances. Here belong salicylic spirit, isopropylbenzodioxane, 1-propenoxybenzol, isopropylbenzofuran. The research is based on model solutions of individual compounds. The research resulted in estimation of molar absorption coefficients, intensity of the forming compounds absorption bands. The list of volatile with water vapor compounds and those forming colored compound with 4-aminoantipyrin has been made up.

## **P088-A1**

### **Heavy Metals Analysis in Small River Bottom Sediments**

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Bottom sediments reflect long-term accumulation and transformation processes of water reservoir substances. High heavy metals concentration in bottom sediments can threaten the reservoir ecosystem, since at particular conditions they can move into water, resulting in secondary pollution of bottom water.

Heavy metals redistribution processes between water and bottom sediments are of prime importance for rivers with small flow quantity and irregular stream canal. Such rivers are typical for Bashkortostan eastern regions, where several ore-dressing and processing enterprises are located.

The Buida river water and bottom sediments analysis has been carried out, since the river is directly affected by Uchaly ore-dressing and processing enterprise.

Heavy metals move both as soluted and suspended matters, proportion depending on water and sediments pH in a river and on the nature of the element.

Secondary pollution processes have proved to affect copper and zinc redistribution between water and bottom sediments at leaching metals from bottom sediments into water.

## P089-A1

### Atomic Absorption and Energy Dispersive X-Ray Spectrometries for Determination of Elements Content of Wild Mushrooms

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The minor elements (Ca, Zn, Cu, Fe, K, Mn, Mg, P, Se) and heavy metal (Cd, Cr, Ni, Pb, Ti, Sr, Co, Bi) contents of eight wild mushrooms (*Amanita vaginata*, *Amanita rubescens*, *Amanita phalloides*, *Armillariella mellea*, *Armillariella tabescens*, *Agaricus campestris*, *Hypholoma fasciculare*, *Hypholoma pudorinus*) and soil samples, from the Dambovită county Romania, were analysed. Elements were determined by Atomic Absorption spectrometry and Energy Dispersive X-ray spectrometry (EDXRF) in 24 samples of eight fungal species and 16 underlying soil samples. The elements, especially heavy metals, in soil were characteristic of the acidic soils of the Romanian forest or plain lands and are influenced by industrial pollution. The EDXRF measurements were made using the Elvax spectrometer having an X-ray tube with Rh anode and a solid state Si-pin-diode X-ray detector with a 140 µm Be window and 200eV at 5.9 KeV (Fe<sup>55</sup> line) energy resolution. The AAS measurements were performed using an Atomic Absorption Spectrometer, equipped with an AVANTA GBC flame and hollow cathode lamps (HCL). In fruiting bodies of these mushrooms, the highest mean concentration of macroelements (dry mass basis) was found for N, K, P, Ca, Zn, Fe. All macroelements were concentrated in considerably higher levels in the fruiting bodies than the soil. The mean concentration of heavy metal (Cd, Cr, Ni, Pb, Ti, Sr, Co, Bi) is higher at mushrooms which are collected on plain lands near urban settlements. For example, the highest cadmium content was observed in *Armillariella mellea* 3.8 ppm, collected on plain lands. Lead was determined at highest concentration in *Amanita vaginata* 89.3 ppm, *Amanita phalloides* and *Amanita rubescens*, 84.9 ppm and 86.7, respectively. Analytical possibilities of EDXRF and AAS analytical methods were compared and the heavy metal transfer from substrate to mushrooms was studied. The results of this study showed the fact those wild toxic mushrooms species are metal bioaccumulators. Heavy metal contents of all analysed mushrooms were generally higher than previously reported in literature.

**Keywords:** wild mushrooms, EDXRF, AAS, essential elements, heavy metal

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## **P090-A1**

### **Evaluation of Multi-Walled Carbon Nanotubes as Solid-Phase Extraction Adsorbents of Pesticides from Different Kind of Soils**

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Pesticide application over soils constitutes a threat to soil quality and their ability to provide environmental/agricultural services. In general, when pesticides enter the soil environment, they are quickly and strongly bound to soil components and their absorption and desorption to these components govern their fate. Concerning the analysis of pesticides in this type of matrix, it is very frequent to develop an extraction with a suitable organic solvent accomplished by agitation or sonication and, afterwards, a clean-up step using solid-phase extraction (SPE) cartridges prior to their gas chromatography (GC) determination, which is the most frequent method of choice.

One of the new materials that are currently being studied as SPE stationary phases are carbon nanotubes (CNTs) which have also found different applications in analytical chemistry (i.e. in the design of novel gas sensors, enzymatic biosensors, voltammetry and DNA probes) since their discovery in 1991. Their strong binding affinity for hydrophobic molecules, their internal tube cavity and surface area as well as their ability to establish  $\pi$ - $\pi$  electrostatic interactions are the main characteristics that have attracted their use in SPE.

In this work, a new, simple and cost-effective method based on the use of multiwalled carbon nanotubes (MWCNTs) as solid-phase extraction (SPE) stationary phases is proposed for the determination of a group of seven organophosphorus pesticides (i.e. ethoprophos, diazinon, chlorpyrifos-methyl, fenitrothion, malathion, chlorpyrifos and phosmet) and one thiadiazine (buprofezin) in different types of soil samples (forestal, ornamental and agricultural) using GC with nitrogen phosphorus detection (NPD). The method was validated in terms of linearity, precision, recovery, accuracy and selectivity. Matrix matched calibration was carried out for each type of soil since statistical differences between the calibration curves constructed in pure solvent and in the reconstituted soil extract were found for most of the pesticides under study.

## **P091-A1**

### **Suitability of Non-Porous Membranes for Solvent Extraction of UV Filters from Water Samples**

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UV filters are commonly employed in sunscreen preparations in order to protect skin damage due to the exposure to sunlight. In addition, UV filter compounds are integrated in many cosmetic (e.g. lotion, shampoo or lipstick) formulations in amounts between 0.1 to 10%. Recent studies have shown that UV filters are reaching surface waters (rivers, lakes and coastal sea water). Several techniques have been used to measure UV filters in the environment. Common preparation techniques are time-, solvent- and labour-consuming. A promising method to overcome these disadvantages is the membrane-assisted liquid-liquid extraction (MALLE). This technique is carried out by using a membrane as interface between the sample and the organic solvent which avoids mixing the two phases. Moreover, the use of non-porous membranes provides selectivity and specificity in terms of permeation and transport through the membrane.

In this research, hydrophobic non-porous membranes have been evaluated for the extraction and concentration of nine UV filters in water samples. Analytes were extracted using only 0.1 mL of an appropriate solvent and determined by LC-APPI-MS/MS.

Membrane bags made of different polymeric materials were examined to enable a fast and simple extraction of the target analytes. Finally, the tailor-made membrane bags were prepared from low density polyethylene for solvent volumes of 100  $\mu\text{L}$ . The fully optimised protocol provides recoveries from 76 to 101% and limits of detection between 0.4  $\text{ng L}^{-1}$  (OD-PABA) to 16  $\text{ng L}^{-1}$  (EHMC). The effective separation of matrix molecules was proved by only marginal matrix influence during the APPI-MS/MS analysis since no ion suppression effects were observed. The analysis of lake water indicated the presence of seven UV filter compounds included in this study at concentrations between 40  $\text{ng L}^{-1}$  (BP-3) and 4381  $\text{ng L}^{-1}$  (OC). In non-treated wastewater several UV filters were also detected at concentration level as high as 5322  $\text{ng L}^{-1}$  (OC).

## **P092-A1**

### **Iatroscan-Measured Dissolved and Particulate Marine Lipid Classes in the Northern Adriatic Sea**

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The Chromarod-Iatroscan thin-layer chromatography-flame ionization detection (TLC-FID) system was used to measure lipid classes in the dissolved and particulate fractions of seawater samples taken in the northern Adriatic Sea during winter 2008. This technique distinguishes sixteen lipid classes including hydrocarbons, wax and sterol esters, fatty acid methyl esters, fatty ketone, triacylglycerols, free fatty acids, fatty alcohols, 1,3- and 1,2-diacylglycerols, sterols, pigments, monoacylglycerols, mono- and digalactosyl diglycerides, mono- and di-phosphatidylglycerols, phosphatidylethanolamines, and phosphatidylcholine. The method serves as a broad diagnostic tool for ecosystem studies, including the evaluation of components of biomembranes from living organisms, determination of caloric capacity of organic matter evaluated from compounds linked to metabolic energy reserves, and determination of the extent of degradation of organic matter by looking at lipids issued from the breakdown of glycerides in dying cells and detritus and also pollution.



## **P093-A1**

### **A New FI-ICP-AES Procedure for the Accurate Analysis of Dissolved Platinum Group Elements in Aqueous Environmental Samples**

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Since the introduction of catalytic converters in automobiles for their exhaust purification in Europe in the past 20 years, they have been considered as the main source for emissions of platinum group elements (PGEs) into the environment. Therefore a new focus has been drawn to the determination of PGEs, mainly platinum, rhodium and palladium, in environmental samples due to their cytotoxic and allergic properties resulting in possible harmful effects on human health and other living organisms. Numerous studies have shown that the use of autocatalysts and increasing PGE concentrations in the environment are clearly linked. At first the metals are emitted as particles in metallic form, which are deposited on the road, transported and washed-out by rain and finally accumulated in the soil. Due to transformations of the metals in the environment, reactive and bioavailable species are obtained, which hereby accumulate in plants, reaching the food chain in a final step.

Determination of PGE in environmental, biological or medical samples is hampered by numerous interferences in the most sensitive analytical techniques combined with extremely low PGE levels. To overcome these problems the use of enrichment and or matrix separation procedures is recommended.

This contribution presents an FI-ICP-AES procedure for the element specific measurement of PGE in natural environmental liquids. The method is based on a microwave-assisted UV-digestion of aqueous sample solutions for decomposition of dissolved organic carbon, enrichment of the formed anionic PGE-complexes on an activated alumina column, and subsequent on-line analysis of the pre-concentrated sample using ICP-AES. Analytical characterization of the proposed procedure was performed using spiked extracts of soil samples. The developed technique was applied to investigate the adsorption behavior of dissolved PGE on soil samples of different origin.

## **P094-A1**

### **Flow Injection Based Ultra-Trace Analysis of Platinum Group Elements by ICP-MS**

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Due to the increased use of platinum group elements (PGE) in car exhaust systems, in medical applications and as catalyst metals, determination of PGE has received special interest over the past 20 years. Nevertheless, ultra-trace analysis of PGE in environmental and biological samples remains challenging, since even after suitable mineralization procedures numerous interferences impede accurate quantification strategies.

Improved analytical schemes therefore include appropriate steps to eliminate interfering elements and to pre-concentrate the low abundant analytes. Several procedures have been published that provide these, but the applied manual sample pre-treatment steps are complex, time-consuming and pose additional risks of contamination or handling errors.

In 1996, Schuster and Schwarzer designed a pre-concentration scheme for AAS-analysis based on complexation of Pd and Pt with N,N-diethyl-N'-benzoylthiourea (DEBT), which is previously immobilized on a reversed phase microcolumn. However, since complex-formation with DEBT is non-selective, the adaption of this existing method for quantification by ICP-MS must include elimination of interfering cations which would hamper accurate measurement.

A microwave digestion procedure was developed by Rudolph et al (2006) to mineralize samples of differing origin. The conversion of PGE to anionic chloro-complexes is followed by a sample pre-treatment routine involving cation exchange for matrix removal.

The presented FI-manifold combines the DEBT-based pre-concentration routine with the cation exchange necessary for elimination of cationic matrix constituents. Based on a fully automatized Agilent *AESOP* pre-concentration setup, it provides high reproducibility and minimizes manual sample handling. To overcome the problems associated with the use of organic solvents, the ICP-MS system (ICP-QMS *Elan 6100 DRC II*) was equipped with a Peltier Chiller "Organic-Setup" for sample introduction. Thereby, on-line coupling to the ICP-MS system was possible even though a methanolic eluant was employed. The system was applied to analysis of road dust and soil samples.

## **P095-A1**

### **Thallium in Fractions of Soil from an Area of Lead-Zinc Ore Mining and Processing**

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Thallium is an element which is highly toxic to animals, plants, microorganisms and humans. Frequently it enters the environment as a result of the processing of lead-zinc ores. The concentration of the element in soil strongly depends on the lithology of the parent rock from which the soil was derived. However, a crucial factor for the potential toxic effect of thallium in the investigated soils is thallium mobility. This can be determined by sequential extraction of soil. The aim of this work was to compare thallium concentration in fractions of soils derived from different parent rocks in areas where lead-zinc mining and processing have been carried out.

Soils formed on ore-bearing dolomites, dolomites, limestones and marls, claystones, siltstones and conglomerates, glaciofluvial sands and gravel, slope-wash sands, loams and loesses, all from a zinc-lead ore exploration area, as well as two soils formed on dolomites, limestones and marls from a reference area, were investigated in terms of thallium distribution between soil fractions. Sequential extraction of soil was performed according to the BCR protocol, with an additional initial stage of extraction with water. Apart from labile thallium, thallium entrapped in the residual parent matter was also determined.

Thallium determination was carried out using flow injection- differential pulse - anodic stripping voltammetry.

In all cases of soil formed on dolomites, limestones and marls, claystones, siltstones and conglomerates, glaciofluvial sands and gravel, slope-wash sands, loams and loesses, the major fraction is thallium entrapped in indigestible parent matter (62–93%). In the case of soils formed on ore-bearing dolomites, thallium is almost equally distributed between reducible (32%) and oxidizable (27%) fractions, as well as a fraction entrapped in hardly digestible parent matter (36%).

## P096-A1

### Online Coupling of Bead Injection Lab-On-Valve Analysis to Gas Chromatography: for Determination of Trace Levels of Polychlorinated Contaminants in Solid Waste Leachates

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On-line sorptive preconcentration exploiting renewable solid surfaces, so-called bead injection (BI) [1], in the miniaturized Lab-on-Valve (LOV) platform [2,3] is for the first time hyphenated to gas chromatography (GC) for automated determination of trace level concentrations of polychlorinated organic environmental pollutants [4].

Microfluidic handling of solutions and suspensions in LOV is accomplished by programmable flow with a multi-syringe flow injection (MSFI) setup. The method involves the incorporation of minute amounts (3 mg) of reversed-phase copolymeric beads with hydroxylated surface (Bond Elut Plexa, Varian) into the channels of a polyetherimide LOV microconduit, thus serving as a transient microcolumn packed reactor for preconcentration of organic species. The analyte loaded beads are afterwards eluted with 80  $\mu$ L of ethyl acetate into a rotary injection valve, and subsequently introduced via an air stream into the programmable temperature vaporizer (PTV) injector of GC. The used beads are then backflushed and delivered to waste. The GC separation and determination is synchronized with the preconcentration steps of the ensuing sample.

The potentials of the devised BI-LOV-GC assembly with electron capture detector for downscaling and automation of sample processing were demonstrated in the determination of polychlorinated biphenyls in raw landfill leachates and a leachate containing the Aroclor 1260 congener mixture [4]. By sampling 12 mL leachates to which 50 vol. % methanol was added to minimize sorption onto the components of the flow network, the automated analytical method features relative recovery percentages > 81%, limits of quantification within the range 0.5-6.1 ng L<sup>-1</sup>, relative standard deviations better than 9% at the 50 ng L<sup>-1</sup> level and 25-fold decrease in cost of SPE consumables as compared with on-line robotic systems or dedicated setups.

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## P097-A1

### Detection of Formaldehyde, a Ubiquitous and Carcinogenic Air Pollutant: Colorimetric Warning for Homes and a Portable Device for Instantaneous and Quantitative Measurements

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During the last decade, the increase of the awareness of the importance of indoor air quality and its potential impact on human health has stimulated an interest in formaldehyde, a carcinogen for humans<sup>[1]</sup>. Because of its numerous emission sources (plywood, adhesive resins, cosmetic, etc...), CH<sub>2</sub>O is a ubiquitous indoor pollutant whose concentrations can vary from a few ppb to more than 100 ppb in homes<sup>[2]</sup>. As most of humans spent 80 to 85 % of their time indoor, the World Health Organization recommends, for a chronic exposure during a whole life, a formaldehyde concentration as low as 10 µg/m<sup>3</sup> or 8 ppb (part per billion)<sup>[3]</sup>.

Over this domain of concentration, there is no low-cost and simple detection system which can be used as a warning in homes. On the other hand, the few sensors, which are commercially available have a few drawbacks in terms either of selectivity, simplicity of the sampling, heavy maintenance or high cost.

These findings have prompted us to develop new chemical sensors to provide a colorimetric pollution warning for homes and a portable device for the instantaneous and quantitative determination of the formaldehyde content in air. These sensors are based on the use of porous matrices acting as sponges to trap the targeted pollutant and doped with Fluoral-P for a selective detection of formaldehyde<sup>[4]</sup>. For colorimetric pollution warnings, various matrices are synthesized, which display a progressive colour change *visible by eyes* upon a continuous exposure over days or weeks to the targeted pollutant. A different strategy was developed for the portable device, which is based on the use of nanoporous thin films and fluorescence detection. The prototype, equipped with simple and low cost optical devices, can measure sub-ppb concentration of formaldehyde in air.

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## **P098-A1**

### **Gas Chromatography-Ion Trap-Tandem Mass Spectrometry Determination of Illegal Abuse Drugs in Water Samples**

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Zuccato and co-workers reported in 2005 that cocaine and its metabolite (bezoyllecgonine) occurred in Italian sewage and river water reaching the  $\mu\text{g/L}$  level, as it had actually been predicted four years before by Daughton [1]. Since then, concentrations of cocaine and other illicit drugs have been reported in several other European countries and several liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been developed for the determination of several illegal drugs in water samples. However, the main inconvenient of these methods are the strong matrix effects occurring in wastewater analysis and the high acquisition costs of LC-MS/MS instruments. Therefore, the goal of this work was the development of an alternative method based on gas chromatography-ion trap-tandem mass spectrometry (GC-IT-MS/MS). To this end, several derivatisation reactions were tested. Silylation with N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) produced the overall best results and was thus, further optimised with a real sample extract. The best derivatisation conditions were derivatisation during 1 h at 80 °C with sample/MSTFA ratio of 1/1. Furthermore, other factors, such as sample preservation to avoid drug's degradation or the solid-phase extraction (SPE) enrichment and elution were studied. In the final method, filtered samples (adjusted to pH ~8.4) were spiked with deuterated internal standards and enriched as soon as received in the lab on SPE Oasis HLB 200 mg cartridges. These cartridges could then be stored at -20 °C for at least 3 weeks without any analyte degradation. Then, cartridges were eluted first with 2 mL ethyl acetate and then with 8 mL acetone (cannabinoids). The acetone was blown down to dryness before combining the residue with the first eluate in order to avoid amphetamines forming non-volatile enamines and further reduced to a final volume of 0.1 mL before derivatisation and determination by GC-IT-MS/MS. With this new methodology, 500 mL samples could be pre-concentrated without breakthrough of the analytes and 14 illicit drugs/metabolites could be determined at the 0.8 (amphetamine) to 26 (heroin) ng/L level (LOD for S/N=3 from spiked surface water samples), including amphetamines, opiates, cannabinoids and methadone. The application of the method revealed bezoyllecgonine at 400 ng/L, cocaine at 200 ng/L and carboxy-THC and morphine at 100 ng/L and codeine at 60 ng/L levels.

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## **P099-A1**

### **Compensating for the Matrix Interference during the Analysis of Antibiotic Residues in Water and Wastewater Samples by Liquid Chromatography-Tandem Mass Spectrometry**

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Of current concern worldwide are the emerging pollutants such as pharmaceuticals and personal care products, of which antibiotics are a major category due to their widespread use in large quantities and with high frequency. The occurrence of antibiotics in the environment has therefore received considerable attention, due to the potential development of antimicrobial resistance among microorganisms. This presentation describes the development of an improved method for the analysis of ten antibiotic compounds using solid-phase extraction (SPE) followed by liquid chromatography–electrospray ionization tandem mass spectrometry (LC-MS/MS). The target analytes were tetracycline, oxytetracycline (tetracyclines), sulfathiazole, sulfamethazine, sulfadiazine (sulfonamides), erythromycin-H<sub>2</sub>O, roxithromycin, spiramycin (macrolide), ofloxacin, and norfloxacin (quinolones). Optimization of the LC (e.g. column, mobile phase) and MS (collision and cone energy) enhanced the separation and peak areas of the target analytes. As a result of these experiments, increased selectivity and sensitivity were obtained compared to what have been reported in the literature. Furthermore, the matrix effects were examined for internal standards and the target analytes in five different water sample matrices, with increasing signal suppression effects in the order: ultrapure water, tap water, river water, sewage effluent, sewage influent. A combined application of the internal standards and matrix-matched standard calibration was shown to be successful for compensating for matrix effects. The recovery of the target analytes in sewage effluent as well as river water was found to be satisfactory, which was further enhanced by adjusting the effluent pH to 2. The method was successfully applied to the analysis of samples taken from surface water and wastewater samples in the UK and China.

**Keywords:** Antibiotics; Liquid chromatography-tandem mass spectrometry; Solid-phase extraction; Sewage effluent; Matrix effects.

## **P100-A1**

### **A Screening Method for Direct Determination of Arsenic Species in Marine Organisms by GF AAS**

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Marine organisms have been intensely used as bioindicators of contamination in aquatic ecosystems. Arsenic is an interest element and is commonly found in organic (dimethylarsinic acid, DMA and arsenobetaine, AB) and highly toxic inorganic (As III and As V) species. Due to these different forms and the toxicity, sometimes speciation is more important than total determination. For this reason, the present work proposes a simple and fast procedure for direct organic and inorganic As screening in fish tissue by graphite furnace atomic absorption spectrometry (GF AAS). Liquid, slurry (SLS) and solid (SS) sampling GF AAS were used. Spectrometer was operated with As hollow cathode lamp ( $\lambda = 193.7$  nm,  $I = 4.0$  mA, bandpass = 0.8 nm). Pyrolysis and atomization temperatures were 300°C and 2500°C, respectively. In all cases, 10  $\mu$ l of the chemical modifier containing 5  $\mu$ g Pd + 3  $\mu$ g Mg was necessary. Total arsenic concentration was determined by analysis of 20  $\mu$ l of tuna fish tissue (BCR627) suspension prepared by weighing 8.4 mg of BCR627 in 5 ml of water. After that, the suspension was submitted to centrifugation at 3000 rpm for 2 min. Organic As species was determined by analyzing 20  $\mu$ l of supernatant by GF AAS. Solid residue was dried at 60 °C and analyzed by SS-GF AAS to allow As inorganic species determination. Same study was performed using 5.0% v/v HNO<sub>3</sub> as solvent, however water showed the best results: Recommended value  $\pm$  SD (mg kg<sup>-1</sup>, n=5): AsB + DMA = 4.06 $\pm$ 0.23; Inorganic As = 0.74 $\pm$ 0.023 and Total As = 4.80 $\pm$ 0.023. Found value  $\pm$  SD (mg kg<sup>-1</sup>, n=5): AsB + DMA = 4.07 $\pm$ 0.50; Inorganic As = 0.66 $\pm$ 0.18 and Total As = 4.67 $\pm$ 0.16. The developed procedure become possible the discrimination of organic (AsB and DMA) and inorganic As species without use of separation techniques.



## **P101-A1**

### **Optimization of a Simple Method for the Determination of Common Booster Biocides in Marine Sediments by Means of MAE and LC-MS/MS**

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From seventies to our days organotin compounds has been commonly employed as biocides in antifouling paints added to ship hulls. Due to toxic effects that these compounds present over non-target marine species like molluscs, their use has been forbidden in EU [1]. In order to replace them, other biocides used as herbicides were introduced. They are known as booster biocides and are added to improve the efficiency of antifouling paints based in copper oxides. Booster biocides are also high toxic to non target species [2].

Commonly used methods for the extraction and preconcentration of these analytes from marine sediments often too time consuming, involve multi-step procedure and prone to loss of analytes. Modern trends in analytical chemistry are developed towards the simplification of sample preparation, reducing analysis time and improve the quality and sensitivity of the analysis.

In this work, a method combining microwave assisted extraction (MAE) followed by solid phase extraction (SPE) step for clean-up and preconcentration, is optimized before the chromatographic determination by LC-MS/MS of four common booster biocides (Diuron, TCMTB, Irgarol 1051 and Dichlofluanid) in marine sediments.

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## **P102-A1**

### **Bioaccessibility Assessment of Platinum (Pt) and Lead (Pb) in Urban Soils**

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Platinum-Group-Metals (PGM) are generally considered as efficient catalytic converters for automobile: platinum (Pt) and palladium (Pd) oxidizing CO to CO<sub>2</sub> and HC to H<sub>2</sub>O, and rhodium (Rh) reducing NO<sub>x</sub>. The release of PGM into the roadside environment caused by the use of catalytic converters represents a new type of emission source with a widely spread distribution. The city of São Paulo has a population of approximately 11 million inhabitants; the Metropolitan Region, who totalizes 38 cities, has a population of 20 million inhabitants. Considered one of the main cities of Brazil, its population and economic growth allowed that the pollution, caused mainly for vehicles and industries, reached critical levels. Due to these anthropogenic activities, the toxic metal content, as lead, is generally high. In this study, soil samples were collected from 2 parks (Ibirapuera and Independência) in São Paulo city and analyzed for contents of Pb and Pt. The samples were sieved in order to separate soil particle fractions representing deliberate (<2 mm) and involuntary (<50 µm) soil ingestion by children. However, the human digestive system cannot dissolve all of a toxicant present in the matrix, and cannot absorb it nearly 100% efficiency. *In vitro* bioaccessibility method had been applied in order to estimate oral bioavailability of Pt and Pb using a physiologically-based extraction procedure. A scanning-electron microscope equipped with an EDX unit was used in this research. All the SEM imaging was done in the backscattered-electron mode in which higher average-atomic-number compounds appear brighter than those with lower average atomic numbers. Quantification measurements were performed by SS ET AAS and ICP-MS. Total concentration determined by SS ET AAS for Pb in some samples were above alert value for São Paulo State (72 mg kg<sup>-1</sup>) for both Independência and Ibirapuera Park. Bioaccessible lead in gastric fluid was around 65% and 10% in intestinal fluid. Bioaccessible platinum in gastric fluid was not possible to quantify by ICP-MS, but it was detectable in intestinal fluid (4.8 ng g<sup>-1</sup> for Independência Park and 10.7 ng g<sup>-1</sup> for Ibirapuera Park). SEM/EDX studies confirmed Pt as contaminant in topsoil of studied parks. The Pt and Pb bioaccessibility appears to depend on chemistry, particle size, the mechanism of dissolution and geochemistry of soils.

[FAPESP, CNPq]

## **P103-A1**

### **Determination of Arsenic in Danube Water: Results of Four Regional Interlaboratory Studies**

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On the basis of international, European and regional experiences four interlaboratory studies ("WATER ANALYSIS – 2003", etc.) were organised in the period 2003-2008 for the region of South-East Europe. Over 60 laboratories from Greece, Montenegro, Republic of Srpska – Bosnia and Herzegovina, Romania and Serbia and took part in them. The International Scientific Committee with professor dr. Anastasios Voulgaropoulos as the Chairperson organised and evaluated these interlaboratory studies. The used methodology was the same as in international interlaboratory studies organised by the Institute for Reference Materials and Measurements (IRMM-European Commission Joint Research Centre) within the International Measurement Evaluation Programme (IMEP) [1]. The task for participants was to determine some or all trace elements (Al, As, Cd, Cu, Mn, Fe, Pb, Zn) and some other parameters in samples based on the water from river Danube near Belgrade. In this paper results for determination of arsenic were presented. All regional interlaboratory studies were generally successful and useful for participants. There was a good agreement between the results of most laboratories. Systematic errors were observed in some cases and these laboratories need to improve their performance. The quality of results improved gradually in the period 2003-2008.

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## P104-A1

### Prognostication of Indicator Electrode Choice in Potentiometric Titration

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It is important to make a right choice of indicator electrode in potentiometric titration. It can be predicted knowing analytical reaction and nature of ions existing in solution.

The best electrodes for complexometry, complexonometry, precipitating titration can be metal indicator electrodes which ions form the most stable compound with using reagents. These ions and electrodes give different electrochemical systems where indicator reactions proceed and their potentials are determined by conforming equilibriums.

For example, on potentiometric titration of  $\text{Ag}^+$  ( $\text{Hg}_2^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Pd}^{2+}$  and others) by reagent (R) with silver (Hg, Pd and others) indicator electrode electrochemical system of I kind  $\text{Ag}^+/\text{Ag}$  appears before the beginning of titrations and before the equivalent point (eq. p.). During titration process the most stable compounds of electrode material ions ( $\text{AgR}$ ) are forming. Reactions proceed on the electrodes  $\text{Ag}^+(\text{Hg}_2^{2+}, \text{Hg}^{2+}, \text{Pd}^{2+}) + e \Leftrightarrow \text{Ag} \downarrow (\text{Hg}, \text{Pd})$ . Electrode potentials are calculated by equilibriums  $E_{\text{Ag}^+/\text{Ag}} = E^\circ_{\text{Ag}^+/\text{Ag}} + \lg a_{\text{Ag}^+}$  and others. After eq. p. excess of titrants exits and

electrochemical systems of II kind electrode appear  $\text{R}^-, \text{AgR}/\text{Ag}$ ;  $\text{R}^-, \text{HgR}/\text{Hg}$ ;  $\text{R}^-, \text{PdR}_2/\text{Pd}$ . Following electrochemical reactions proceed  $\text{AgR} + e \Leftrightarrow \text{Ag} \downarrow + \text{R}^-$  and others. Potentials can be calculated by equilibriums  $E_{\text{R}^-, \text{AgR}/\text{Ag}} = E^\circ_{\text{Ag}^+/\text{Ag}} + 0,059 \lg \text{IP}_{\text{AgR}}(\text{SP}_{\text{AgR}}) - 0,059 \lg a_{\text{R}^-}$ . Under the change of electrode

systems the biggest potential jumps result. On the titration of strange ions relatively material of the electrode Ag indicator electrode acts as I kind electrode before the beginning of titration  $\text{Ag}^+/\text{Ag}$ ; during the titration to eq. p. as III kind electrode  $\text{Me}^{n+}, \text{MeR}_n, \text{AgR}/\text{Ag}$ , with electrode reaction  $\text{Me}^{n+} + n e + n \text{Ag} \Leftrightarrow \text{MeA}_n + n \text{Ag}$  and equilibrium of potential  $E_{\text{Me}^{n+}, \text{MeR}_n, \text{AgR}/\text{Ag}} = E^\circ_{\text{Ag}^+/\text{Ag}} + 0,059 \lg \text{IP}_{\text{AgR}} -$

$\frac{0,059}{n} \lg \text{IP}_{\text{MeR}_n} + \frac{0,059}{n} \lg a_{\text{Me}^{n+}}$ . After eq. p. system of II kind electrode appears.

For complexonometric titration following system of electrodes are used I kind  $\text{Hg}_2^{2+} (\text{Hg}^{2+})/\text{Hg}$ , II kind  $\text{Y}^{4-}, \text{HgY}^{2-}/\text{Hg}$ , III kind  $\text{Me}^{n+}, \text{MeY}^{n-4}, \text{HgR}^{2-}/\text{Hg}$ . For red-ox reactions platinum electrode are used if that titrant is a reducer. It is possible to use ionic-selective electrode on conforming ion.

As evidenced by foregoing values of potential jumps are dependent on equilibrium constants of reactions of determined ions and similar the electrode material ions. Knowing them it is possible to choose the most appropriate indicator electrode giving the biggest potential jumps under titration. It can be electrodes of I-III kind, red-ox, ion-selective electrodes.

## **P105-A1**

### **About Possibilities of Rubeanic Acid as a Potentiometric Titrant**

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Among sulfur-containing organic reagents using for potentiometric titration of sulfur forming metal ions the compounds containing sulfur and nitrogen atoms are interesting. Rubeanic acid (RBA, dithiooxamide) is one of these compounds. RBA has good analytical properties, it is stable, has good solubility in ethanol, chloroform, acetone, alkalis, concentrated sulfuric acid, but don't soluble in water. Ethanol solutions of RBA don't change their concentrations during a few months. RBA exists in three forms thionic, thionic-thiolic, and thiolic. Thiolic form is reactive form. Crystallized form of the reagent has thionic form, solutions in ethanol and sulfuric acid consist thionic-thiolic form, alkali solutions of RBA has the most concentration of thiolic form.

Ethanol solutions of dithiooxamide widely use in potentiometric titration  $\text{Ag}^+$ ,  $\text{Hg}_2^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pd}^{2+}$  ions with Hg and Ag indicator electrodes. The biggest potential jumps were fixed with Hg indicator electrode. All listed above ions form with RBA stable chelates in acid medium.  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Fe}^{2+}$  ions don't react with RBA ethanol solutions in acid medium.

Possibilities of dithiooxamide can be expanded using different solvents which increase thiolic form concentration in RBA solution and using alkali solutions of interacting ions with different ligands which give less stable complexes than rubeanates, because it shifts hydrolysis to sub acid or alkali medium.

Acetone solution of RBA consists 30% more thiolic form than the ethanol solutions because of potential jumps 30% higher.  $\text{Sb}^{3+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Te(IV)}$  were titrated with small potential jumps in concentrated sulfuric acid, but for other ions jumps were too small. Sulfuric acid solutions of RBA are not very stable. In alkali solutions of RBA potential jumps are increased in 1,5...2,5 times in comparison with ethanol solutions, but they are stable during 3...5 days only.  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  ions were successfully titrated by alkali solutions of RBA.

The most effective method of potential jumps increasing is the using of ligands and creation of optimal medium of titration ( $\text{pH}=10,00...11,00$ ) which gives possibility to analyze  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Fe}^{2+}$  ions by RBA ethanol solution with sufficient value of potentiometric jumps. The highest potential jumps were fixed under titration by alkali solutions of RBA in such conditions.

## P106-A1

### Criteria of Prediction and Practical Use of Reagents in Titrimetric Analysis

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The most suitable prediction criterion in titrimetric analysis are theoretical curves of titration, equilibrium constants of reactions and proceeding degrees. The comparative analysis of these criterion have shown that the best and the most informative criterions are proceeding degrees of individual reactions (PD %) and two – component mixtures proceeding degrees (PD %).

Threshold proceeding degrees and limit proceeding degrees of individual reactions (PD<sub>th.</sub>=99,80 %, PD<sub>lim.</sub>=99,71 %) were theoretically calculated by us.

Under the threshold proceeding degrees the titration jump originating in the titration process in standard conditions have value enough and the error of determination is not more than 0,1 - 1 % . Under of limit PD the titration jump is not observed, and titration is not possible. PD'<sub>th.</sub>=99,40 %, under this two titration jumps are observed and the errors of determination are from 0,1 to 2,0 % . PD'<sub>lim.</sub>=99,20%, under this total jump is observed and it is impossible to determinate two components in tandem.

PD, PD' are calculated for sedimentation and complex forming reactions  $nMe^{m+} + mA^{n-} = Me_n A_m$ ;  $Me_1^{m+} + Me_2^{n+} + (m+n)A^{-} = Me_1 A_m + Me_2 A_n$  by equilibriums

$$PD = 100 - \frac{C_{Me^{m+}, A^{n-}} (n \cdot C_{Me^{m+}} + m \cdot C_{A^{n-}}) \cdot 100\%}{C_{Me^{m+}} \cdot C_{A^{n-}}},$$

$$PD' = 100 - IP_{Me_1 A_m} \frac{m}{n} IP_{Me_2 A_n} \frac{m}{n} C_{Me_1^{m+}}^{-1} C_{Me_2^{n+}} \frac{m}{n} V_{(Me_1^{m+} + Me_2^{n+})} \frac{m-n}{n} (V_{(Me_1^{m+} + Me_2^{n+})} + mV_{A^{-}})^{\frac{n-m}{n}} 100\% ,$$

$C_{Me^{m+}, A^{n-}}$  - equilibrium concentrations of  $Me^{m+}$  and  $A^{n-}$  mol/l;  $C_{Me^{m+}}, C_{A^{n-}}, C_{Me_1^{m+}}, C_{Me_2^{n+}}$  - starting concentrations of ions mol/l;  $IP_{Me_1 A_m}, IP_{Me_2 A_n}$  - ionic products of chelated complexes;  $V_{(Me_1^{m+} + Me_2^{n+})}, V_{A^{-}}$  - volumes ml;  $m, n$  - stoichiometric coefficients of chemical reaction.

Using PD<sub>th.</sub>, PD<sub>lim.</sub>, PD'<sub>th.</sub>, PD'<sub>lim.</sub> and equilibrium constant of reactions it is possible to predict procedures of individual compound titrations, specificity and selectivity of their titrations, multi component mixtures titration and error of determination of each compound. This method of prediction was examined by the most widely using reagents of titrimetry such as silver nitrate, EDTA, potassium diethyldithiocarbamate and others. Using this method, optimal conditions of ions determinations can be predicted if all reactions proceeding in the titrations are known. We have developed programs of prediction of individual compounds titration, their selectivity and multi component mixtures titration.

## P107-A1

### Prognostication and Practical Use of Potassium Diheptyldithiophosphate in Potentiometric Titration of $\text{Sn}^{2+}$ , $\text{Bi}^{3+}$ , $\text{Ag}^+$ Ions

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Prognosis of possibilities of use of potassium diheptyldithiophosphate as a potentiometric titrant is based on certain ionic product (IP) of the reagent complexes, proceeding degrees of individual reactions (PD%) and two – component mixtures (PD%), theoretical curves of potentiometric titration. However the most descriptive evaluation in our opinion is the evaluation by using proceeding degrees (PD%) which calculate on equilibrium constant of reactions.

Proceeding degree of chemical reaction of any stoichiometry  $n\text{Me}^{m+} + m\text{A}^{n-} = \text{Me}_n\text{A}_m$  can be calculated by equilibrium

$$\text{PD} = 100 - \frac{C_{\text{Me}^{m+}, \text{A}^{n-}} (n \cdot C_{\text{Me}^{m+}} + m \cdot C_{\text{A}^{n-}}) \cdot 100\%}{C_{\text{Me}^{m+}} \cdot C_{\text{A}^{n-}}},$$

$C_{\text{Me}^{m+}, \text{A}^{n-}}$  - equilibrium concentrations of  $\text{Me}^{m+}$  and  $\text{A}^{n-}$  mol/l;  $C_{\text{Me}^{m+}}, C_{\text{A}^{n-}}$  - starting concentrations mol/l;  $m, n$  - stoichiometrical coefficients of chemical reaction.

Using ionic product from literature data we have calculated proceeding degrees of 20 complexes with diheptyldithiophosphate. According these data it is possible to determinate 16 sulfide forming ions with PD more than threshold value under which potential jump can be registered yet and titration is possible. On IP of the reagent complexes determined by us the range of coherence of proceeding of reactions of ions ( $\text{Hg}^{2+}$ ,  $\text{Hg}_2^{2+}$ ,  $\text{Ti}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Pd}^{2+}$ ,  $\text{Se}^{4+}$ ,  $\text{Te}^{4+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Cu}^+$ ,  $\text{Sb}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{In}^{3+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Ga}^{3+}$ ) was calculated.

For reaction  $\text{Me}_I^{m+} + \text{Me}_{II}^{n+} + (m+n)\text{A}^- = \text{Me}_I\text{A}_m + \text{Me}_{II}\text{A}_n$  PD' is calculated by equilibrium

$$\text{PD}' = 100 - \text{IP}_{\text{Me}_I\text{A}_m} \text{IP}_{\text{Me}_{II}\text{A}_n}^{-\frac{m}{n}} C_{\text{Me}_I^{m+}}^{-1} C_{\text{Me}_{II}^{n+}}^{\frac{m}{n}} V_{(\text{Me}_I^{m+} + \text{Me}_{II}^{n+})}^{\frac{m-n}{n}} (V_{(\text{Me}_I^{m+} + \text{Me}_{II}^{n+})} + mV_{\text{A}^-})^{\frac{n-m}{n}} 100\%,$$

$\text{IP}_{\text{Me}_I\text{A}_m}, \text{IP}_{\text{Me}_{II}\text{A}_n}$  - ionic products of chelated complexes;  $C_{\text{Me}_I^{m+}}, C_{\text{Me}_{II}^{n+}}$  - starting concentrations

$\text{Me}_I^{m+}, \text{Me}_{II}^{n+}$  ions mol/l,  $V_{(\text{Me}_I^{m+} + \text{Me}_{II}^{n+})}, V_{\text{A}^-}$  - volumes ml;  $m, n$  - stoichiometrical coefficients of chemical reaction.

According received data the determination of 106 two-, 374 three-, 754 four-, 855 five-, 529 six-, 162 seven-, 19 eight-component mixtures is possible.

Prognosis of choosing of indicator electrodes has been made. Hg and Ag electrodes, ion-selective electrode on conforming metal ions and sulfide ion, platinum red-ox electrode can be used. Indicator electrodes made from Hg and Ag act as electrode of I kind  $\text{Ag}^+/\text{Ag}$  before the equivalence point on its ions determination, and as electrodes of III kind  $\text{Me}^{n+}, \text{MeR}_n, \text{AgR}/\text{Ag}$  on strange ions determination.

$\text{Sn}^{2+}, \text{Bi}^{3+}, \text{Ag}^+$  were potentiometrically titrated by potassium diheptyldithiophosphate, optimal interval of pH in which relative standard deviation is not more than 1% were determinate. For  $\text{Sn}^{2+}$  pH interval is 0,70 – 3,00; for  $\text{Bi}^{3+}$  pH interval is 0,30 – 2,15; for  $\text{Ag}^+$  pH interval is 1,45 – 6,00. Optimal concentrations for  $\text{Sn}^{2+}$  4,400 – 194, 000; for  $\text{Bi}^{3+}, \text{Ag}^+$  2,00 – 400,00 mkg/ml.

## **P108-A1**

### **Measuring the Redox Potential in Milk and its Implication to Sensing and Diagnostics**

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Milk is a complex biological liquid that contains many different substances ranging from metal ions to enzymes. Furthermore, milk has wide biological activity, which persists even after pasteurization. Although milk is highly controlled from the time it is collected until its distribution, there are no adequate methods for monitoring its quality, in particular after it is bottled.

It is a known fact that the quality of milk is affected by its redox state. We have developed a novel analytical approach for analyzing the milk by terms of redox or poisoning capacity. Such method, we believe, will not only serve the dairy industry but also the consumer, by providing a simple way of diagnosing the quality of milk without the need for prior knowledge.

Several approaches were made so far to diagnose milk (especially monitoring which is important to the industry); however, they do not correctly address the redox issue.

The redox or oxidation-reduction potential (Eh) which is an intrinsic indication of biological media, can be defined as the measure of the ability of a system to gain or lose electrons. The redox potential is also likely to be important in the production of flavor compounds in milk and milk products.

In this study we demonstrate a better way of measuring the redox state of milk by a potentiometric method based on the introduction of different redox mediators that shuttle charge from a wide range of materials to a solid electrode material. These mediators are used to overcome one of the fundamental problems in potentiometry, i.e., slow kinetics between potential determining substances and the electrode surface. The redox couples: hexacyanoferrate(III/II), hexachloroirridate(IV/III) and iodine/iodide have been employed in a redox titration mode. Furthermore an attempt to fabricate a selective electrode based on this method has been made.



## **P109-A1**

### **Selective Detection of Dopamine Using Glassy Carbon Electrode Modified by a Combined Electropolymerized Permselective Film of Polytyramine and Polypyrrole-1-Propionic Acid**

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A sensitive and selective electrochemical method for the determination of dopamine using a combined electropolymerized permselective film of poly-tyramine and poly-pyrrole-1-propionic acid on a glassy carbon (GC) electrode was developed. Selectivity for electrochemical detection can be realized by forming an electropolymerized film with a uniform and controllable thickness to block the passage of such interferences to the active area of the sensing electrode. Conducting polymers like polypyrrole and its derivatives have been widely utilized; however, their limited permeability hinders the diffusion of the target analyte. In contrast, nonconductive polymers have emerged as attractive candidates for generating considerable thinner membranes due to their self-limiting formation. Electropolymerized polytyramine is a strongly adhering polymer film with excellent permselectivity against anionic species. In addition, the hydrophilic poly(pyrrole-1-propionic acid) (PPA) film is able to realize rapid diffusion, fast response with high resulting response signal. Therefore, a combination of these two films could provide selectivity for the measurement of dopamine without compromising its detection sensitivity. The formation of a "layer-by-layer" film has allowed for selective detection of dopamine in the presence of 3, 4-dihydroxyphenylalanine (L-DOPA), DOPAC, ascorbic acid, uric acid, epinephrine and norepinephrine. The modified electrodes exhibited a detection limit of 100 nM with linearity ranging from  $5 \times 10^{-6}$  to  $5 \times 10^{-5}$  M.

## **P110-A1**

### **Enzyme-Modified Nanoporous Gold-Based Electrochemical Biosensors**

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On the basis of the unique physical and chemical properties of nanoporous gold (NPG), which was obtained simply by dealloying Ag from Au/Ag alloy, an attempt was made in the present study to develop NPG-based electrochemical biosensors. The NPG-modified glassy carbon electrode (NPG/GCE) exhibited high-electrocatalytic activity toward the oxidation of nicotinamide adenine dinucleotide (NADH) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which resulted in a remarkable decrease in the overpotential of NADH and H<sub>2</sub>O<sub>2</sub> electro-oxidation when compared with the gold sheet electrode. The high density of edge-plane-like defective sites and large specific surface area of NPG should be responsible for the electrocatalytic behavior. Such electrocatalytic behavior of the NPG/GCE permitted effective low-potential amperometric biosensing of ethanol or glucose via the incorporation of alcohol dehydrogenase (ADH) or glucose oxidase (GOD) within the three-dimensional matrix of NPG. The ADH- and GOD-modified NPG-based biosensors showed good analytical performance for biosensing ethanol and glucose due to the clean, reproducible and uniformly distributed microstructure of NPG. The stabilization effect of NPG on the incorporated enzymes also made the constructed biosensors very stable. After 1 month storage at 4 °C, the ADH- and GOD-based biosensors lost only 5.0% and 4.2% of the original current response. All these indicated that NPG was a promising electrode material for biosensors construction.

## **P111-A1**

### **Towards Immobilised Anion Sensors**

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A series of fluorescent “on off” anion sensors based on the 4-amino-1,8-naphthalimide moiety have been synthesised with a view to allowing immobilisation of these sensors to a surface. NMR and fluorescence studies on the synthesised molecules indicate that the sensors respond to the common anions dihydrogen phosphate, acetate and fluoride. Furthermore, the addition of fluoride resulted in a colour change (likely due to deprotonation of the 4-amino moiety). In all cases fluorescence was switched off in the presence of the anion. Immobilisation of the sensors on a surface has also been achieved via a terminal double bond that reacts with silicon *via* well established silanol chemistry. Characterisation of the surface immobilised molecules and their performance in anion sensing is currently underway.

## **P112-A1**

### **Gold Microelectrode Array for Voltammetric Analysis**

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A gold microelectrode is proposed for voltammetric determination as sensitive, low-cost, non-toxic and well-stable in time.

The microelectrode array was obtained by electrodeposition of gold from  $\text{AuCl}_4^-$  on the given composite support at 0.0 V for 60 seconds. The support consisted of polyethylene mixed with carbon in 5:1 ratio. The electrode's modified surface was characterized by Scanning Electron Microscopy (SEM) and Cyclic Voltammetry (CV). The dependence of peak current on scan rate was investigated. The comparative studies were carried out using a bare composite support and a gold microelectrode. The data obtained using  $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$  system demonstrated a higher reversibility of this behavior of gold microelectrode array than a bare composite support and proved electrocatalytic effect of gold particle modification that led to increase of signal-to-noise ratio.

The behavior of some electrolytes on the electrode was also investigated. Some organic acids and their salts are of great interest because of their photoactive ability and oxygen can be removed photochemically from the solution to be analyzed. The electrode was utilized for the straight voltammetric determination of  $\text{Cr}^{6+}$  from simulated solution. Linear calibration plot was obtained in the concentration range of  $10^{-7} - 10^{-6} \text{ mol dm}^{-3}$  and the detection limit of  $9.62 \times 10^{-8} \text{ mol dm}^{-3}$  was achieved. Also the electrode was applied in the trace determination of  $\text{Fe}^{3+}$  and  $\text{As}^{3+}$ .

## **P113-A1**

### **SPR Imaging Biosensor for Aspartyl Cathepsin Determination: Sensor Development and Application for Biological Material**

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Cathepsins D (3.4.23.5.) (CatD) and E (3.4.23.34) (CatE) are intracellular aspartic endopeptidases of the pepsin superfamily. Cathepsin D has been isolated from spleen, liver, uterus, thyroid, small intestine, lung and erythrocytes. Cathepsin E is mainly present in cells of the immune system.

CatD and CatE determination may have diagnostic value, especially in cancer diagnosis .

Surface Plasmon Resonance (SPR) spectroscopy is one of the promising methods for investigating the interaction of proteins. One of the proteins – the probe – is immobilized on a gold sensor, and the other reacts with the immobilized protein. A formed complex may be observed in the SPR imaging system. The SPR imaging system converts the SPR effect into an image. SPR imaging (SPRI) is also a useful tool for protein determination.

The aim of this work was to develop an SPRI sensor for aspartyl cathepsin determination and to apply it to such determination in biological material. The development of the sensor is based on specific interaction between aspartyl cathepsins and pepstatin. Pepstatin is an aspartyl cathepsins inhibitor.

At pH below 4.5, pepstatin forms a 1 : 1 complex with CathD and CathE ( $10^{-10} \leq K \leq 10^{-9}$  M).

In order to prepare the SPRI sensor, pepstatin was immobilized on 50 nm gold film with the use of immobilization techniques based on thiol chemistry. Cysteamine (2-mercaptoethylamine) was used as a linking thiol [1,2]. Conditions for aspartyl cathepsin determination were optimized. The concentration of aspartyl cathepsins in human plasma, leucocytes, erythrocytes and nasal polyps was determined.

## **P114-A1**

### **Electrochemical Biosensing Platforms Using Phthalocyanine-Modified Carbon Nanotube Electrodes. New strategies for Electrochemical Nucleic Acid Detection**

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In the study of phthalocyanine chemistry, an area of particular interest in recent years has been the formation and characterization of polymeric compounds in various forms and the use of these compounds to carry out well known PC applications involving catalysis, analysis, etc. A novel assay for the electrochemical detection of guanine and single stranded DNA based on carbon nanotube paste electrodes (CNTPE) modified with cobalt phthalocyanine has been investigated. The modification of a CNTPE with this compound results in excellent amplification of the guanine oxidation response. The electrochemical behavior of the modified electrode and the mechanism of the oxidation of guanine and ss-DNA were investigated using cyclic voltammetry and differential pulse voltammetry. The electrochemical studies indicate that the charge transport is diffusion controlled. Detection limits of 100 and 300 ng mL<sup>-1</sup> were obtained for guanine and ss-DNA, respectively, by using the electrocatalytic oxidation signal corresponding to the Co(II)/Co(III) redox process. Cobalt-phthalocyanine-modified carbon nanotube electrodes are shown to be excellent indicators for electrocatalytic amperometric measurement of guanine and single stranded DNA in aqueous solution. The morphologic characterization of the modified electrode surface by scanning electron microscopy is also illustrated. Applications on real samples are presented. The advantages of convenient fabrication, low-cost detection, short analysis time and combination with nanotechnology for increasing the sensitivity make the modified electrodes worthy of special emphasis in the non-labeled detection of DNA hybridization reaction and in development of DNA based biosensors for toxic chemicals, toxins and pathogens determination.

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## **P115-A1**

### **Self-Assembling Peroxidase-Chitosan Complex in the Analysis of Water Insoluble Samples**

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Horseradish peroxidase is one of the widely used enzymes in analytical biochemistry. However efficient use of peroxidase in clinical, nutritional and environmental analysis still has to overcome a number of limitations, particularly its low operation stability in organic media which is necessary to determine poorly water-soluble analytes in water-insoluble samples.

To overcome the above mentioned limitations we have proposed the approach based on the inclusion of peroxidase in the nanostructured self-assembling complex with natural polyelectrolyte - chitosan. The novel biocatalyst is characterized by narrow distribution in average hydrodynamic radius of particles (25-30 nm) and is twice more active than the native enzyme in the presence of 30% dimethylsulfoxide (DMSO), and functions as a native enzyme in the presence of 60% DMSO. The reasons of unique high activity of peroxidase-chitosan complex in water-organic medium were studied by the methods of enzymatic kinetics, FTIR and zeta-potential measuring.

The self-assembling enzyme-polyelectrolyte complex was used as a basis for design of optical sensors for the determination of biologically active compounds (phenols and phenothiazines) – substrates of peroxidase. The technology of the formation of the transparent films from the water-organic media on the surface of optical glasses and in the wells of polystyrene plates was developed. It was shown that the biorecognising films generated from water-DMSO medium have a higher transparency than the films generated from water solutions. The morphology of the films on the basis of peroxidase-chitosan complex was studied by AFM.

The optical biosensors for the determination of biologically active compounds are sensitive and selective due to the conjugated reactions with chitosan matrix and an effect of “substrate-substrate” activation, respectively.

The sensors based on peroxidase-chitosan complex were used for the determination of phenols and phenothiazines in water-insoluble samples – ointments and organic extract (heptane-DMSO) from human blood serum. (*RFBR projects N 07-03-00556 and 09-03-00823*).

## **P116-A1**

### **Colorimetric Responses of Transparent Polymers Doped with Metal Phthalocyanine for Detecting Vaporous Amines**

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Low molecular weight organoamines ( $C_2$ - $C_6$ ) are hazardous volatile organic compounds and detection of low molecular weight organoamines has gained a lot of attention. This study reports colorimetric responses of several metal phthalocyanines (MPcs), and MPc-doped polymers to primary amines. Among all MPcs tested, only iron phthalocyanine (FePc) shows promise as a good candidate for detecting primary amines. In toluene, FePc shows a distinct color change from colorless to deep green upon addition of hexylamine. This colorimetric response is also highly specific. FePc does not respond to secondary amines, tertiary amines or other interferents tested in the study. We also doped several transparent polymers, poly(vinylalcohol) (PVA), Norland Optical Adhesive 65 (NOA65), and poly(dimethylsiloxane) with FePc and studied their colorimetric responses to hexylamine. Although they all exhibit color changes from transparent to green upon exposure to hexylamine, we find that only PDMS doped with FePc shows a selective and reversible response to hexylamine. Thus, FePc-doped PDMS may be coated onto protective suits or safety goggles to offer real-time information about amine exposure.

**Keywords:** *colorimetric sensor, amine vapor, iron phthalocyanine, polymer.*



## P117-A1

### A Deuterium-Palladium Electrode as a New Sensor in Non-Aqueous Solution: Potentiometric Titration of Weak Acids in N,N-Dimethylformamide and N-Methylpyrrolidone

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N,N-dimethylformamide and N-methylpyrrolidone are extensively used as a medium in potentiometric determinations of many acidic substances with a glass electrode as the indicator electrode. However, this electrode shows certain undesirable feature: the potential response of a glass electrode in non-aqueous is often very slow. In addition, the electrode has a limited useful life when employed in non-aqueous titrations because the solvents dehydrate the glass membrane, thereby reducing its affinity for, or response to, hydrogen ions. In the basic range in non-aqueous solution, a glass electrode also exhibits an alkaline error.

These disadvantages of glass electrodes have led to intensive research for alternative pH electrodes in non-aqueous solution. In this study, the possibility of applying D<sub>2</sub>/Pd as a sensor (sensor carrier is palladium, the metal that is chemically inert in organic solvents) for the potentiometric determination of weak organic acids in N,N-dimethylformamide and N-methylpyrrolidone as solvents. The indicator D<sub>2</sub>/Pd electrode was made of conventional palladium wire (2 cm long, diameter being 0.5 or 1.0 mm). The wire was spiral-folded and sealed into a glass tube by means of platinum and connected with the pH meter using a contact made of copper wire. The platinum used for the connection with the Pd wire was never exposed to the solution; hence, it did not have an effect upon the potentials measured. The prepared Pd electrode was heated in oxidation flame, and after that, the wire was saturated with gaseous deuterium, obtained by electrolyzing deuterium oxide at 1 mA electric current.

The investigated electrode showed a linear dynamic response for p-toluenesulfonic acid in the concentration range from 0.1 to 0.001 mol L<sup>-1</sup>, with a Nernstian slope of 78.0 mV in N,N-dimethylformamide and of 64 mV per decade in N-methylpyrrolidone. The behavior of D<sub>2</sub>/Pd electrode as an indicator electrode was checked by titration of a series of organic acids of different strengths and various structure.

The potential in course of the titration and at the equivalence point (TEP) was found to be rapidly established. The response time was less than 10 - 11 s, and the lifetime of the electrode is long. Sodium methylate, potassium hydroxide and tetrabutylammonium hydroxide proved to be a very suitable titrating agent for these titrations. The experimental results obtained for the proposed electrochemical sensor and a conventional glass electrode were in good agreement. The advantages of the electrode are long-term stability, fast response, reproducibility, and easy preparation.

## P118-A1

### Hybrid Signal Processing in Voltammetric Determinations on Non-Modified Solid Electrodes

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Electrochemical stripping techniques still attract considerable attention for trace metal analysis and for measuring several important organic compounds, due to their unique capabilities of pre-concentrating the analytes at the electrode surface and associated favorable low limits of detection. The proposed approach is based on the assumption that the adequate signal processing which considers the fluctuations of the individual current of the electrode may eliminate the influence of this effect on the final result of determination.

The presented hybrid algorithm consists of realization of three numerical steps. First step, experimental curve is transformed and presented in relation to the signal obtained for the supporting electrolyte [1]. Signals ratio of voltammetric signals is based on the fact that the ratio of the global signal to the electrolyte signal reaches maximum in the region where the contribution of the analyte is the highest. Second step, the orthogonal signal correction (OSC) [2] is applied. It is a preprocessing technique used to remove systematic variation from the response matrix that is unrelated, or orthogonal, to the property matrix. OSC was introduced in order to avoid the removal of information that is important for prediction. Third step, adaptive-degree polynomial filter [3] is used. The adaptive-degree polynomial filter (ADPF) for data smoothing is further improvement of the least-squares regression formalism introduced to the experimental data processing by Savitzky and Golay. The adaptive approach is based on the statistical testing of the fitting quality of polynomial function to the experimental data in the smoothing window sliding along the curve. It allows the automatic choice of the proper degree of the polynomial function in the various parts of the experimental curve.

Application of the proposed algorithm causes the improvement of the calibration model and as a result quality of the determination is higher. The usefulness of the method was tested by means of voltammetric determination of metal ions in natural waters on metallic and carbide electrodes.

Calculations were performed using Matlab for MS Windows, version 7.3.

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## **P119-A1**

### **Biosensor Technology for the Screening of Cancer Risk Biomarkers Related to Diet**

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Colorectal cancer is one of the most common cancers found in the developing countries. Recently, it has been indicated that a diet with a high intake of red and processed meat increases the risk of colorectal cancer. This is mainly because high red meat consumption is known to enhance the endogenous formation of *N*-nitroso compounds, which are potent carcinogens. Studies have detected the presence of O<sup>6</sup>-carboxymethyldeoxyguanosine (O<sup>6</sup>CMdG), a DNA adduct due to the nitrosation of glycine, in human exfoliated colonocytes and human blood. O<sup>6</sup>CMdG in DNA, is resistant to repair proteins and may be a potential urinary biomarker of colorectal cancer risk. The aim of our present research is to develop analytical methodologies for the measurement of this adduct in urine and correlate it to dietary studies. Samples are from previous studies of human volunteers carried out at MRC Dunn Human Nutrition Unit at Cambridge in which high red meat diets were consumed over a period of 15 days and various samples collected including 24 h urine samples.

The ideal method for the routine screening in the general population of cancer risk biomarkers should be simple, fast, easy to perform and cost-effective. Biosensor technology fulfil this need, in particular thick film technology using screen-printing procedure allows inexpensive mass-production of disposable electrochemical sensors for point-of-care analyses. An immunosensor based on an indirect competitive assay is being developed and validated for the detection of O<sup>6</sup>CMdG in synthetic urine. The detection of this DNA adduct is based on competition for binding to a polyclonal antibody with an ovalbumin conjugate, followed by incubation with a secondary antibody labelled with horseradish peroxidase.

## P120-A1

### Detection of Hg(II) Using Aminoacidic Units Anchored on Polyaryleneethynylenes Platforms

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Polyaryleneethynylenes (PAE)s, are oligomeric and polymeric materials possessing a conjugated backbone bearing a regular sequence of aromatic and acetylenic units. These species present very interesting optical properties and an easy processability that are tunable with the proper choice of the aromatic moiety (phenyl, thienyl, pyridyl, anthryl, etc.) inserted in the conjugated path and/or their decorating side arms. In this work we took the advantage of combining an original synthetic method and the ability of aminoacids to interact selectively with metal ions to produce fluorescent material able to selectively detect Hg(II) ions. In particular, oligomeric and polymeric PAEs bearing a different number of leucine or glycine units, anchored as side arms on the aromatic units, were synthesised and their fluorescence spectra recorded. All these species are displaying drastic changes both in the absorption and the emission spectra upon interaction with Hg(II). Different complexation stoichiometries were found with log K ranging around 10 in some cases indicating a high affinity towards Hg(II). A flow-injection set-up was used to run dose/response curves for Hg(II) and  $I_{50}$  values (amount of Hg(II) able to quench 50% of fluorescence) were found to be among 0,15 and 1 equivalent with lower detection limits for the polymeric compounds. The lowest calculated LOD was 50 ppb using incubation time of 1 min at room temperature. Remarkable selectivity was observed in all cases, with no quenching effect in the experimental conditions for  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $Cu^{2+}$ ,  $Mg^{2+}$ ,  $Ni^{2+}$ ,  $Mn^{2+}$ ,  $Co^{2+}$ ,  $Zn^{2+}$  at ratios  $M^{2+}/Hg^{2+}$  up to 1000:1. Either the oligomeric and polymeric materials prepared appear very promising toward their use as sensing material for the development of optical sensors for the rapid assay of Hg(II).

**Acknowledgment** This work has been supported by MIUR through PRIN 2007 project.

## P121-A1

### Rapid Screening of poly(ethylene glycol) Polymers by High-Performance Liquid Chromatography with Piezoelectric Detection

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Polyethylene glycols (PEGs) are synthetic polymers obtaining from the condensation of ethylene oxide and water. Polyethylene glycol polymer is non-toxic, odourless, neutral, lubricating, non-volatile and non-irritating and is used in a variety of pharmaceuticals and in medications as a solvent, dispensing agent, ointment and suppository bases and tablet excipient. There is a clear necessity to determinate and characterize this type of polymers in order to identify them as additive in commercial products, as residue in industrial processes, and in waste waters [1-3]. The techniques typically used to characterize these synthetic polymers encompass a broad spectrum of physical (colorimetry, turbidimetry), spectroscopic (Fourier transform IR) and separation methods (gas chromatography, size exclusion chromatography, gel permeation chromatography, high-performance liquid chromatography and capillary electrophoresis).

In this work, a simple flow injection (FI) device connected with a piezoelectric detector was used for the determination of PEGs. The detector is based on resonance frequency shifts when different concentration of these polymers was injected into the system. First, in order to demonstrate the determination capability of this detector, individual analytical studies such as linearity, precision, detection and quantization limits were carried out for PEGs with different molecular weights from 100 to 6000. Secondly, the piezoelectric detector was coupled with a liquid chromatography system with a C18 column (Kromasil, 5  $\mu$ m, 150x46mm) to retain PEGs in function of their molecular weight. It was possible to carry out a PEG polymeric screening depending of the composition of the mobile phase (methanol:water). Finally, the proposed method was applied to evaluate the presence of these polymers in pharmaceutical products and waste waters.

**Acknowledgements:** Financial support from the Spanish Ministry of Innovation, Science and Technology (Project CTQ2007-61830) and the Junta de Comunidades of Castilla-La Mancha (PAC08-0015-0722) are gratefully acknowledged.

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## **P122-A1**

### **Chlortetracycline-Imprinted Materials for its Potentiometric Determination in Food and Biological Samples**

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As a result of the stressful conditions in aquaculture facilities there is a high risk of bacterial infections among cultured fish. Chlortetracycline (CTC) is one of the antimicrobials used to solve this problem. It is a broad spectrum antibacterial active against a wide range of Gram-positive and Gram-negative bacteria [1]. Numerous analytical methods for screening, identifying, and quantifying antibacterial residues such as CTC in edible animal products have been developed over the years. An alternative and advantageous method should rely on expeditious and efficient procedures providing highly specific and sensitive measurements in food samples. Ion-selective electrodes (ISEs) could meet these criteria. The only ISE reported in literature for this purpose used traditional electro-active materials [2]. A selectivity enhancement could however be achieved after improving the analyte recognition by means of molecularly imprinted polymers (MIPs).

Several MIP particles were synthesized and used as electro-active materials. ISEs based in methacrylic acid monomers showed the best analytical performance in terms of slope (62-68 mV decade<sup>-1</sup>) and detection limit (4.0-5.5x10<sup>-5</sup> and mol/L). The electrodes displayed good selectivity. The ISEs are not affected by pH changes ranging from 2.5 to 13. The sensors were successfully applied in the analysis of serum, urine and fish samples.

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## **P123-A1**

### **Man-Tailored Biomimetic Sensor of Molecularly-Imprinted Materials for the Potentiometric Measurement of Oxytetracycline**

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Oxytetracycline (OXY) belongs to the group of the tetracycline antibiotics. This drug is used in various human and veterinarian applications, being the preferred tetracycline drug in aquaculture. In particular, the wide use of tetracyclines in meat and fish food production species led to environmental and food spread of antimicrobials, and may result in the emergence of antibiotic-resistant bacteria [1-2]. Therefore, reliable analytical methods are required for monitoring OXY in aquatic environments as well as in biological samples and in commercial drugs. To detect it, new biomimetic sensors based on molecularly-imprinted polymers (MIPs) are proposed. These sensors exhibited a near-Nernstian response in steady state evaluations; slopes and detection limits ranged 42 – 63 mV/decade and 2.5 – 31.3 µg/mL, respectively. In flowing media, the biomimetic sensors presented good reproducibility (RSD of ± 0.7%), fast response, good sensitivity (65 mV/decade), wide linear range ( $5.0 \times 10^{-5}$  –  $1.0 \times 10^{-2}$  mol/L), low detection limit (19.8 µg/mL), and a stable baseline for a  $5 \times 10^{-3}$  M citrate buffer (pH 2.5) carrier. The sensors were successfully applied the analysis of drugs and urine. This work confirms the possibility of using MIPs as ionophores for organic ion recognition in potentiometric transduction.

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## **P124-A1**

### **Periodically Interrupted Amperometry. A Way of Improving Detection Limit of Film Coated Electrodes**

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Amperometric enzyme electrodes are made of base sensing element and enzyme containing stationary reaction layer coating its surface. In conventional amperometry the electrode potential is kept constant, and the current is detected. Recently a new measuring method has been worked out in our laboratory, the so called periodically interrupted amperometry (PIA). It was proved very useful in lowering the detection limit of membrane coated electrodes, like amperometric biosensors.

In conventional amperometry the continuous electrode reaction decreases the local concentration of electroactive species. In this way the stationary layer at the surface of the sensing element gets more or less depleted.

Periodic interruption of the electrolysis, allows time for reloading the layer at the close vicinity of the sensing element. This could result in higher current and lower detection limit.

In our presentation the basic principle of the PIA method will be discussed. In the experimental work to be presented amperometric enzyme electrodes were prepared using platinum disk base sensing element. PIA measuring program was applied and optimized, selecting the optimal measuring ( $t_M$ ) and resting ( $t_R$ ) time periods and the data collecting parameters.

Conventional amperometric and PIA methods were compared. In blood samples putrescine is in lower  $\mu\text{M}$  range. Amperometric putrescine enzyme sensor hardly can measure concentrations in that range with conventional technique. Therefore lowering the detection limit is important if application in clinical diagnosis is considered. Using the PIA mode acceptable signal / noise ratio could be obtained. The PIA detection extends the measurement range of membrane coated amperometric sensors.

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## **P125-A1**

### **The Role of Suppression in Ion Chromatography: a Comparative Study with Education Purposes**

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Ion chromatography is an ideal analytical separation technique for inorganic anions and cations [1-2]. Analytes can be separated by means of its different retention times in an anion or cation-exchange column respectively using isocratic elution and by injecting a very small sample volume. In most of cases, detection implies the measurement of conductivity of analytes in a thermostated conductivity cell at the end of the column. Actually, typical ionic chromatographic analysis yield separations and quantifications at ppbs levels in both cases.

Ion chromatography without eluent conductivity suppression has been employed successfully [3]. Nevertheless, for better detection of charged species the eluent conductivity has to be eliminated with a suppressor system. First time of this item was in 1975 [4]. This way, conductivity of analytes is enhanced and simultaneously eluent conductivity is minimized (typical values of eluent conductivity about 1  $\mu\text{s/cm}$ ). Nowadays several companies have developed systems of suppression.

To the best of our knowledge, no papers dealing with different educational aspects of ion chromatography with and without suppression have been reported.

In this work we proposed that student do a comparative study of different ways of suppression proposed by different marks, which are patented in some cases, with those without suppression. The characteristics and advantages and disadvantages of such ways of suppression were evaluated by undergraduate students for meaningful and interactive learning.

The evolution in time of suppressors is too studied for improving students' attitudes toward, and their conceptual understanding of, ionic chromatography.

The proposed study is suitable as teamwork exercise for enhancing some analytical chemistry concepts and is also useful to instructors by providing information and feedback on the degree of assimilation of the taught material by the students.

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## **P126-A1**

### **A Simple Process Analysis to Verify the Complete Neutralization of Bovine Serum and Viral Antigens Inactivated with BEI**

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Although the iodometric analysis is supposed to be a good way of verify the neutralization of BEI (binary ethylenimine) in viral antigens, it is not the case for bovine serum. Actually, not even a single article has addressed this issue for all this time, to suggest an analysis able to verify the BEI neutralization in bovine serum. The bovine serum is a component in the production of synthetic cell culture media. An effective BEI neutralization is primordial. Free BEI is cytotoxic to cell culture. In addition, free BEI could reduce the antigen's immunogenicity in viral antigens, and it can be carcinogenic for animals or human been.

The BEI is a cyclic amine that was obtained throughout the reaction of BEA (2-bromoethylamine hydrobromide) with NaOH, which cyclizes to the aziridine. Traditionally, sodium thiosulphate is used to neutralize the residual BEI.

Solubility of AgX salts and photo sensibility are some characteristics of the Ag<sup>+</sup> ion, which was taken to develop this quick method, to prove the existence or not of S<sub>2</sub>O<sub>3</sub><sup>-2</sup> anions in the samples of bovine serum and viral antigens. The presence of this anion indicates that all BEI is neutralized.

The propose technique is simple, and it follows three main steps: collect a sample (serum or antigen) in a tube; add on it AgNO<sub>3</sub> solution; finally, add a saturate solution of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.

In samples not neutralized, a white precipitate, mainly of AgBr and AgCl is obtained, after AgNO<sub>3</sub> addition. The reaction is reversible when S<sub>2</sub>O<sub>3</sub><sup>-2</sup> anion is present in high concentrations. Then after to add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, all precipitates are soluble.

However, in samples completely neutralized, the Ag<sup>+</sup> ion reacts with free S<sub>2</sub>O<sub>3</sub><sup>-2</sup> anions (derivate from neutralization), forming Ag<sub>2</sub>S (dark precipitation) in an irreversible reaction. Thus, the precipitate remains after Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> addition, even if it is in high concentrations.

We have seen that for serum, the propose analysis has better results than those obtained with iodometric titration (standard method). This method has visual complications which makes it impossible to check the final point of the analysis.

For viral antigens, the results reached were as good as obtained with iodometric titration. However, the propose method has an advantage: the results were obtained faster, with a very simple technique.

## **P127-A1**

### **"History and Methodology of Analytical Chemistry" as the Educational Course in Russian Universities**

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Analysts in Russia are trained mainly in universities. The professional training includes some special subjects. However, the analytical chemistry (AC) is rarely dealt with as a whole; undergraduates don't study the history of this science, its general aspects and methodology. Undoubtedly, teaching of corresponding disciplines by graduates is to improve their professional level, competence and style of thinking. The principles of selecting the content of this educational course are substantiated in the report. These principles were realized in recently published textbook (Zolotov Ju.A., Vershinin V.I. History and Methodology of Analytical Chemistry [in Russian]. 2007. Moscow: Academia - 464 p.). This book has no direct analogues because well-known monographs (Szabadvary etc) are not intended for students. New educational course construction demands systematical approach to the methodology of this science. AC is considered by us in three different modes: a) as a certain direction of investigations and practical activity, b) as a system of knowledge about analytical methods and about analysis in toto; c) as a social phenomenon. The object and the subject of AC, its main goals and special problems, interdependence of analytical science and analytical service and some other themes should be included in the curriculum. It is quite necessary to expose the theoretical basis (common basis for all methods), which has been discussed by leading analysts for a long time. It is inadmissible to reduce the history of AC to any list of untied discoveries or to the summary of separate histories for some methods. Appropriate processes should be connected with the general history of science and technology. We must demonstrate the role of chemical analysis in everyday life (diagnostics etc) in particular, as usual AC textbooks neglect this moment.

The main theoretical problem is a lack of a generally accepted system of time-stages for the history of analytical chemistry and for chemical analysis itself. Convenient systems will be presented in the report. For teaching, both systems may be combined. Such combined system includes four stages: a) period of fire assaying; b) creation of chemical methods; c) period of theoretical justification of chemical methods and advent of basic physical methods; d) unification of all analytical methods as a result of metrology and chemometrics development.

Omsk State University has many years' experience of teaching such educational course. Graduates in chemistry study this subject during last year, after all other special subjects. The course amounts to 2 credits; students attend lectures (20 hours) and seminars (12 hours), prepare individual essays and discuss them at the conference, the student himself selects the subject of essay.

By this time new textbook publishing has resulted in introduction of similar educational courses in certain universities of Russia, especially for the masters in chemistry. We hope that our experience would be interesting for other European countries.

## **P128-A1**

### **Reliable Quantification of GMOs – How to Get Correct PCR Results**

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The quantification of the content of GMOs in food and feed is required for the control of labelling thresholds in the frame of the implementation of different regulations all over the world. At present the analytical method of choice is real-time PCR, despite many still existing fundamental obstacles. One difficulty concerns the calibration of such measurements, aggravated by the limited availability of corresponding calibrants. Another challenge consists in the control of the complete analytical process starting from an analytical test portion of the food or feed up to the data evaluation. During recent years IRMM has developed new systems of reference materials which allow both the calibration as well as the quality assurance of GMO analysis. This presentation will explain the analytical requirements on such reference materials and their accompanying information as well as their proper application for GMO quantification. Moreover, the scientifically sound estimation of measurement uncertainties for PCR measurements and resulting consequences for the regulatory decision making will be discussed.

## **P129-A1**

### **Biosurfactant Production Monitoring Using the Indirect Tensammetric Technique**

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Numerous bacterial strains produce biosurfactants. This is observed in the case of *Pseudomonas fluorescence*, which is a gramme-negative bacteria strain. In this work, the strain was isolated from a solution biodegrading oxyethylated alcohol. The strain, in a medium containing beef extract, peptone and urea, as well as mineral nutritional components, produces biosurfactant.

The aim of this work was to develop tools for the monitoring of biosurfactant concentration.

Biosurfactant produced by *P. fluorescence* behaves like a typical non-ionic surfactant, and creates an analytical signal in the indirect tensammetric technique (ITT). The calibration curve of the biosurfactant is a typical  $\Gamma$ -type curve following the Langmuir isotherm. Due to the lack of a standard, the results are evaluated using Triton X-100 as the standard.

The biosurfactant is extracted using ethyl acetate. In this way it can be isolated from the water matrix.

It is also adsorbed in the PTFE capillary trap and can be isolated from the water matrix this manner.

Elution is performed with methanol or ethyl acetate. In contrast to non-ionic surfactants, the biosurfactant does not react with the modified Dragendorff reagent.

The developed method was applied to biosurfactant production by *P. fluorescence* under OECD 301 E test conditions.

## **P131-A1**

### **An Investigation into the Chemical Composition of Irish Wood Fuel in Regards its Use as a Renewable and Sustainable Energy Source**

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Due to growing concerns for the environment such as green house gas emissions, air and water pollution and diminishing amounts of available fossil fuels, there is a constant demand for renewable and sustainable energy sources. Wood is an important renewable energy source for Ireland, as it has a significant energy potential in Ireland's unexploited forestry resource. Wood energy is sustainable, renewable and carbon neutral, it can be produced locally to generate heat for domestic, commercial and industrial markets. Unfortunately regardless of the technology used, any combustion will lead to air emissions and some level of wood ash. Before we consume this natural resource it is essential for further research into the use of wood biomass, by looking at its chemical components to see how it can affect the environment.

The chemical properties of wood are dependent on many factors. Evidence suggests that the geographic source of the wood, as well as species, play an important role in determining the chemical make-up. This research investigated the chemical composition of Sitka spruce. Sitka spruce is a predominant wood species in Irish forestry, as it grows productively under a wide range of conditions. Several different harvesting assortments: whole tree (WT), round wood (RW), energy wood (EW) and firewood (FW) of freshly felled and seasoned Sitka spruce from various geographical locations across Ireland, were studied to investigate the effect of geography and harvesting times. This information should contribute in the determination of the chemical parameters that will lead to the production of an efficient, eco-friendly and unproblematic combustion wood.

ICP spectroscopy and bomb calorimetry were utilised to determine the heavy metal content and the calorific values of the wood samples, respectively. Other properties such as the chlorine, nitrogen carbon and hydrogen concentration and ash content have also been investigated.

## **P132-A1**

### **Problems Associated with Uncertainty Estimation in Analytical Procedures Using Chromatographic Techniques**

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Chromatographic techniques are very frequently used in analytical procedures for the separation, determination and identification of a wide spectrum of analytes present in samples with complex and sometimes variable matrices. However, the estimation of uncertainty of the final results does not include the uncertainties associated with the actual chromatographic process. In effect, such results cannot always be treated as a reliable sources of analytical information.

Uncertainty is a basic characteristic of any measurement; uncertainty is always present, at every step of a procedure. Estimation of uncertainty leads to better measurement reliability, renders data from interlaboratory studies comparable, and helps to assess the statistical significance of the difference between the measurement and a relevant reference value.

Uncertainty of measurement is a component of uncertainty in all the individual steps of an analytical procedure. Hence it is necessary to determine the sources and types of uncertainty for all these steps.

In a typical chromatographic analysis, the main elements of uncertainty are associated with:

- the amount of sample used for a determination,
- the recovery value of the analytical procedure, including the recovery of an analyte from a sample and the recovery associated with the accuracy of final determinations,
- the repeatability of determinations for a true sample (represented by the repeatability of signals),
- the concentration associated with the upper detection limit,
- calibration of the analytical instruments.

There is an urgent need to convince all chemists, including practising analysts using different chromatographic techniques in their work, that estimating the uncertainty of measurements will enhance the reliability of analytical information.

## P133-A1

### Improving the Visualization of the Pareto-Optimal Front in the Optimization of Multiresponse Analytical Procedures

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It is usual in Analytical Chemistry that experimental procedures depend on several experimental factors and also that these experimental factors affect several analytical responses of interest. Further, in general, the experimental factor(s) affect the analytical responses in different ways. Therefore, the usual situation when looking for experimental conditions to optimize several analytical responses is that there is a conflict among responses and a compromising solution is necessary.

One possible alternative is to look for the experimental conditions that provide optimal (maximum or minimum) response values in at least one of the responses being considered. The set of such solutions is known as the Pareto-optimal front, which accounts for the extent of the trade-off among responses. The Pareto-optimal solutions have shown to be useful in analytical procedures<sup>1</sup>. However, for a high number of experimental factors and/or responses, the exploration and analysis of the Pareto-optimal front is difficult.

In this work, we present an adaptation of the parallel coordinates plot<sup>2</sup> to improve the visualization and exploration of the Pareto-optimal solutions. It is applied in three analytical problems of detection of residues in foodstuffs of animal origin regulated (forbidden) by the European Union.

Concretely, we look for experimental conditions that allow:

1. The chromatographic separation<sup>3</sup>, by gas chromatography–mass spectrometry, GC/MS of the diastereoisomers,  $\alpha$  and  $\beta$ -estradiol, hormones that coelute and have the same mass fragments. We need to decide the temperature programme of the column to improve the resolution between the peaks of both isomers but maintaining their widths narrow enough.
2. The simultaneous determination<sup>4</sup> of steroid hormones estrone and 17- $\alpha$ -ethinylestradiol by GC/MS. In this case, the quantity of the silylation agent, time, and temperature of the reaction have to be chosen so that the base peak area of each derivatized analyte is maximized.
3. The determination of malachite green and leucomalachite green in fish by liquid chromatography tandem mass spectrometry (LC-MS/MS). In the preparation of the spiked samples of fish, the volume of hydroxylamine, the time the analytes are incubated with the matrix in darkness, and the shaking time are to be decided to simultaneously maximize the areas of the two peaks.

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## **P134-A1**

### **Bringing the Chemical Knowledge into Empirical Chemometric Models - Applications on Food Systems**

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It is often desired to measure a number of samples from a set of experiments with several instrumental methods. One of the objectives in such situations is to find out what is the chemistry in the system or process that is being observed that is described commonly by all instruments, and what are the contributions from individual instruments. At the same time, one wants to apply the chemical background knowledge to confirm the interpretation from the models. Cross model validation is a useful method to find the significant variables between two or more sets of instrumental measurements and visualize the common information. By dividing the information into what is common and what is unique, a multi-block model can be established to give detailed information about which instruments that describe the various chemical properties. The chemical background knowledge can be presented as qualitative information in models of the so-called L- or U-shape. This enables a visualisation of the underlying chemistry to confirm the findings in the empirical models. The methods were applied on Raman, FT-IR and NIR spectra of foods, and the results show that that empirical data analysis and chemical knowledge can be combined in a holistic framework

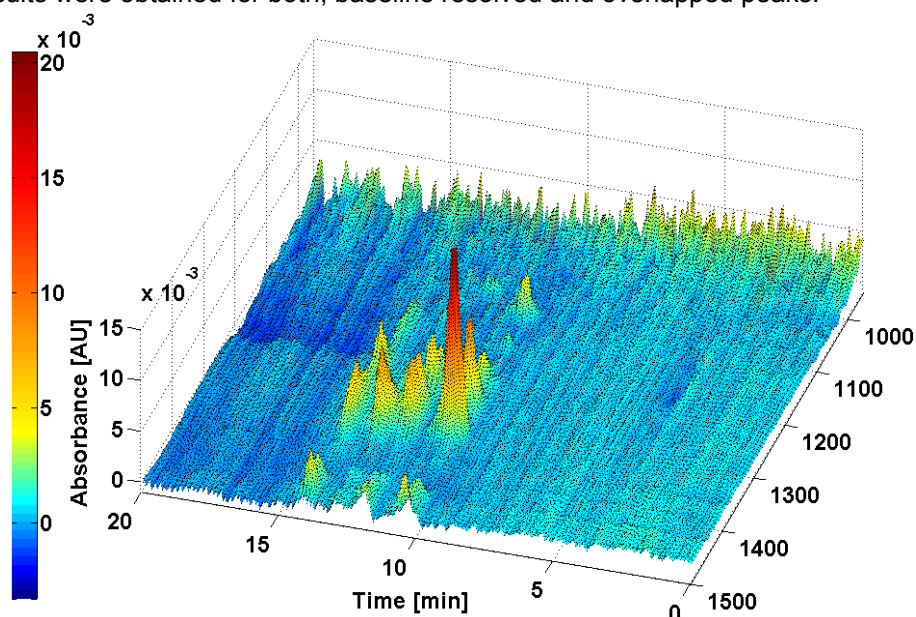
## P135-A1

### Chemometric Background Correction in On-Line Liquid Chromatography - Fourier Transform Infrared Spectrometry (LC-FTIR) Applying a Point-to-Point Matching Algorithm

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A new chemometric background correction method for the on-line coupling of gradient liquid chromatography and Fourier transform infrared spectrometry has been developed. The method is based on the use of a point to point matching algorithm. In order to select an appropriate reference spectrum for the compensation of the spectral contribution of the changing mobile phase composition, each absorption spectrum of the sample data set is compared with those of a reference data set. This reference data set consists of a (previously) recorded blank gradient run. The parameter used for this comparison is the spectral similarity, calculated as the spectral correlation coefficient. An adequate spectral range for the comparison is determined with minimal user-interaction which facilitates considerably the application of the whole method. The background correction method has been successfully tested on a chromatographic separation of four nitrophenols running acetonitrile (0.08% v/v TFA):water (0.08% v/v TFA) gradients with compositions ranging from 35 to 85% v/v acetonitrile. Accurate results were obtained for both, baseline resolved and overlapped peaks.



A 3D plot of background corrected spectra of a standard injection measured in 20 minutes (right). The injected standard solution contained  $5 \text{ mg ml}^{-1}$  of 4-NP,  $4 \text{ mg ml}^{-1}$  of 3m4-NP,  $10 \text{ mg ml}^{-1}$  of 2,4-dNP and  $8 \text{ mg ml}^{-1}$  of 2 NP. Retention times were 10.4, 12.1 and 13 and 14.1 min for 4-NP, 3m4-NP, 2,4-dNP and 2 NP, respectively.

## **P136-A1**

### **Interval Multi-Way Partial Least Squares (iNPLS) for Quantification of Allergens in Perfume Using Comprehensive Two-Dimensional Gas Chromatography (GCxGC)**

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Partial least squares (PLS) regression is a method for building multivariate calibration models. The algorithm works on first-order data, where for each sample/object a vector of variables are obtained. In some analysis, an interval of the variables results in models more reliable, so the interval partial least square (iPLS) was proposed. The iPLS splits the first-order data set into a number of intervals and calculates PLS models for each interval. An extension of PLS toward higher orders is called multi-way partial least squares (NPLS). Until now, there is no algorithm to perform interval selection in NPLS. In this work, an interval multi-way partial least squares (iNPLS) procedure is proposed. The algorithm splits the second-order data set into intervals in both dimensions, so a new reduced matrix is built from the initial one, and calculates NPLS models for each new matrix. This method was evaluated as a quantitative tool for Comprehensive Bidimensional Gas Chromatography (GCxGC). Preliminary studies were performed using synthetic samples (ethanol solutions of 1-octanol, undecane, 2-octanone, ciclohexanone and toluene with concentrations ranging from 0.0 and 3.0 % v/v). For all compounds, the iNPLS was able to locate the correct region on the two-dimensional chromatograms where the target peaks were located; also, the root mean square errors of cross-validation (RMSECV) of iNPLS was lower than the NPLS using the whole data. This approach was further applied to the quantification of three allergens (geraniol, citronellol and benzyl alcohol) in perfume samples. Samples were low price replacements for brand name perfumes, widely available on the local market. The samples were spiked with the analytes, resulting on concentrations up to 100 ppm. The RMSECV obtained with iNPLS were 9.75, 9.49, and 12.67 %, while the RMSECV obtained for NPLS was 40.41, 49.47 and 39.62 % for geraniol, citronellol and benzyl alcohol, respectively.

## **P137-A1**

### **Multivariate Control Charts Based on Net Analyte Signal (NAS) for Characterization of Polymorphic Composition of Piroxicam Using Near Infrared Spectroscopy**

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The quality assurance of the product during the whole development and manufacturing cycle of pharmaceuticals through increased level of understanding process is the main aspect to be considered within process analytical technology (PAT). Therefore, development of new tools using, e.g., multivariate methods for quality control in different steps of manufacturing process are of great importance. In this context, near infrared spectroscopic and multivariate statistical control charts based on net analyte signal (NAS) were applied to polymorphic characterization of Piroxicam samples. X-ray powder diffraction (XRPD) was used as a reference technique. The piroxicam has three polymorphic forms. Forms II and III were prepared from form I. The form II (needles) was obtained by the crystallization from saturated Piroxicam solution in absolute ethanol at room temperature while form III was obtained by the saturated piroxicam solution in absolute ethanol on dry ice. It was prepared 62 samples containing the active principle (Piroxicam) in the range of 20.0-40.0 % (w/w) in excipient. The samples were divided into four groups. The A sample set (with form I) refers to the “under control” samples; while the B (with form II) and C (with form III) sample sets are “out control” samples. The sample set named D is also “in-control”. The methodology was used to identify the polymorphic composition of sets B, C and D. The advantage of methodology is that the systematic variation in the product due to the Piroxicam is separated from the remaining systematic variation due to excipient in the matrix. The use of multivariate technique in rapid evaluation polymorphic composition of Piroxicam samples is demonstrated. It is presented an alternative method for quality monitoring of the drug after crystallization process within PAT concept.

## **P138-A1**

### **MEMS-Based Single Detector Grating Spectrometer – a Compact and Cost-Effective Sensor Approach for NIR and MIR On-Line Analysis and Process Control**

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The inherent IR-absorption properties of many substances make IR-spectroscopy a powerful tool for direct analyte detection. Over the last few years, several MEMS-based NIR-spectrometer designs have been published. Still, there is a lack of affordable, on-line IR-spectroscopic sensor instrumentation especially for the mid-IR range, where important analytes like CO<sub>n</sub>, NO<sub>x</sub> and hydrocarbons can be detected and distinguished.

State of the art on-line sensors in the mid-IR measure either only at a small number of wavelengths (NDIR design) e.g. using filters, or require large and precisely guided optical components (conventional IR-spectrometer design). The disadvantage of the first design is the loss of inherent spectral information, whereas the second is bulky, costly and has limited in-process applicability. Together, the limitations of both concepts represent a significant obstacle, severely limiting the practical applicability of spectrally resolved IR-sensors.

Our approach aims at replacing large and inert optical components in spectrometers with micro-electro-mechanical (MEMS) devices. Combining the advantages of full spectral information and real-time measurement (single scan < 10 ms) into a compact and cost-effective device recommends our spectrometer for on-line analysis. The spectrometer design is based on a Czerny-Turner type monochromator with a blazed single crystalline silicon scanning reflective grating (3 × 3 mm<sup>2</sup> aperture) and an optical resolution of ~10 nm at 50 μm entrance aperture. Due to the fast and precisely oscillating MEMS-grating only a single-element detector is needed, the spectrometer is almost immune to shock and vibrations and high potential for further miniaturisation is given. The MEMS-spectrometer covers limited spectral ranges (max. one octave at a time) between 1.2 – 5.0 μm, making it possible to use smart chemometrics and conduct reliable multi-analyte and/or interference detection.

The potential of our spectrometer as a sensor approach for chemical applications is evaluated for a multi-component gas analysis. The results will be presented at the conference.

## P139-A1

### QSRR Applied to Ion Chromatography of Ionic Liquid Cations

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The Quantitative Structure-Retention Relationships (QSRR) are statistically derived relationships between chromatographic parameters and the descriptors characterizing molecular structure of the analytes. A typical QSRR study comprises the following steps: (1) composition of the experimental data set, (2) molecular structure entry, (3) structure descriptor calculation, (4) analysis of regression relating to the experimental data and to the structural descriptors [1]. The most popular approach of those relationships is carried out for logarithm of *n*-octanol-water partition coefficient, log *P*, which is

a hydrophobicity measure. The interest in the hydrophobicity parameter is especially because of its influence on absorption, transmembrane transport, bioavailability, hydrophobic drug–receptor interactions, metabolism, pharmacological activity or toxicity of molecules, which all typically occur in the biological effects [2].

Ionic liquids (ILs) are salts comprised of molecular ions with a melting point below 100°C. ILs show unusual physical and chemical properties, which are caused by a combination of cations and anions. Such properties make them essential in biotechnological applications and in the pharmaceutical and chemical industry and from this view of point their hydrophobicity parameter is important factor influences their use [3].

In the present study, the QSRRs have been studied with the aim of predicting the hydrophobicity parameters and retention of IL cations in ion chromatography system. The eleven ionic liquid imidazolium and pyridinium cations were analyzed on cation-exchange column under the isocratic elution mode of acetonitrile/methanesulfonic acid mixtures. Their retention parameters were used to derive QSRR for logarithms of retention factors, log *k*, normalized to a hypothetical zero percent organic of modifier eluent, log *k<sub>w</sub>*, treated as a dependent variable. As the most statistically significant two-parameter QSRR regression equations related log *k<sub>w</sub>* to analyte water accessible molecular surface area (*A<sub>WAS</sub>*) and dipole moment (*μ*).

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## **P140-A1**

### **Profiling of Volatile Fatty Acids in Biomethanation Broths by Steam Distillation and Anion-Exchange Chromatography**

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Biomethanation has become a popular way to produce energy from low-cost organic matrices like silage, agricultural or industrial wastes. However, despite numerous studies describing the basic mechanisms of anaerobic digestion, a substantial work remains to be done to elucidate the critical factors allowing a proper functioning of reactors. Thus, one of the most critical issues is to determine which chemical or physical parameters could be used as early diagnostic tools during biogas production.

Volatile fatty acids (VFA) are important intermediates of the biomethanation process but they tend to stress microbial communities when present in too high concentrations. To that extend, monitoring the amount and profile of VFA could be relevant to detect early signs of improper functioning while evaluating the digester efficiency.

In this work, a simple and versatile method was developed for the quantification of VFA from biomethanation broths. This technique uses steam distillation as preparation step, followed by anion-exchange chromatography with suppressed conductivity detection. This procedure eliminates the main part of matrix compounds that may interfere during chromatographic analysis, which enhances significantly the robustness of the method. Moreover, additional information can be obtained from the collected steam distillate by volumetric titration for total VFA determination. This method was used to quantify short-chain fatty acids in batch anaerobic digesters exposed to increasing feeding rates. The results showed that the profile of VFA is modified when the organic loading rate exceeded a critical value. In digesters functioning well, acetate was the predominant VFA whereas in overfed digesters, butyrate and valerate showed the highest concentrations, and the global amount of VFA rose substantially. In conjunction, an acidification of the digestate and a significant decrease in methane production were noticed.

In conclusion, the profile and amount of VFA can be considered as excellent indicators of dysfunction or overfeeding during the biomethanation process.

## **P141-A1**

### **Evaluation of Signal and Noise in the Tuning of an ESI IT Mass Spectrometer by Multivariate Pattern Recognition Tools**

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When mass spectrometry is not combined to separation techniques, the evaluation of signal and noise in a complex mass spectrum is not trivial. The tuning of the spectrometer based only on the increase of the signal of a selected number of  $m/z$  values does not ensure the achievement of the best experimental conditions: often when signal increases, also noise has the same behaviour. The scope of this work is the development of a new approach for separating signal and noise (for evaluating the S/N ratio) from complex mass spectra. The final aim is to generate a target function for the automatic tuning of the instrument. Two different methods were applied:

- the first method is based on the separation of each mass spectrum recorded in the overall  $m/z$  range, in two contributions: one due to signal and one due to noise. This method is a generalisation of standard tuning procedures commonly used in literature based on the increase of both a single or pools of selected  $m/z$  intensities.
- the second method is based on Principal Component Analysis (PCA): the information accounted for by the first principal component (PC) is related to the average signal, while the residuals account for noise.

For both methods, a target function related to the signal and a S/N ratio were obtained. The comparison of the two methods was then carried out by evaluating the stability of the target functions along time and the variation of the functions as a function of concentration of the standard mixture used.

The method based on PCA provides a target function stable along time, not dependent on the presence of discontinuities in the system operations and showing a trend consistent with an increasing or decreasing concentration of the standard used.



## **P142-A1**

### **Off-Line Py/GC-MS and On-Line Py-GC-ICP-AED of Herbaceous and Woody Biomass for Preliminary Evaluation of Thermochemical Conversion to Liquid Fuels**

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Pyrolysis is a thermal decomposition conducted in the absence of air, exploited to convert vegetable biomass into a liquid fuel (bio-oil). Besides a heavy fraction eluding GC analysis, a large fraction of bio-oil is made up of hundreds of GC detectable compounds which have a strong impact on its fuel and ecotoxicological properties. Although qualitatively similar being mostly derived from the degradation of lignocellulose, their distribution is variable and determined by several factors, including the origin of substrate (e.g. biomass type, cultivation and harvesting procedures). Analytical techniques capable to provide fast and reliable information on the distribution of pyrolysis products are of great importance in strategy development. Analytical pyrolysis (Py) can be conducted under controlled conditions with a microscale apparatus interfaced (*on-line*) or not (*off-line*) to the GC system for the analysis of the evolved products.

In this study, on-line Py-GC-ICP-AES and off-line pyrolysis with sorbent trapping followed by GC-MS analysis were applied to study the pyrolytic behaviour of woody and herbaceous biomass on a quantitative base. Different crops utilised for energetic purposes (e.g. poplar, switchgrass, sorghum, corn stove) were compared from the point of view of agronomical productivity, yields and quality of pyrolysis oil.

The two techniques provided complementary information on the chemical composition of biomass pyrolysates. Py-GC-AED furnished a direct quantitation of elemental carbon useful to calculate overall yields of different fractions. Yields of individual compounds were obtained by off-line Py/GC-MS. Pyrolysates were characterised by common (e.g. predominance of hydroxyacetaldehyde and acetic acid) and distinctive features (e.g. abundance of hydroxymethylfurfural from shorgum). with the largest differences observed between herbaceous and woody biomass.

## **P143-A1**

### **Determination of Organic Layers on Metal Surfaces**

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To protect metal surfaces e.g. of steel sheets used in automobile manufacturing against aging during storage and against mechanical stress in the finishing process the surface is normally coated with an organic film <sup>[1]</sup>. In the final processing it is necessary to control the thickness of the organic film to avoid damage. The thickness of the organic protection film is between 0.5 – 3 g/m<sup>2</sup>.

Often the thickness of the layer is measured by the use of infrared spectroscopy. This method fails in certain cases when there is not a homogenous film on the metal surface.

The typical organic coatings however show fluorescence. So there is the question whether it is possible to develop an alternative method to determine the thickness of the organic film with use of fluorescence spectroscopy. Fluorescence spectroscopy is in contrast to infrared spectroscopy not absorption but an emission technique <sup>[2]</sup> and by this it should be more robust against inhomogeneities of the organic layer. Because there is no method described in literature for determining the thickness of organic surface layers with fluorescence spectroscopy fundamental experiments were done to see in how far this is possible. Different types of steel sheets and organic layers were investigated. The results will be presented.

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## P144-A1

### Carbon Nanotube Separation and Characterisation Based on Asymmetrical Flow Field Flow Fractionation Coupled to Multi-Detection

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For 10 years, anthropogenic nanoparticles have driven the attention of a large part of the scientific community. The most known of them are carbon nanotubes that represent the new industrial revolution. Indeed, carbon nanotubes show great potential for material applications. They may be used as nanoprobe, nanowires, electrode components, catalyst supports, in polymer composites, and perhaps for hydrogen storage [1-5]. They combine particular electronic structures, high surface area, electrical conductivity and excellent strength.

Due to their complexity and heterogeneity, one of the major challenges in analytical chemistry is their size and shape characterisation (i.e. length, diameter and chirality) as well as the determination of their electronic properties. This knowledge is become essential in order to better control their manufacturing processes, improve their physico-chemical performances and better understand their environmental impacts. Even if different analytical techniques are available to separate (SEC, Capillary Electrophoresis, ...) and give an image of CNT (AFM, ...), they are often limited in term of resolution and size-range they can reach [6-9].

This work shows the potentiality of Asymmetrical Flow Field-Flow Fractionation (As-FI-FFF)-based hyphenated techniques associated to a multi-detection approach to obtain a complete size and shape characterisation of carbon nanotubes [10]. The multi-detection approach is based on the hyphenation of As-FI-FFF with UV, Multi Angle Laser Light Scattering (MALLS) and differential refractometer (DR). So As-FI-FFF-UV-MALLS-DR gives physico-chemical data on carbon nanotube such as sizes (especially length and diameter) by the gyration and hydrodynamic radius determination. In order to validate such coupling and the associated information, different NTC fractions were imaged by Atomic Force Microscopy (AFM). The comparison of both analytical methods shows that As-FI-FFF separates carbon nanotubes principally on the basis of length, leading to fractions with relatively uniform lengths. Finally As-FI-FFF is confirmed to have excellent fractionation power and resolution. Moreover As-FI-FFF coupled to a multi-detection including MALLS and Inductively Coupled Plasma Mass Spectrometry (ICPMS) is shown to provide relevant and reliable information, such analytical procedure being cheap and quick with regard to the characterization challenge of CNT.

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## P145-A1

### Aqueous Synthesis of CdS Nanocrystals with High Luminescence and Temporal Stability

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Semiconductor nanocrystal quantum dots have been explored as fluorescent biological labels due to their photostable, size-tunable, narrow bandwidth photoluminescence and chemically functionalizable surfaces.<sup>1</sup> The unique optical properties of QDs make them appealing as fluorophores in a variety of biological investigations, in which traditional fluorescent labels based on organic molecules fall short of providing long-term stability and simultaneous detection of multiple signals.<sup>2</sup> Currently, there are lots of essays trying to improve the methods of synthesis of QDs and it can be considered a powerful area that involves several fields of science.<sup>3</sup>

CdS QDs have photoluminescence properties which make them suitable in applications such as biomedical labeling, solar energy conversion, optoelectronic... Organic solvent approach for these QDs synthesis is complex and harmful to the environment and the "as-prepared" QDs cannot be directly used in biological applications due to their hydrophobic character. We propose a reproducible, low-cost and environment friendly aqueous synthesis which provide water soluble and biocompatible samples.

Our studies report an easy strategy to synthesize highly luminescent, water soluble and biocompatible CdS NCs by the reaction of  $\text{Cd}^{2+}$  and  $\text{S}^{2-}$  in the presence of mercaptoacetic acid (MAA) as capping agent (stabilizant), under normal pressure and atmospheric temperature. We also systematically investigate the influence of various experimental variables, including the pH value, Cd-to-S ratio as well as Cd-to-MAA ratio, on the optical properties and the growth rate of CdS NCs. Through the temporal evolution of the UV-VIS absorption and PL emission spectra we have studied mean particle size and size distribution of CdS NCs. In our work, we have synthesized CdS QDs with particle sizes between 3 and 6 nm of diameter.

This highly luminescent water-soluble QDs can be expected to be very promising biological labels.

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## **P146-A1**

### **Synthesis of CdSe Quantum Dots in Water. Study of Variables Affecting Size, Quantum Efficiency and Stability**

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Quantum dots (QDs) are semiconductor nanocrystals comprising elements from the periodic groups II–VI, III–VI or IV–VI and with sizes typically in the range 1-10 nanometers. QDs have received significant attention in the past two decades due to their unique chemical and physical properties (broad absorption spectra, narrow emission spectra, large fluorescence quantum yields, etc.). There is quite a wide range of available methods of CdSe nanoparticles preparation but these techniques have many inherent limitations, such as the utilization of high toxic precursors, in particular organometallic cadmium and selenium compounds, high temperatures, high-energy irradiation, and others [1-4].

Our studies have been focused on the development of a synthesis of water-soluble CdSe quantum dots with nanometer sizes and with high luminescence (fluorescence). Due to the difficulties of the reaction in organic medium, our method for the preparation of CdSe QDs consists in using CdCl<sub>2</sub> and Na<sub>2</sub>SeSO<sub>3</sub> as precursors and mercapthoacetic acid as a stabilizing agent in aqueous medium.

The Na<sub>2</sub>SeSO<sub>3</sub> has a very slow kinetic hydrolysis at 4°C. In these conditions the nanocrystals have a high quantum efficiency (IF~1000u with slits of 3 nm). The stability of the QDs is longer than 100 days.

In conclusion, we have developed, controlling the variables affecting the synthesis, a simple method for the preparation of CdSe QDs with sizes between 3-6 nm, with different fluorescence emission wavelengths for the same excitation wavelength.

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## **P147-A1**

### **Optical Properties of Highly Aligned DNA Nanofibers**

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One of advantages for DNA is that intercalating organic dyes can be highly aligned inside the DNA double helix structure. However, this utilization might be difficult on single DNA molecules deposited on a substrate. For most commonly used substrates like mica or silicon oxide, interactions between molecule and surface are very strong and induce very large compression deformations on deposited DNA molecules. For bundles of DNA molecules called DNA nanofibers, their interior where there is no interaction with a surface, are expected to keep native double helix structures. Thus, DNA nanofibers on a substrate would be attractive building blocks for the nanoscale electronics and photonic devices. Here, we report simple and reproducible method to create highly ordered arrays of DNA nanofibers by solvent evaporation. This process leads to a DNA nanostrand much longer than the contour length of lambda DNA (16.3  $\mu\text{m}$ ) and facilitates manipulating a single nanofiber under microscope observation and measuring its optical properties.

## P148-A1

### Layer-by-Layer (LBL) Method as an Approach in Creating Gold Nano-Particle Modified Polymer Monolithic Columns

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Monolithic materials are widely used in separation and biochemical science for many different types of analysis<sup>1</sup>. Monolithic columns have been shown to be an attractive alternative to classical packed columns. Organic polymer monolithic columns are easy to produce and different surface chemistries can be realised. There is still an ongoing need for new methods of surface modification of the bare polymer scaffold to create selective surfaces required for specific interactions.

In this work, a LBL method for creating the necessary surface chemistry is suggested as an alternative to classical covalent surface modification. The LBL method has been well established in nano-technology<sup>2,3,4</sup>. It utilises electrostatic interactions between polyelectrolyte layers deposited one by one onto the surface of the monolith. Gold colloidal solutions were used to create gold nano-layer on the monolithic scaffold. Classical methods<sup>5,6</sup> have been used to produce either positively or negatively charged gold nano-particles in aqueous solutions. Colloidal gold solutions were characterised using dynamic light scattering.

Gold nano-layer coated polymers are potentially be useful stationary phases for the trapping and separation of sulphur containing substances<sup>7</sup>. The strong attraction between the gold surface and thiol groups also suggests utilising self-assembled monolayers (SAMs) of thiol containing functional ligands.

Monolithic polymers of 2-aminoethyl methacrylate, glycidyl methacrylate, 2-acrylamido-2-methylpropane sulphonic acid, cross-linked with ethylene glycol dimethacrylate, were synthesized using either thermal- or photo-polymerisation. These monomers were chosen as they have easily chargeable functional groups. The LBL method was achieved by flushing through the charged monolith successive solutions of poly-electrolytes each having an opposite charge to that of the previous layer and gold nano-particles embedded in the final layer or in the monolithic scaffold.

The surface morphology of the gold nano-layer modified monoliths was characterised by scanning electron microscopy (SEM) and optical microscopy. Capacitively coupled contactless conductivity (C<sup>4</sup>D) detection was used to characterise any changes in conductivity of monoliths after modification.

Selectivity was tested with low molecular weight, thiol containing compounds. The detection methods used included contactless conductivity and absorption-photometric detection. The potential of the approach is discussed herein.

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## **P149-A1**

### **Stability Evaluation of Nanometer-Sized Cu, Ag and Au Particles Using Asymmetrical Flow Field-Flow Fractionation (AF<sup>4</sup>)**

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The tunable optical absorption of nanoparticles via the particle size in the range of 10 nm – 100 nm is utilized in spectroscopy, biochemistry, catalysis and medicine. Gold particles are frequently used as location markers in studies of biological specimen. Silver sols serve as optical absorbers with tailored optical absorption properties, making other sols of other metals such as gold or copper particles highly favored substrates for surface-enhanced Raman scattering spectroscopy (SERS).

As the particle properties are strongly related to size, a precise determination of absolute size as well as particle size distribution is of great importance to verify stable conditions of particle synthesis. In addition, metal sols are sensitive to temperature, light and the presence of ions in the solvent and may alter rapidly their size. For that reason, the stability of metallic sols was systematically evaluated in order to obtain reproducible particle size.

Electron microscopy (EM) is an excellent technique to derive particle size distributions. However, as particles are deposited on a filter prior to analysis, this is a very time consuming method. Since the analysis in EM takes place under vacuum, vacuum, it is difficult to ascertain the state of aggregation in the original dispersion.

To ensure rapid and precise measurements of particle size as well as size distribution directly in dispersion, asymmetric flow field-flow fractionation (AF<sup>4</sup>) was chosen to characterize nano-sized silver, gold and copper particles. A method for the high-density particle was developed, including optimization of flow rates and carrier composition. The results were verified by image analysis obtained by EM analysis. In combination with UV/Vis absorption measurements, size distributions of the sols are correlated with optical absorption. Various preparation conditions are investigated and were especially dedicated to the presence of salts in the solvent. The particle stability was monitored over a more than a week.



## P150-A1

### Quartz Crystal Microbalance: a Promising Method for the Characterization of Copolymer Maleic Anhydride-Styrene with Photochrome Dye

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Quartz Crystal Microbalance (QCM) has found numerous applications in many fields including thin-film measurement, chemical analysis, gas sensor, humidity sensor and biosensor. Especially, the development of QCM systems for use in fluids or with visco-elastic deposits has dramatically increased the interest towards this technique.

QCM device will be used for measurements of adsorption of synthesized copolymer. The gold sensor has been recognized as excellent indicator of mass changes because of the direct relationship between mass change and resonant frequency response. New maleic anhydride (MA) copolymer with styrene (S) modified by condensative coupling reaction with azo photochrome dye ( $\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{N}=\text{N}-\text{C}_6\text{H}_4-\text{CH}_3$ ) was obtained and the results concerning structures were published. The selection of the maleic anhydride copolymer is justified by MA tendency for forming alternant copolymers, what permits to obtain architectures with big degree of structural regularity as well as of the anhydridic group reactivity in reaction with primary amines. During the adsorption process the QCM measures a frequency change that can be associated to a mass change due to adsorption of the copolymer.

Quartz crystal microbalance is used to monitor in real-time the polymer adsorption followed by azoic dye adsorption and then copolymer adsorption as well as optimization of interaction processes and determination of solution effects on the analytical signal. Solutions of azoic dye ( $5 \cdot 10^{-4}$   $\mu\text{g/L}$ ,  $5 \cdot 10^{-5}$   $\mu\text{g/L}$  and  $5 \cdot 10^{-6}$   $\mu\text{g/L}$  in DMF) are adsorbed at gold electrodes of QCM and the sensor responses are estimated through decrease of QCM frequency. Also, the response of the sensor at MA-S copolymer (solution  $5 \cdot 10^{-4}$   $\mu\text{g/L}$ ,  $5 \cdot 10^{-5}$   $\mu\text{g/L}$  and  $5 \cdot 10^{-6}$   $\mu\text{g/L}$  in DMF) is fast, large, and reversible.

This research showed the fact that the quartz crystal microbalance is a moderne alternative to study some physical and chemical properties of synthesized copolymer. Additionally, infrared spectroscopy and nuclear magnetic resonance (NMR) has been used to compare the properties of the polymer, copolymer and dye.

**Keywords:** Quartz crystal microbalance, maleic anhydride-styrene, photochromic dye, adsorption, sensor

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## P151-A1

### Conductometric Analysis Interfacial Layer Silica with Covalently Bonded Acidic Groups

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During past decade's vast publications were devoted to preparation and variety applications of silica-based materials with covalently bonded acidic groups, such as carboxylic, phosphonic, sulfonic and others groups. Most of the chemical properties of these materials depend on the condition of their synthesis and the support morphology. The reason for such dependence is multifunctional nature of the interfacial layer. These composite materials often have several types of the organic groups immobilized of the same support and real composition of interfacial layer is unknown. Common chemical analysis of such modified silica's is not informative and "average" composition of immobilized layer can only be found.

0256Abstract00914.pdf This research was focused on application of conductometric analysis as a tool for investigation of the immobilized layer composition. Next silicas with covalently immobilized organic acids, having multifunctional nature of immobilized layer were selected as investigation object: silica with immobilized pyridine carboxylic – (SiO<sub>2</sub>-PyC), iminodiacetic (SiO<sub>2</sub>-IdA) and methylaminophosphonic (SiO<sub>2</sub>-Ph) acids. Usually, these materials obtain from aminopropyl silica and their interfacial layer may contain the residual amino groups. This aminopropyl groups can bind protons from immobilized acids and affect to acid-base and complexing properties of the adsorbents. It was shown that conductometric method is promising tool for determination of total concentration of functional groups as well as for estimation of a number of major and residual groups in the interfacial layer of the silica. Particularly it was demonstrated that part of the immobilized methylphosphonic acid exists on the adsorbent

surface in molecular form ( $H_2L$ ), but the other part is ionized ( $HL^-$ ). It turned out that conductometric titration allowed the concentration of each of these forms were determined. Thus, interfacial layer various samples SiO<sub>2</sub>-Ph consist of 53-81% methylphosphonic groups and 47-19 % of residual aminopropyl groups. By comparison, interfacial layer of SiO<sub>2</sub>-PyC samples consist of about 50 % residual aminopropyl groups. The analysis of SiO<sub>2</sub>-IdA water suspension demonstrated that from total amount of bonded iminodiacetic acid, about 75% were ionized in ( $HL^-$ ) form.

## **P152-A1**

### **Development of a Method for the Determination of Trace and Minority Elements in Petroleum Cokes by WD-XRF**

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Cokes have in their composition a series of elements that determine their usage. Because of that, one of the controls they must undergo is the chemical analysis of these elements, which are mostly present as traces, usually below 5 ppm.

There are some standard methods for the determination of these elements by different techniques such as wavelength dispersive X-ray fluorescence spectrometry (WD-XRF) or inductively coupled plasma atomic emission spectrometry (ICP-OES). However, the standards for the analysis by WD-XRF have detection limits higher than 10 ppm and only few elements of interest are analysed. The analysis method by ICP-OES entails high sample manipulation and long preparation time, with the resulting errors that this implies.

The objective of the present study was the development of a methodology for the analysis of the following elements: Si, Al, Fe, Ca, Ni, V, P, Cr, Ti, Mg, Mn, K, Ba, Sr, Pb, Zn, Cu, Mo, Se, Sn, Ge and Sb in petroleum cokes in ppm concentration, preparing pressed pellets of the sample and optimizing the measurement conditions.

For the calibration and validation of the measurements the following standards reference materials have been used: SRM1632c, SRM2718, SRM2719, AR2771, AR2772, SARM18, SARM19 and CLB-1. Moreover, some cokes were analysed by ICP-OES in order to have the necessary number of standards for the calibration and validation of all the elements of interest.

By setting up this methodology, very low detection limits were obtained for all the elements analysed, this detection limit being lower than 1 ppm in some elements such as Cr, Mn, Se, Cu, P, Sr, Zn, Mo, Sn, Ge and Sb. In addition, a fast and accurate method was reached.

## **P153-A1**

### **Chemometrics Strategies for Standardless Spectral Analysis**

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A system where no a priori information about the chemical components is available is classified as a black system. The aim of performing curve spectral resolution on black systems is to estimate the spectra (qualitative analysis) and concentration profiles (quantitative analysis) of all chemical components present in the mixture.

We apply recently proposed methods for mixture decomposition into statistically independent components (MILCA, SNICA) to practical problems in analytical spectroscopy. They are applied to experimental standardless qualitative and quantitative analysis by means of UV-visible, luminescence and IR spectroscopy.

Composition of waste waters in petroleum industry enterprises and widespread environmental contaminants defined our choice to study aromatic compounds and polyaromatic hydrocarbons (PAHs). We examine up to six component mixtures with various concentrations of benzene, toluene and o-xylene and its isomers for analysis. We also tested various multicomponent mixtures of PAHs in solid and liquid state by means of IR and UV and luminescence spectroscopy. We examine complex mixtures of water and fat-soluble vitamins B6, B9, B12, PP, C, E, D, A as well. Similarly, we analyzed multicomponent mixtures of metals based on the decomposition of mixtures of their EDTA complexes.

In all cases the relative quantification error in concentrations does not exceed 7%, the locations of absorption bands were determined with the 0.5 nm accuracy, which is comparable with instrumental errors.

Approbation of ICA decomposition algorithms on real objects is of great practical interest, especially where mixture composition is not known exactly. We apply MILCA to analysis of vitamins and metals in complex 10 multivitamin drugs. Spectra of individual vitamins were extracted and concentrations of compounds in initial drug were obtained with 10% relative error. We also examine different fuels (gasoline, diesel fuel) and carry out quantitative and qualitative analysis of 5 aromatic compounds and 3 sulfur-containing organic substances comparable with chromatographic determination.

## **P154-A1**

### **Dependence of Intensity Feedback and Spatial Frequency on Spectral Density of a Pattern Formation**

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The Nonlinear optical systems with feedback and associated phenomena such as pattern formation [1] and cavity solutions have been continuously drawing attention for various reasons [2]. Pattern formation in optically nonlinear feedback system is a topic of extensive research in recent times. Naturally pattern start from noise, from which some frequencies experience higher gain, and, through the feedback in the cavity, they stabilize and form a pattern. Such patterns appear in laser cavities as well as in passive cavities also, employing optical nonlinearities [3-4]. The cavity threshold is always characterized by a sharp increase in the modulation depth. If the nonlinearity is at low (high) saturation, an increase in feedback leads to forward (backward) crossing over of the two thresholds, i.e. to switching the pattern on (off). In the present study we have studied effect of intensity feedback and spatial frequency on the spectral density in a temporally incoherent cavity below the cavity threshold. It has been observed that the spectral density at constant spatial frequency increases with the increase of intensity feedback and at certain intensity feedback value it increases suddenly and the spectral density becomes very large at very small value of intensity feedback when the cavity length is large (greater than the threshold value ).

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## **P155-A1**

### **Vibrational Spectroscopy of Some Monomeric Diazines-N-Oxides Molecules i.e Pyrazine- N,N'-02 , Pyrimidine- N,N'-02 and Pyridazine N,N'-02**

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The diazine rings are building blocks of many important natural and synthetic compounds, e.g., nucleotides. The simplest diazines: pyrazine, pyrimidine and pyridazine have been carried out of b3lyp/6-311++g\*\* levels. The computational results Optimized geometrical parameters, charge distributions and vibrational wavenumbers were calculated using ab initio quantum chemical method has also been carried out to help assign the fundamentals of these molecules. Each vibration has been assigned using observed wavenumbers in the IR and Raman spectra and their relative intensities, depolarization ratios of the Raman lines, the calculated frequencies, vector displacements IR intensities and Raman scattering activities and depolarization ratios for the Raman lines using Gaussian 03 program [1]. The calculations were performed at the Hartree–Fock (HF) level [2] using 6-31++G(d,p) basis set. Density functional (DFT) [3] with the Becke's three-parameter exchange functional (B3) [4,5] in combination with Lee et al. [6] correlation and/or Vosko et al. correlation functional [7] was also used to carry out computations using 6-31++G(d,p) and 6-311++G(d,p) basis sets due to their better accuracy over HF method. The geometries were optimized by minimizing the energies with respect to all the geometrical parameters without imposing any molecular symmetry constraints. Substitution of O atom(s) by the N1 sites of the ring affected by the substitution of O atom at the N<sub>1</sub> site while the length of the other bond changes.

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## **P156-A1**

### **CLIP, a Comprehensive Line Selection Tool for High Resolution ICP-AES Instruments**

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The possibility that spectral interferences occur on analyte lines is quite high in ICP-AES with line-rich elements but also when the number of elements present in a sample is important. Line selection is then a crucial step that must ensure that the lines selected for the analysis are free from spectral interferences, this to ensure the reliability of the results. As line selection includes finding a line free of spectral interferences along with adequate sensitivity, it could be a complex, time-consuming step, implying experiments by overlapping spectra of the different elements expected with varying concentrations.

HORIBA Jobin Yvon already developed a dedicated spectral database, based on the acquisition of spectrum of single element solutions at various concentrations. This base, called S3-base (Single element Spectra, Spectroscopic data) is already used with a dedicated tool, MASTER (Multi-line Analysis, Selection Tool for Enhanced Reliability), allowing the suggestion of lines free from spectral interferences with adequate sensitivity and the display of the combination of the single-element spectra, on the CCD-based ACTIVA instrument.

As the most challenging matrices, in terms of spectral interferences, are often analyzed with high resolution instruments that are PMT-based sequential instruments, and as the time needed for the line selection can be increased due to the sequential nature of the measurements, there was a strong need to get a tool able to shorten the line selection step and that is adapted for all kind of instruments that exhibit various focal lengths, slit combinations and gratings. HORIBA Jobin Yvon developed the CLIP tool (Collection of Line Intensity Profile) that allows the profile displays of the analyte lines and the concomitant elements lines according to their expected relative concentrations. Line selection is then facilitated, saving time for the analyst and the laboratory and ensuring enhanced reliability for the analysis, particularly for complex matrices.

## **P157-A1**

### **MEMS-Based High-Speed FT-IR Spectrometer – a New Platform for Real-Time IR Spectroscopy and Process Control**

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Fourier-Transform IR spectroscopy, as a standard technique in analytical chemistry, is at present largely restricted to the laboratory. Although inherently suitable for direct, non-invasive multi-analyte real-time analysis, e.g. in process control, few FT-IR analysers have progressed beyond at-line applications. Key factors preventing in-process applications are size, robustness, acquisition speed and instrumentation costs. Most of this is due to the need for a precisely moving modulating mirror, a massive optical component requiring exact driving.

This work deals with a new concept for building fully portable FT-spectrometers, where a resonantly driven translational micro-electro-mechanical (MEMS) mirror recently developed by the Fraunhofer IPMS replaces the standard scanning mirrors. Applying such, practically inertia-less, MEMS devices to modulate IR radiation allows building highly compact spectrometers with (sub-)millisecond scan times that are immune to shock and vibrations. Furthermore, they are potentially significantly cheaper in purchase and operation than standard instruments.

In initial experiments, a 2mm<sup>2</sup> rectangular MEMS mirror element suspended on two springs was used. Electrostatically driven by voltages <100V, this device is capable of a resonant sinusoidal forward-backwards oscillation in the kHz range with mechanical amplitudes of  $\pm 100\mu\text{m}$ . Based on a classical Michelson layout and equipped with a Peltier-cooled MCT detector, the resulting spectrometer prototype covers the spectral range 4500 - 1500cm<sup>-1</sup> at a spectral resolution <40cm<sup>-1</sup>.

A key characteristic is the high scanning speed, which allows either measuring spectral changes at ms time resolution, or co-adding several hundreds of scans to one spectrum within the time conventionally required to acquire a single scan. This makes it possible to build direct spectroscopic sensors monitoring chemical reactions, e.g. in a burner flame, in real-time, or achieve signal-to-noise ratios equivalent to many standard instruments. The high scan speed is of particular interest for process control, where a number of consistent subsequent readings are needed to actuate a process intrusion.

The further development of this concept is now the objective of the EU-FP7 project "MEMFIS". Aiming at developing the technology to a practically applicable stage, this involves e.g. the implementation of a new circular micro-mirror with a mirror area of 20mm<sup>2</sup> and an amplitude  $\pm 500\mu\text{m}$ , which will allow building MEMS FT spectrometers with spectral resolutions better than 10cm<sup>-1</sup>.



## **P158-A1**

### **A Novel Approach of Liquid-Liquid Extraction in Sequential Injection System for On-Line Spectrophotometric Determination of Copper**

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A new approach of automated liquid-liquid extraction in sequential injection system based on easy separation of organic and aqueous phases without application of any additional segmenters and separation modules for On-Line spectrophotometric determination of copper in the form of an ion associate with polymethine dye 1,3,3-trimethyl-2-[5-(1,3,3-trimethyl-1,3-dihydroindol-2-ylidene)-penta-1,3-dienyl]-3*H*-indolium using CCD detector has been suggested. The extraction sequential injection system was constructed by connection of two multiposition valves and two syringe pumps for the aqueous–organic mixture flow and for organic flow explicitly. The extraction procedure was realized by air-bubbling in small unit which was simultaneously used for the self-separation of both phases after the extraction. The reaction conditions of ion-associate formation, such as medium acidity, concentration of ligand and dye, as well as physical parameters of extraction sequential injection system were optimized. The method has shown good linearity and repeatability of results, which have been tested for analysis of real water and pharmaceutical samples spiked by copper.

**Keywords:** sequential injection analysis, liquid-liquid extraction, copper

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## **P159-A1**

### **Determination of Trace Amount of Zinc By FAAS after Separation and Preconcentration on to Modified Natural Analcime Zeolite Immobilized TPPZ**

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The zinc ions were separated by solid phase extraction onto modified natural analcime zeolite loaded with 2, 3, 5, 6-tetra (2-pyridyl) pyrazine (TPPZ). Extraction efficiency and the influence of sample matrix, flow rate, pH, and type and minimum amount of stripping eluent were investigated. Solution of zinc ions were passed through a glass column packed with 1.0 g of the sorbent, at pH range of 3.8-4.4. The adsorbed zinc ions were eluted with 5.0 mL of 2 M HNO<sub>3</sub> at a flow rate of 1 mL min<sup>-1</sup>. The calibration curve using the preconcentration system was linear from 0.05 to 2.0 µg mL<sup>-1</sup> in final solution with a correlation coefficient of 0.9991. The relative standard deviation (RSD) for eight replicate determinations at the 0.5 µg mL<sup>-1</sup> zinc was ±0.92%. Detection limit was 2.9 ng mL<sup>-1</sup> in final solution and preconcentration factor was 170. The proposed method was successfully applied for determination of zinc in various standard and water samples.

## **P160-A1**

### **Energy Mass-Analysis of Secondary Sputtered Ions**

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The essential information about the surface characteristics and its changing can be received by secondary ion energy spectroscopy and X-ray diffraction methods.

Energy redistribution in SIMS is an aim of present work. The data of secondary ion emission and X-ray probing of the semiconductor monatomic and the compound surface are revised on example such as the Si,  $\text{Al}_x\text{Ga}_{1-x}\text{As}$ , and ets.

The transition from molecular to a crystal state is characterized by change of coordination number and it is possible to take into account by the correction to atom electronegative number or recalculation of distribution of electronic density of the nearest atoms of a matrix and impurity including implanted ions. The researches of structure Si upper layers by mean of X-ray standing waves method observe the difference in angle reflected and photoelectron distribution.. For units electron-volt appropriate to a maximum on secondary ion energy spectra the characteristic time is about  $10^{-15}$  s.

On the unusual interaction the defects near a free surface is marked. For instance the point defects with the similar signs attract each other and against with the different signs push away ones. The formation of the surface vacancy and interstitial loops after different technological treatments are described.

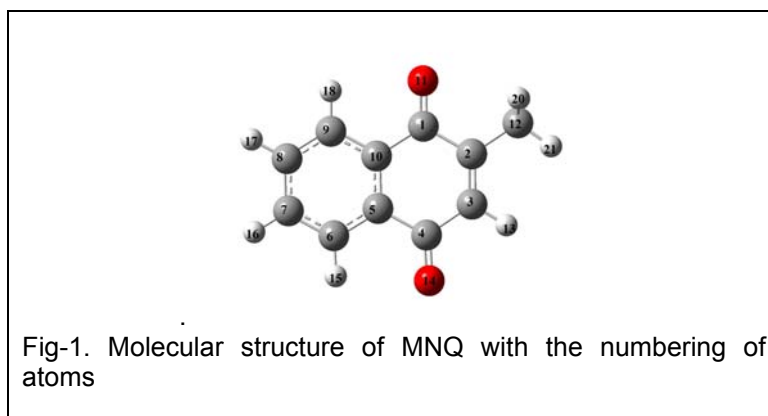
## P161-A1

### DFT Study on the Electronic Structures and Vibrational Spectra of Vitamin K<sub>3</sub>

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Vitamin K<sub>3</sub> {2-methyl-1, 4-naphthoquinone (MNQ) also called known as Menadione} is a made up of a 1, 4-naphthoquinone head group with a methyl substituents at the 2 position. The vitamin K analog menadione (K3), capable of both redox cycling and arylating nucleophilic substrates by Michael addition, has been extensively studied as a model stress-inducing quinone in both cell culture and animal model systems. Vitamin K is used in the body to control blood clotting and is essential for synthesizing the liver protein that controls the clotting. *ab initio* RHF and DFT calculations were carried out to study the molecular structures, charges and vibrational spectrum of vitamin K<sub>3</sub>. Vibrational mode calculations provided convinced theoretical evidence for assignments of fundamental vibrational mode. The second objective of the present work is to compute the optimized geometries, APT charges and harmonic vibrational frequencies along with their IR intensities and Raman activities and depolarization ratios of the Raman lines of the anions of the NA, NQ and MNQ molecules and study the change in the optimized geometries, APT charges and Vibrational characteristics due to conversion of the neutral NA, NQ and MNQ molecules into their corresponding radical anions. And the third objective of the present work is computed the thermodynamic properties (i.e, total energy, dipole moment and etc.) and also many other calculated physiochemical properties can be used as molecular descriptors, and they include HOMO, LUMO, ionization potential, electron affinity, chemical hardness, chemical softness, chemical potential, electronegativity, Electrophilicity index, energy gap and polarizability obtained with based on density functional theory calculations at the B3LYP/6-311++g\*\* level and also presented energy diagram of the molecular orbital energies.



## **P162-A1**

### **CE-MS via ESI Interface: Effect of Geometrical Alignment**

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During almost two decades since its first mentioning in the literature [1], capillary electrophoresis coupled to mass spectrometry (CE-MS) has become an accepted exponent within the group of hyphenated techniques. In the meantime a number of technical improvements have been achieved, substantially facilitating CE-MS experiments. In particular the introduction of commercially available instrumentation for CE-MS has made this technique amenable to a wider circle of users [2]. Focusing on research devoted to technical improvements in CE-MS instrumentation several major trends can be observed: i) the development of miniaturized devices for CE-MS; ii) the construction of novel spraying units primarily to improve sensitivity; iii) the combination of MS detection with coated capillaries or capillaries including sections for sample pretreatment; iv) the combination of CE with high performance MS instrumentation.

Among these topics, research on the design of new improved interfaces for the coupling of CE to MS instruments plays an outstanding role [3]. In this work we present results from recent investigations on the effect of interface geometry on parameters like spray stability and signal intensity. For this purpose a time-of-flight (TOF) MS instrument equipped with a modified micro-electrospray interface was employed. The design of this instrumentation allowed the use of spraying devices (either with or without a sheath liquid) mounted in axial or orthogonal position with respect to the MS orifice. Subsequently a test mixture consisting of nine (fluoro)quinolones was analyzed by CE-MS using three different interface layouts (sheath-flow interface: axial, 45°, orthogonal; sheathless: axial, orthogonal). Thereby the influence of the type of interface as well as the interface alignment on the robustness of the system and the sensitivity for these analytes was investigated.

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## **P163-A1**

### **Spectroscopic Study of Interaction of Aggressive Solvents' Vapors with Dye and Metal Filled Thin Polymer Films**

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Thin two and three components' films have been obtained by co-deposition in vacuum with standard vacuum deposition technique. The matrix used for the deposited films was polytetrafluoroethylene (PTFE) or polyparaphenylene sulphide (PPS). In order to obtain two components' films the bare films have been filled with various concentrations of acidochromic dyes (squaraines, stillbenes and polymethines). Part of these two components' films have been filled with an additional inorganic component (Au and As<sub>2</sub>S<sub>3</sub>) to create three components' film.

The goal of our investigation was to study the sensitivity of optical properties (absorption and luminescence) of the deposited films to aggressive environment. Spectroscopic analysis of the deposited films was performed with Cary 5 Uv-Vis-Nir spectrophotometer before and after treatment with several aggressive vapors (dichloromethane, methanol, acetic acid and trifluoroacetic acid).

Absorption and luminescence spectra of the two components' films based on PTFE as matrix and filled with squaraines or stillbenes have shown any changes after treatment with the vapors. It seems that PTFE matrix strongly enhances stability of several acidochromic dyes even at high concentration of dye. This effect can be used for enhancement of the stability of various optical devices, based on the dyes.

For the films based on PTFE matrix filled with polymethine, and for the films based on PPS matrix filled with acidochromic dyes it was observed transformation of the absorption and luminescence spectra under vapor action. Then these two components' films have been filled with an additional inorganic component. Adding of inorganic component leads to formation of inorganic nanoclusters with high surface energy, as adsorption centres for the solvent molecules. The inorganic nanoclusters enhance changes of optical properties of the films after treatment of aggressive vapors. It seems that three components' films are more sensitive than two components' ones toward vapors of dichloromethane, methanol, acetic acid and trifluoroacetic acid.

## **P164-A1**

### **Vibrational and Comparative Studies of Aniline, Benzoic Acid and p-Amino Benzoic Acid**

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*ab initio* calculations have been performed using quantum chemical method to compute optimized geometries, atomic charges, harmonic vibrational frequencies along with intensities in IR and Raman spectra and depolarization ratios of the Raman bands for Aniline (AN), Benzoic acid (BA) and Para-amino benzoic acid (PABA). It is found that the BA molecule possesses planar structure with the phenyl ring plane as the plane of the molecule, however, the PABA molecule possesses over all  $C_1$  symmetry, whereas, the AN molecule possesses  $C_s$  point group symmetry with the plane of symmetry as the plane passing through the N and  $C_4$  atoms and perpendicular to the phenyl ring plane. Some significant structural changes are observed in going from one molecule to another. The modes 1 and 16 suffer significant changes in going from the AN to BA to PABA molecules. The C-H out-of-plane bending modes 10 and 17 are also found to change significantly in going from one molecule to another. The  $NH_2$  wagging and torsion modes and the COOH rocking mode also suffer considerable changes in going from one molecule to another. In AN /BA the C-X(X= $NH_2$ /COOH) modes correspond to the modes 11/11, 18b/18b and 20a/7a whereas in the PABA molecule the C- $NH_2$  modes are 10b, 18b and 20a while the C-COOH modes are the modes 17b, 18a and 7a. Thus, which of the C-H modes become(s) C-X modes depends on the nature(s) of the substituent(s) and its/their position(s). The  $\nu(C-COOH)$  changes drastically (by  $415\text{ cm}^{-1}$ ) in going from the PABA to BA molecules.

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## P001-A2

### Advantages of Soybean Peroxidase over Horseradish Peroxidase as Enzyme Label in Chemiluminescent Immunoassay

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To increase the sensitivity of enzyme immunoassay (EIA) the enhanced chemiluminescence reaction (ECR) is traditionally used. In this case horseradish peroxidase (HRP), luminol/H<sub>2</sub>O<sub>2</sub> and *p*-iodophenol are used as enzyme label, substrates and enhancer, respectively. However, this reaction has severe drawback connecting with an inactivation of HRP by products of ECR and quick quenching the enzyme-induced chemiluminescence. Contrary to HRP, highly stable soybean peroxidase (SbP) produces a long-term chemiluminescent signal upon a catalytic oxidation of luminol. Moreover, we showed that 3-(10'-phenothiazinyl)propane-1-sulfonate (SPTZ) was a potent enhancer of SbP-induced chemiluminescence. To the best of our knowledge, SPTZ is the first enhancer of SbP to be discovered. Optimal conditions for SbP-catalyzed oxidation of luminol in the presence of SPTZ were determined. The SbP-SPTZ system showed better sensitivity and lower detection limit with respect to HRP-4-iodophenol system. The addition of 4-morpholinopyridine (MORP) to the SbP-SPTZ system decreased the lower detection limit value of SbP to 0.03 pM, which was 35 times less than that typical for HRP-4-iodophenol system. The SbP-SPTZ-MORP system was employed in sandwich chemiluminescent ELISA (CL-ELISA) for determination of human thyroglobulin, one of markers of thyroid gland cancer. Comparison of the CL-ELISA kits based on SbP and HRP demonstrated that use of the former enzyme allowed us to develop the assay with a higher sensitivity and lower detection limit. These results open up very promising perspectives for using the SbP-SPTZ-MORP system in ultra-sensitive immunoassays.



## P002-A2

### Molecular Forms of the Insulin-Like Growth Factor (IGF) - Binding Proteins in Human Serum and Placental Cell Membranes Separated by Affinity Chromatography

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Insulin-like growth factors (IGFs) are involved in the regulation of cell growth and metabolism. In biological fluids and tissues, they are bound to six IGF binding proteins (IGFBP-1 to -6). IGFBP modifications, such as glycosylation, phosphorylation and proteolytic cleavage, and/or association with other proteins may modulate the affinity for IGF binding.

We investigated a diversity of molecular forms of IGFBPs and their association with major human serum proteins using chromatography matrix with immobilized antibodies against these proteins (IgY-12). Proteins in the human serum and the solubilized placental cell membranes were analyzed by immunoblotting. Separated protein fractions derived from the serum were assayed for IGFBP-1, IGFBP-2 and IGFBP-3, whereas in the placental membranes only IGFBP-1 was investigated.

The majority of IGFBP-1 from serum, intact and complexed (220 kDa) with other proteins, was detected in the bound fraction, together with an IGFBP-1 doublet in the region 45-100 kDa. Thus, IgY-12 matrix, with its immobilized anti- $\alpha_2$  macroglobulin ( $\alpha_2$ M) antibodies, bound the majority of IGFBP-1, suggesting that the circulating IGFBP-1 is predominantly in complexes with  $\alpha_2$ M. The passage of the serum through the IgY-12 column caused degradation of IGFBP-2. Complexed forms of IGFBP-2 were also noticed. In the case of IGFBP-3, neither the intact protein nor its fragments bound to the affinity column, while IGFBP-3 complexes were mostly retained. Since the anti-transferrin (Tf) antibodies were immobilized on IgY-12 matrix, the protein band at approximately 100 kDa originated from the Tf/IGFBP-3 complex.

As for the placental membranes, intact IGFBP-1 was seen in both bound and unbound fractions. A protein of approximately 60 kDa was detected in the unbound fraction. It corresponded to a dimeric IGFBP-1. IGFBP-1 complexes of the masses above 220 kDa were seen in both fractions, indicating that a certain amount of IGFBP-1 is most likely bound to  $\alpha_2$ M.

### Toward Molecular Imprints for Proteases

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Process fluids derived from mammalian cell culture fermentation are contaminated with proteases due to the lysis of cells during fermentation and the subsequent cell-separation process, which may affect the product quality and product shelf life of biopharmaceutical drug proteins.

The proteolytic activity in cell cultures is largely removed during downstream processing. However, trace amounts of proteolytic activity may remain. Such residual proteolytic activity can be silenced using a variety of specific protease inhibitors<sup>[1]</sup>. However, due to the considerable toxicity of such protease inhibitors and the associated significant costs, their application to biopharmaceutical manufacturing processes is not desired. Thus, it is of ultimate interest to remove proteases from the final product, rather than suppressing their activity. Consequently, we study the synthesis of molecularly imprinted polymers (MIPs) for selectively binding (acidic) proteases. Serving as molecularly selective biomimetic receptors with tailored binding sites, molecular imprinted polymers may be applied for binding and removing proteases, and consequently, reducing their activity in degrading proteins<sup>[2]</sup>. So far, molecular imprinting techniques have been applied to a variety of macromolecules including nucleic acid bases<sup>[3]</sup>, peptides, and proteins<sup>[4]</sup>, and have proven a valuable biomimetic receptor concept in medicine, diagnostics, proteomics, environmental analysis, and in drug delivery. In comparison to natural receptor materials such as e.g., antibodies, MIPs provide comparable binding constants<sup>[5]</sup> at increased mechanical and chemical robustness, i.e., withstanding high temperature, pressure, and extreme pH conditions along with enhanced storage capabilities, and may be manufactured in large quantities ideally at lower costs than their natural analogues<sup>[5]</sup>. Furthermore, it is anticipated that they enable more rapid separation processes, and should thus be ideally amenable to industrial applications in biotechnology<sup>[6]</sup>.

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## P004-A2

### Selective Enrichment of Phospholipids Using Tin, Titanium and Zirconium Dioxide Material

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Phospholipids (PLs) structurally consist of a polar head group (zwitterionic phosphonate moiety) and a large hydrophobic part (one or two fatty acid ester groups). In biological systems their most important role is the formation of bilayer membranes.

In this study, three metal oxides, i.e. stannia (SnO<sub>2</sub>), titania (TiO<sub>2</sub>) and zirconia (ZrO<sub>2</sub>) have been evaluated comparatively in order to selectively enrich PLs from the lipid part of biological or food samples. The PL retention mechanism is based on a highly selective Lewis acid-base interaction with the metal ions.

The enrichment process was optimized using standard phosphatidyl cholines (PCs), such as Lyso-PC-18, Lyso-PC-16, AA-PC, PO-PC, PL-PC, MM-PC, and PP-PC. The experimental conditions have been evaluated to quantitatively retain and preconcentrate the PCs prior to their analysis by liquid chromatography - electrospray ionization mass spectrometry (HPLC-ESI-MS/MS) in positive mode. Different acidic conditions such as formic acid, acetic acid or trifluoroacetic acid at various concentrations have been tested for bonding and washing steps to enhance the selectivity of the material without compromising PL recovery. For the elution step different concentrations of aqueous or methanolic ammonia have been studied. Non-selective competitive binding of fatty acids (FAs) was evaluated by application of mixtures of PCs and FAs at various ratios to the metal oxide materials and evaluation of the matrix effect on the retention of the PCs.

Finally, the effectiveness of the studied metal oxides was tested by use of the three materials for selective enrichment of PLs from real samples such as plasma samples and different food matrices.

## P005-A2

### Direct Electrochemical Determination of Living Cells of Pathogenic Yeasts (*Candida Albicans*)

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The most simplistic approach for the detection of microorganisms are direct methods, which do not require additional reagents or special sample preparation procedures. Thus, we applied Linear Sweep Voltammetry (LSV) in the anodic potential range to suspensions of the opportunistic fungal pathogen *Candida albicans* and of the non-pathogenic yeast *Saccharomyces cerevisiae* and obtained an oxidation peak at + 750 mV. The peak potential was independent on the respective yeast strain, but was shifted with peak scan rate indicating an irreversible electrochemical reaction. This was confirmed by the lack of a cathodic peak in cyclic voltammograms. The peak current increased linearly with yeast cell numbers in the range from 0.33 to  $1.6 \times 10^7$  cells / mL. To elucidate the electron transfer step, which underlies the electrochemical signal, we investigated the influence of the electron transport chain inhibitors rotenone, antimycin A and potassium cyanide on the signal. Only cyanide decreased the signal, pointing to cytochrome c oxidase (COX) as relevant protein complex. Thus, we further applied single gene deletion mutants of nuclear COX subunits of *S. cerevisiae*. Deletion of subunits COX5a or COX5b eliminated the signal almost totally. These subunits influence the accessibility of heme  $a_3$  for electron transfer from heme a, so that we suggest that the electrochemical signal is due to an electron shuttling mechanism from the heme a to heme  $a_3$  electron transfer step in cytochrome c oxidase (COX) to the outer parts of the cell wall to allow the interaction with electrodes.

## P006-A2

### Determination of Insoluble Avian Eggshell Matrix Proteins

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The organic components of bones and other mineralized tissues have a high impact on the organization and deposition of calcium, and consequently influence the mechanical properties of those tissues. Avian eggshells have a relatively simple structure: the outermost layer is a relatively thin cuticle, followed by a thick calcified layer composed of calcite, which forms elongated structures named palisades and they are terminated by rounded inner ends named the mammillae. The extractable proteins of this matrix were studied previously and many of them were identified. However insoluble (non-extractable) proteins are sparsely studied.

We studied water-insoluble proteins by gradually decalcification of eggshell by EDTA to obtain three distinct layers (cuticle, palisade and a mammillary layer). The insoluble proteinaceous films from these layers were chemically treated by CNBr. The arising mixtures of long peptides were gradually precipitated by salt and these fractions were digested by trypsin. After that, HPLC-MS/MS (ion trap mass spectrometer) analyses of the resulting peptide mixtures were performed. Peptides/proteins were identified by software SpectrumMill and databases IPI chicken and SwissProt.

The analysis of the whole matrix of eggshell (without precipitation steps) enables to determine 6 proteins only (ovocalyxins 32 and 36, ovocledin 17 and 116, clusterin, and ovalbumin). The use of pre-treatment of individual eggshell's layers and gradually precipitation by salt distinctly increases the number of proteins identified – 36 proteins were determined. Besides previously described proteins we can mention protein similar to Kunitz-like protease inhibitor, collagens type I (two chains) and type III, EDIL3, fibronectin, sulfhydryl oxidase, tubulin alpha 1, lysozym, Dickkopf-related protein 3, various keratins, ovotransferrin. Relatively abundances of proteins were determined by exponentially modified protein abundance index (emPAI) in all eggshell layers. In cuticle layer 8 proteins were identified, whereas at palisade layer 20 and at mammillary layer 28 proteins were described.

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## P007-A2

### Development of a Microchip-Based Total Bioassay System for Oral Medicine

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A bioassay using cultured cells is one of the most important analytical methods in a search for new drugs, a safety evaluation of foods and chemical compounds, and basic biochemical studies. We have reported integration of several bioassay systems into a microchip to realize reduction of assay time, cell and reagent consumption, and troublesome manual operations. In this study, a microchip-based total bioassay system for oral anticancer agents and estrogens was developed, and overall activity of the agents, i.e. intestinal absorption, hepatic metabolism, and bioactivity to target cells, can be assayed with simple operation.

The microchip was composed of a slide glass, a membrane filter and two PDMS sheets which had microchannels made by lithography using SU-8. Human intestinal model cells were cultured on the membrane in the microchip. Human hepatocellular carcinoma cells were cultured on the microcarrier beads, and then packed in the microchannel. Human breast carcinoma cells were cultured on the fibronectin-coated glass surface of the microchannel as target cells.

All processes of the microchip-based total bioassay system were done by introduction of an assay drug. Overall activity of the agent could be assayed successfully. The system realized reduction of assay time and cell consumption. We concluded that the developed system had an ability to evaluate the overall characters of the agents, i.e. both bioavailability and bioactivity to the target cells.

## P008-A2

### The Development of Simultaneous Assay of the Trace Amounts of Horseradish Peroxidase Using Sodium Sulfite/BTB/Hydrogen Peroxide Autocatalytic Reaction System and its Evaluation

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□Introduction□A horse radish peroxidase(HRP) is an antibody label of enzyme immunoassay. Determination of HRP using a sodium sulfite/BTB/hydrogen peroxide autocatalytic reaction system is performed by induction time until a color of the reaction solution suddenly changing. This reaction is accelerated by HRP because the HRP acts as a catalyst. In this study, many specimens simultaneous determination of HRP concentration was performed using autocatalytic reaction. In addition, the effective evaluation method of the result was found.

□Experimental□A sodium sulfite solution including BTB and hydrogen peroxide were added in HRP sample solution at the same time. Four specimens of 0, 10, 20, 50 ppm were simultaneously measured in one time. The first time was measured one hour later from reagents prepared, and the measurement was performed seven times every 20 minutes. The induction time was measured by a video camera.

□Results and discussion□The induction time was shortened when HRP concentration increases. The relationship between average of the induction time( $n=7$ ) and the negative logarithmic of HRP concentration became good straight line in 10-50ppm. The individual induction time in each concentration moved for a short time in the side so that time passed. Then, the relative standard deviation (RSD) of induction time was 3.91, 3.57, 4.00, 4.27% ( $n = 7$ ) about each HRP concentration of 0, 10, 20, 50ppm. It is thought that sulfite has instability in solution. As a result, it was found that this time decreasing can cancel to exchange the induction time into a "division value". The division value is expressed in a quotient of induction time in each HRP concentration and the blank test. The RSD of division value in the measurement was 0.00, 1.10, 1.26, 2.83 %( $n = 7$ ) about each HRP concentration of 0, 10, 20, 50ppm.

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**Sorption-Photometric Determination of Bovine Seruic Albumin in Biological Liquids**

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Detection of protein quantity in biological liquids is important for clinical practicing. Thus at pre – diagnosis of diabetes the quantity of albumin is determined; its increasing is one of the earliest signs of diabetes, then albuminuria makes 30 – 30000 mg/day. If proper therapy is recommended at the beginning of initial albuminuria, development of pathological changes of organs may be prevented, also destroying of kidneys at last terms of diabetes. In relation with this fact, the problem of determination of albumin microquantity in urine is actual.

It is known, that for peptide bond of protein keto – and enolic form are typical (it depends on pH – level of solution). Charged macromolecules can create ionic associates with ionogenic surfactant, which are used for their further investigation, for example, by resonance light – diffusion method.

The aim of work was to develop optimal conditions for extracting of proteinic associates from water solutions on modified silica with polyoxyethylene isooctylphenol (SiO<sub>2</sub> – TX). As a protein we used bovine seruic albumin (BAS). We suggested that formation of ionic associat (IA) between BSA and surfactant (SAS) in combination with its specific adsorption on SiO<sub>2</sub> – TX may let us increase the efficiency of IA – extraction from water solutions and reach higher concentrations coefficients comparing with known methods of solid phase extraction.

In our experiments we used SAS of anionic and cationic nature: cetyltrimethylammonium bromide (CTMAB), tetradecylpyridyne bromide (TDPB), dodecylbenzyltrimethylammonium chloride (DDBDMAC), sodium dodecylsulphate (DDS), octadecylpyridine bromide (ODPB), sodium hexadecylsulphate (HDS). Degree of BSA – extraction from solution in presence of different cationic SAS was studied in the pH range from 2 to 8. Degree of BSA – extraction on SiO<sub>2</sub> – TX at increasing of pH = 8 becomes 15 %. While using cationic SAS, degree of BSA – extraction grows extremely, in presence of ODPB and CTMAB – to 80%, DDBMAC – to 60%, and TDPB makes it increased in smaller degree.

Degree of albumin extraction from water solution is correlated correspondently to increasing of number of methylen groups in aliphatic radicals of SAS. Thus degree of associate extraction of cationic pyridine SAS from BSA – solution is considerably higher in presence of long – chained ODPB, comparing with short – chained TDPB.

The fact is, that during extraction of IA BSA on SiO<sub>2</sub> – TX in presence of cationic and anionic SAS at static conditions and while protein concentration is 100 mkg/ml, sorption equilibrium appears in a few minutes (3 – 10 min). Maximum sorption value of SiO<sub>2</sub> – TX over IA of BSA is 32.8 mg/g in presence of ODPB, in presence of CTMAB – 27.5 mg/g, and 26.9 mg/g in presence of DDS. Distribution coefficients reach to 2.5×10<sup>3</sup> ml/g. In further investigations for development of methods of BSA –pre-concentration and its detection DDS was used, because in its presence the highest degree of BSA – extraction (from 90 to 100%) is observed in time of sorption equilibrium (5 – 10 min).

It is shown, that eluant with volume ratio of NaOH – solution to acetonitrile (3:7) at c(NaOH)=0.5 M is optimal for quantitative desorption of IA BSA. IA is quantitatively eluated in 1 – 4 ml of this solvent, and solution after filtration may be used for photometric detection of BSA by Laurie's method.

This advanced methodic allows us to detect BSA in urine on 4 mg/l – level. The limit of detection is in twice lower than by Laurie's method.



## P010-A2

### Chromatographic $pK_a$ Values of Angiotensin II Receptor Antagonist in Acetonitrile–Water Media

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For many analytes, the analytical response in the technique is a function of the acid–base properties of the analyte and solvent medium. The HPLC retention parameters of acids and bases strongly depend on the acid–base  $pK$  of the analyte and the pH of the mobile phase. Data on dissociation constant in water – organic modifier binary mixture are of utmost interest for the understanding of the retention behavior of the compounds. Very often, the main difficulty in the determination of dissociation constants of drugs is their aqueous solubility that forces the use of chromatographic techniques. LC-UV technique requires very low analyte concentration and allows suitable absorbance measurement in the hydroorganic mixtures even the drugs with low aqueous solubility for determination of dissociation constants. Nowadays, acetonitrile–water (MeCN–water) mixtures are widely used in high-performance liquid chromatography (HPLC) for this purpose.

Angiotensin II receptor antagonist represent a new pharmacological class of antihypertensive drugs. These compounds are safe and effective agents for the treatment of hypertension and heart failure, either alone, or in combination with the diuretics. These antagonists have been launched recently and any advance in the development of analytical methods for their determination or in the knowledge of the properties of these compounds provides a great contribution. A good knowledge of the acid base behavior of angiotensin II receptor antagonists in hydroorganic mixtures is essential to predict the influence of pH on several fundamental parameters such as selectivity and retention.

In this study,  $pK_a$  values of the angiotensin II receptor antagonist (ARA II); irbesartan, losartan, telmisartan and valsartan have been determined by means of the data obtained from reversed phase LC studies in acetonitrile water binary mixtures. A Gemini C18 column (150 mm×4.6 mm, 5  $\mu$ m) was used for all the determinations. Retention factor values of ARA II were experimentally determined at different percentages of acetonitrile–water binary mixtures (50%, 55% and 60%; v/v) at pH 2.75 - 8.00 in each mobile phase considered. The methodology suggested uses pH values in the mobile phase instead of those in water and takes into account the effect of activity coefficients. The simultaneous determination of  $pK_a$  values together with the  $k_{HA}$  and  $k_A$  from  $k/pH$  data was carried out by using the software NLREG 4.0. In this study precision of the methodology was evaluated. The results obtained indicate that LC methodology is a useful procedure in the determination of dissociation constants from  $k/pH$  LC data.

## P011-A2

### Development of an RP-HPLC Method for the Analysis of Induced Oxidation of Met Residues of Filgrastim

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Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology in *Escherichia coli*. The product is nonglycosylated and thus differs from G-CSF isolated from a human cell. Oxidation of methionine groups is one of the major degradation pathways of proteins. Filgrastim contains four methionine groups at positions 1, 122, 127 and 138 and each of them can be oxidized to methionine sulfoxide. The covalent addition of an oxygen atom to the sulphur atom of Met leads to the change of chemical properties of proteins, thus cause loss of biological function. Our goal in this study was to develop and validate RP-HPLC method for the analysis of oxidized forms of filgrastim in protein formulations. The solution of 0,1 % H<sub>2</sub>O<sub>2</sub> was used for the oxidation of filgrastim, the samples were kept at ambient temperature for 24 hours, and the analysis were performed at various oxidation times. The experiments were carried out by RP- HPLC using LiChrospher® WP 300 RP-18e, (150 x 4 mm i.d.) column. The mobile phase used was: solvent A ( 0,1 %(v/v) TFA in a mixture of water: acetonitril = 90:10) and solvent B (0.08 %(v/v) TFA in a mixture of acetonitrile: water = 90:10), at gradient mode. The temperature was 50°C, with UV detection at 215 nm. Under the proposed chromatographic conditions four peaks were detected with retention times of 17, 18, 19, min for oxidized and 20 min for nonoxidized form. Good validations parameters were achieved. The proposed RP-HPLC method is suitable for detection of oxidized forms and allows the following of the oxidation of methionine groups in filgrastim.

## P012-A2

### Identification of Neural Stem Cells by Infrared Spectroscopic Imaging

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Neural stem cells are currently in the focus of research since they offer a promising new approach to otherwise therapeutically intractable disease such as neurodegenerative diseases and tumor diseases. The in-situ monitoring of the differentiation of neural stem cells is not possible using conventional microscopic modalities. Recent developments in Fourier Transform Infrared (FTIR) imaging spectroscopy as well as in Raman imaging provide a new powerful technique for investigations of cells and tissue. This technology combines spatial resolution down to the size of a single cell with the high chemical selectivity of a vibrational spectrum and opens the possibility to classify cells and tissue without additional labeling.

Human adult hippocampal neural stem were cultivated and characterized using infrared spectroscopic imaging. A classification model based on linear discriminate analysis was developed to distinguish the differentiation of the stem cells to neurons, astrocytes as well as to identify not differentiated stem cells without labeling. The classification is based upon spectral features which mainly arise from proteins and nucleic acids. A spectral training set was formed with spectra from cells which were identified by a subsequently labeling with antibodies according to a standard histological protocol. Differentiated cells could be classified with a high accuracy whereas not differentiated stem cells did exhibit some misclassifications. The spectral classification shows no general confusion between the three types. The results of this pilot study demonstrate that this non-subjective classification procedure has identified absorption features that correspond to suspected markers of the identification of stem cells as the basis for classification.

## P013-A2

### Electrochemical Methods in Investigation of Living Cell-Metal/Metal Oxide Nanoparticles Interaction

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The aims of the study are: development of stripping voltammetry and potentiometry (with mediator system) variants specially adopted to investigate living cells-nanoparticles interaction and to use the methods as sources of information on above mentioned processes and toxicity of nanoparticles that can be particle size and concentration dependent.

The study of toxic effects of Ag, Au and Fe<sub>3</sub>O<sub>4</sub> nanoparticles is presented here. Alveolar cell line (W38) served as biomaterial investigated. Toxicity of nanoparticles Ag, Au (10 and 25 µg/ml) and Fe<sub>3</sub>O<sub>4</sub> in concentrations 1 and 5g/l were introduced into nutrient media. Au and Ag nanoparticles were prepared by the reduction of their salts with sodium citrate and Fe<sub>3</sub>O<sub>4</sub> were prepared by coprecipitation corresponding ions with NH<sub>4</sub>OH medium radiuses of iron oxide, Fe<sub>3</sub>O<sub>4</sub>, Ag and Au were 10, 20 and 12 nm respectively. Mixture of nanoparticles and cells was incubated during 24 hours. After that medium containing nanoparticles was removed and substituted for pure nutrient medium. All over the exposure period the cells-nanoparticles interaction including endocytosis observed with the use of optical and/or electron microscopes. Simultaneously viability of the cells was evaluated. A significant decrease in viability of living cells exposed for 120 hours to Au, and Ag was observed. Fe<sub>3</sub>O<sub>4</sub> does not lead to changing of cells viability.

Cells-nanoparticles interaction is resulted in decrease of integrated antioxidant activity. The lowest oxidative stress is observed in the cells exposed to Fe<sub>3</sub>O<sub>4</sub>.

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## P014-A2

### Selective Differential Pulse Voltammetry Method of Dopamine Determination in Biological Samples Containing Ascorbic Acid

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Dopamine, a catecholamine, has very important functions in central nervous, cardiovascular and other organism systems of the human beings. Extreme abnormalities of the dopamine levels in biological fluids as serum, plasma, cerebrospinal fluid, and so on, are symptoms of several medical conditions with incidence on behavior and cognition, motor activity, motivation and reward, sleep, mood, attention and learning.

The most convenient approach of dopamine determination in aqueous media and biological fluids is grounded on the ability of electrochemical oxidation at low potentials but the serious interference of the ascorbic acid makes most micro- and ultramicroelectrodes reported so far in the literature useless for this purpose. Nearby, a new type of carbon paste electrode, incorporating an anionic surfactant, is described. The tests performed by cyclic voltammetry have shown that the electrode developed by us discriminates efficiently between the cationic form of dopamine and the anionic electroactive species existing normally in biological fluids at the physiological pH value of 7.4. On this principle, a differential pulse voltammetry method has been optimized. It has the detection limit comprised in the submicromolar range and the capability to remove completely the interference of the ascorbic acid and to diminish significantly the interference of the uric acid. That method has been tested with good results on real samples of plasma and serum and is appropriate for important applications.

## P015-A2

### Kinetic Study of Cytochrome P450 2C9 Reaction with Diclofenac by MEKC Combined with a Sweeping Technique

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The early knowledge about the metabolism of a new drug and its affinity to certain drug-metabolising enzymes are especially important in the drug development process. The drug discovery thus continually creates a demand for a development of a new generation of high throughput assays. The majority of these studies are oriented on cytochromes P450 (CYP) because these enzymes play a key role in drug metabolism at human. The aim of this work was to demonstrate the possibility of using MEKC for kinetic study of CYP2C9, one of the most important isoforms in human liver, with diclofenac as a probe substrate. In view of the fact that several studies showed that diclofenac hydroxylation deviated slightly from typical Michaelis-Menten kinetics at low substrate concentration, the combination of sweeping with MEKC was applied in this study. The enzymatic reaction thus could be simply monitored by the analysis of the reaction mixture without any pre-treatment; moreover high sensitivity and repeatability of the separations were preserved.

A 50  $\mu\text{m}$  fused-silica capillary (56 cm effective length) was used to carry out all separations. 60 mM SDS in 20 mM phosphate 20 mM tetraborate buffer pH 8.6 was used as BGE. Injection was accomplished by an application of 50 mbar pressure to the sample vial for 48 s. Separation was performed at 24 kV (positive polarity), with the temperature of capillary 25  $^{\circ}\text{C}$  and detection at 200 nm.

As the result Michaelis constant  $4.62 \pm 0.38 \mu\text{M}$ , maximum reaction velocity  $18.35 \pm 0.63 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{nmol}^{-1}$  and Hill coefficient  $1.22 \pm 0.07$  were determined, which were in agreement with the literature data. Value of Hill coefficient confirms a presence of weak positive cooperativity in low substrate concentration region. The explanation could be found in binding of two substrates in or near the active site of CYP2C9.

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## P016-A2

### Rapid and Innovative Formation of Giant Unilamellar Vesicles

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Here we demonstrate a new and fast method for producing giant unilamellar vesicles (GUVs) via surface acoustic wave's excitation. These cell-sized unilamellar vesicles are very attractive model systems for studying many cellular processes and have increasing applications in drug delivery, in food industry, in medical therapy and genetic engineering.

Many research efforts are focused to develop techniques for preparation of giant unilamellar vesicles. Most of these methods have one big disadvantage; they require very low salt concentration, which strongly affects the studies on such artificial cell assembly. Only few techniques developed GUVs under physiological conditions but these methods are time-consuming or not robust. Using the mechanical deformations induced by the surface acoustic waves we have successfully produced giant unilamellar vesicles with a diameter of 10-50  $\mu\text{m}$  in only 10 minutes, with high yield. Our method is robust, is independent from the properties of the hydration medium and is successful working on physiological ionic strength. The preparation of GUVs using surface acoustic waves system open large opportunities for new type of biophysical studies like DNA-lipid interactions, lipid domain formation, partition of membrane proteins into lipid domains and many applications in medical therapy and genetic engineering.

## P017-A2

### Using of Electrochemical Methods and Paramagnetic Particles for Detection of Adiponectin

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During the extensive research in the past two decades it was shown, that adipose tissue is a very active producer of a wide spectrum of biologically active compounds called adipocytokines. Adiponectin belongs to the most important ones. This adipocytokine is involved in regulation of saccharides and lipids metabolism, it enhances glucose and free fatty acids utilization and increases their transport into muscle, liver and adipose cells. In hepatocytes it acts as gluconeogenesis suppressor. The analytical determination of adiponectin during *in vivo* and *in vitro* experiments requires a very precise bioanalytical approach due to its very low concentrations in biological systems. For this purpose a unique analytical method utilizing modified paramagnetic microparticles coupled with electrochemical detection was proposed. Prepared microparticles were tested for adiponectin isolation in following experiment. The samples with adiponectin concentrations from 0 to 100 ng/ml were prepared and the magnetic particles were incubated with these samples for 6 h at 37°C at 450 rpm. At lower concentrations the yield of adiponectin isolation was lower than at higher. The limit of detection for our method was about 0.5 ng/ml with RSD 25%). At adiponectin concentrations within the range from 5 to 50 ng/ml the amount of captured adiponectin was linear and the detected signal of adiponectin increased linearly with increasing concentration. This indicates that this electroanalytical system is very suitable for adiponectin analysis.

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## P018-A2

### Fully Automated Isolation and Detection of Swine H1N1 and Avian H5N1 Influenzas by Using of Paramagnetic Particles Coupled with Microelectrochemical Chip

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In our work we targeted the proposition of strategy based on magnetisable microparticles for viral nucleic acid detection. We utilized microparticles modified by streptavidin that demonstrate excessive affinity to biotin. We took advantage of this phenomenon and modified nucleotide probes specific to virus (influenza A/H1N1 and H5N1) by biotin. The probes prepared like this were mixed with streptavidin-modified paramagnetic particles. Interaction between biotin and streptavidin is very rapid; after 120 s the particles were washed and ready for next utilization. In our consequent experiments viral nucleic acids were applied to prepared paramagnetic particles. In consecutive step the hybridization of nucleic acids with specific probe bounded onto surface of paramagnetic particles proceeded. Acquired solution was immediately analysed by the use of electrochemical detection. For this purpose the miniaturised carbon electrodes as well as carbon electrodes modified by carbon nanoparticles were utilised. While setting of optimal conditions it was possible to detect 75-80% of added viral nucleic acid. Limits of detection of nucleic acids were in nanomolar concentrations. In addition the whole proposed process was tested in fully automated arrangement from sample application to qualitative and quantitative evaluation.

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## P019-A2

### Lab-on-Chip Analytical System with Integrated Self-Assembled Magnetic Nanoparticles for Efficient Capturing of Biomolecules or Cells from the Complex Biological Material

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Recent years have indicated great progress in miniaturization of bioanalytical instrumentation fully integrated "µTAS" or "Lab-on-a-chip" systems on a top; systems dedicated to the integration of the total sequence of lab processes to perform chemical analysis. The fluidic operations as sample preparation, injection, manipulation, dilution, reaction, separation and detection can be easily performed inside a single chip of only millimetres to a few square centimetres in size, handling of extremely small fluid volumes. The analysis using multifunctional microfluidic chips generally provides benefits as low cost, high throughput, fast analysis, sensitivity and reproducibility.

Moreover with the advancement of microfluidic technology, a few methods to integrate magnetic microbeads into a chip channel have been introduced. Magnetic particles work as tiny magnets in water – based suspension. When no magnetic field is applied, the nanoparticles do not have any orientation, and float about in a liquid medium. They are therefore easy to introduce into a microchannel. When a magnetic field is applied, the particles form rigid columns. Spontaneously self-assembled sieve within the chip enable to separate desired cells or biomolecules.

We have designed and developed microfluidic multiplexed devices for rapid detection of Alzheimer's disease markers. Device provides not only basic analytical parameters but also merits as increased sensitivity and specificity, possibility to automate. The goal was to detect pathological form of Aβ peptides and/or hyperphosphorylated Tau protein in CSF at an early stage of disease. The main approach was to prepare the highly efficient immunosorbent based on immobilization of specific antibody to the superparamagnetic micro/nanospheres for magnetically active microfluidic device without adversely affecting the antibody's function to capture antigen. It combines a high loading capacity and fast affinity reaction, thanks to a high surface to volume ratio and extremely thin diffusion layer.

Also we attempt to develop a new integrated microfluidic system for the fully automated capture, display and typing of rare Circulating Tumor Cells (CTC) in blood. Thanks to a reversible, self-assembled array of biofunctionalized magnetic particles and to nanoflow control technologies (patented „Ephesia“), cells will be captured with high specificity and efficiency, and organized into a regular array allowing automated in-situ immunophenotyping.

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## P020-A2

### The Separation of Isoforms of Human Lipoprotein Particles by Capillary Electrophoresis

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Plasma lipoprotein particles, which are transport vehicles for cholesterol and triglycerides in the human blood, are generally classified by density, size, or protein composition. Particles are spherical, composed of a polar shell and a hydrophobic core. Low-density lipoprotein (LDL) particles are the major cholesterol carriers in the circulation, with the physiological function of carrying cholesterol to the cells. High-density lipoprotein (HDL) particles are the key cholesterol carriers in the reverse cholesterol transport, i.e., the transfer of accumulated cholesterol molecules from the arterial intima to liver for excretion. Separation of LDL and HDL particles is highly important in clinical diagnostics today. In the case of capillary electrophoresis the heterogeneity of the lipoproteins and their unfavorable and strong adsorption onto fused silica capillary wall make their separation extremely challenging. Attempts have been made to diminish the adsorption has by dynamic [1] or permanent [2] coating of capillaries. In this study sugars [3] (monosaccharides, disaccharides and sugar alcohol) were exploited to alleviate the adsorption of lipoprotein particles onto the uncoated fused-silica capillary wall allowing their separation by capillary electrophoresis. In addition, the effect of sugars on the size of lipoprotein particles was clarified by asymmetrical flow field-flow-fractionation, zeta-sizer and molecular dynamic simulation studies.

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## P021-A2

### Type I and Type III Collagen Surfaces Studied by Atomic Force Microscopy

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Collagens are one of the major components involve in the formation of extracellular matrix. They are structural proteins of which 29 types have been identified [1]. Type I and type III collagens are widely spread in the body and they take part in the development of several diseases, such as atherosclerosis and diabetes.

Over the last few years, the atomic force microscope (AFM) has been commonly used to study the structure and molecular properties of human materials. AFM provides images on the nanometer scale by using a highly sensitive and tiny probe that is essentially pulled across a surface coated with human material, and topographical images can be obtained down to a nanometer scale. In addition, AFM allows biomolecules to be imaged also under physiological conditions.

The aim of this work was to image type I and type III collagen surfaces on mica and fused-silica surfaces with atomic force microscopy and compare their ability to bind decorin during the fibril formations. Furthermore, the surfaces of collagens modified by sugars were imaged in order to clarify the adsorption patterns of the glycated collagens.

Our studies indicated that the size and shape of collagen type I and III fibrils are affected by the addition of decorin and surface employed. The presence of decorin during fibrillogenesis causes not only the reduction of fibrils diameter, but also collagen layer is more uniform. Moreover, our results showed that the surface type effected different collagen adsorption pattern. Glycation led to the rearrangement of collagens in thinner fibrils and lower density coverage.

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## P022-A2

### Separation of Tryptic Peptides of Native and Glycated Bovine Serum Albumin by Open-Tubular Capillary Electrochromatography Using Salophene-Lanthanide-Zn<sup>2+</sup>Complex-Modified Capillary

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CEC is a hybrid technique useful for the analysis of a broad spectrum of proteins and peptides. In this work we tried to increase the separability of tryptic hydrolysates of bovine serum albumin and to investigate the glycation of this compound via OT-CEC. This method was applied to study of glycation of BSA. Glycation (non-enzymatic modification) significantly affected the properties of peptides and proteins and it is of great importance from a physiological point of view. Analysis of the arising compounds is very difficult because they cross-link peptides, and during electrophoretic separation they stick to the capillary wall. For this reason we tried using a capillary coated with salophene-lanthanide-Zn<sup>2+</sup> complexes that are effective at detecting neutral sugars as well as glycolipids and phospholipids. Optimum separation conditions were found: capillary 37 cm, 50 μm I.D. coated by salophene-lanthanide-Zn<sup>2+</sup> complex, voltage 10 kV, optimized background electrolyte consisting of 100 mmol/L borate buffer, pH 9 with 1 mmol/L ZnSO<sub>4</sub>. Tryptic peptides of glucose-glycated BSA gave a similar map to tryptic peptides of native BSA. There were minor significant changes in the profile (number of peaks), however, changes in the area/height of some peaks did occur. More distinct changes were observed after the modification of BSA with ribose.

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## P023-A2

### Gas Chromatographic Separation of Amino Acid Enantiomers via Derivatization with Heptafluorobutyl Chloroformate and Chirasil-L-Val Column

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A novel heptafluorobutyl chloroformate reagent was examined in enantioseparation of amino acids by gas chromatography. Twenty proteinogenic amino acids, plus ornithine, cystine and 4-fluorophenylalanine (internal standard) were treated with the reagent and separation properties of the arising derivatives assessed on a Chirasil-Val capillary column. The arising N,O,S-heptafluorobutoxycarbonyl O-heptafluorobutyl ester derivatives are simply prepared in aqueous media and easily extracted into an isooctane phase, directly amenable to GC analysis at elution temperatures below 200°C. Nineteen amino acid enantiomers were efficiently separated with improved resolution over to other chloroformate-based methods in 43 minutes, except proline and, further, arginine and cystine which were not eluted from the Chirasil-Val column. The new approach was demonstrated on analysis of amino acid enantiomers in natural peptides.

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## P024-A2

### Laserablation/Inductively Coupled Plasma/ Mass Spectroscopy for Quantification of Conversion Layers on Coated Steel Panels

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Blank and zinc coated steel panels are among the most important products of the Voestalpine Stahl GmbH. In majority of cases, this steel panels are coated with organic paintings, which should enhance the corrosion resistance as well as the appearance of the material. Prior the painting process the steel panels get treated with a conversion solution, which itself enhances the adhesion onto the metal and prevents corrosion creep below the coating. Up to now, the on the metal surface precipitated very fine conversion layer could only be detected respectively measured before the painting process. This Quantification is usually done by acid-etching with following analysis of the etchant or by the XRF-method.

The aim of this diploma thesis is to verify, if the conversion layer can also be quantified after the painting process. Therefore the Laserablation/inductively coupled plasma/ mass spectroscopy device should be used. Additionally the results of the LA/ICP/MS should be compared with measurements of the glow discharge optical emission spectroscopy.

## P025-A2

### Solid-Phase Microextraction for Gas Chromatographic Determination of Dimethylselenide and Dimethyldiselenide in Milk and Milk-by Products Using Atomic Emission Detection

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The metabolization of inorganic selenium salts by bacteria and other microorganisms and the subsequent detection of the alkyl volatile species dimethylselenide (DMSe) and dimethyldiselenide (DMDSe) are well documented. The possibility of that the selenium content in milk and milk by-products may be converted to the cited volatile compounds is examined, carried out by milk bacteria or those used to produce yogurt, such as *Lactobacillus bulgaricus*, *L. casei* or *L. acidophilus*, between others.

A method based on solid-phase microextraction (SPME) followed by gas chromatography with microwave-induced plasma atomic emission detection for the determination of DMSe and DMDSe in milk and milk-by products is optimized. For this purpose, a CAR/PDMS fiber was exposed to the headspace of 2 mL sample volume, placed into a 15 mL SPME-vial and in the presence of 0.6 g NaCl, for 5 min at 40 °C. In the case of solid samples, 1 mL of water was added to 2 g of sample. The thermal desorption of the analytes was carried out at 310 °C for 1 min, being separated on a DB-624 column, applying a program temperature which elutes DMSe and DMDSe at 3.03 and 4.96 min, respectively. The standard additions calibration was required for quantification purposes. The detection limits ranged from 70 to 110 pg mL<sup>-1</sup> for DMSe and from 80 to 400 pg mL<sup>-1</sup> for DMDSe, depending on the sample. None of the twenty three samples analyzed contained the studied compounds. A kinetic study on the stability of DMSe and DMDSe in different fortified samples was carried out under different storage conditions. Higher recoveries were obtained at 4 °C rather than at ambient temperature, especially in the case of DMDSe. When inorganic selenium was added to investigate its possible methylation to produce DMSe and/or DMDSe in the studied matrices, this methylation process was discarded.



## P026-A2

### SPE instead of Preparative-TLC for Purification and Enrichment of Phytosterols

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Suitable preparative methods in the analysis of phytosterols help to obtain accurate results. Thin-layer chromatography (TLC) is a conventional preparative method in analysis of phytosterols, but this method has some drawbacks. Solid-phase extraction (SPE) is a simple and inexpensive chromatographic method. SPE has been widely used in the preparation of lipid classes prior to further analyses by GC and GC-MS. Phytosterols comprise a major proportion of the unsaponifiables in vegetable oils. They are divided into three main classes: 4-desmethylsterols, 4-monomethylsterols, and 4,4'-dimethylsterols. Methylsterols usually occur in relatively low amount and therefore it is necessary to separate and enrich them by preparative chromatographic methods before quantification by GC. Phytosterols also occur either in free form or esterified with fatty acids and other conjugates. Separation of free and esterified phytosterols by preparative methods before quantification by GC provides detailed information on their distribution and stability. TLC methods and their drawbacks in the preparation of phytosterols before analysis by GC and GC-MS and advantage of SPE method as an alternative method to conventional TLC methods, is discussed.

**Key words:** Phytosterols, phytosterol oxidation products, solid-phase extraction, SPE, thin-layer chromatography, TLC

## P027-A2

### Direct Chiral Determination of Amino Acids by Two Dimensional (LC-LC) High Performance Liquid Chromatography

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The role of alpha amino acids in biological system is well known. Some D-alpha amino acids have essential functions in living systems as neuromodulators; the human body has a limited capacity to metabolize these enantiomers.; several diseases such as Alzheimer and renal disorders have also been associated with the presence of these compounds.

Formation of D-amino acids in foods is related with some treatments such as heating or some biological processes such as bacterial actions or ripening. The enantiomer's ratio is taken as an indicator of the quality of food products.

Nowadays, the consumption of ready-to-eat (RTE) foods is becoming more frequently. The preparation of RTE products involves several operations in which different pathogenic microorganisms can be incorporated to this food. Different technologies such as E-beam irradiation are available to prevent the growth of pathogens, but they may produce radiolysis of proteins as well as racemization of amino acids which can decrease the nutritional RTE food .quality.

The potential effect of E-beam irradiation on chiral transformations of amino acids in dry cured Iberian ham was evaluated. The free amino acids studied were tyrosine, phenylalanine and tryptophan, which are especially sensitive to radiation and are used commonly as markers of chiral.

A fast and controlled extraction method based on the use of ascorbic acid, was developed in order to extract free amino acids from samples irradiated with doses up to 8 kGy.

Then, a two dimensional high performance liquid chromatography method was applied. Amino acids were firstly separated in a primary column (C<sub>18</sub>) using a mixture of ammonium acetate buffer (20 mM, pH 6) and methanol (6%) as a mobile phase. Then, part of each peak was transferred by heart-cutting through a switching valve to a teicoplanin chiral column; methanol (90 %) / water (10%) was used as a mobile phase. UV detection was at 260 nm. Detection limits were between 0.16 mg·L<sup>-1</sup> and 3 mg·L<sup>-1</sup> for each enantiomer. Under these conditions, no significant racemization was detected even at high radiation doses.

## P028-A2

### Comparison between HTLC and HPLC for the Analysis of Foodstuff Using ELSD and ICP-AES Detectors

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The determination of organic compounds in food requires sensitive and rapid methods. In the last years High-Temperature Liquid Chromatography (HTLC) has gained interest because it shows very interesting chromatographic properties as compared to High Performance Liquid Chromatography (HPLC). Among them we can highlight: shorter retention times, better analytes separation and the ability to use only water as mobile phase. These characteristics make possible to adapt HTLC to powerful detectors such as Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Note that in this case the use of organic solvents may degrade the thermal characteristics of the plasma and may also give rise to chromatograms with a high background level.

The main goal of the present communication was to compare two new hyphenations: HTLC-ICP-AES with HTLC-ELSD for the determination of organic compounds (i.e., carbohydrates). A second goal was to compare HTLC against HPLC for these detectors. In this work, the ICP-AES detector was used to detect the analytes by measuring the carbon 193.03 nm atomic emission line. The chromatograms were thus obtained by plotting the emission intensity versus time. The tested compounds were several carbohydrates (i.e., glucose, fructose, sucrose, maltose and lactose). A porous graphitic carbon column (hypercarb) was used and temperatures ranged from 20 up to 200 °C.

The results showed that column heating up to 150 °C allowed shortening the time of analysis with regard to room temperature (HPLC) without degradation in terms of resolution. Moreover, column heating was also advantageous in terms of signal-to-noise ratio (S/N) for both ELSD and ICP-AES. Furthermore, with HTLC-ICP-AES hyphenation it is possible to suppress the nebulizer of the spectrometer, thus minimizing the off-column peak dispersion. Limits of detection (LODs) obtained by HTLC-ELSD were included within the 1 to 3 mg L<sup>-1</sup> range. According to previous results, LODs obtained for carbohydrates by HPLC-ICP-AES were close to 5 mg L<sup>-1</sup>. Therefore, HTLC-ICP-AES is a promising method for the determination of organic compounds in food.

## P029-A2

### Potential of Near Infrared Spectroscopy for the Analysis of Volatile Components in Cheeses

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Near Infrared Spectroscopy (NIRS) was used for the determination of volatile compounds in cheeses allowed to ripen for different times using a remote fibre-optic reflectance probe. To do so, cheeses with known and varying percentages of cow's, ewe's, and goat's milk were elaborated and used as reference material. The volatile compounds determined were: acetaldehyde, ethanol, 1-propanol, 2-butanol, 2-pentanol, 3-methyl-1-butanol, 2-butanone, 2-pentanone, 2-heptanone and 2-nonanone. The regression method employed was the modified partial least squares (MPLS). The robustness of the method was confirmed by applying it to twenty new samples of different compositions and ripening times which did not belong to the calibration group. Likewise, the correlations between the factors of influence studied and the volatile compounds were carried out. The results of the NIRS method are comparable with those of the gas chromatography-mass spectrometry method with purge and trap.

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## P030-A2

### Quality of Austrian Beef with Respect to its Elemental Composition

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“Bio” is a well known catchword in Austrian supermarkets. Especially health-conscious consumers tend to buy usually more expensive products of which the origin is traceable and the production follows “Bio”-guidelines. In the present work we have investigated meat from five Austrian beef labels (ALMO, Bio Ochse, Bio Kalbin, Ja! Natürlich Jungrind, Qualitätsmastkalbin) for its element composition. These five labels are well known for their beef production according to “Bio”-guidelines. From each beef label, meat samples from eleven animals of the roast beef were collected. After freeze-drying the samples were mineralized with nitric acid in a microwave heated autoclave. Inductively coupled plasma mass spectrometry was used for the simultaneous determination of major and trace elements. The accuracy of the results was tested with the CRM *Bovine Muscle Powder* (NIST 8414).

The determined element concentrations were utilized to find differences in the five beef labels. Additionally we have investigated the influence of feeding, origin of the animals, age, race, and sex on the element concentrations.

## P031-A2

### **A Single Reaction Interface Flow System for Spectrophotometric Determination of Aluminum, Phosphorus and Total Iron in Sugar-Cane Musts and Molasses**

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A flow system exploiting a single reaction interface (SIFA) is proposed for spectrophotometric determination of aluminum, phosphorus and total iron in musts and molasses. The concept do not rely on the utilization of well defined sample and reagent volume, but rather on the establishment of a single interface where mutual penetration of sample and reagent zones takes place prior detection. Controlled dispersion and reaction zone formation are therefore not so influenced by sample and reagent volumes but determined mainly by the extension of the overlap of two zones. This aspect guarantees a simplified system configuration, a low maintenance, a cost effective operation and the design of a rugged flow system.

The spectrophotometric methods are based on the reaction of aluminum with chromeazurol S, iron(II) with 1,10-phenantroline, and phosphate with molybdate and further reduction of the formed heteropoly acid yielding the molybdenum blue. All methods are implemented in the same manifold, thus the proposed system can be considered as a polyvalent one. Solenoid pumps are used for improving mixing conditions with consequent lessening of the reagent consumption. Influence of the chemical and physical parameters on the performance of the system was evaluated in order to permit optimization of the analytical procedures by the univariate procedure. The proposed system allows the determination of aluminum, phosphorus and total iron in musts and molasses after water dilution and pH adjustment. Detection limits of 0.1, 15.0, and 2.5 mg L<sup>-1</sup>, good analytical precision (r.s.d. < 1.6%, n = 5), and sample throughputs of 130, 304 and 145 h<sup>-1</sup>, respectively, were obtained. Results were in agreement with those obtained by ICP-OES at a 95% confidence level.

## P032-A2

### PCB Contamination in Fish Species from Rivers in the North of Luxembourg: Potential Impact on the Eurasian Otter (*Lutra Lutra*)

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Polychlorinated biphenyls (PCBs) were widely used in dielectric fluids in capacitors and transformers, as components of adhesives and plastic materials and also as pesticide extenders. Although PCBs have been banned since the 1980s, they still persist in the environment and exert negative impacts on the wildlife. PCBs are known for their bioaccumulation in the aquatic food chain and for their wide range of toxic effects.

The Eurasian otter (*Lutra lutra*) is a semi-aquatic mammal, its diet consists mainly of fish (50-94%). Otter populations declined strongly since 1970 in Western and Central Europe, and for the moment, the otter is considered as very rare in Luxembourg. Part of this decline may have resulted from the increased contamination of aquatic systems by PCBs.

Concentrations of PCBs (13 congeners) in fish species from the rivers of the North of Luxembourg have been analyzed by GC-MS. Fish species collected were the stone loach (*Barbatula barbatula*), the chub (*Squalius cephalus*), the barbel (*Barbus barbus*), the brown trout (*Salmo trutta fario*), the roach (*Rutilus rutilus*) and the yellow eel (*Anguilla anguilla*). Concentrations of PCBs measured in fish varied between 5 and 388 ng.g<sup>-1</sup> (wet wt.), depending on the site and the fish species. Fish collected at Wallendorf on the Our River and at sites on the Wiltz River and Clerve River showed the highest concentrations in PCBs. These results were further compared with results obtained in a previous similar study in 1994 in Luxembourg, indicating a general decrease of PCB contamination. The total PCB level expected in the liver of the otter through fish feeding in these rivers was also estimated following a mathematical model. According to our results, the contamination of fish by PCBs in the North of Luxembourg may be considered as problematic but not critical for otter survival.

## P033-A2

### Determination of Pesticides in Bee Products by Gas Chromatography-Mass Spectrometry

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Among the pesticides commonly used by Austrian beekeepers to combat the varroa mite, an external parasite of honey bees, acrinathrin, amitraz, bromopropylate, coumaphos, *p*-dichlorobenzene, diethyltoluamide, 2,4-dimethylaniline,  $\tau$ -fluvalinate, tetradifon and thymol play a major role. The use of these chemicals has led to contamination of bee products such as honey, beeswax and propolis, ultimately affecting human health.

In this work a technique for extraction of the ten aforementioned pesticides from honey by solvent extraction (SE) with hexane followed by gas chromatography-mass spectrometry (GC-MS) was validated by carrying out linearity, sensitivity, reproducibility, and recovery studies. The results from these studies ascertained that the proposed method is suitable for routine analysis and quantification of the ten pesticide residues in honey samples.

This method was tested for applicability to ethanolic propolis extracts as well as solid propolis samples. While the determination of pesticides in ethanolic propolis extracts was not successful at sufficiently low levels due to serious interferences from the matrix, it was possible to develop a method for solid propolis samples. After SE, clean-up of the extract was achieved by liquid-liquid extraction with acetonitrile followed by treatment with Florisil. Subsequently, quantification of the pesticides was carried out by GC-MS. With the present method four of the abovementioned pesticides were detected in the analyzed propolis samples. The detection limits for the ten compounds ranged from 0.08 to 0.71 mg kg<sup>-1</sup> propolis and the quantification limits were between 0.25 and 2.36 mg kg<sup>-1</sup> propolis.



## P034-A2

### Determination of Benzoic Acid and Sorbic Acid in Food Products by Capillary Electrophoresis

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Benzoic acid (BA) and sorbic acid (SA) are common food additives, which are used to prevent the growth of yeasts, moulds, and bacteria in food and beverages. They are considered as generally safe food additives. However, these preservatives in high doses may be harmful to consumers due to their tendency to induce allergic reactions. Consequently, in order to protect consumer health, the development of convenient and inexpensive analytical methods of these additives is of great importance for food safety.

Capillary electrophoresis (CE) is a powerful separation technique that can provide high resolution efficiency and is becoming a standard tool for the analysis of many compounds. The advantages of the method for food samples are short sample preparation and analysis time, low electrolyte and sample consumption. In CE, even if the sample contains a matrix, it can be injected with minimal sample preparation without decreasing the separation performance. The capillary between injections is rapidly flushed with fresh buffer. It is therefore a suitable method for the analysis of food products.

In this study, a simple, rapid and reliable CE method for determination of BA and SA in food products using direct UV detection was optimized and validated. The acids were separated in fused silica capillary (38 x 50  $\mu\text{m}$ ) at 28 kV. Background electrolyte was 20 mmol/L borate buffer, at pH 9.3; internal standard was cinnamic acid. Under the optimized conditions acids were separated in 3.5 min. The concentration ranges of the calibration curves were 0.005 – 0.4 mmol/L. The correlation coefficients for each standard curve exceeded 0.999. Good reproducibility and recovery results were obtained using internal standard. Detection limits of BA and SA (Signal-to-noise ratio=3) are 0.315  $\mu\text{g/mL}$  and 0.362  $\mu\text{g/mL}$  respectively.

## P035-A2

### High Performance Anion Exchange Chromatography with Pulsed Amperometric Detector (HPAEC-PAD) for Determination of Fructooligosaccharides in Food Products

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Fructooligosaccharides (FOS) naturally occurs in several kinds of vegetables. They are also added as functional food ingredients for their property to deliver the benefits of dietary fiber exhibiting prebiotic function, or are employed as fat replacer.

Traditional analysis of FOS fraction is based on enzymatic assays which are long-time consuming and only give results about average fiber content. Besides, they do not provide information about the polydisperse distribution of FOS having different degree of polymerization (DP), which is of great importance for intrinsic properties such as digestibility, prebiotic activity, caloric value, sweetening power, water binding capacity.

Our work proposes an innovative HPAEC–PAD method for FOS qualitative profile evaluation and quantitative determination in food. The lack of standards having a DP higher than five is an obvious problem when investigating FOS. Another issue, connected to the use of the amperometric detector, is that its response has been found to be not linear since it is affected by molecular weight and structure. We faced those difficulties and performed a method which allows separation and quantitative determination of compounds whose standards are not available, obtaining the possibility of dosing even FOS of unknown degree of polymerization. Full method validation in terms of detection and quantitation limits, linearity, precision and recovery has been carried out. Comparison of obtained data with those from traditional methods has been carried out and showed good data matching.

The method has been successfully applied to the analysis of FOS added to synbiotic fermented milks monitoring eventual variations induced by microorganisms during product shelf-life. Besides, investigation of FOS distribution in onions of different cultivars has been carried out. In particular, our attention has been focused on onions with protected geographical indication (IGP) as Var. *red tropeana*, attempting to propose FOS distribution as a possible quality marker.

## P036-A2

### Comparison of Monolithic and Conventional Particle Columns for the Analysis of Soy Isoflavones by HPLC

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Analysis of soy isoflavones is generally carried out by HPLC using conventional particle based columns, requiring approximately 60 min with a complex gradient to separate all main isoflavones. The most recent trend in HPLC analysis is toward fast, high sensitive and high-resolution separations. In this context, monolithic columns are an attractive alternative to reduce analysis time because they allow higher flow rates to be used.

In this study we evaluated the performance of a monolithic and a conventional column for the analysis of the 12 main soy isoflavones and the influence of several parameters, such mobile phase composition, solvent nature (aqueous methanol or aqueous acetonitrile), amount of acetic acid in the mobile phase, and elution modes (isocratic and gradient).

The use of the conventional column resulted in extremely long analysis times while being unable to resolve some isoflavones in all evaluated conditions. The monolithic column revealed a highly superior performance when compared to a conventional particle based column with similar properties. Under isocratic conditions the monolithic column was capable of separating most isoflavones independent of the solvent used, although acetonitrile achieved better separation in less time (40%) than methanol. Even though isocratic elution performed relatively well, the use of a linear gradient (0-100%) proved to be highly superior. Notably, a very simple gradient with methanol or acetonitrile achieved an acceptable separation of all isoflavones in less than 11 min and 5 min, respectively. Regardless of solvent nature and elution mode, the amount of acid used had a great impact on retention time and resolution indicating that the percentage of acid in the mobile phase is critical to achieve high speed separations (<10min).

## P037-A2

### Fast Analysis of Total Isoflavones in Soy Foods by High Performance Liquid Chromatography Using a Monolithic Type Column

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The main isoflavones found in soybeans are genistin, daidzin, glycitin and their respective acetyl, malonyl and aglycone forms. Analysis of all chemical forms usually takes 30-60 minutes using complex gradients, which severely limits sample throughput. However, glucosides can be hydrolyzed, greatly simplifying analysis, while providing information about the total concentration of these compounds in the sample. Also, the recent appearance of HPLC monolithic columns, which operate at extremely high flow rates, can be explored to further reduce analysis time. Therefore, the objective of this work was to develop a fast method for the determination of total isoflavones using a monolithic type column.

Chromatographic analysis were performed using a monolithic column (Chromolith RP18e, 100mm, 4.6mm) and water with 0.1% acetic acid (solvent A) and methanol with 0.1% acetic acid (solvent B) as mobile phase. Isoflavones were identified by comparison of retention times with authentic standards and UV spectra using a PDA detector. Several chromatographic parameters, such as mobile phase composition, flow rate and temperature, were step by step optimized in order to obtain a fast method for separation of isoflavone aglycones.

The optimized mobile phase composition was identified as 65:35% (solvent A:B), while higher temperatures (35°C) provided better peak resolution and symmetry for all aglycones. Flow rate was one of the most important variables affecting analysis time. Using the previous optimized conditions, a flow rate of 5.0 mL/min resulted on an excellent separation and resolution of all peaks in less than 4 minutes. One of the main advantages of the method, besides speed, is the considerably shorter required stabilization time when compared to conventional methods using particle based columns. Different soy products (soybeans, soy flour and soy beverages) were successfully analyzed using the developed method with a relative standard deviation lower than 5% in retention time and peak areas.

## P038-A2

### Comparison of Sorbents for Solid-Phase Extraction of Soy Isoflavones

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Solid phase extraction (SPE) is an increasingly useful sample preparation technique. With SPE, many of the problems associated with liquid/liquid extraction can be prevented, such as incomplete phase separations, incomplete recoveries, and disposal of large quantities of organic solvents. Selection of the most adequate solid phase is one of the most time consuming part of the method development. There are several new polymeric sorbents available and almost no information on the performance of these adsorbents for isoflavone extraction.

The aim of this work was to characterize the extraction properties eight SPE cartridges (namely BondElutC-18, BondElut ENV, Discovery DSC-18, Strata C-18E, Strata SDB-L, Strata X, LiChrolut EN, Oasis HLB) with a wide range of sorbents from different suppliers for the SPE of soy Isoflavones. A large variation on cartridges performance was observed, especially regarding retention and breakthrough volume during sample load and washing steps. While some cartridges retained all isoflavones, others had severe losses. The cartridges LiChrolut EN and Strata C-18E, presented a very low and variable retention capability. In contrast, cartridges DCS-18, BondElutC18 and SDB-L retained approximately 100% of most isoflavones. The cartridges Strata X, HLB and BondElut ENV, had an excellent performance, retaining approximately 100% of all isoflavones with no observable losses.

The breakthrough volume was also determined by increasing the sample amount to an extent that losses can be observed, indicating saturation of the sorbent bed. The C18 based sorbents (BondElutC-18 and DSC) presented a low breakthrough volume (<35 mL) while polymeric sorbents showed a much higher breakthrough volume (ENV, SDBL and HLB: 55 mL; Strata X: 95 mL). The HLB cartridge had an unexpectedly high breakthrough volume since it has the lowest sorbent bed mass (60 mg) compared to another cartridges (200–500 mg) indicating an excellent performance over conventional sorbents.

## P039-A2

### Sample Conservation of Soy Isoflavone Extracts

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The stability of samples is one of the most important aspects of any analytical methodology. This is especially important in the case of soy isoflavones. There are 12 main isoflavones in soy beans: genistin, daidzin, glycitin, and their respective malonyl, acetyl and aglycone derivatives. Some isoflavones have a relatively unstable character, and degradation or interconversion between chemical forms can take place during storage, affecting concentration and profile of these compounds in the sample. Therefore, it is essential to consider the stability of soy isoflavones extracts under storage conditions to allow better planning of routine analysis of large number of samples and avoid analytical errors resulting from inadequate storage before analysis.

We have studied several storage conditions during 4 months to overcome potential degradation of soy isoflavone extracts, including different temperatures (room temperature, 5°C and -32°C), addition of an antioxidant agent (2,6-Di-tert-butyl-4-methylphenol) and of an  $\beta$ -glucosidase inhibitor (D-(+)-gluconic-delta-lactone) at different concentrations (0.01-0.05% and 1-10 mM, respectively) as well as the combination of the above mentioned conditions.

It was observed that storage for up to 4 months at room temperature promoted a gradual conversion of malonyl derivatives of all isoflavones to the corresponding glucosides. Storage of samples at 5°C reduced the extent of this effect, while preservation of isoflavone concentration and profile was only achieved by storing the extracts at lower temperatures (-32°C). However, the use of the antioxidant agent (above 0.01%) reduced degradation at room temperature which was not so obvious at lower temperatures. There was no significant effect of the addition of the enzymatic inhibitor in the preservation of isoflavone profiles, independently of concentration and temperature, leading us to believe that the main cause of degradation during storage is due to oxidative reactions.

## **P040-A2**

### **Confirmation of Aroma Restoration into the Reconstituted Orange and Apple Juices**

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According to the requirement of the EC-Fruit Juice Directive fruit juice from concentrate must display organoleptic and analytical characteristic at least equivalent to those of an average type of direct juice. In the manufacture of fruit juices, evaporation in the juice concentration process and thermal treatment in the pasteurizing process are critical factors that may contribute to flavor loss or deterioration. The aim was to propose a simple method for aroma analysis and to select individual volatiles and their typical characteristic to confirm the aroma restoration. A simple, efficient and sensitive method of solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) has been used for the evaluation of volatile compound profile and quantification of individual alcohols, esters and monoterpenes in the samples. Orange and apple juices from concentrates has in many cases lower values of esters and terpenes than is mentioned in the literature or would correspond to the proper concentrates dilution and whole aroma recovery.

## **P041-A2**

### **Filbertone Analysis as the Tool for the Assessment of Hazelnut Spread Authenticity**

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The recipe for hazelnut spreads varies in different trademarks and countries. In addition to a certain amount of roasted hazelnut paste, that varies from 0 to approx. 35 %, these products mainly consist of sugars, vegetable oils and artificial flavorings. However, the quality of hazelnut spread is based on the content, origin and composition of the hazelnuts used in their production. The aim of the study was to propose a method for the estimating of hazelnut paste content in hazelnuts spreads. The procedure was based on the SPME/GC/MS determination of filbertone ((E)-5-methyl-hept-2-en-4-one), unique and characteristic nutty, roused, hazelnut like aroma component of hazelnuts. The obtained results of analyses of raw materials, model samples with known hazelnut content and real samples from the market showed that the sensitivity achievable with the proposed procedure was enough to detect hazelnut paste in the product of up to 0.1 %, however the high variability in which filbertone occurs in some raw materials made the real hazelnut content determination difficult and inaccurate.



## P042-A2

### Fluorescence Microscope Images Combined to Chemometric Tools for Recognition of Citrus Varieties

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A total of 48 leaves from 12 greenhouse-grown plants (6-8 months of age) of different varieties of sweet orange (*Citrus sinensis* L. Osbeck) 'Hamilin', 'Pera', 'Laranja Natal' and 'Valência' were evaluated.

The varieties of Citrus showed some particularities that the direct identification is difficult, by visual inspection or other analytical techniques. These points are important considering the manufactures of juice industry need large quantities of the plants and they cannot take any risk about equivocated plants or possible genetic mutations after grafting procedure. In addition, it can cause differences of the quality, flavor, and production of Citrus trees.

In this study, the fluorescence phenomena involve mainly, the color variation profiles from excited fluorescence emissions of the plants using ultraviolet radiation.

The fluorescence images of leaves were obtained using a Zeiss stereomicroscope Stereo Lumar.v12 coupled to digital camera AxioCamMR5 and mercury-vapor short-arc lamp. During the fluorescence microscope imaging it was used 545 ms of exposure and the magnification was 20 times. The mercury-vapor short-arc lamp was utilized to excite the fluorescence.

For each image analysis it were selected a region of 300 x 300 pixels (size of original image 1292 x 968 pixels). This criterion was important to keep the same characteristics during the chemometric analysis.

The colorgram (histogram of frequency colors) was obtained for fluorescence images of following colors: green, red, blue, luminosity, relative red, green and blue, hue, saturation and intensity generating a total of 2560 variables.

Principal Component Analysis (PCA) and Parallel Factor Analysis (PARAFAC) were applied in the data. Both methods confirmed the influence of blue, green and hue colors in the successful classification of the varieties.

This study has paved the way for the taxonomy applications. The potential of this analytical method is to investigate the specific signature of each Citrus plant variety.

## P043-A2

### Comparison of Different QuEChERS for Organochlorine Pesticides Determination in Strawberries

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Organochlorine pesticides (OCPs) are an important group of contaminants and they had widespread use around the world. Despite their banning several years ago, the analysis of OCPs residues are of special interest as their high chemical stability results in their persistence and bioaccumulation in the environment [1].

The aim of this study is to develop an analytical method to determine 12 OCPs (hexachlorobenzene, aldrin, 4,4'-dichlorobenzophenone, endosulfan I, dieldrin, p,p'-DDE, endrin, endosulfan II, p,p'-DDD, o,p'-DDT and methoxychlor) in strawberries obtained from integrated pest management (IPM) farming.

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method [2] has been readily accepted for many pesticide residues analysis in food [2] so it was selected for strawberries sample preparations. The procedure involved the extraction of 10g of grinded and homogenized strawberries samples in a Teflon centrifuging tube with 10mL acetonitrile. By adding different chemical the pesticides are extracted from the matrix into organic phase with vortex mixer. Three types of QuEChERS have been studied: i) 4g anhydrous magnesium sulfate, 1.0g sodium chloride, 1.0g trisodium citrate dehydrate, 0.5g disodium hydrogencitrate sesquihydrate; ii) 6g anhydrous magnesium sulfate, 1.5g sodium chloride, 1.5g trisodium citrate dehydrate, 0.75g disodium hydrogencitrate sesquihydrate and iii) 4g anhydrous magnesium sulfate and 1g sodium chloride.

After centrifugation, the supernatant was placed into a tube containing 150mg primary secondary amine (PSA) sorbent plus 150mg anhydrous magnesium sulfate and 50 mg C18, which constituted a cleanup procedure, called dispersive solid-phase extraction. After a second shaking and centrifugation step, the acetonitrile extract was transferred to autosampler vials for analysis by gas chromatography with electron capture detection (GC-ECD).

Recoveries obtained were between 39.12-161.57% for p,p'-DDE and lindan, respectively, and repeatability less than 10% for all of fortified pesticides. The highest recoveries for the studied OCPs were obtained with QuEChERS extraction tubes with 4 g anhydrous magnesium sulfate, 1.0g sodium chloride, 1.0g trisodium citrate dehydrate, 0.5g disodium hydrogencitrate sesquihydrate.

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## P044-A2

### Determination of Inorganic Species in Bivalve Molluscs

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Bivalve molluscs that live in coastal environments like mangrove swamps ingest plankton, microorganisms and organic material through the filtration of about 10 to 50 L/h of water, with low or no selective capability, allowing the accumulation in their visceral mass of all the biological and abiotic agents present in the water where these organisms live. Considering they are vulnerable to contamination by metallic ions, due to pollution generated by urban and industrial sources, the determination of trace elements in this kind of sample is of great interest in analytical chemistry. This work describes the simultaneous determination of As, Cd, Cr, Cu, Mn, Ni, Pb, Se and Zn in tissues of bivalve molluscs. A comparison between the metals content of male and female samples was done. The analyses were performed by ICP OES, after adequate sample treatment using a digester block.

Nebulization flow rate (0.6-0.9 L/min) and radiofrequency power (1.2-1.5 kW) were optimized and the best analytical signals were obtained when 0.8 L/min and 1.4 kW were used, respectively. The best ratio between acid and peroxide was investigated for sample treatment. The digestion efficiency was evaluated considering the total organic carbon content present in the digested material with values from 0.40 to 0.64% being obtained.

The accuracy of the proposed methods was good, considering the recoveries obtained by comparison with the values of certified reference material (Oyster Tissue, SRM 1566b - NIST) (87-110 %). The concentration of the analytes, considering 240 samples of bivalve molluscs, collected in the mangrove of Sao Francisco do Conde, Brazil, showed that the mollusks have contents of As, Cd, Mn and Zn higher than those allowed by Brazilian legislation. On the other hand there is no significant difference (at 95% confident level) between male and female species.

CNPq, FAPESP

## P045-A2

### Comparative Study of Elemental Content in Muscle and Liver of Fish from Aqua Culture and Sea

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Multielementar analysis were performed directly in muscle and liver samples from sea perch and gilt head bream from aqua culture and sea by energy dispersive X- ray fluorescence (EDXRF) and by Atomic Absorption Spectrometry (AAS). The samples were collected in sea and aquaculture industries from several points of the Portuguese coast, in nearly eighty (80) analyzed samples. The corresponding elemental concentrations of K, Ca, Fe, Ni, Cu, Zn, As, Se, Br, Rb, Sr and Pb (for EDXRF) and Cd, Pb and Hg (for AAS) all samples were then obtained, in order to find some elemental correlations with size, mass and location.

For EDXRF, samples were lyophilized and analyzed without any chemical treatment. From each sample, a powder was obtained in a mortar of polyester mill. For analysis, each of three pellets, without any substrate, was glued on a Mylar film, and put on a sample holder directly on the X-ray beam for elemental determination. An X-ray dispersive fluorescence spectrometry system was used for this study. For AAS, it was used a Varian Spectr AA 220 Z. Samples were digested with nitric acid (HNO<sub>3</sub>) and hydrogen peroxid in a microwave. A minimum of two replicate analyzes were performed for each sample. Concentrations were calculated from linear calibration plots obtained by measurements of the absorbance of standard solutions

The gilt head bream range between 250 to 850 g and between 27 to 35 cm, and the sea perch range between 300 to 950 g and between 30.5 to 42 cm.

Very low levels were obtained for toxic elements in all the analyzed samples independently of the place and the species. The highest levels for toxic elements were found in muscle tissue samples.

Comparing the same species from different places from northern and southern Portuguese coasts significant differences were found between concentrations for almost all the studied elements in both tissues. For different species of the same area differences in elemental levels were also found for almost all elements.

## P046-A2

### Determination Methods of Nitrate and Nitrite. An Ecological – Economical Aspect

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Nitrate is a naturally occurring compound in vegetables, in addition it is used as a food additive in certain fish, meat and dairy products. Accurate methods for determination of nitrite and nitrate are needed for both legal and food safety aspects.

The classical method to determine nitrate and nitrite in foods is by colorimetry after cadmium column reduction of nitrites to nitrites. This method is easily automated but hence according to Codex regulations Cd is not anymore allowed, other methods should be preferred. Efforts into this direction are the use of enzymatic reduction by NADPH and implementation of ultracentrifugation instead of Carrez precipitation (ISO/IDF). The method is still time consuming and enzymes have a restricted exposure times.

Liquid chromatographic methods for simultaneous detection of nitrate and nitrite in foods have been developed. However, HPLC requires expensive columns and long run times leading to high quantities of organic solutes and high costs. Foods are mostly complex matrixes and the presence of interfering substances leads to complicate determinations especially at low levels such as milk and some meat products.

Capillary electrophoretic methods are gaining acceptance as robust, fast and ease of use alternatives in analytical laboratories to conventional wet chemical and instrumental techniques. The electrolytes are normally water based and the amount of organic waste is minimal compared with HPLC, leading to lower expose to harmful substances for both environmental and laboratory technicians point of view.

In this work we report comparison of different techniques for determination of nitrate and nitrite levels of different vegetables and foods. The CE method is evaluated against other techniques for its suitability for routine simultaneous analysis of nitrate and nitrite from different food matrixes. Ecological and economical aspects are discussed in detail.

The aim of this work is to compare different techniques and develop more convenient ecological and cost-effective methods for routine determinations of nitrite and nitrate and to compare, assess and validate the benefits of different methods.

## P047-A2

### Investigation of Cyanuric Acid Contamination during the Cleaning of the Glassware for the Sample Preparation

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The melamine-tainted milk scandal in China posed one of the most serious global food safety crises in recent years. It might not be possible to determine a safe level for infant formula containing both melamine and cyanuric acid compounds due to lack of reliable toxicological information of melamine-cyanurate coagulated compound so far. However cyanuric acid concentration in foods must be analyzed with melamine together due to the very low water solubility of melamine-cyanurate (1/1620 of melamine water solubility) causing the serious renal failure by increased formation of kidney stone. Melamine and cyanuric acid in foodstuffs, were analyzed using LC-ESI MS/MS in multiple reactions monitoring mode. During the replicate analysis of melamine and cyanuric acid for the method development, recurring cyanuric acid contamination was observed in blank samples. There might be more than two contamination sources such as strong alkali detergent used for the dish washing machine and the cross-contamination during automatic dish washing from other glasswares used for preparing high concentration of cyanuric acid standard solution. Authors would report the most recent result for the investigation of the cyanuric acid contamination sources to eliminate the any possibility of the false positive quantification result of cyanuric acid.

## P048-A2

### Exposure and Risk Assessment of Melamine in Representative Korean Foods for Infants and Children

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In 2008, baby formula containing melamine was found to be responsible for a large outbreak of renal failure in infants in China. A total of 294,000 infants were hospitalized, and at least 6 babies died due to ingestion of the tainted formula. Melamine contains high levels of nitrogen (>60%), which is used as an indicator of protein content. Therefore, high levels of melamine in infant formula were thought to be the result of deliberate contamination in an attempt to increase its apparent protein content. Following inspections by China's national inspection agency, assorted products from at least 22 dairy manufacturers across China were found to have varied levels of melamine (range: 0.09~6196.61 mg/kg). Melamine co-exposure with cyanuric acid can induce acute melamine-cyanurate crystal nephropathy, which can lead to renal failure at much lower doses than if either compound were ingested alone. However, currently, there are very few data on melamine analogues other than cyanuric acid. At an expert meeting of the WHO and FAO held to review toxicological aspects of melamine and cyanuric acid on December 14, 2008, a new tolerable daily intake (TDI) of melamine was established that could be applied to the entire population, including infants. Therefore, a risk assessment of the various theoretical melamine contamination levels in infant formula and selected representative foods (other than infant formula and sole-source nutrition products) is urgently needed for Korean babies and children up to 7 years of age. Although the undetectable level regulation for infant formula may be low enough to guarantee the safety of babies under the age of 1 year (including premature babies), the melamine standard of 2.5 ppm for foods other than baby formula could be insufficient to protect the 95th percentile population aged 1~2 years because of this demographic's high consumption of milk, yogurt, and soy milk (hazard index = 1.79). Because TDIs are chronic values intended to protect an individual over his/her lifetime, occasional modest ingestion in excess of the TDI is not likely to be a health concern. However, children aged 1~2 years may have renal systems that are comparatively more sensitive to the crystallization of melamine and its analogues. Therefore, governmental jurisdictions may need to practice more prudent management of food items that could raise the melamine exposure for this population.

## P049-A2

### Determination of Free Radical Scavenging Activity of Cabernet Sauvignon, Merlot and Pinot Noir Wines Producing of Different Clones of Grapes and its Correlation with Polyphenolic Constituents

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Wines contain a wide range of polyphenolic constituents, primarily flavonols and anthocyanins that have showed anticancer, and anti-inflammatory effects *in vitro*, as well as the ability to block cellular events predisposing to atherosclerosis and coronary heart disease (Temple, 2000). The amount and types of phenolics present in wines may play an important role in controlling oxidation in the human body. However, the concentration and composition of phenolic compounds in red wine is variable depending on grape species, variety, season, and a wide range of environmental and management factors such as climate, soil conditions, and may be modified during vinification (production of wine). Recent research indicates that different clones, with distinctive enological characteristics, have been identified in many grape cultivars (Arozarena et al., 2002).

For these reasons, the objective of our research was to consider the changes in the free radical scavenging activity and content of phenols, flavonols, tartaric esters and anthocyanins of several clones of Cabernet Sauvignon, Merlot and Pinot noir wines producing of grapes grown in the same Serbian vineyard, 2008 year. The antioxidant activity of the wine samples was analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>·</sup>) assay. The phenolic composition of experimental wines made from grapes of those clones was checked, to determine if differences in the free radical scavenging activity of wines made with them are affected by characteristics of those clones. The results show, the free radical scavenging activity is correlated with the content of total phenolics, tartaric esters, flavonols and monomeric anthocyanins, expressed as mg/l wine, in experimental samples. The wines present slight differences in their antioxidant activity and their polyphenolic constituents, depending of the used clone. This fact may be related to several factors, some of them connected to reactions undergone by phenols, flavonols and anthocyanins in wines. It could be concluded that the differences observed in the phenolic composition of Cabernet Sauvignon, Merlot and Pinot noir wines made with grapes of different clones are related to agroclimatic factors, linked to year of production, and genetic differences among clones.

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## P050-A2

### Determination of Phenolic Composition and Antioxidant Activity of Selected Serbian Fruits

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*Natural antioxidants*, particularly in fruits have gained increasing interest among consumers and the scientific community. Epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Temple, 2000). This beneficial effect is primarily attributed to the occurrence of phenolic composition, carotenoids, vitamins and minerals. Over 8000 phenolic compounds have been identified from plant materials. These compounds have been categorized into different groups such as phenolics, flavonoids, flavonols, anthocyanins, tannins etc. The quantity and the composition of phenolic compounds in fruits are influenced by genotype, storage conditions, extraction procedure, and environmental conditions (Robbins, 2003).

The objectives of this study were to determine the content of total phenolics, flavonoids, flavonols, tartaric esters, anthocyanins, polymeric color (tannins) and antioxidant activity in originally Southern Serbia's fruit extracts of strawberry (*Fragaria vesca*), blackberry (*Rubus fruticosus*), raspberry (*Rubus idaeus*) and sour cherry (*Prunus cerasus*). The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging assays and was ranged from 90.87% in strawberry to 94.13% in cherry extract. The contents of investigated phenolic compounds showed highly correlation with the total antioxidant capacity of investigated fruits. The highest phenolic compounds contents were observed in a raspberry and the lowest were observed in a strawberry among selected fruit extract. The polymeric colour (tannins) ranged from 20.81% in cherry to 50.73% in raspberry fruit extract. Evaluation of antioxidant compounds showed its considerable levels in frozen fruits and suggested their health-promoting properties. The effects of light and temperature on total phenol content in methanolic extracts of selected fruits were examined in an experimental setting designed to mimic storage conditions. Total phenolics in control samples kept in the dark at 8°C were not significantly different from those exposed to the light and kept at 20°C during 60 days. In conclusion, all of these results show that the investigated fruit extracts can be used as an easily accessible source of natural antioxidants and as a possible food supplement.

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## **P051-A2**

### **The Monitoring of Pb, Cd, Hg and Cr(VI) in Food Packagings**

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The goal study monitored the amount of residue of the Cd, Pb, Hg, and Cr(VI) in food packagings. We investigated total 370 samples in the circulated from domestic. . Amounts of Cd Pb, Hg, and Cr(VI) in food packagings were detected at low concentration or not detected and the level of metals were below to standard of Korea Food Code and EU. And also these metals were controled of below 100mg/kg for total concentration in Korea and EU. Hg was detected and the result of quantitative analysis range from 0~0.06mg/kg, Cr(VI) was 0~0.1mg/kg, Pb was 0~26.3mg/kg and Cd was 0~1.3mg/kg.

And we developed the analytical methods of metals and verified the suitable of the methods through validation. Hg was developed using Mercury Analyzer and Cr(VI) was developed using diphenylcarbazide method with UV-VIS spectrophotometer. Pb and Cd was analyzed with ICP-AES(Inductively Coupled Plasma Atomic Emission Spectroscopy). The limits of quantification of metals, respectively, Hg : 2.1µg/L, Cr(VI) : 5µg/L, Pb : 27.82µg/L, Cd : 0.15µg/L.

## P052-A2

### Comparison of Different QuEChERS for Organochlorine Pesticides Determination in Strawberries

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Organochlorine pesticides (OCPs) are an important group of contaminants and they had widespread use around the world. Despite their banning several years ago, the analysis of OCPs residues are of special interest as their high chemical stability results in their persistence and bioaccumulation in the environment [1].

The aim of this study is to develop an analytical method to determine 12 OCPs (hexachlorobenzene, aldrin, 4,4'-dichlorobenzophenone, endosulfan I, dieldrin, p,p'-DDE, endrin, endosulfan II, p,p'-DDD, o,p'-DDT and methoxychlor) in strawberries obtained from integrated pest management (IPM) farming. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method has been readily accepted for many pesticide residues analysis in food [2] so it was selected for strawberries sample preparations. The procedure involved the extraction of 10g of homogenized strawberries samples in a Teflon centrifuging tube with 10mL acetonitrile. The pesticides are extracted from the matrix into organic phase with vortex mixer. Three types of QuEChERS have been studied: i) 4g anhydrous magnesium sulfate, 1.0g sodium chloride, 1.0g trisodium citrate dehydrate, 0.5g disodium hydrogencitrate sesquihydrate; ii) 6g anhydrous magnesium sulfate, 1.5g sodium chloride, 1.5g trisodium citrate dehydrate, 0.75g disodium hydrogencitrate sesquihydrate and iii) 4g anhydrous magnesium sulfate and 1g sodium chloride. After centrifugation, the supernatant was placed into a tube containing 150mg primary secondary amine (PSA) sorbent plus 150mg anhydrous magnesium sulfate and 50 mg C18, which constituted a cleanup procedure. After a second shaking and centrifugation step, the acetonitrile extract was transferred to autosampler vials for analysis by gas chromatography with electron capture detection (GC-ECD).

Recoveries obtained were between 39.12-161.57% for p,p'-DDE and lindan, respectively, and repeatability less than 10% for all of fortified pesticides. The highest recoveries for the studied OCPs were obtained with QuEChERS extraction tubes with 4g anhydrous magnesium sulfate, 1.0g sodium chloride, 1.0g trisodium citrate dehydrate, 0.5g disodium hydrogencitrate sesquihydrate.

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## P053-A2

### Application of High Resolution Continuum Source Flame Atomic Absorption Spectrometry to Sequential Analysis of Fe and Mn in Instant Coffee Substitutes

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The estimation of nutritionally important mineral elements in food and beverages can be carried out by different spectrometric techniques, most frequently by line-source flame atomic absorption spectrometry (LS FAAS). The applicability of LS FAAS has been further extended with the development of high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS) [1]. The major features of this technique are the use of a single radiation source for all analytes and wavelengths, the visibility of the spectral environment around the analytical line at high resolution, and its unsurpassed background correction capabilities that make it suitable for the direct analysis of complex samples.

The aim of this study was to optimize and apply sequential multi-element determination of Fe and Mn in instant coffee substitutes by HR-CS FAAS. Fe and Mn are among micronutrients most frequently required in food analysis. A total of 50 instant coffee substitutes, representing the brands available in the Portuguese market, were analyzed. The beverages were prepared by dissolution in hot water and filtration. All measurements were carried out using an Analytik Jena ContraAA 300 spectrometer equipped with a xenon short-arc lamp operating in hot-spot mode (XBO 301, 300W, GLE, Berlin, Germany). Air-acetylene oxidizing flame for the atomization was used and main absorption lines of both elements (248.3270 and 279.4817 nm, for Fe and Mn, respectively) were selected. Metal contents were determined with multi-element calibration curves in the range 0.11-5.50 mg/L and 0.026-1.30 mg/L for Fe and for Mn, respectively. Data are discussed in accordance with the beverage composition (coffee, chicory, malt, barley and rye) as well as their contribution to daily ingestion. This study will be extended to sequential determination of other minor elements in instant coffee substitutes.

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## **P054-A2**

### **Imaging the Inner Structure of Corn Starch Granules Using Atomic Force Microscopy**

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Starch granule is made mostly of two materials, amylose and amylopectin which are macromolecular polymer of glucose. Although the molecular structures of the starch have been investigated in detail, the hierarchic structures of a starch granule in molecular level are still unknown. Recently, a blocklet model that the starch granule consists of two kinds of blocklets with different size (~500 nm and ~50 nm) and crystallinity (hard and soft) was proposed and the both sizes of blocklets have been observed by scanning electron microscopy (SEM). Atomic force microscopy (AFM) images of both the granular surfaces also supported the blocklet models but only the smaller size (~50 nm) of blocklet has been detected in the section surfaces.

Thus, we developed a resin-embedded section method specific to the starch and performed the structural investigation of the growth ring by AFM. As the results, both the big and small blocklets were clearly observed within the growth rings in the section of corn starch granule by AFM. Furthermore, we found a new structure with fibrous form (10~12 nm in height) at a center of a granule, which might be a cluster structure of amylopectin molecule with a diameter of 10~15 nm. The resin-embedded method combined with AFM can be used to analyze structure of various biological sample including botanical starch granules and the other food-materials with hierarchical structure from nanometer to micrometer.

This research was supported by "Food Nanotechnology Project," MAFF, Japan.

## **P055-A2**

### **Evaluation of Shelf Life and Heat Treatment of Fruit Baby Food**

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Model storage experiments of fruit baby food were carried out. The sets of samples were stored at various storage temperatures (40, 55, 70 and 90°C for 4 weeks), during the storage the selected chemical markers were followed and correlated with the sensory evaluation, the markers were: DPPH, total polyphenols, ascorbic acid, 2-furaldehyde, 5-hydroxymethyl-2-furaldehyde (HMF) and colour (L, a\*, b\*, ΔE). The kinetic data (reaction rate constants, activation energies, Q10, z values etc.) were calculated, the applicability of the followed parameters for the shelf life prediction or for the evaluation of the heat treatment intensity was considered.

## **P056-A2**

### **Quality and Authenticity of Salt-Water Fish Fillets**

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The frozen fillets of different species of salt-water fish were analysed. The three different calculating methods for the fish meat content in frozen fish fillets were compared. The presence of additives (polyphosphates and salt in the couple with citric acid) was measured. Model technological experiments were carried out. The influence of technological procedures (repeated freezing, soaking under mechanical stress) on content of fish meat in frozen fish fillets was studied. During the technological model experiments the selected chemical markers were followed: dry matter, ash, total phosphorus, fat, nitrogen (Kjeldahl).

## P057-A2

### Method Development for Simultaneous Determination of Sudan Group Colorants in Foodstuffs by Liquid Chromatography–Tandem Mass Spectrometry

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The group of color additives known as Sudan dyes and their degradation products are considered to be genotoxic carcinogens for animals. Although the EU and USA does not permit the use of Sudan colors as food pigments [1], these dangerous substances are still used in order to mimic or intensify the color of different chili powders and attention have been given to the achievement of new analytical techniques and instruments for the Sudan dyes trace analysis [2-6]. In the present work a rapid and efficient liquid chromatography–tandem mass spectrometry method was developed for the determination of Sudan dyes (Sudan I through IV) in foodstuffs using a liquid chromatograph-mass spectrometer Agilent Series 1200 system with a triple quadrupole analyzer. A column with ZORBAX SB-C18 5 stationary phase was used and formic acid-acetonitrile mixture (A) and a formic acid in water (B) were employed as mobile phases. Successful separation was obtained for all four Sudan Red colorants (Sudan I, II, III, IV) using an optimized gradient elution within 3 min. For the extraction of colorants from different food matrices a simple pretreatment procedure was applied. The method was applied to the determination of these compounds in chili spices, spice mixtures, curry powder and tomato based sauces.

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## P058-A2

### Antioxidant Activity and Total Phenolic, Organic Acid And Sugar Content in Selected Turkish honeys

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This study reports the antioxidant activities and total phenolic, individual organic acid and sugar contents of selected honeys from the Black Sea region and Aegean coast of Turkey. Antioxidant activities of the honeys were examined by two different methods, namely scavenging of free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ferric reducing/antioxidant power (FRAP). Total phenolic contents were determined by using Folin-Ciocalteu reagent as catechine equivalent. There was a significant linear correlation between total phenolic contents and antioxidant activities of the all samples. Among the honey products studied, chestnut honeys and pine honey were the richest in total phenolic amount and showed the highest antioxidant activities. The individual organic acid and sugar contents were determined by capillary zone electrophoresis. Gluconic acid was found as the predominant organic acid in all honey samples ranging between 1.50-13.8 g/kg. Glucose content changed between 22.35 and 42.24 g/100g, and fructose content changed between 31.02 and 64.22 g/100g. Fructose/glucose ratios of honey samples found as between 1.18 and 1.75.

## **P059-A2**

### **Determination of Carnosic Acid and Rosmarinic Acid in Sage by Capillary Electrophoresis**

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A simple and rapid capillary electrophoresis method was developed for the identification and quantitative determination of two antioxidative compounds – carnosic acid and rosmarinic acid – in the extracts of commercial Sage (*Salvia officinalis*) tea-bags. Capillary zone electrophoretic separation of carnosic and rosmarinic acids was performed using 40 mmol/l borate, at pH 9.6 as the running buffer. Weighed sage samples were extracted from tea-bags by sonification and the extracts were directly injected without any purification and pre-separation process. Coumarin was used as internal standard for quantitation and the limits of detection for carnosic acid and rosmarinic acid were obtained as 2.79 and 3.18 µg/ml, respectively using UV detection at 210 nm.

## P060-A2

### Development of Inductively Coupled Plasma Atomic Emission Spectrometry for Arsenic Determination in Wine

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Simple and fast method for determination of arsenic species and total arsenic in different wine samples by hydride generation-inductively coupled plasma atomic emission spectrometry (ICP-OES) was described. In order to find optimal conditions for analysis of arsenic species various effects on signal intensity were investigated. They included: concentration of the reaction medium (hydrochloric acid) and the reducing agent (sodium tetrahydroborate(III)) for the arsines generated from solutions of arsenite, arsenate and dimethylarsenic acid (DMA). Various generator power (RF) and flow of the carrier gas were also investigated. Effect of ethanol addition on spectral line intensity was especially investigated as well as the possibility for arsenic determination in wine samples without prior sample preparation. Optimal conditions for DMA determination were: 0.005 M HCl and 0.02 % NaBH<sub>4</sub>; for As(III): 4 M HCl and 0.1 % NaBH<sub>4</sub> and for (As(III) + As(V)): 8M HCl and 2 % NaBH<sub>4</sub>. Total arsenic was determined after microwave digestion. The method was applied on arsenic determination in 6 wine samples originated from Serbia. The results were compared to the standard addition method and good correlation indicated that there was no effect of the wine base. Relative standard deviation was 0.13%. The limit of detection was 0.05 µg/L. Arsenic concentration in wine samples depends on various factors including the effect of aerosol on grapes, climate conditions, soil quality, pesticide usage and the wine storage conditions. It is difficult to predict oxidative states of arsenic in wines, organic and inorganic part as there are different data in literature.

## P061-A2

### Identification of Meat Spoilage from FTIR Data Using the Symbiosis Data Analysis Framework

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Symbiosis is a web-based data integration and analysis system that allows collaborative analysis of large datasets using an easily expandable toolkit of chemometric techniques. The system is being developed to support the FP7-funded SYMBIOSIS-EU project ([www.symbiosis-eu.net](http://www.symbiosis-eu.net)). In this poster we present some novel research as a case study of Symbiosis in use. The aim of the research was to evaluate the most effective data analysis workflow for identifying meat spoilage in minced beef from data obtained from Fourier transform infrared spectroscopy (FTIR). FTIR has previously been shown to be capable of detecting microbial metabolites that are indicative of meat spoilage, and therefore holds promise as a rapid method for identifying spoiled meat.

The Symbiosis framework comprises a database capable of accepting heterogeneous data, and an interface to the R statistical programming language that allows R scripts to be applied to the stored data. Symbiosis maintains a library of R scripts for common analysis techniques, but additional scripts can easily be added. For this application, FTIR data was uploaded to the system and a number of different pre-processing and classification approaches evaluated. Evaluation was achieved using a thorough model validation approach. The results show that it is indeed possible to discriminate between fresh meat and spoiled meat, though borderline cases do pose some difficulty. In conclusion, this work proved to be a useful exercise both for evaluating best way to deal with the FTIR data in this particular application, and for testing the Symbiosis framework. The software continues to be developed and we welcome any suggestions for additional functionality or applications.

## P062-A2

### Development and Optimization of a Cleanup Procedure for the Determination of PCBs in Mussel Samples by Gas Chromatography-Triple Quadrupole Tandem Mass Spectrometry

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PCBs are characterized by a diffused presence in the environment, due to the combination of their characteristics of persistence and the widespread use that has been done in the past. In fact, even though PCBs are banned under the 2001 Stockholm Convention, they are still frequently detected in various food samples owing to their lipophilic and bioaccumulation properties. Since these compounds exhibit a high affinity for lipophilic matrices, mussels have been considered an interesting target for this investigation, in order to estimate their potential use as biological indicators and/or accumulators of environmental contaminants. Moreover, PCBs contaminated mussels are routes for direct entry into human beings, and then it is important to monitor and identify these pesticides and to prevent these residues from constituting a risk for human health.

Several methods for the determination of PCBs in food are available in the European Committee for standardization guideline (EN 1528-1-2-3-4), where different strategies for extraction, cleanup and quantitative analysis of PCBs are indicated.

In this work a new extraction cleanup method for PCBs in mussel samples has been developed. It is based on the lyophilization of the mussels and the successive treatment of the fat dried sample by n-hexane, concentrated sulphuric acid and ENVI-carb. The final residue, obtained by solvent evaporation, has been dissolved in isooctane and analysed by a gas chromatograph triple quadrupole in Selected Reaction Monitoring (SRM) mode. The optimization of the collision energies in terms of sensitivity, selectivity and structural information for quantitative purposes has allowed for each analyte the selection of a precursor ion and two significant MS/MS signals.

The method has been validated by the analysis of several spiked real samples and a certified material (BCR 682), and the relevant performance parameters, such as recoveries, repeatability, reproducibility, have been evaluated.

Keywords: Mussels; PCBs; GC/MS Tandem; extraction cleanup.

## P063-A2

### Comparative Analysis of Wort Oligosaccharides by Means of HPLC-RI and -MS Techniques Employing Different Types of Chromatographic Columns

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Carbohydrates play very important role in huge amount of processes taking place in living cells. The efficient approach to the structure elucidation of oligosaccharide isomers involves combination of HPLC with soft techniques of MS.

Our study was focused on the chromatographical behavior of (iso)maltooligosaccharide samples performed on three advanced columns. A porous graphitized carbon (PGC) stationary phase, as well as phases based on HILIC interactions (aminopropyl, zwitterionic) were investigated. Under the optimized conditions effective and reproducible separation of the oligosaccharides with the different degree of polymerization were obtained.

The method performance was demonstrated for the analysis of wort samples by applying of subsequent chromatographic steps on a pair of columns. Thus, selected fractions obtained from the first column were subjected to second one. Our results indicate that pre-separation on amino column followed by the chromatography on PGC phase was the best choice for LC-MS analysis of complex mixtures of oligosaccharides.

The pool of separated oligosaccharides was detected by on-line and/or off-line electrospray mass spectrometry. The structural analysis and identification of these compounds was based on tandem MS experiments and on the comparison with linkage-specific fragmentations of dextrin standards. The isobaric species were characterized by the abundance and the relative ratio of  $[M-H-nHex]^-$  and  $[M-H-nHex-H_2O]^-$ , as well  $[M-H-nHex-60]^-$ ,  $[M-H-nHex-78]^-$ , and  $[M-H-nHex-90]^-$  ions.

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## P064-A2

### Macrominerals Composition of Sardine (*Sardine Pilchardus*) by Microwave-Assisted Digestion and Flame Atomic Absorption Spectrometry

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Dietary habits are among the major society concerns, and it is considered fundamental the quality and amount of the nutrients ingested for good health and well being. Among the products available, fish is currently considered one of the most interesting. Fish is healthy, nutritious, and highly essential in a balanced diet, being an important source of essential minerals, proteins and lipids of high biological value (with long chain polyunsaturated fatty acids) and also liposoluble vitamins [1].

Throughout the year, fishes are subjected to considerable environmental changes and fluctuations in the availability and compositions of their feed, which can affect their morphological and chemical composition. Minerals contents in seafood are slightly higher than those in terrestrial animals and can present potential as therapeutic components in the diet.

The total contents of minerals such as sodium, potassium, calcium, magnesium, and phosphorus and microelements such as selenium, fluorine, iodine, cobalt, manganese, and molybdenum in raw marine fish muscle are roughly in the range of 0.6–1.5% wet weight [2]. In the present study, fresh samples of Sardine (*Sardine pilchardus*; a highly appreciated fish in Portugal) were characterized concerning their macromineral content (Na, K, Mg and Ca). The relevant morphological characteristics were also recorded. Homogenised edible samples were digested in a microwave-assisted digestion procedure (CEM, Mathews, NC, USA) and quantitative determination was performed by flame atomic absorption spectrometry (FAAS).

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## P065-A2

### FTIR Wine Analysis Evaluation Using an Interlaboratory Test

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Commercially available Fourier Transform Infrared (FTIR) instrumentation with versatile and innovative software, designed specifically for grape and wine analysis, has recently received much attention [1]. In the last decade the number of these equipments in medium and large wine laboratories has increased enormously.

This technology has brought countless analytical advantages, such as time saving and high resolution. Its application to wine analysis provides excellent results in terms of precision and accuracy [2]. Nevertheless, care should be taken during validation procedures [3]

In Portugal, a significant large number of laboratories (38) set up a workgroup (CIVP – Portuguese Wine Interlaboratory Circuit) to create an interlaboratory test for both still and fortified wine. This test has been carried out in a monthly basis since 2001. In the present format (since March 2007) the test is carried out for both FTIR wine analysis and common routine methods. A total for 25 parameters are analyzed and compared (ordinary parameters, organic acids and some inorganic and chromatic parameters). This enables not only the evaluation of the performance of each laboratory but also of the exactnesses of the FTIR technology.

The results from March 2007 to February 2009 were used to compare the precision of the FTIR wine analysis and also the exactness when compared to ordinary reference methods, for all 25 parameters. Precision for both analytical methodologies are very similar. Very good exactness results were obtained, especially for the most common parameters in routine wine analysis, the ones for which this kind of equipment is very reliable and intended for.

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## **P066-A2**

### **Changes of Antioxidant Capacity of Robusta and Arabica Coffee During Roasting and Storage**

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Coffee is believed to be the most important source of natural antioxidants in the diet. The antioxidant capacity of coffee is based mainly on the phenols, but during the roasting phenols are degraded and new products with antioxidant capacity are formed. A major contributor to the antioxidant activity was identified as N-methylpyridinium, which is formed by degradation of trigonelline during the roasting process. The several parameters including water content, antioxidant capacity, the trigonelline, chlorogenic acid and polyphenols content, brown pigments formation were followed during the usual roasting procedure and storage of Arabica and Robusta coffees. During the roasting and subsequent storage the total antioxidant capacity (TAC) decreased to the about half level and the a slow decrease continued also during the storage of roasted coffee beans of the both varieties. No trends were observed in the total polyphenols content, probably due to the not very specific analytical procedure. Trigonelline decomposed, it was found about 60 % of initial value at the end of the roasting process and also a degradation continued slowly within the storage. Free phenolic acids decomposed in the initial stages of roasting, than the level varied around 20 % of initial content.

The correlation matrix was calculated for the all measured parameters. Correlation was found between the TAC and trigonelline, the TAC value correlated also with colour and water content in coffee.

## P067-A2

### Fat Content and Fatty Acid Composition of Traditional Dry Cured Sausages from Portugal

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Fermented sausages are traditional products used for meat preservation, since the earliest civilizations. Dry cured sausages are common in Portugal, being characterised by a high sensory quality due both to the intrinsic characteristics of the raw material selected from pigs and condiments. Besides, they are key products in gastronomic tourism from several regions of Portugal.

The aim of this project was to determine the total fat content and composition of eighteen samples of dry cured sausages from different geographical origins of Portugal. Special attention was devoted to its fatty acid composition (cis/trans isomers, saturated, monounsaturated and polyunsaturated fatty acids and the n6/n3 ratio). Total fat was quantified by the Soxhlet method (using petroleum ether as solvent). For the determination of the fatty acid composition, total lipids were extracted according to Folch *et al.*, using dichloromethane instead of chloroform. Fatty acids were determined by GLC/FID/capillary column as methyl esters (FAMES) prepared by esterification with methanolic potassium hydroxide solution followed by BF<sub>3</sub>/MeOH and extraction with *n*-heptane.

Total fat contents ranged from 25 to 46 g/100g. Concerning the fatty acid profile, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 *cis*9) and linoleic acid (18:2n6) were the most representative fatty acids in all samples, as expected for a typical profile of processed pork meat products.

Monounsaturated fatty acids (MUFA) were the major fatty acids present and ranged from 4 to 25 g/100g. *Trans* isomers were present in mean levels of 0.66 g/100g. The ratio n6/n3 is about 12.

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#### Acknowledgments:

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## P068-A2

### Bioaccessibility of Calcium and Phosphorus in Milk Samples

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Milk is an essential macro and micronutrients source for growth, development and health, and as one of the animal protein sources for all age's humans. This work aimed to study *in vitro* bioaccessibility with simulated gastric digestion in the Ca and P levels in samples of different kind of milk (UHT cow's milk, raw cow's milk, raw sheep milk, UHT goat's milk and soya's "milk"). The experiment was based on simulation of gastrointestinal digestion with pepsin-HCl during the gastric stage and pancreatic-biliary salts during the intestinal stage. The fraction of an element diffusing through a semi-permeable membrane during the intestinal stage is measured to predict the element's dialyzability. Microwave sample digestion was used in order to access the total amount of analytes. Referring to total, the percentages of dialyzed calcium were around  $10 \pm 1\%$ ,  $20 \pm 1\%$ ,  $12 \pm 1\%$ ,  $17 \pm 1\%$  and  $16 \pm 1\%$ , to raw sheep's milk, raw cow's milk, UHT cow's milk, UHT goat's milk and soya's "milk" samples, respectively. The results of dialyzed phosphorus were  $10 \pm 1\%$ ,  $30 \pm 1\%$ ,  $32 \pm 1\%$ ,  $36 \pm 3\%$  and  $20 \pm 1\%$  for raw sheep's milk, cow's milk, UHT cow's milk, UHT goat's milk and soya's "milk" samples, respectively. It may be observed higher amount of phosphorus obtained by dialysis than calcium, except for the raw sheep's milk sample, with similar results. Calcium ranked dialysis percentage was: raw cow's milk > UHT goat's milk > soya's "milk" > UHT cow's milk > raw sheep's milk. Phosphorus ranked dialysis percentage was: UHT goat's milk > UHT cow's milk > raw cow's milk > soya's "milk" > raw sheep's milk. The results confirmed the methodology efficiency. *In vitro* method allows a preliminary estimate of the bioavailability of nutrients.

FAPESP, CNPq, CAPES

## P069-A2

### Mycotoxin Determination in Cereals by Liquid Chromatography-Tandem Mass Spectrometry with Atmospheric Pressure Photoionization Source

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Mycotoxins are secondary metabolites produced by various species of filamentous fungi, growing under a wide range of climatic conditions on agricultural commodities (cereals, spices, fruit, oleaginous seeds, coffee, nuts, etc.) in the field and during storage [1]. Mycotoxin occurrence in food, beverages and feed is caused by direct contamination of plant materials or their products [2], or by a carry over of mycotoxins and their metabolites into animal tissues, milk and eggs after intake of contaminated feed [1].

The main five genera of fungi, *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Claviceps*, produce mycotoxins belonging to eight groups that are relevant in food industry: aflatoxins, citrinin, fumonisins, ochratoxins, patulin and other small lactones, trichothecenes, resorcyclic lactones and ergot alkaloids. Mycotoxins whose maximum levels in cereals are listed in the European Regulation [3], i.e., the main four aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, T-2 toxin and HT-2 toxin, were investigated. One g of wheat or millet was extracted with 6 mL of mixture acetone/water/acetic acid 80/19/1 under sonication for 20 minutes. After centrifugation, 4 mL of the extract were collected and solvents removed under a nitrogen stream at 40° C. Then, the residue was reconstituted with water/methanol 80/20 and, after filtration and without any further clean-up step, an aliquot was analyzed by liquid chromatography-tandem mass spectrometry employing an atmospheric pressure photoionization (APPI) source. Compared to an electrospray interface, the APPI presented the advantage to have a reduced signal suppression due to matrix coeluting components. Method limits of quantification resulted suitable for application of this multiresidual method to the cereals selected, in compliance with European Commission limits.

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## Chemical View on Walnuts from Country-Side of Kragujevac

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It is well established that consumption of walnuts has benefits to human health. The walnuts kernel contains ~60% oil, in which linoleic, oleic and linolenic acids predominate, many essential amino acids, sterols, carbohydrates and vitamins.

Phenolic compounds from walnut fruits have a positive influence on human health such as a decrease of coronary heart diseases, prevention of several kinds of cancer and antimutagenic activities. *In vivo* studies reported that daily consumption of walnuts significantly lowers total and LDL cholesterol, and that omega-3 fatty acids reduce inflammation, which is a key component in the processes that turn cholesterol into artery-clogging plaques.

Walnuts are a very good source of biometals. Some of them are essential cofactors in a number of enzymes important in antioxidant defenses.

In the present work, we have investigated the walnuts (*Juglans regia* L.), which naturally occurs in Šumadija (central Serbia), and had not been chemically analyzed until now. The concentration of metals in the walnuts kernel samples from Kragujevac country-side, were investigated by flame atomic absorption spectrometry. Dry samples were chopped, homogenized and then fired in the firing furnace at 550 °C. The rest was solved in concentrated HNO<sub>3</sub> and hydrogen peroxide solution, moved to already measured vessels of 50 ml and underwent to analysis.

GC-MS analyses of lipophilic compounds from walnuts kernel oil were performed by using Agilent 6890N (G 1530N) (Serial# CN10702033) gas chromatograph, fitted with a HP-5ms column and Agilent mass selective detector 5975B (Agilent Technologies, USA) (G 3171A) (Serial# US65125280). The injector and detector temperature were 290 °C, and the oven was programmed from 150 to 290 °C. The injector volume was 2 µl, with a split ratio 20:1.

Very interesting sample is one that contains significant amount of vitamin E and stigmasterol (for difference from other samples in which unsaturated fatty acids are predominant) and manganese, known as "antistress" mineral.

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## P071-A2

### Simultaneous Determination of Halophenols and Haloanisoles in Wine Using Dispersive Liquid-liquid Microextraction

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The enological industry needs to produce high quality wines capable of standing out in the highly competitive current market. One of the most extended problems in wine making with a negative impact on wine quality is the appearance of some compounds producing the so-called cork taint, characterized by moldy-musty off-flavor.

Haloanisoles (2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA) and 2,4,6-tribromoanisole) are the main compounds responsible for cork taint. They originate from a defensive reaction of some microorganisms through the biomethylation of their corresponding halophenols, present in wine due to the use of polluted materials in cellars. Since halophenolic compounds could develop into haloanisoles, their determination is of great interest to the wine industry. It must be taken into account that, due to their high polarity, halophenols must be derivatized prior to chromatographic analysis.

Dispersive liquid-liquid microextraction (DLLME) is a novel extraction method in which a extraction mixture of disperser and extraction solvents is rapidly injected into aqueous samples, forming a cloudy solution then centrifuged to separate the organic extract.

In this work, a DLLME-derivatization procedure was developed and optimized for the simultaneous determination of haloanisoles and halophenols in wine by means of gas chromatography with electron capture detection. An exhaustive study was carried out in order to select the appropriate extraction mixture by examining all combinations of disperser (methanol, acetone and acetonitrile) and extraction solvents (chloroform, chlorobenzene and carbon tetrachloride) considered. Salt effect was also studied so that it could be obviated in a subsequently experimental design to optimize the simultaneous extraction and derivatization processes. In this context, a Central Composite Design (CCD) was applied to analyze the influence of four factors (i.e., disperser and extraction solvent volume, acetic anhydride (derivatization reagent) and  $K_2CO_3$  solution) and find out their optimum values.

## P072-A2

### Rapid Detection of *Flavobacterium psychrophilum* with Magnetic-Fluorescent Beads and Flow Cytometry

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*Flavobacterium psychrophilum*, the aetiologic agent of bacterial coldwater disease and rainbow trout fry syndrome, has emerged as one of the most significant bacterial pathogens in salmonid aquaculture worldwide. We have been studying the detection of harmful bacteria using immunomagnetic separation and flow cytometry (FCM). The bacteria were first reacted with fluorescein isothiocyanate (FITC)-labeled antibody and then with magnetic-conjugated antibody, and collected with a magnet and detected by FCM. These methods are effective, but complex because they require two antigen-antibody reactions. These methods can also be used to detect specific bacteria, but cannot be used to determine bacteria viability. Therefore, in the present study, we used fluorescent magnetic beads and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). Bacteria were specifically collected by fluorescent magnetic beads with only one antigen-antibody reaction. Prokaryotes that are able to reduce CTC are considered to be respiring bacteria, because CTC is involved in the electron transport chain and is reduced instead of oxygen. CTC turns into a red fluorescent formazan that is detectable by FCM. *F. psychrophilum* were stained by CTC and labeled with fluorescent magnetic beads. Double-stained bacteria (red fluorescence by CTC and green fluorescence from fluorescence magnetic beads) were detected with FCM. Bacterial cell numbers were determined by FCM and compared with those measured using a traditional colony counting method in the range of  $10^2$ ~ $10^8$  cells per milliliter. A good correlation was observed between the range of  $10^2$ ~ $10^8$  colony-forming units per milliliter. The FCM assay could provide a bacterial cell count within 1 min and the total assay time, including sample preparation, was less than 3 hours.

## P073-A2

### **A Bottom Up Metabolic Profiling Approach for the Detection of Potential Stress Markers in Pinot Noir Grapevine Leaves**

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Identifying stress markers is a major topic in plant biology. In our case the long term goal of the project "Physiological Fingerprinting in Viticulture" is to provide tools for the improvement of quality of grapes by measuring grapevine vitality in the vineyard. To achieve this several physiological as well as chemical parameters (water potential, chlorophyll fluorescence, gas exchange, sugar and polyphenol content) are measured. In addition to known stress markers (sugars, polyphenols) we aim to find new ones which are associated with the physiological state of grapevine. In this study we used headspace - solid phase microextraction (HS-SPME) coupled with gas chromatography - mass spectrometry (GC-MS) for the identification of stress associated metabolites. An in-house GC-MS - library was compiled covering more than 300 volatile metabolites occurring in grapevine plants respectively in the leaves of plants of the same botanical order (Rhamnales) by literature review. For identification of compounds we used the AMDIS software package (Automated Mass Spectrometry Deconvolution and Identification System) which compares the mass spectra of the sample with the library and checks if the LTPRI (Linear Temperature Programmed Retention Index) fits within predefined limits. Our strategy is first to identify as many substances in a leaf sample as possible and subsequently compare the expression levels of these metabolites in two different physiological states. With principal component analysis (PCA) it is possible to differentiate two very similar Pinot Noir clones (18 Gm and 1-84 Gm) which are varying only 4 base pairs.

We could identify 70 substances in the leaf samples, whereas, - to our best knowledge - 30 of them are described to occur in grapevine leaves for the first time. Trans-Geraniol is one of the identified substances and occurs in significant higher concentration in the 18 Gm clone.



## P074-A2

### Validation of High Performance Liquid Chromatographic Method, for the Simultaneous Determination of Pesticides in Potato Samples

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Pesticides in foodstuff are becoming a major issue due to their intensive use in agriculture. Thus an appropriate control of their residues in food samples has to be carried out.

In this study, a HPLC-DAD method has been developed for the simultaneous analysis of: acetamiprid, Imidacloprid, Thiacloprid, as neonicotinoid insecticides, tebuconazole as a fungicide and tetramethrin, deltamethrin and bifenthrin as pyrethroid insecticides, in potato samples.

Ultrasonic assisted extraction procedure, using ethyl acetate as extractant, has been applied to samples previous HPLC determination. The chromatographic conditions used for pesticides analysis were: Symmetry C-18 column (75x4.6 mm, 3.5  $\mu$ m), gradient elution mode with a mobile phase of H<sub>2</sub>O:ACN, flow rate of 1 mL/min and the pesticide Bendiocarb was used as internal standard.

Validation of HPLC-DAD method was made in terms of: linear concentration range, limit of quantitation, repeatability, accuracy, stability and robustness. The validated method was applied to potato blank and fortified potato.

**Keywords:** Ultrasonic extraction; pyrethroids; neonicotinoid, fungicide; potato sample; HPLC-DAD.

## P075-A2

### Trace Elemental Analysis of Strawberries and Strawberry Juices

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Strawberries (*Fragaria × ananassa*) are fruits being very much consumed around the world. They represent a good source of ascorbic acid, anthocyanins and flavonols and which have a high antioxidant activity. Thus they are a useful contributor to healthy nutrition. However not the organic components are of great importance, but also the trace and ultratrace elements contained in the berries as well as in the juices prepared from them. On the one hand the concentrations of toxic elements have to be controlled, on the other hand the amount of essential elements is of interest. In this research work strawberries grown in Croatia and in Austria and strawberry juices produced in Croatia, Slovenia and Austria were analyzed for their trace elemental content. After a digestion procedure the concentrations of 27 elements, namely Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sc, Se, Si, Sm, Sn, Sr, Tl, and Zn were determined by inductively coupled plasma – optical emission spectrometry (ICP-OES).

Reference ranges for the elements investigated were established for berries and juices. The results for the strawberries from Croatia were compared to those from Austria in order to see their geographical differences. Furthermore the results for the fresh strawberries and the strawberry juices were compared.

## P076-A2

### Multiresidual Detection of Five Coccidiostats Used in Zootechny

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A relatively simple, selective liquid chromatography-tandem mass spectrometry (LC-MS-MS) method has been developed and validated to analyze residues of five coccidiostats in eggs : clazuril, diclazuril, robenidine, nicarbazin, toltrazuril and its metabolites. The coccidiostats are medicinal substances of synthesis or fermentation that are administered against the coccidiosis both for prophylactic chemotherapy and for health care.

After a single extraction with acetonitrile and dilution of the organic phase in water, the extract is passed through a polymeric SPE cartridge. The elution is performed by methanol after a washing step with water-methanol (95:5). The eluate is evaporated to dryness under nitrogen stream (50°C) and dissolved in 500  $\mu$ L acetonitrile:water (70:30). Samples are then injected into the LC-MS-MS system on a C<sub>18</sub> column in MRM mode.

The developed method has been validated according to Commission Decision 2002/657/CE. The validation parameters, as linearity, precision, recovery, specificity, decision limit (CC $\alpha$ ) and detection capability (CC $\beta$ ) have been determined. CC $\alpha$  varies from 2,39  $\mu$ g/kg<sup>-1</sup> for nicarbazin to 6,34  $\mu$ g/kg<sup>-1</sup> for clazuril and CC $\beta$  varies from 4,79  $\mu$ g/kg<sup>-1</sup> for nicarbazin up to 7,67  $\mu$ g/kg<sup>-1</sup> for clazuril.

## P077-A2

### The Benefits of High Resolution Mass Spectrometry in Screening Analysis of Mycotoxins in Food

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Screening of pesticides, mycotoxins and veterinary drugs is of great importance in regulated environments such as food and animal feed analysis. Due to the broad variability of physico-chemical properties of the screened residues it is critical to employ very simple sample preparation procedure to maintain the recovery of the broad range of analytes. This however unavoidably leads to the fact that final extracts injected into the chromatographic system contain significant amounts of coextracts. For the chromatographic analysis it is therefore necessary to use the system with high selectivity but still capability to identify potential unknowns.

Traditionally these types of screening experiments have been carried out using SRM scanning with triple quadrupole instruments. This approach has certain limitations: (I) no post acquisition re-interrogation of data (II) limited number of compounds per analysis (III) little possibility to scan for unknown compounds at high levels. Because of these limitations, there is currently a trend towards full scan MS experiments in residue analysis. Current screening approaches employ high performance ToF instruments, with mass accuracies of < 5ppm and resolutions max 15,000, coupled to Ultra High Performance Liquid Chromatography (U-HPLC). However, most of the techniques and instruments currently available suffer from either poor mass accuracy and its variability and more significantly from resolution not sufficient to separate analytes of interest from coeluting species. Especially the mass resolution plays an important role in the successful identification of the most of present residues in samples containing high amounts of matrix coextracts.

The poster will focus on the main problems and issues related to the application of the screening MS techniques using accurate mass technology and will introduce the new system based on the proven Orbitrap™ technology. Mass spectrometers based on the unique performance of this type of mass analyser are routinely achieving mass resolution up to 100,000 and mass accuracy below 2 ppm. Those parameters significantly improve the efficiency and accuracy of the residue screening methods and allow successful screening of various residues at even very low concentration levels. This fact will be documented on practical examples from the field of the analysis of priority mycotoxins in the samples of food and feed.

## P078-A2

### Study of the By-Products Formed from UV/Ozone Treatment of Aniline in Aqueous Solution at pH above 9

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The industrial wastewaters as a rule contain toxic substances which are dangerous for humans even in small doses. Among various wastewater treatment options, ozone-based advanced oxidation processes (AOPs) are likely key technologies for degrading the pollutants. As known, early ozonation of wastewater containing aniline result in by-products increased the biotoxicity. The goal of this investigation is to study the by-products formed under the ozonation of aniline in aqueous solution at pH above 9 under UV-irradiation.

Reference solutions of aniline were prepared from a.r. grade substances. They were irradiated with a low-pressure mercury lamp at room temperature. The content of ozone in the ozone-air mix in the reactor inlet was  $4.5 \cdot 10^{-4} \text{ kg} \cdot \text{hr}^{-1} \cdot \text{dm}^{-3}$  and in the outlet was  $1.3 \cdot 10^{-6} \text{ kg} \cdot \text{hr}^{-1} \cdot \text{dm}^{-3}$ . The concentrations of aniline and products of their oxidation was determined by both optical and IR-spectroscopy. The optical absorption spectra were registered on Shimadzu Corp. spectrophotometer. The diffuse reflectance IR-spectra in the  $5000\text{-}450 \text{ cm}^{-1}$  region were registered by the FTIR spectrometer "System-2000" by Perkin Elmer.

The main products of aniline decay under treatment are biatomic phenols, humus substances and carboxylic acids. Nitrobenzene and monoatomic phenols are not detected. Biatomic phenols, that are more toxic than aniline, are formed during the treatment of the aqueous aniline solution in the first stage (< 7 hrs). When oxidized after the 7 hrs treatment biatomic phenols change into humus substances and carboxylic acids. After the 13 hrs treatment biatomic phenols in measurable quantities are not detected. Specific discharge of ozone per 1 mol aniline oxidation in its reference solution after the 15 hrs of ozonation is 0.26 mol. Contamination factor of the aniline solution after the 15 hrs treatment is ~90% and does not actually depend on initial aniline concentration.

## P079-A2

### Non-Steroidal Anti-Inflammatory Drugs Determined in Wastewaters by Magnetic Matrix Solid Phase Dispersion-HPLC

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The determination of non steroidal anti-inflammatory drugs (NSAIDs) residues in the environment is a field of special interest nowadays mainly due to the risks to human health and aquatic environmental. The critical point in the studies of the effect of NSAIDs in ecosystems is the correct selection of the quantification method. There is a continue increase on the improvement for isolation of NSAIDs from environmental matrices in order to generate methodologies efficient, economic with a higher sampling rate.

In this work, the effect of pH, sample volume and support polarity was evaluated in the pre-concentration of acetaminophen, naproxen, diclofenac and ibuprofen using magnetic matrix solid phase dispersion and determination by HPLC in wastewater effluents. Environmentally relevant pharmaceuticals were chosen according to human consumption in Mexico.

The magnetic supports were initially dispersed and mixed in the samples with the help of Triton X-100. The magnetic support (containing the analytes) was magnetically isolated and finally the NSAIDs were eluted with methanol before the injection in the HPLC system. The highest recovery percentage (>90%) were obtained using the support containing octadecyl (C8) at pH 3. The lowest limit of detection achieved in the pre-concentration of 1 L sample were around 1-2  $\mu\text{g L}^{-1}$  with repeatability below 5% in all cases. The method was applied in the analysis of wastewater samples.

The described method has as main advantage the pre-concentration time around 30 minutes (1.0 L of sample) whereas, for the conventional methods, as the solid phase extraction (SPE) the pre-concentration time is 10 h for same sample volume.

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## P080-A2

### Exploring Beneficial Use of Mosquito as an Environmental Friendly Detector of Hazardous Volatile Organic Compounds

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Volatile organic compounds (VOC) are common chemicals in human, animal and plant ecology. Some of VOC have important role in mosquito communication, categorized as semiochemical, which may lure, alarm and repel them upon detection. There are instances where the vaporized VOC lure mosquitoes at low concentration but repel at high concentration, e.g. lactic acid and acetone. These entomological facts triggered interest to investigate possible biological fate of mosquito upon forced exposure to VOC beyond the repelling concentration. Female *Stegomyia (Aedes) aegypti* aged 2 to 7 days were exposed to selected VOC known to be hazardous to human at level of concentration which is permissible for human exposure (PEL) in a 15 mL air-tight glass tube. The selected VOC and its PEL were diethyl ether (100 mL/m<sup>3</sup>), xylene (50 mL/m<sup>3</sup>), acetone (500 mL/m<sup>3</sup>), chlorobenzene (5 mL/m<sup>3</sup>), methanol (200 mL/m<sup>3</sup>) and hexane (20 mL/m<sup>3</sup>). The behaviour of mosquito upon sublethal toxic exposure, e.g. rubbing of antenna using front legs, aggressive movements, and motionless state until its inability to fly or stand (knockdown) was observed. The investigation showed that knockdown time of mosquitoes exposed to diethyl ether, xylene, acetone, chlorobenzene and methanol were 1.86, 1.94, 1.96, 5.12 and 15.66 minutes, respectively. However, the female *Stegomyia aegypti* did not knockdown within the 60 minutes observation when exposed to hexane. Despite of being a noxious insect, the good sensory system possessed by mosquito to find human seems also capable to detect the presence of other VOC. It is also noticeable the selected VOC were also hazardous to mosquito, except for hexane.

## P081-A2

### The Effect of Soil Contamination Level and Plant Origin on Contents of Arsenic and its Compounds in *Mentha Aquatica* L

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Water mint (*Mentha aquatica* L.) belongs to the arsenic tolerant plant species suitable for cultivation in Central European climate conditions. Two identical plant species of different origin, i) commercially market-available mint plants and ii) plants habituated at the arsenic contaminated former mining area in southern Tuscany (Italy) were investigated for their arsenic uptake and transformation in our pot experiment. The total arsenic concentrations in the experimental soils varied from 21 to 1573 mg As kg<sup>-1</sup>, the mobile fractions of arsenic were determined in 0.05 mol.L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> extracts and did not exceed 2% of total soil arsenic. The mint plants originating from the contaminated area showed higher biomass yield together with slightly lower content of arsenic in both leaves and stems of water mint. However, the higher biomass yield resulted in higher total removal of arsenic from the experimental pots. The mint originated from the contaminated area removed ~400 µg of arsenic per pot, whereas the commercial plant removed significantly lower amount (~300 µg of arsenic per pot). In 0.02 mol.L<sup>-1</sup> ammonium phosphate buffer extracts of mint plants, only arsenite and arsenate, but no organoarsenic compounds were identified in both stems and leaves. Arsenate was the predominant arsenic compound and reached up to 80% regardless of the origin of the mint plants. Evidently, the arsenic uptake by mint plants was more significantly affected by soil contamination level than by the plant origin. Although *M. aquatica* seems to be able to grow in contaminated soils without symptoms of phytotoxicity, its efficiency to remove arsenic from contaminated soil is limited as can be demonstrated by total offtake of As from individual pots not exceeding 0.1 %. Moreover, the application of plants originating from the contaminated site did not result in sufficient increase of potential phytoextraction efficiency of *M. aquatica*.

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## P082-A2

### Organic Matter Concentrations and Characteristics in Bulk Precipitations in Croatia

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Organic matter, especially its reactivity and physico-chemical properties, play a crucial role in complex biogeochemical processes in aquatic environments. The interactions between the atmosphere and aquatic systems are of considerable importance for the fate, transport and cycling of organic matter and other microconstituents in the environment, having thus a decisive role in global changes and in environmental protection. Organic matter in atmosphere is a result of primary anthropogenic and biogenic emissions and/or of transformation processes of different precursors. The monitoring of chemical composition of rain has attracted attention of numerous investigators, but there is still lack of knowledge about chemistry of precipitation. The dissolved organic carbon (DOC) is the most relevant and most frequently used collective parameter for organic matter content. Surface active substances (SAS) contribute to the amount of organic compounds and play a role in the control of atmospheric processes, enhancing the potential of rain drops for washing out organic matter from the atmosphere and mobilizing micropollutants on the global level.

In this work the dissolved organic carbon (DOC) and content of surface active compounds (SAS) have been measured in the samples collected in two cities in Croatia (1999 – 2009), one in continent and the other in coastal zone in the Middle Adriatic. The capacity of organic ligands to complex metal ions and the oxidation state of the organic carbon present were determined as additional parameters for qualitative and quantitative characterization of organic matter in precipitations, what is of special interest for understanding the pertaining physico-chemical processes.

The results will be compared with those for water bodies (of marine and freshwater origin as well as seasurface microlayers) in the investigated area.

## P083-A2

### Determination of Isotopic Fractionation of B Isotopes in Soils and Plants by Quadrupole ICP-MS and its Application in Fingerprinting of Wine

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Isotope fractionation can cause isotope ratios for certain elements to vary from place to place on Earth depending on the geochemistry of the soil. Isotope ratios can therefore be used as chemical indicators for the identification and classification of agricultural products or archeological artifacts according to geographical origin. Such fingerprinting procedures have many applications in various fields. The fundamental assumption is that the isotope ratio of a particular element in the product or artifact will reflect the composition of the provenance soil.

We have shown in previous work<sup>1</sup> that  $^{11}\text{B}/^{10}\text{B}$  ratios can potentially be used to classify wine according to geographical origin without proving that the fundamental assumption stated above is indeed valid. In the current work the link between the B isotope ratios in wine and that in the provenance soil, was verified. The 2<sup>nd</sup> important condition is that isotope fractionation should not occur during nutrient uptake by the grape vine. This was investigated by running hydroponic experiments in which the B isotope ratio of boric acid in the nutrient solution given to grape vines was varied. The results show that the B isotope ratio in new growth quickly responds to changes in the B isotope ratio in the growth medium thus proving the link between soil and plant with respect to B isotope ratio. Matrix effects caused by components in the digested wine samples resulted in too high  $^{11}\text{B}/^{10}\text{B}$  ratios. The role of matrix effects was thoroughly investigated and it was found that in order to eliminate matrix effects completely it was necessary to separate B from digested wine, plant, and soil samples by ion exchange before isotope ratio determinations by ICP-MS.

The methodology was used to classify wines from 6 wine-producing regions in Europe and South Africa.

<sup>1</sup> PP Coetzee and F Vanhaecke. (2005) *Anal Bioanal Chem* 383 977-984

## P084-A2

### Use of a Method of Sampling Tar in Syngas for Analysing Hydrocarbons in Vehicle Exhausts

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During regular maintenance of vehicles, a lot of attention is paid to the analysis of exhaust fumes. One of the parameters under scrutiny in exhaust emissions is CH, or total hydrocarbons. Composition of exhausts depends on the technical condition and power of the motor, quality of the catalyst system, quality of fuel, regulation of the petrol-air proportion. There are practically no techniques designed for a fine-grained analysis of engine emissions. Therefore it was decided to try and apply the technique of sampling tar in syngas for the analysis of hydrocarbons in the car exhaust fumes. For this, the device specially designed for tar sampling in syngas was placed in the exhaust tube of a car. The device consists of two adsorbent cartridges loaded with aminopropyl-bonded silica adsorbent and activated coconut charcoal.

Exhaust fumes in one of the tested cars contained 133 identified compounds, mainly aromatic, aliphatic, and alicyclic hydrocarbons. Benzene, toluene, and xylenes make up 56.17 % of the total mass of all compounds, while all aromatic derivatives 86.67 %. Taking into account a big proportion of aromatic derivatives in emissions, and a possibility of using activated coal for sampling saturated and unsaturated hydrocarbons, it is concluded that the above-mentioned two-sorbent device can be used for sampling hydrocarbons in car exhaust fumes. The necessity of using two sorbents is proved by the distribution of compounds between the sorbents. More volatile compounds mainly pass the first sorbent and get adsorbed on the second one; semi volatile compounds spread evenly between the sorbents, and heavy compounds mainly get adsorbed on the first one. It was also noticed that aromatic hydrocarbons more effectively adsorb on the aminopropyl-bonded silica sorbent while aliphatic and alicyclic hydrocarbons easily pass this sorbent and get adsorbed on activated coconut charcoal.

## P085-A2

### **BTEX Background Level in the Tricity Area. Field Comparison of Different Types of Passive Samplers in Air Quality Monitoring**

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Problems with urban air quality are related to at least two key trends: the dramatic increase in traffic in urban areas and the corresponding increase in traffic emissions as a proportion of total air pollution, as well as mounting evidence that air pollution has more significant effects on health than previously thought.

Passive sampling involves the measurement of the concentration of an analyte as an average weighted over the sampling time. The concentration of a given analyte is integrated over the entire exposure period, making this approach immune to accidental variations in the analyte concentration. Information obtained in this way is suitable for long-term characterization of analyte concentration levels in a given environmental compartment. Moreover, diffusive monitoring provides a simple and cost effective method for collecting large numbers of samples required in many air monitoring programs. As a result, large-scale atmospheric air quality monitoring is possible to conduct and it is not prohibitively expensive.

The aim of our study is to examine BTEX background levels in the Tricity area using passive sampling for analyte collection. The knowledge of BTEX concentration in atmospheric air allows the assessment of present and future condition of the atmosphere, and can help explain changes in the environment due to human activity. The results obtained by passive air monitoring could be also used to examine the spatial and seasonal distribution patterns of BTEX in the area of the Tricity. Our previous study indicate that sources other than traffic contribute significantly to atmospheric BTEX levels in the Tricity and the nearby city of Tczew. Examining the correlation between benzene and toluene concentrations and between the remaining BTEX compounds it is possible to point to main industrial emissions sources of BTEX to the atmospheric air of the Tricity.

## P086-A2

### **Functional Speciation of Metals Associated to Environmental Microparticles, Nanocolloids and Macromolecules: Size Characterization by Asymmetrical FIFFF Coupled to ICP-MS**

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T1: Bioanalytics, Samples Preparation, Environmental and Health, Clinical Analysis, Food Quality and Analysis

A unified methodology for the characterization of environmental microparticles, nanocolloids and macromolecules by Asymmetrical Flow Field Flow Fractionation (AsFIFFF) will be presented. The procedure is optimized to minimize the possible alteration of the original size distribution and metal associations of the species characterized. Prior to separation by AsFIFFF, samples are subjected to two pre-fractionation procedures: gravitational settling of the solid suspension, which removes particles larger than 50  $\mu\text{m}$ , and a centrifugation of the settled sample to remove particles larger than 1  $\mu\text{m}$ .

In the settled sample, those microparticles larger than 1  $\mu\text{m}$  are eluted in steric mode in the AsFIFFF, whereas colloids and macromolecules coeluted in normal mode. Both pre-fractionation procedures are used to provide information on microparticles. The centrifuged sample allows the proper characterization of both colloids and macromolecules applying different operational conditions in the AsFIFFF system. The methodology proposed has been applied to compost leachates.

The coupling of As-FIFFF with ICP-MS ensures a detection capability in accordance to the concentrations expected. Compost leachates show associations of heavy metals with macromolecules (in the range of a few kDa), colloids (20-60 nm) and microparticles (5-20  $\mu\text{m}$ ).

Finally, an on-line isotopic dilution (ID) technique coupled with AsFIFFF-ICP-MS has been applied for the determination of these metallic associations for some elements (copper and lead).

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## P087-A2

### **Determination of Bioavailable Cd and Pb in Soils of Keller Peninsula, King George Island, Maritime Antarctica Using Thermospray Flame Furnace Atomic Absorption Spectrometry (TS-FF-AAS)**

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The Antarctica presents a fragile ecosystem and great scientific efforts have been done in order to evaluate the impact promoted by scientific bases located in this continent, tourism activities and mainly the world climate changes. In the Brazilian scientific station (EACF) several groups promote scientific expeditions to study, for example, the animals and soil near the EACF. During the period of summer of 2007/2008 one campaign was done to collect soils located near and far from the EACF. Collect points near the helicopter landing area (HLA) and diesel tanks (DT) were privileged, as well as, points located up to 4 km far from the station. A total of 49 samples were collected in 15 points. The soils were dried at 60 °C, powdered and sieved at 212 µm. The bioavailability of Cd and Pb were determined using DTPA solution for metal extraction. Cd and Pb were determined by Thermospray Flame Furnace Atomic Absorption Spectrometer (TS-FF-AAS). Using TS-FF-AAS a Ni tube is placed above an air/acetylene flame and the liquid extract was totally introduced directly into the Ni tube to promote high sensitivity. Using this combination it was possible to obtain limits of detection of 1.48 and 44 µg/kg for Cd and Pb, respectively. In the case of Cd it was not observed any difference between the studied areas and the Cd average ranged from 16 to 18 µg/kg for points around EACF and far from it, respectively. On the other hand, for Pb the points around the DT presented concentration (average = 7668 µg/kg) 20 times higher than those observe in sampling points far (average concentration = 375 µg/kg) from the EACF. These results were important to verify the impacts characterized by the presence of the diesel tanks in that region and the Pb levels indicate that pollution source.

## P088-A2

### Surveying a Wastewater Treatment Process through Image Analysis and Partial Least Squares

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Image analysis is, nowadays, an important complement to analytical survey procedures in biotechnological processes. Furthermore, as the quantity of the resulting data can become overwhelming, it is usual for these data to be fed into a multivariate statistical analysis procedure. In the case being considered, an activated sludge reactor was operated for wastewater carbon and nitrate removal, during 35 surveyed days.

COD contents, as well as nitrogen contents, in terms of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , were surveyed in the feeding effluent, reactor bulk and settler. COD and  $\text{NH}_4^+$  removal percentages were determined, as well as  $\text{NO}_3^-$  and  $\text{NO}_2^-$  increase inside the reactor, resulting in the determination of 16 analytical parameters. Furthermore, regarding the biomass characterization, a total of 40 image analysis parameters were initially determined, from which 15 were discarded, as they presented cross-correlations over 0.9 with other image analysis parameters. The remainings were set in 4 groups, covering free filamentous bacteria contents, aggregates contents, aggregates size and aggregates morphology. Finally, and with respect to the aggregates characterization, these were divided in 3 classes (large, intermediate and small aggregates) according to their size.

A Partial Least Squares analysis was then performed to the dataset, composed of 35 observations (sampling days), in which 26 days (around 75% of the dataset) were included on the training dataset and the remaining 9 days on the validation dataset. The obtained results allowed to establish an overall reasonable prediction ability (R values above 0.75), for the  $\text{NH}_4^+$  removal percentage and  $\text{NO}_2^-$  increase inside the reactor. Furthermore, the parameters found to be more correlated with the  $\text{NH}_4^+$  removal were identified as belonging to the aggregates size and contents, whereas for the  $\text{NO}_2^-$  increase, was clearly the  $\text{NO}_2^-$  contents in the feed effluent, followed by the aggregates morphology.

## P089-A2

### Sorption-Desorption Behaviour of Atrazine on Soils Subjected to Different Organic Long-Term Amendments

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The adsorption and desorption of atrazine on soils subjected to different organic long-term amendments, thus containing different organic matter amounts and properties, were measured using batch equilibrium technique.

Freundlich equation reasonably described the adsorption of atrazine on soil samples and a higher  $K_F$  value ( $2.20 \text{ kg}^{-1} (\text{mg l}^{-1})^{-N}$ ) was obtained for soil fertilized with compost, presenting a higher organic matter content. However, the adsorption constant related to the soil organic carbon content ( $K_{FOC}$ ) was higher for a soil amended with farmyard manure. A correlation between the  $K_{FOC}$  values and the percentage of aromatic carbon in the organic matter was observed. The highest  $K_{FOC}$  value was obtained for the organic matter of the soil with higher aromatic content. This content induces a higher hydrophobicity of humic substances, so the results obtained were an indication of hydrophobic interactions as a key role in binding of atrazine to organic matter. On the other hand, the farmyard manure soil presented also the higher content of carboxylic units. These can be responsible for hydrogen bonding between atrazine and organic matter. The higher predominance of hydrogen bonds in adsorption of atrazine on soils, compared to hydrophobic interactions, weaker type of bonding, can be responsible for the lower desorption capacity observed on farmyard manure soil. The higher importance of stronger binding forces in atrazine adsorption mechanism on soils can be responsible for a reduction in the leaching into drinking water resources and run-off to rivers and other surface waters. Hence organic fertilizations, and especially farmyard manure, can be effectively used to minimize the residual toxicity of atrazine-treated agricultural fields, with the advantage of combining their use as detoxifying agents and organic fertilizers.



## P090-A2

### Implementation of Solid Phase Extraction with Micellar Desorption and HPLC for Determination of Fluoroquinolones in Natural Waters

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The pharmaceutical drugs used during medical treatment may partly be excreted in an unmetabolized form, enter municipal sewage systems, and can even survive the passage through the sewage treatment plants [1]. Then, the residues can reach sea waters, surface waters or groundwaters.

These substances are among the so-called “emerging” contaminants and can be considered “pseudo” persistent pollutants due to their continual introduction into the environment [2]. For that, in the recent years has increased the number of publications related to the development of new methods for the determination of different analytes in several matrices.

Fluoroquinolones (FQs) are within of the antibiotics compounds group and are a new and synthetic generation of quinolones antibacterials family, used in human and veterinary medicine [3]. The FQs are efficient against a widely range of infections process, but they are resistant to microbial degradation and may be persisting within environmental waters [4]. Moreover, their environmental concentrations are very low, for that these analytes need to be extracted and preconcentrated prior their analysis. These compounds have been detected at the  $\mu\text{g}\cdot\text{L}^{-1}$  and  $\text{ng}\cdot\text{L}^{-1}$  levels in environmental liquid samples [5].

In this work we applied a rapid liquid chromatography method with fluorescence detection for the determination of five fluoroquinolones: levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin and sarafloxacin in water from different sources. The extraction procedure was based on solid-phase extraction with micellar desorption. The method allows us to determine successfully FQs in water samples.

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## P091-A2

### A Simple Approach for BDE-100 Determination by LLE-GC-(EI)MS

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Polybromodiphenyl ethers (PBDEs) are a group of chemicals used in industry as flame retardants. The commercial mixtures, known as tretaBDE and pentaBDE, include among others, the pentabromodiphenyl ether (BDE-100), which is presently legislated by the European Union with maximum admissible levels in surface waters of 0,2 ng/L (Directive 2006/0129 from 17.07.2006). This extremely low value is due to the high toxicity of this kind of compounds.

The determination of BDE 100 in waters requires a preconcentration step, which may be liquid-liquid extraction (LLE), solid phase extraction (SPE), stir bar sorptive extraction (SBSE) or other. Capillary gas chromatography (GC) with electron-capture (ECD) or mass spectrometry (MS), either with electron impact ionization [1] or negative chemical ionization detection [2] are the chosen techniques for this type of analysis.

In this work a simple approach was validated in order to allow a rapid screening of the presence of these contaminants in waters. BDE-100 was used as model that may be further extended to the analysis of the other PBDEs.

The sample volume, the type of extracting solvent by LLE and the chromatographic conditions (SIM mode or MS/MS, as well as the possibility of using internal standard) were optimized. The calibration curve was carried out with standards extracted in the same conditions as samples and the validation parameters were obtained: linearity range, detection and quantification limits, repeatability and accuracy (by recovery percentage of standard additions). Matrix effects were compensated by matrix-matched calibration whenever needed.

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## P092-A2

### Determination of Alkylphenolic Ethoxylated Surfactants (APEOs), their Degradation Products (DPs) and Bisphenol A (BF-A) by SPE-HPLC-ESI-MS/MS Method in Coast Sewage Water Samples in Gran Canaria Island (Spain)

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In the recent years, chemical compounds able to mimic the natural hormones, disrupting the normal processes of reproduction and development of animals, including humans, have aroused in the scientific community an enormous interest [1]. Among these substances, the presence of alkylphenols (APs) and bisphenol A (BPA), which have demonstrated estrogenic potency and chronic toxicity by in vitro and in vivo bioassay studies [2], has caused a high concern due to their wide use, ubiquitous occurrence and persistency in the environment [3]. Unlike the natural estrogen “imposters”, these compounds present high levels of bioaccumulation in fatty tissues due to their high log  $K_{ow}$  values [4], which impedes a rapid excretion of the organism.

This work presents a quantitative method for the simultaneous determination of Bisphenol-A, Octylphenol, Nonylphenol and the corresponding ethoxylates (1 to 12) in sewage treatment plants (STPs) emissaries on the coast of Gran Canaria island (Spain).

Identification and quantitation were accomplished by high performance liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS). The method enabled high-reliable identification by monitoring the corresponding ammonium adduct  $[M+NH_3]^+$  for AP<sub>n</sub>EOs and the deprotonated molecule  $[M-H]^-$  for Nonylphenol, Octylphenol and Bisphenol-A. The method is applied to determine levels of the target analytes in coast sewage water samples from Gran Canaria island (Spain).

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## P093-A2

### Use of an *in situ* Aqueous Derivatization Reaction with Headspace Generation-Programmed Temperature Vaporization-Gas Chromatography-Mass Spectrometry for the Determination of Ibuprofen in Water Samples

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In recent years there has been increasing concern about the presence of pharmaceutical compounds in water. Their presence can be explained in terms of both their use in medicine and the inefficiency of water purifying systems in removing them. To this must be added expired drugs that users sometimes flush into the sewer system. Ibuprofen is one of the most used pharmaceuticals in the world. Due to its properties (high polarity and sparingly volatile), gas chromatography usually required a prior derivatization reaction. The aim of the present work is to propose a method for the determination of ibuprofen in aqueous samples. To do so, an *in situ* derivatization reaction in aqueous medium was employed in the vial of a headspace sampler (HS), after which instrumental measurements were made with gas chromatography-mass spectrometry (GC-MS). As the injection system we propose a programmed temperature vaporizer (PTV) where, in solvent vent mode, better results can be obtained than with the conventional split and splitless injection modes. Since the derivatization reaction takes place in the HS vial, after the mixing of reagents and the sealing of the vial, the whole process takes place on-line, with no need for intermediate steps. The simplicity and speed of the method –analysis throughput: 10.5 min- together with the limit of detection obtained (0.23 µg/L), bearing in mind that no preconcentration step or later clean-up step are required, make this a good alternative for the analysis of ibuprofen in aqueous samples of urban waste water.

## P094-A2

### AT-LINE Micro Extraction by Packed Sorbent (MEPS) – Gas Chromatography Mass Spectrometry for the Determination of Sun Screen Agents and Polycyclic Musk Compounds in Water

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UV-filters and polycyclic musk compounds frequently applied in household and personal care products attract increasing public concern since recent research results gave hints to potential bioaccumulation and health risks. Their environmental concentration, particularly of UV-filter compounds, varies seasonally and ranges from low  $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$ . Thus, the monitoring of these substances in water samples requires analytical procedures involving analyte enrichment and sensitive analysis.

An at-line analysis protocol was developed that allows the determination of four UV-filters (i.e. EHMC, 4-MBC, BP-3 and OC), and the polycyclic musk compounds Galaxolide<sup>®</sup> (HHCB) and Tonalide<sup>®</sup> (AHTN) in water. The fully automated method includes the enrichment of the analytes with Micro Extraction by Packed Sorbent (MEPS) coupled directly to gas chromatography-mass spectrometry with large volume injection. Two MEPS sorbents, C8 and C18, were examined to extract the target substances from sample volumes of 800  $\mu\text{L}$  and 2 mL, respectively. The analytes were extracted by 100  $\mu\text{L}$  steps and each extracted sample aliquote was discarded into waste. After washing and drying, the analytes were desorbed by two portions of ethyl acetate which were directly injected into the cooled large volume injector of the GC-MS instrument.

Analyte recoveries for the C8 and C18-sorbents were between 46 -120% and 44-115%, respectively. The limits of detection (LOD) ranged from 34 to 96  $\text{ng L}^{-1}$  and enable a fast and sensitive analyte monitoring at common environmental levels.

The optimized MEPS procedure includes washing and recalibration steps which finally allows the multiply use of a MEPS sorbent up to 70 analyses with the same sorbent. C8- and C18 sorbent provided linear calibration curves for the analytes up to a concentration level of 20  $\text{ng mL}^{-1}$ . The fully automated micro extraction GC-MS protocol was evaluated for the influence of matrix substances typical for wastewater. Real water samples were analyzed by the MEPS-GC-MS method and compared to standard SPE.

## P095-A2

### Non-Instrumental Test for TNT Rapid Screening in Water Samples

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Detection of 2,4,6-trinitrotoluene (TNT) is an important environmental, security and health concern for the global community. TNT enters the environment in waste waters and solid wastes resulting from the manufacture of the compound, the processing and destruction of bombs and grenades, and the recycling of explosives; different military and terrorist activities (e.g., manufacturing, waste discharge, testing and training) have resulted in extensive contamination of soil and ground water by TNT. Consequently, sensitive and reliable analytical methods are needed to evaluate the presence of TNT at field conditions.

The goal of this study was to develop an approach for the non-instrumental determination of TNT in water samples with cut-off levels at 5 µg/l. A new immunologically-based tube test for non-instrumental detection of TNT in water samples was developed. The method combines the pre-concentration of analyte by immunoextraction and its detection by ELISA, using Sepharose 4B-immobilized monoclonal anti-TNT and N-(4-methyl-3,5-dinitrophenyl)-glutaric acid monoamide-horseradish peroxidase conjugate (4-ADNT-GA-HRP) as tracer. The immunoaffinity gel was placed in a standard 1-ml SPE column through which a 5 ml aliquot of water sample was passed. Following, free antibody binding sites were detected by application of the tracer. Four minutes after addition of the chromogenic substrate the results were visually evaluated by occurring or stayed away blue colour development for negative and positive samples, respectively. Total time for assay was about 18 min for six samples. Under optimized conditions a cut-off level for TNT at 5 µg/l was found.

An approach for fast non-instrumental detection of TNT was devised and optimized for screening of water samples. The described gel-based immunoassay has proved useful for rapid on-site screening of TNT in a fairly simple manner, and without any sample preparation like extraction, centrifugation, filtering, etc. The cut-off level for TNT was estimated at 5 µg/l.

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## P096-A2

### Determination of Low Levels of PAHs, PCBs and Pesticides by Means of SBSE-TD-GC-MS in Snow and Seawater from the Antarctica

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The presence of persistent organic pollutants (POPs) in Antarctica has been attributed to the long-range transport of pollutants produced not only by human activities, but also due to global distillation, a phenomenon which involves the evaporation of POPs at warm low latitudes and deposition at cold higher latitudes. In this sense, polar territories are considered as the regions playing a very significant role in global environmental processes [1]. In Antarctica the presence of contaminants can threaten living resource since several POPs accumulate in the tissues of organisms. The increase in the levels of these compounds in various environmental matrices in these locations remains in the centre of researchers' attention [2].

POPs are generally found at trace levels in water samples and require, therefore, extraction and preconcentration steps. Recently and due to the importance that miniaturisation has reached in the analytical chemistry field, extraction techniques such as Stir Bar Sorptive Extraction (SBSE) have attained great importance in the preconcentration of analytes from water samples since they minimise the use of organic solvents (green chemistry) and analysis time and improve the sensitivity of the global analytical method [3]. Besides, stir bars can be used to store samples when the analysis cannot be performed immediately.

In the present work the most significant findings on the presence several POPs polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and chlorinated pesticides in the marine/terrestrial ecosystem at Livingston Island (Shetland South) are presented. Snow and seawater samples were collected in the surroundings of the Spanish Base Juan Carlos I during an expedition in Antarctica (January-February 2009). PCBs, PAHs and chlorinated pesticides were monitored in the samples mentioned by SBSE-TD-GC-MS method previously optimised [4, 5]. The previously optimised method was slightly modified in order to obtain better limits of detection (LODs) and stability of the preconcentrated analytes on the polydimethylsiloxane (PDMS) polymer was studied in order to estimate an approximate storage time.

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## P097-A2

### Determination of Estrogenic Compounds by Accelerated Ultrasonic Derivatization and PTV-LVI-GC-MS in WWTPs Samples

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Environmental or industrial chemicals and phytoestrogens interfering with the hormonal or endocrine system are defined as endocrine disruptors [1]. These compounds are able to mimic estrogens or inhibit estrogenic response. Phenolic compounds, pesticides, tributyltin (TBT), phytoestrogens and synthetic estrogens are examples of those compounds. In this work, previous optimization and validation of the method, determination of estrone (E1), 17 $\beta$ -estradiol E2, mestranol (MeEE2), 17 $\alpha$ -ethynyl estradiol (EE2) and estriol (E3) estrogens in influent and effluent waste water samples after solid phase extraction followed by accelerated ultrasonic derivatization and PTV-LVI-GC-MS determination has been carried out.

Derivatization process of estrogens is usually performed after heating in heat blockers or microwave-ovens [2]. In this work and in order to accelerate the time consumed by heat blockers, a cup booster ultrasound device has been used. Cup booster ultrasound devices consist of miniaturized ultrasound baths (<12 mL) that can control amplitude, sonication cycles and time, increasing efficiency and precision. Optimal conditions were as follows: 125  $\mu$ L of pyridine (derivatization solvent), 25  $\mu$ L BSTFA+1% TMCS (derivatization reagent) and 10 min of sonication at 80 % of amplitude power and 9 cycles.

Large volume injection (LVI) using a programmable temperature vaporizer (PTV) can be used in order to improve the limits of detection (LODs). LVI-PTV variables such as CIS4 initial temperature (40-80  $^{\circ}$ C), venting time (0.4-5 min), venting flow (50-100 ml/min), venting pressure (2-7.7 psi) injection volume (20-45  $\mu$ L), purge flow to split vent (30-100 ml/min), splitless time (0.5-1.5 min) and injection speed (2-6  $\mu$ L/s) were optimized by means of experimental design approaches (Plackett-Burman and central composite design).

Figures of merit (linearity, reproducibility, recoveries, limits of detection and limits of quantification) of the optimized method were studied using spiked natural water samples free of the analytes of interest. Finally this method was applied to real influent and effluent of waste water treatment plants of the Metropolitan Bilbao (Bilbao, North of Spain) and Gernika (Urdaibai, Reserve of the Biosphere, North of Spain).

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## P098-A2

### Soil Chemical Composition of an Abandoned Zn/Pb Mine: a First Step for the Environmental Risk Assessment of the Chronic Pollution in the Surrounding Abiotic and Biotic Communities

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For centuries the human being activities have sometimes promoted severe deterioration of the surrounding environment. Some of the most important have been intensive mining activities [1] with potential and environmental risks [2-3]. In times when the environmental awareness is growing, the development of studies in order to understand their impacts, that often last for years, is needed. The present work has tried to determine the environmental impacts of an abandoned Blende/Galena mine situated in the Karrantza Valley (Biscay, North of Spain). In this land mine, the mode of exploitation consisted in the extraction, piling and washing of the ore. Overall, these activities supposed an increase in soil heavy metal concentration [4] that often means a multi-elemental pollution source [5].

In order to reach the soil chemical characterization objective both non-destructive and destructive analytical techniques were used. First, the molecular forms present in the sampled soils were investigated mainly by using Raman spectroscopy, while XRF measurements display the elemental composition. The soil heavy metal quantification was carried out by means of the ICP-MS after HCl/HNO<sub>3</sub> digestion. Other analytical techniques such as FT-IR in the ATR mode were also applied in order to complete the information obtained.

The data were shown in 3D diagrams using The Surfer 8.2 Surface Mapping System so that the pollutant trends and relationships could be visualized. In addition, the soil quality evaluation values, VIE-A, VIE-B and VIE-C, adopted in the Basque Country [6] were used to check the potential risks of each of the pollutants. As it was predictable, the results shown the highest heavy metal concentrations on those places where the mining activity was considerable. However, the rest of the area was also impacted, which points out the need of studying the different processes that may be taking place so that their impact on the environment can be predicted.

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## P099-A2

### Methodological Study of Chemical Fractionation Procedures and Species Occurrence of Risk Elements in Urban Particulate Matter

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More precise estimation of the bioavailability and potential toxicity of important trace elements in environmental samples is based on the knowledge of the distribution of their chemical forms. In our work the three-step sequential extraction procedure has been applied to the homogenized urban dust sample collected in the main ventilation shaft in an automobile tunnel in Prague. The mobility of As, Cd, Cr, Mn, Ni, Pb, the potential applicability of the procedure for air samples and conditions for Cr(VI)/Cr(III) species determination, were investigated. Depending on concentration levels, ICP-OES and GF AAS were employed in measurements of analyte concentrations in dust fractions and in fractions where common air filters were included. For testing of stability and presence of Cr species in obtained three dust fractions, a coupled technique connecting on-line HPLC with element-sensitive detector ICP-OES has been used. The anion exchange column was used for separation of Cr species. Optimal conditions for the separation were following: mobile phase 50 mmol·l<sup>-1</sup> CH<sub>3</sub>COOH and 10 mmol·l<sup>-1</sup> NaClO<sub>4</sub> (pH 7.0; flow rate 1.5 ml·min<sup>-1</sup>), injected 200 µl of the sample, addition of 30 µg·ml<sup>-1</sup> CDTA to the sample for a transfer of Cr(III) to anion complex. This combined technique allowed to determine 10 ng of Cr(III) and 13 ng of Cr(VI) in the sample (absolute LOD). It was found that portions of investigated elements in mobile and mobilizable dust fractions were changed in presence of filters. All three extraction agents used for a fractionation of elements negatively influenced the stability of Cr(VI) species in the solution immediately after their contact with the sample. Probably this is a reason why only species of Cr(III) were determined in leaches of particulate matter samples. At present a testing of alkalic inoxidable leaching agents is performed.

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### Preparation and Comparison of Lead-Selective PVC Membrane Sensors Based on Crown Ethers 18C6 and DC18C6

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Despite the vast industrial applications of lead, it is categorized as one of the most toxic elements [1]. Therefore, a precise, accurate and rapid measurement of lead content of environmental samples is of vital importance. Ion selective electrodes provide an appropriate route to achieve this goal [2]. The selected ionophore (carrier) plays a crucial role in the applicability and ability of such sensors.

Due to their ion size selective nature, crown ethers are among the most studied macrocyclic compounds in analytical chemistry investigations. These compounds have been vastly used as sensory molecules for fabricating ion-selective potentiometric sensors [3].

Following to our studies concern to the of ionophoric properties of crown ethers [4] and preparation of ion sensors for lead monitoring [5], we report here, on the application and comparison of two crown ethers 18C6 and DC18C6 as carriers for fabricating Pb<sup>2+</sup> ion-selective electrodes.

The 18C6 based electrode comprises of ionophore (9%), PVC (30%), plasticizer (DBP 59%), anion excluder (oleic acid 2%), shows a nearly Nernstian slope of 30.0±1.0 mV/decade. The best performance for the sensor based on DC18C6 was found by using a composition of ionophore (9%), PVC (30%), plasticizer (DBP 59.5%), additive (NaBPh<sub>4</sub> 1.5%). This potentiometric sensor exhibits a Nernstian slope of 29.7±0.5 mV/decade. The working concentration range of these electrodes was 1×10<sup>-6</sup>-1×10<sup>-3</sup> M and 1×10<sup>-5</sup>-1.0×10<sup>-2</sup> M, respectively. The response of the electrodes was independent to the pH variations in the range of 4.3-5.3 and 4.6-5.2, for the electrode based on 18C6 and DC18C6, respectively. The detection limit of the first sensor was 5.6×10<sup>-7</sup> M and that of the second was found to be 6.3×10<sup>-6</sup> M. The dynamic response time of the electrodes to achieve a steady potential was very fast (6 s and 12 s for 18C6 and DC18C6 based electrodes, respectively). The stability of both ligands was about one month. The selectivity relative to several mono-, di- and tri-valent metal ions was examined by using the match potential method. The selectivity towards lead ions presented by the electrode based on 18C6 was superior to that presented by the other electrode. Both electrodes were used successfully as an indicator electrode for potentiometric titration of lead solutions by standard solution of ammonium chromate. It is noteworthy that the DC18C6 based Pb-ISE could be used as sensor in the mixed water/X (up to 90/10, X=MeOH, EtOH and CH<sub>3</sub>CN) solvents. The applicability of the sensors was assessed for lead measurements in various synthetic and real samples.

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**Preparation and Characterization of Polyaniline–MMT Clay Nanocomposite on Aluminum Alloy 3004 via Electropolymerization: Electrochemical Studies of Corrosion Protection**

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Homogeneous and adherent polyaniline–montmorillonite (MMT) nanocomposite and pure polyaniline coatings were electrosynthesized on aluminum (Al) alloy 3004 (AA 3004) by using the galvanostatic polarization method. A higher applied current density in the polymerization stage proved to be the best condition to adopt for the synthesis of more compact and strongly adherent coatings on Al.

The synthesized coatings were characterized by UV–visible absorption spectrometry, Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD) patterns and scanning electron microscopy (SEM). Optical absorption spectroscopy revealed the formation of the emeraldine form of polyaniline.

The improved corrosion protection effect of polyaniline–MMT nanocomposites compared to pure polyaniline was demonstrated by performing a series of electrochemical experiments of potentiodynamic (e.g., Tafel plots) and impedance (e.g., Nyquist plots) measurements on Al in 3.5 wt% aqueous NaCl electrolytes. The  $I_{\text{corr}}$  values decreased from  $6.55 \mu\text{A cm}^{-2}$  for uncoated Al to  $0.158$  and  $0.102 \mu\text{A cm}^{-2}$  for polyaniline and polyaniline–MMT nanocomposite-coated Al, respectively, under optimal conditions. Also, the corrosion rate (CR) of Al was significantly reduced as a result of the reduction in  $I_{\text{corr}}$ . The CR of the polyaniline and polyaniline–MMT nanocomposite-coated Al were found to be  $5.17 \times 10^{-4} \text{ mm yr}^{-1}$  and  $1.12 \times 10^{-4} \text{ mm yr}^{-1}$ , which are about 40 and 190 times lower than those observed for uncoated Al, respectively. The most effective protection against corrosion in an aqueous neutral corrosive medium was accomplished when the applied current density was  $15 \text{ mA cm}^{-2}$ .

According to the results, the polyaniline–MMT nanocomposite coating enhanced the corrosion protection effect compared to pure polyaniline coating.

## P102-A2

### Opuntia Extracts as a Natural Source Inhibitor for Mild Steel Corrosion in 2 M HCl Using Polarization and Electrochemical Impedance Spectroscopy Methods

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Acid solutions are commonly used for the removal of undesirable scale and rust in metal finishing industries, cleaning of boilers and heat exchangers. To prevent unexpected metal dissolution and excess acid consumption in the process of cleaning, therefore, inhibitors will be inevitable to be put into use [1]. The present study is an attempt to find a cheap and environmentally safe inhibitor for mild steel corrosion in the acidic solution i.e. HCl. The use of Opuntia Ficus Indica modified stems extract as a mild steel corrosion inhibitor in 2.0 M HCl solution was investigated by weight loss experiment, potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) methods. The weight loss results showed that opuntia is an excellent corrosion inhibitor for mild steel immersed in 2.0 M HCl after 24 h. Potentiodynamic polarization curves indicated that the plant extracts behave as mixed-type inhibitors. EIS measurements show an increase of the transfer resistance with the inhibitor concentration. The inhibition action of the extract was discussed in view of Langmuir adsorption isotherm. To explain the adsorptive behavior of the molecules on the mild steel surface, a semi empirical approach involving quantum chemical calculations using Hyperchem 7.0 was undertaken. The HOMO electronic density of the molecules was used to explain the inhibiting mechanism. The effect of temperature on the inhibition efficiency (IE) was studied. It was found that the presence of extract increases the activation energy of the corrosion reaction.

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## P103-A2

### Chemical Sensors for the Detection of Chlorine and Nitrogen Trichloride at ppb Level

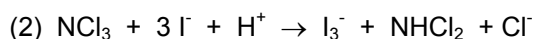
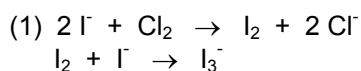
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The detection and analysis of toxic gases such as chlorine (Cl<sub>2</sub>) or nitrogen trichloride (NCl<sub>3</sub>) at ppb level are of great importance not only in semiconductor industries but also in swimming-pools and in food-processing plants. In these last places, Cl<sub>2</sub> is used as a disinfectant to minimize the risk to users from microbial contaminants. NCl<sub>3</sub> issued from the reaction of chlorine with nitrogen compounds provokes significant eye and respiratory irritations in swimmers and pool-attendants and an epidemiologic study has recently shown that it could induce asthma [1]. The NCl<sub>3</sub> content can vary quickly during the day depending on the number of swimmers and the temperature of the pool. It is therefore important to be able to follow its concentration in order to renew rapidly the pool atmosphere when needed. However, there is no commercially available and low-cost system which can instantaneously measure ppb concentrations of NCl<sub>3</sub>.

Our challenge is the development of a cheap and sensitive chemical sensor, able to cover a wide domain of concentration. We achieve this goal by using nanoporous silicate films doped with various probe-molecules such as organic iodide salts. The choice of the probe molecules is based on well known reactions, which occur in aqueous solutions and produce triiodide ions, I<sub>3</sub><sup>-</sup>, which display an intense absorbance at 286 and 353 nm.



The same reactions occur in the thin films doped with iodide ions with an enhanced reactivity due to the confined medium, thus leading to a very fast formation of I<sub>3</sub><sup>-</sup> ions. The kinetics of the reaction is studied as a function of many parameters such as the concentrations of the probe-molecule and pollutant, the gas mixture flux and its relative humidity.

## P104-A2

### Electrochemiluminescent Microfluidic Sensor Fabrications Technology

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In this study a microsystem technology for electrochemiluminescent (ECL) microfluidic sensor creation is considered. A microfluidic platform was designed on a planar polymer material-substrate, which includes such functions as pumping, sample/reagent loading, separation, and ECL measurement.

The mathematical model of probe motion in microfluidic chip allowed to determinate separation channel length for optimal resolution is provided.

A method of laser ablation (LA) of polymers was introduced as a tool for rapid fabrication of microfluidic devices. The laser system used was a commercial Trotec 8003 Speedy C40 laser system. The channel dimensions were measured by electron microscope PEM-106. Some specific features of LA is discussed. On this basis a new approach was been offered for microstructures-channels for capillary creation with controlled dimensions (width and depth from 100 to 300  $\mu\text{m}$ ). In combination with a simple bonding method, it is possible to produce working microsystems with channel dimensions up to 50  $\mu\text{m}$ . Time between changing the design and testing the finished structure is less than two hours. This makes the laser set-up a highly flexible and inexpensive tool for rapid prototyping in microfluidics.

The technology of ECL transducer microfluidic device creation was developed on the basis of investigation of electrochemiluminophore-reagents properties incorporated in ordered thin Langmuir-Blodgett (LB) films. Electrochemical and ECL investigation of obtained LB films allowed characterization of their stability on the ITO electrodes surface and consequently transducer capabilities. Furthermore, electrochemical and ECL investigations of developed transducer allowed determining optimal conditions of its operation concerning detection of bioorganic substances in aqueous solutions. Sensors properties of ECL transducer were confirmed by definition of amino acids.

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## **P105-A2**

### **Polymer Stabilized Cholesteric Liquid Crystal as Colorimetric Sensors for Amine Vapor Detection**

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In this study, we reports the utility of polymer stabilized cholesteric liquid crystal (PSCLC) made from cholesterol derivatives and UV curable polymer NOA61 for colorimetric sensors to detect hazardous amines vapor. Upon amines vapor exposure, perturbed molecular orientation of cholesteric liquid crystal leads to a visible distinct color change in PSCLC. Interestingly, PSCLC shows selectivity to hexylamine over secondary amine, tertiary amine, or other vapor analytes tested which have similar molecular weight at 400 ppmv. Sensitivity of PSCLC to other primary amines vapor ( $C_3 - C_{10}$ ) is logarithmically proportional with the molecular weight of primary amines molecules. For toxic decylamine ( $C_{10}$ ) vapor, PSCLC response reaches detection limit of 2 ppmv. This PSCLC colorimetric sensor is suitable to detect primary amines vapor and can be coated on protective equipment as thin polymer based sensor to offer information about amine exposure. Moreover, it also can be further developed as colorimetric microarray for molecular recognition.

*Key words: cholesteric liquid crystal, amines vapor, sensors, colorimetric*



## P106-A2

### Investigation of the Mobility of Ions in Fly Ash and Bottom Ash from Wood Combustion by Extraction Experiments

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Biomass incinerators produce heat by burning plant materials as wood, straw or hay. During the incineration process the organic input material is decomposed and ashes remain as inorganic residues. The coarse ash (bottom ash) is collected at the bottom of the incinerator. After leaving the incinerator together with the off-gas the fines (fly ash) are collected in the gas cleaning system. The ash fractions contain valuable nutrients for soils like calcium, magnesium, potassium, nitrate and phosphate. Ashes from biomass incineration can therefore be valuable in terms of recycling these components to the soil when the ash is spread on greenlands. In this way the cycle of nutrients for biomass growth can be closed.

In this study the mobility of the mentioned nutritive ash components into the soil was investigated in order to determine the amount of elements set free by humidity and precipitation and getting so available for plant growth.

Extraction experiments on ashes were done using different extraction solutions according to a six step extraction procedure described in a literature where the mobility of cadmium in fly ash from biomass combustion was investigated. The first extraction medium was deionised water, the last extraction step was a digestion of ash components with diluted nitric acid.

All leachates were analysed by ion chromatography (IC). The measured ions were  $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$ . In order to determine the availability of the elements, also their total concentration in the ashes was analysed by IC after a microwave digestion procedure.

It was found, that most of the investigated elements could already be extracted in the first extraction step – the highest amounts being found for potassium, calcium, chlorine and sulphate. This is a satisfying result as these elements are important for the growth of biomass.

## P107-A2

### The Use of Electrochemical Techniques in the Study of Supported Phospholipid Bilayers and Transport of Charged Particles Across them

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The phospholipids bilayers (PLBs), consisting of two layers of lipid molecules, with various incorporated proteins, glycolipids etc., form continuous barriers around cells and cell compartments, separate them mutually and prevent the free transport of ions and proteins. However, various elements and compounds must be transported across the membranes for realization of usual metabolic processes. The transporting processes have been very well described in some cases (diffusion, ion pumps and channels (Na-K-ATPase etc.), endocytosis and exocytosis etc. On the other hand, much less attention has been devoted to the investigation of transport of heavy metals and of their inorganic compounds across such biological membranes between two aqueous (miscible) solutions. Therefore, we concentrate on investigation of such processes on supported model membranes utilizing the electrochemical methods, which seem suitable and promising for quantitative and qualitative characterization of such transports.

Firstly, the attention has been devoted to the construction of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and di-acyl-sn-glycero-3-phosphoethanolamine bilayers on polycarbonate porous supports, on the formation of ion channels using simple ionophores (e.g. valinomycin, which selectively enable ion trans-membrane transport), and to the characterization of such PLBs and of transport processes by electrochemical impedance spectroscopy (simulation by equivalent circuits, estimation of time constants). Further, PLBs, formed on agarose support, have been investigated. Possibilities of voltammetric determinations of heavy metal cations, transported across model and real membranes, have been considered.

#### **Acknowledgements**

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#### **Key words**

Supported phospholipid bilayer; transport of charged particles; voltammetry; electrochemical impedance spectroscopy.

## P108-A2

### Implementation of Agnes with a Thin Mercury Film Rotating Disc Electrode

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*This work is dedicated to the memory of Professor Helena Maria Carapuça, by the inspiration, the limitless support and especially by the always demonstrated friendship.*

The free metal determination with Absence of gradients and Nernstian equilibrium stripping technique (AGNES) [1][2] using a thin mercury film on a rotating disk electrode (TMF-RDE) has been implemented. The thickness of the mercury film and several AGNES parameters have been optimized. A nominal 16 nm film is chosen due to the higher faradaic current relative to the value of the capacitive current. The selected time for the AGNES measurement in the second stage ( $t_2$ ) is within [1-3] ms. A specific mathematical treatment is developed in order to subtract a corrected blank taking into account the degradation of the thin film (presumably, falling down of drops).

The limit of detection for lead(II) is  $7.4 \times 10^{-9}$  M and  $7.2 \times 10^{-8}$  M for a Y of 5000 (deposition time of 150 s) and 1000 (deposition time of 100 s), respectively. Lower LOD, in comparison with the conventional dropping mercury electrode or the Ir-Hg microelectrode, can be attained with this electrode for comparable times. Special care has to be taken to avoid anomalous stripping currents for higher metal concentrations which lead to a loss of linearity in the calibration plot. Good results have also been obtained with TMF-RDE when performing speciation determinations. The free metal concentration, and, thus, the conditional stability constants, determined with AGNES for the two systems considered (the complexation of Pb(II) with monodisperse carboxylated latex nanospheres and the complexation of Pb(II) with iminodiacetic acid (IDA), are in reasonable agreement with values reported in the literature [3][4].

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## P109-A2

### Influence of Surfactants on Deposition Process when Cadmium is Determined on Boron Doped Diamond Electrode

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Mercury based electrodes are widely used for the detection of heavy metals applying anodic stripping voltammetry. However its usage is in a recent times diminishing due to apparent toxicity of electrode material and discouraging environmental legislation. A possible non-toxic alternation to mercury are solid electrode like bismuth film electrode, chemically modified electrodes, carbon paste electrodes, glassy carbon electrodes or gold fibre electrodes are now more frequently applied.

One bare electrode receiving considerable attention is boron doped diamond (BDD), which is extremely robust with a low level of background interference as well as an attractively wide potential window also towards the positive potentials in aqueous media [1].

In this contribution the deposition and determination of cadmium using boron doped diamond electrode is discussed. This mercury free approach is first investigated under silent conditions then with applying an acoustic field to improve the limit of detection. The deposition process is also studied in the presence of highly passivating surfactant Triton X-100. The reason of sensitivity loss and deformation of voltammetric stripping signal is investigated by atomic force microscopy. It was shown that hindered electrodeposition (nucleation and growth) occurs in the presence of surfactants [2].

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## P110-A2

### Miniaturized Mid-Infrared Sensor Technologies

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Mid-infrared (MIR; 3-20 $\mu$ m) spectroscopy is based on the vibrational and rotational modes associated with most organic and anorganic molecules interacting with MIR photons, and provides inherent molecular selectivity particularly attractive for sensing applications. Despite the potential for optical sensing, early applications of MIR spectroscopy were mostly confined to a laboratory environment due to the dimensions of conventional spectroscopic equipment. Considering the already realized miniaturization/integration of UV-Vis and Near-infrared (NIR) optical devices, the opportunities for miniaturized MIR sensors are evident for in-situ and on-site monitoring. However, for establishing portable sensing systems appropriate technologies enabling miniaturizing/integrating each optical building block are required in order to facilitate on-chip MIR sensor technology.

Based on the contributions of our research group [1] toward on-chip IR sensors we will discuss recent progress in miniaturized MIR optical sensor technology utilizing quantum cascade lasers (QCL) in combination with planar GaAs/AlGaAs waveguides [2], which represent the first thin-film MIR semiconductor waveguides.

Aiming at cheap and mass-producible yet sensitive on-chip MIR sensor components, we will furthermore present progress toward structured MIR GaAs/AlGaAs waveguides based on e.g., strip waveguides and microdisk/microring resonators. Resonant optical structures ensure enhanced interaction of photons with the target molecules via the evanescent field at the device surface. Consequently, molecules may be detected with yet unachieved sensitivity by determining e.g., a resonance peak shift, a change in Q-factor, or a refractive index change. In contrast to similar device structures operating in the NIR spectral regime, a significant increase in sensitivity of 2-4 orders of magnitude is anticipated, given the significantly higher penetration depth of the evanescent field in the MIR regime.

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## P111-A2

### Time-Resolved Quasi-Elastic Laser Scattering Method on the Chemical Oscillation at Water/Nitrobenzene Interface with a Sodium-Alkyl-Sulfate System

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It has extensively been reported that an interface of a biphasic system of water and nitrobenzene exhibits a rapid flip motion and a tangential flow, so-called Marangoni convection, accompanying a pulse-like change of its interfacial electrical potential when an aqueous solution of sodium alkyl sulfate such as sodium dodecyl sulfate (SDS) is continuously introduced to the interface through a capillary. The hypothesized mechanism of this phenomenon is based on so-called Marangoni convection triggered by the heterogeneity of the interfacial tension. Here we have employed a time-resolved quasi-elastic laser scattering (QELS) method with a time resolution of 200 ms in order to trace the time-course change of the interfacial tension of the water/nitrobenzene interface, and demonstrated the first experimental evidence of the heterogeneity of the interfacial tension. It has been, moreover, found that the heterogeneity of the interfacial tension changes back to the homogeneity within 1 s and that  $DS^-$  ions are absorbed at the interface simultaneously.

The role of the electrolyte such as LiCl in water phase has been discussed. When the electrolyte is dissolved in the water phase, the surfactants are homogeneously absorbed at the interface immediately after they are conveyed by the tangential flow at the interface. On the other hand, they are solely distributed in both water and nitrobenzene phases without any absorption process on the interface in absence of the electrolyte. It is considered that the electrolytes suppress the repulsion among  $DS^-$  ions and stabilize their absorption at the interface. It has been proved that QELS are one of the most useful techniques to investigate the dynamic process of the molecules at the liquid-liquid interface.

### Electrochemical Methods of PNA/DNA Hybridization Studies

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Recently, there has been an increasing interest for DNA sensors based on the SAMs (Self assembled monolayers) of peptide nucleic acid (PNA) modified electrodes. PNA has a neutral peptide-like backbone with nucleobases that allows the molecule to hybridize to complementary DNA strands with high affinity and specificity.

In this communication, we present hybridization studies with DNA target oligonucleotides on a mixed monolayer of PNA and MCH (mercaptohexanol) on Au electrodes using EIS (Electrochemical Impedance Spectroscopy) and SECM (Scanning Electrochemical Microscopy).

The immobilized PNA probes on the sensor surface are uncharged, and hence, do not affect the charge transfer from the redox mediator  $K_4Fe(CN)_6/K_3Fe(CN)_6$  to the electrode. Once DNA targets hybridize to PNA, the charge density at the sensor surface will be changed. Thus, one can use EIS and SECM to conveniently monitor the PNA/DNA hybridization process in a label-free approach. Moreover electrochemical transduction of the hybridization process was also performed after coupling of a streptavidin–alkaline phosphatase conjugate and the bio-catalyzed precipitation of an insoluble and insulating product onto the surface of a gold electrode. As a consequence, the surface conductivity of the regions where hybridization had taken place decreased. This decrease can be adequately monitored by SECM and EIS.

## P113-A2

### Fluorometric Biochemical Gas-Sensor with UV-LED Based Excitation Technique for Monitoring Gaseous Formaldehyde

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#### Introduction

Formaldehyde in the gas -phase is one of the harmful and injurious VOCs (volatile organic compounds), and has been reported to develop Sick-house syndrome, especially at the hermetic house. In this work, we have constructed a high-sensitive NADH ( $\lambda=340\text{nm}$ ) fluorometric biochemical-sniffer by incorporating fibre-optic device with UV-LED ( $\lambda=335\text{nm}$ ) based excitation system and FALDH (formaldehyde dehydrogenase) enzyme membrane into a diaphragm reaction cell with gas- and liquid-compartments for the (sub-ppb level) formaldehyde monitoring in the gas phase.

#### Experimental

The NADH fluorometric sensor was constructed with an UV-LED ( $\lambda=335\text{nm}$ ), a fibre optic spectrometer, and an optical fibre probe. The UV-LED light source and the spectrometer (or photomultiplier tube) were connected to the optical fibre probe by Y-shaped optical fibre. A band-pass filter (BPF:340nm) and a long-pass filter (Cf=400nm) were placed. The FALDH immobilized membrane was attached on the optical fibre probe as the separating diaphragm between the gas- and liquid-compartments.

Measurement of the gaseous formaldehyde concentration was carried out by PB (w/ NAD<sup>+</sup>) rinsing to the liquid compartment with the optical probe. The excitation UV light was conducted to the sensing terminal of the optical fibre probe. Various concentrations of gaseous formaldehyde were supplied from a gas generator to the gas-compartment. The fluorescent signals of NADH, produced by enzymatic reaction of FALDH, were then guided to the spectrometer (or photomultiplier tube) and recorded using a laptop PC.

#### Results and Discussion

The change of fluorescent intensity induced NADH generation was observed by the application of formaldehyde vapour. The peak wavelength of the fluorescence was 491nm. The fluorescent intensity of the bio-sniffer was related to the concentration of the gaseous formaldehyde (lower detection limit: 500 ppt). The fluorometric bio-sniffer was possible to monitor the concentration change of formaldehyde vapour with good sensitivity (T95 = 1min) and ultrahigh gas-selectivity.



## P114-A2

### Following Milk Coagulation with Animal and Vegetal Rennet Using an Acoustic Wave Sensor

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Portuguese cheese with the protected designation of origin "Serra da Estrela", also known simply as "Serra" is produced exclusively from raw ewe milk and vegetal rennet: flowers of *Cynara Cardunculus*. Replacement of *Cynara Cardunculus* by animal rennet would lead to remarkable changes in cheese properties [1]. Besides, kinetics of the coagulation depends on the rennet used. Other ewe cheeses as well as goat cheeses from the same region are produced with commercial animal rennet.

Acoustic wave sensors based on piezoelectric quartz crystals are usually regarded as mass sensors, although in liquid the frequency of oscillation depends on the square root of the product between density and viscosity, in concordance with the Bruckenstein and Shay equation. [2] This dependence can be used to follow milk coagulation.

In this work, coagulation of milk was followed by measuring the equivalent circuit resistance, obtained connecting a piezoelectric quartz crystal to a network/spectrum/impedance analyser, as well as by the monitorization of the series frequency using an inexpensive oscillator and a frequency meter. Oscillator and crystal cell were home-made which reduces drastically the instrumentation cost, making the methodology accessible to all laboratories.

The possibility to follow real time milk coagulation enable kinetic studies and detection of the differences in coagulation of ewe, goat and cow milk at defined temperatures, with animal rennet or *Cynara Cardunculus*. Besides, the remarkable sensitivity of the sensor allows measurements to be performed at the early stages of coagulation.

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## P115-A2

### Application of Deuterium-Palladium Electrode in Potentiometric Determination of Acids in Tetrahydrofurane

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Potentiometric titration of acids : benzoic, acetylacetone,  $\alpha$ -naphthol, palmitine, 1-nitrozonaphthol-2 and maleine in tetrahydrofurane as solvent, using the electrode pairs  $D_2/Pd - SCE$  and  $D_2/Pd - H_2/Pd$ , was investigated.

Standard methanol solution of potassium hydroxide, sodium methylate and tetrabutylammonium hydroxide as titrant was applied.

The potential during the course of the titration including around the equivalence point, was established rapidly and it was stable.

The application of all three standard solutions as titrant using  $D_2/Pd - SCE$  and  $D_2/Pd - H_2/Pd$  as electrode pairs, shows the satisfactory potential jumps of the end point in the potentiometric titration of acids. The potential jumps of the end point in potentiometric titration using investigated electrodes with standard solution of sodium methylate are 2.5 – 13 times higher than potential jumps obtained using glass – SCE electrode pair.

The results of potentiometric determinations of acids were compared with those obtained by glass – SCE or visual determination using indicator thymol blue and they showed a good agreement,  $\pm 0.2\%$ , for all three standard solutions.

## P116-A2

### Wireless Biosensor for Real-Time Blood Glucose Monitoring in Sea Fish “flatfish”

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A wireless biosensor system was developed for continuous measurement of blood glucose levels in flatfish. Cultured flatfish are bred in aqua-farms in a high-density environment. The resulting poor water quality and various physical stresses may lead to mass outbreaks of infectious disease. Variations of blood glucose levels may represent stress in fish. Thus, monitoring of blood glucose levels is very important for managing fish health. To implant the biosensor in the interstitial fluid under the scleral surface of the eyeball (EISF), a relationship between EISF and blood glucose levels was investigated. EISF glucose levels were strongly correlated with those in the blood and were approximately the same as blood glucose levels in the range of 7 to 25 mg dl<sup>-1</sup>. For continuous EISF glucose monitoring in flatfish, a needle-type biosensor was prepared. A working electrode was made using platinum iridium wire and glucose oxidase was immobilized to the electrode. The biosensor was inserted into the ISF of the eyeball sclera of flatfish for sensor implantation. A 650-mV potential (vs. Ag/AgCl) was applied by a wireless potentiostat to the working electrode for the amperometric glucose measurement. We investigated whether glucose in the EISF can be determined *in vivo*. The estimated glucose levels using a one-point calibration method were correlated with actual blood glucose levels. Using a wireless biosensor system, we could monitor blood glucose levels in flatfish under free-swimming conditions for 16 hours.

## P117-A2

### Spectral Modeling of Mid-Infrared Hollow Waveguide Gas Sensors

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Optical simulations enable modeling an entire chemical gas sensing platform based on hollow waveguides (HWGs) operating in the mid-infrared (MIR) spectral regime using a three-dimensional representation of the sensor components, and taking the spectral response to virtual analytes into account. Furthermore, a strategy for including the spectral response of dielectrically coated HWGs is demonstrated. Utilizing experimentally obtained spectroscopic data recorded at well-defined conditions, the complex refractive indices of selected target analytes (i.e., methane, butane, and isobutylene) have been derived based on a refined harmonic oscillator model. In turn, these parameters have enabled directly assigning the dielectric functions of these analytes to virtual objects representing the analyte within the modeled sensor setup. In a next step, spectroscopic sensor response functions have been simulated as absorbance spectra across selected wavelength regimes utilizing spectrally resolved ray-tracing techniques, and have been compared to experimental data.

The present study is – to our best knowledge – the first attempt to simulate an entire FT-IR/HWG gas sensor system along with its spectral analyte response function, thus enabling directly correlating parameters of the optical sensor configuration with the obtained analytical signal, i.e., the absorption spectrum of individual or multiple gas phase molecular analytes in the mid-infrared spectral regime.

Next to modeling methane, butane, and isobutylene via spectral ray-tracing procedures using a virtual spectrometer, this concept was translated to modeling previously obtained experimental data at a FT-IR/HWG sensor setup. While the definition of a virtual HWG is different from the actual physical representation of a real HWG, the obtained results assigning the response of a virtual analyte to the hollow core of the simulated HWG confirms remarkably close resemblance between modeled and experimentally obtained analyte spectra. Consequently, even with several simplifications a convenient approximation of a real-world optical gas sensing scenario has been achieved.

## P118-A2

### A New Fluorescent Imprinted Polymers Prepared with Vinyl Group Containing Derivative of Acridine as a Signaling Monomer

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There has been a renewed interest in low-cost rapid techniques for measuring actinides and other heavy metal elements in environmental water. One uranium species of interest is the uranyl ion, because it can be found in soils and in low-pH water runoff in and around nuclear waste sites and processing facilities. Most of the reported spectrophotometric methods are tedious and time consuming because they involve prior separation of uranium from impurities by solvent extraction. Therefore, there is need for a simple and selective spectrophotometric method for the determination of uranium in the presence of some other metal ions.

Molecular imprinting is a template polymerization process using self assembly of target molecules or ions with functional monomers followed by copolymerization with a cross-linker [1]. After removal of the template molecule, the resulting polymers contain binding cavities that exhibit specific binding characteristics to the template and structurally related compounds [2]. Imprinted polymers have been designed not only as molecular recognition materials but also as sensors containing transduction elements that couple a readable signal to a binding event [3]. These types of molecularly imprinted materials can be used to directly detect and quantify the target analyte and could be widely applicable in assays [4].

In this study, we designed and synthesized vinyl group containing derivative of acridine (VAcr) as a new fluorescent functional monomer that has a polymerizable acrylate moiety and a fluorescent coordination bonding moiety. Uranyl was used as a model template, and we examined the utility of VAcr as fluorescent functional monomer in constructing uranyl-specific binding sites that emit a spectroscopic signal on binding. Prior to the preparation of imprinted polymers, the utility of the fluorescent functional monomer, VAcr, was tested. The binding characteristics (affinity and selectivity) of the polymers were evaluated by fluorescence measurements using the imprinted polymers as a recognition receptor.

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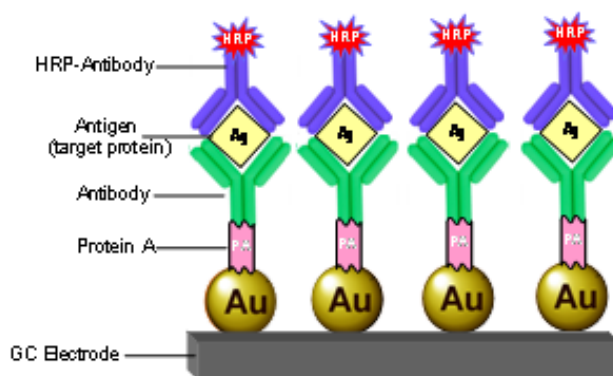
## P119-A2

### Amperometric Immunosensors Based on Nanobiocomposite Materials

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This work deals with the development of new amperometric immunosensors based on the working principle of the ELISA assays. The peculiar properties of nanobiocomposite materials based on gold nanoparticles were exploited for the immobilization of Antibody/Antigen/Antibody-HRP sandwich on the glassy carbon electrode surface.



The electrochemical transduction is mediated by thionine. The activity of such a mediator is fundamental for the electron transfer from the electrodic surface to the active redox center of HRP. Thionine was tested both in solution and after immobilizing it through electropolymerization. The immobilization protocol was applied successfully to different biological systems, the assays requiring very small amounts of sample (10  $\mu$ l). Interesting findings of the developed sensors indicate that their sensitivity and response range are strongly dependent on the size of the nanostructured gold substrate. In fact, gold nanoparticles were deposited on the GC surface by a suitable electrodeposition procedure that made possible to effectively control their size. The best results, in terms of homogeneity, assessed by scanning electron microscopy, reproducibility of the nanostructured layer and sensitivity were obtained with 100 nm-sized nanogold particles. These materials were obtained by double potential step electrolysis.

Other redox mediators (aminophenols) are also being studied in order to assess possible effects on the sensitivity of the assays.

Studies aimed at the amplification of the response by means of dendrimer-based linking of the primary antibodies have also been undertaken. The developed immunosensors could be exploited as powerful screening tools for analysis in clinical and forensic fields.

## P120-A2

### Hand-Held Optical Instrument for CO<sub>2</sub> in Gas Phase Based on Sensing Film Coating Optoelectronic Elements

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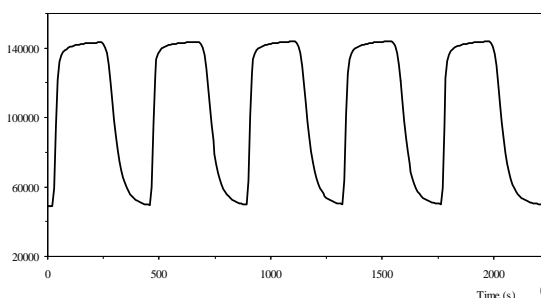
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The fast technological development of new LED and solid-state photodetectors is allowing the design of more compact optical instrumentation<sup>1</sup>.

We present different strategies in order to include a CO<sub>2</sub> optical gas sensor in a low-cost portable electronic instrument. The sensing chemistry is based on plastic solid state sensor membranes that work by quenching of luminescent platinum octaethylporphyrin complex by secondary inner-filter coming from a colorimetric pH indicator,  $\alpha$ -naphthophthalein. To avoid the strong quenching of luminescence by O<sub>2</sub> we used as O<sub>2</sub>-impermeable membrane polymer PVCD.

As a result of this study, the configuration with both optoelectronic components coated with sensing chemistry (LED with the luminophore and photodetector with the pH-active dye) presents a better CO<sub>2</sub> response in terms of sensitivity and reproducibility. The portable measurement system resulting from the integration of coated LED and photodetectors was characterised in terms of calibration, sensitivity, short and long term stability, transient response and thermal dependence with comparable results to laboratory instrumentation and other sensing films described in literature. The sensor calibration curve has been modelled according to a previous theoretical model with two coefficients, which has been



included in the microcontroller of the measurement system to provide a direct reading of the gas concentration in the display. Sensor full range is from 0 to 100 % of CO<sub>2</sub> concentration. The response and recovery times were 31 s and 129 s, respectively. Temperature dependence has been successfully fitted to an Arrhenius type function that has been included into the MCU of the instrument, to calculate and display the CO<sub>2</sub> concentration correcting the temperature dependence. The characterisation demonstrated the

reliability and good performance of this type of solution directed to integrate chemical sensors in electronic and optoelectronic devices.

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We acknowledge financial support from Ministerio de Educación y Ciencia, Dirección General de Enseñanza Superior (Spain) (Projects CTQ2005-09060-CO2-01 and CTQ2005-09060-CO2-02) and Junta de Andalucía (Spain) (Projects P06-FQM-01467, P08-FQM-3535).

## P121-A2

### **Voltammetric Determination of Carbamazepine with a Multi-Walled Carbon Nanotubes-Modified Glassy Carbon Electrode**

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Nowadays the analysis of pharmaceutical substances plays vital roles in the quality control of drugs and of environmental water samples, because these can have an extensive impact on public health. Therefore the development of new, simple, sensitive and accurate methods for the determination of this kind of substances is of great importance and interest.

Among the several common pharmaceutical substances used today, and which can be found to be easily dissipated in the environment as emerging pollutants, the antiepileptic drug carbamazepine (CBZ) is one of the most serious problems due to its large use in some regions.

In this work, a multi-wall carbon nanotubes/dihexadecyl hydrogen phosphate film-modified glassy carbon electrode (MWCNTs/DHP-GCE) is described for the quantification of CBZ by linear sweep voltammetry (LSV). In 0.1 mol L<sup>-1</sup> phosphate buffer solution (pH 6.89), CBZ undergoes several oxidation processes during a potential sweep from -0.700 to 1.300 V versus Ag/AgCl, 3M KCl. The voltammetric response of CBZ in this electrode is significantly improved, when compared with the bare GCE. Around 1.080 V an oxidation peak current can be used as analytical signal for CBZ determination. This signal changes linearly with the concentration of CBZ in a range from 1.3 x 10<sup>-7</sup> to 9.3 x 10<sup>-7</sup> M. The proposed quantification method of carbamazepine was successfully applied to commercial medicinal tablets and wastewater samples.



## P122-A2

### Introducing a New Advanced Data Processing Software for Mass Spectrometry Using Spectral Deconvolution and Chemometric Data Analysis

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A new chemometric based software programme (TargetView) is discussed for identifying the presence of known compounds within a sample analysed by GCMS. The software is a post analysis programme which incorporates dynamic baseline compensation, spectral deconvolution and multivariate data analysis (PCA, pattern recognition).

Many applications exist where post run screening for specific compounds is desirable. Examples of this include carcinogenic/toxic compounds released from building/construction materials; chemicals defined within REACH, allergens within fragrances, hazardous air pollutants etc.

TargetView can be configured to detect lists of compounds, and ultimately produces a simple printed report and/or text file listing those identified. External user programmes will have access to this data for customisation.

The analysis of GCMS data using TargetView follows a defined protocol.

A dynamic background compensation (DBC) algorithm is initially applied which minimises non peak specific background mass ions.

Subsequent deconvolution of baseline-free peak spectra is followed by chemometric data analysis (PCA) to create a class file. A second class file is generated from target spectra. The target and deconvoluted class files are cross matched using forward, reverse or combined search strategies for identification.

When a target compound is found a match co-efficient is calculated (0 to 1) and a plot of match value against peak apex RT is created. A report is ultimately generated showing positive compound identification

One application for this software is within the building/construction industry where regulatory lists of compounds have been defined based on compound toxicity and/or carcinogenicity.

To test software performance material samples were analysed for emissions using thermal desorption preconcentration and GCMS analysis. The TIC profile from these samples are complex, containing many compounds with considerable co-elution and background matrix effects. Under these circumstances the benefit of spectral deconvolution prior to PCA analysis will be shown leading to improved identification and higher match coefficient values.

## P123-A2

### About Estimating the Limit of Detection of Heteroscedastic Analytical Systems

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The limit of detection (LOD) is a fundamental figure of merit in trace analysis, since it indicates the lowest concentration or quantity that can be measured with reasonable statistical certainty. Different approaches are available for its estimation, but only few allow analyzing data characterized by a significant change of precision with concentration. Among these, the ISO 11843-2 approach is certainly the most suitable. However, its implementation may look unapproachable to operators less trained in developing their own spreadsheet by software packages. This contribution is aimed to verifying if more friendly approaches are available for getting acceptably accurate estimates of the LOD. Pragmatically, they were set-up by adapting to heteroscedasticity two of the approaches already available in the literature, but compatible only with homoscedastic analytical systems, namely that based on the standard deviation of the blank and the Hubaux and Vos one.

The performances of these alternative approaches were compared to those of the ISO one by analyzing different combinations of 15 calibrations relevant to the determination of Cr(VI) in water samples by Adsorptive Stripping Voltammetry (AdSV). This analyte/method combination was chosen since, unless in cases of abnormal contamination events, Cr(VI) is present at trace/ultratrace levels in these samples, thus making mandatory an accurate estimation of the LOD and, moreover, previous experimental findings proved the heteroscedasticity of the relevant results. The comparison allowed evidencing that the differences between the various LOD estimates critically depend i) on the quality (numerousness) of the available data and ii) on the chosen method for estimating the weights necessary for performing the weighted linear least square regression of the data. However, they also showed that, even when using the ISO approach, the only safe way to present reliable estimates is performing a number of measurements normally unacceptable under standard operative conditions.

## **P124-A2**

### **Assessment of Medical Laboratory Quality Management in Relation to ISO 15189:2007 in Thailand**

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The objectives of this study were to assess the quality management of 95 medical laboratories at regional and general hospitals under the jurisdiction of the office of the permanent secretary, Ministry of Public Health, Thailand, in relation to ISO 15189:2007 and determine the relationships between role perception, leadership and organization climate with medical laboratory quality management. This study was a cross-sectional study. Data were collected by self-assessment questionnaire distributed to 95 medical laboratory unit chiefs from August 2008 to January 2009. The response rate was 100%. Statistical analysis used were Pearson's product moment correlation coefficient and stepwise multiple regression.

Results revealed that role perception, leadership and organization climate were significantly related to overall quality management of ISO 15189:2007 ( $p < 0.001$ ). Leadership of the medical laboratory unit chiefs and organization climate were the variables that could explain the quality management of ISO 15189:2007 at 17.1% ( $R^2_{adj} 0.171$ ).

It is recommended that medical laboratory unit chiefs should enhance their leadership and be responsible for design, implementation, maintenance and improvement of the quality management. Top management should ensure that the organization structure, organization policy, surrounding and technology which contribute to a quality management system have been made in relation to requirements of ISO 15189:2007.

## P125-A2

### hyperSpec: Chemometric Analysis of Spectroscopic Data in R

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*hyperSpec* is a software package making the statistics software R[1] a convenient platform for analysis of spectroscopic data. *hyperSpec* enables import and export, plotting (spectra, maps/images, calibration curves, depth profiles), and handling/preprocessing of the spectra. Chemometric methods are supplied by R: algorithms for regression, classification, cluster analysis are readily available as well as means for validation and determination of confidence intervals, etc.

Spectra can be stored with arbitrary amounts of meta-information such as position, constituent concentrations, diagnoses, etc. Spectral images/maps need neither be rectangular nor evenly spaced, and may contain spectra without spatial information. Chemometric analysis of spectroscopic data needs specialized and customized methods (e. g. validation schemes for varying numbers of repeated measurements, robust statistics). *hyperspec* is extendible and interacts with other R packages.

R is a statistical programming environment: sophisticated statistical routines are readily available and well tested. R is developed with a quality assurance work cycle and participated in a statistics software test[2]. A standardized interface for data types and methods allows flexible interaction between specialized data (like *hyperSpec* data sets) and specialized chemometric methods. *hyperSpec* allows scripting: calculations can be run as batch jobs. There are also functions for user interaction. Graphical user interfaces tailored for specific tasks can be built.

We present two examples: cluster analysis of a Raman map of chondrocytes in cartilage, and linear calibration of fluorescence emission of quinine.

*hyperSpec* is hosted at <http://r-forge.r-project.org/projects/hyperspec/>.

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## P126-A2

### PAT Solutions for the Packed Raw Substances Control

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The possibility of routine testing of pharmaceutical substances directly in warehouses is of great importance for manufactures, especially in consideration of PAT demands. Rapid, nondestructive analysis of chemical and physical properties of substances is achievable using NIR instruments. Fiber optic probe can measure remote samples in diffuse reflectance or transmission modes. Raw materials are quickly tested for identity and quality conformance. Once a pattern recognition model for a substance has been developed, routine testing takes place in a few seconds, making it possible to test every drum of incoming ingredient to verify the identity.

At the same time the application of fiber probe diffuse reflectance NIR spectroscopy is a particularly challenging problem when measurements are carried out through closed polyethylene bags. This could produce spectral artifacts that are comparable with the substance physical or chemical fingerprints. Additional troubles arise due to the changes in the position of the probe.

In spite of such circumstances the presented two-step approach helps the operator to recognize the perfect raw substances reliably. Generally, at a given Type I error, a pattern recognition results is a dichotomous decision: either accepted, or rejected. We suggest [1] applying trichotomy recognition: accepted, rejected, or extra measurements are required. The latter decision is made if a new spectrum bears the signs of the abovementioned artifacts.

The approach includes the following main issues:

- the appropriate splitting of the initial calibration objects into two classes employing a global PCA model
- the construction of two separate PCA models with an apt number of principal components (PCs)
- the application of Soft Independent Modeling by Class Analogy (SIMCA) with ad hoc calculated acceptance levels [2] for the reliable identification

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## P127-A2

### **An Innovative Instructional Activity to Discuss Sampling and Sample Homogeneity in Analytical Chemistry Undergraduate Courses**

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The conventional curriculum of Analytical Chemistry undergraduate courses emphasizes the presentation of techniques, methods and procedures used for instrumental analysis. Despite their importance, it's necessary to consider the fundamental concepts of Analytical Chemistry in a conceptual perspective. Sampling preparation can be considered as the most critical step to ensure the accuracy and the representativeness of the analytical results, regarded as capital analytical properties. Innovative instructional activities must be devised to fostering the conceptual understanding of the fundamentals of chemical analysis. We present a planned instructional activity to discuss sampling in details. Sample homogeneity, particle sizes and sample mass size are the parameters explored to show how they can affect the precision and the accuracy of analytical results. M&M candy packages are used and their content present candies of six different colors packaged randomly. As the percentage of each color is defined by the manufacturer, the sampling can be studied by counting the different colors candies and comparing the data with a reference value (informed by the manufacturer). The influence of the sample mass size is considered comparing three different M&M's milk chocolate package containing 52, 104 and 200 g. The candies are mixed and different sampling strategies are devised from the students' comments, according to the vessel to be used (cups of 200, 50 and 15 ml). They can note that the smaller sample size (15 ml cup) produces less accurate experimental results, in comparison to the reference value. The influence of sample mass size was evaluated using peanut and mini milk chocolate M&M, respectively bigger and smaller than milk chocolate M&M. The students can observe that the bigger sample size produces less accurate and precise experimental results (n=3). At last, statistical tools are discussed and the student t-test is presented as an important statistical evaluation of the experimental results.

## **P128-A2**

### **A Model of International Quality Systems Symbiosis towards Chemical Use for Sustainable Future**

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Careful use of chemicals has brought many benefits to human daily life in form of medicines, personal care products, industrial chemicals etc. Unfortunately, unwise use of chemical did results adverse effects to human and environment. Nevertheless, history has showed society worldwide did learn from their mistakes in relation to chemical use. This is evident based on the global establishment of various quality systems (QS) and quality management systems (QMS) developed by internationally represented bodies, e.g. Organisation for Economic Cooperation and Development (OECD) and International Organization for Standardization (ISO). These QS (e.g. GLP, GMP, and GCP) and QMS (e.g. ISO9001, ISO13485, ISO14001, ISO15189, ISO/IEC 17025, and OSHAS18001) have its own scope of application and originally some were not intended specifically for chemical safety. However, due to consistent community awareness and political will globally for the past three decades, in reality the adoption of these systems in stages by chemical producers within their economies had created inclination to ensure safe and effective use of chemical based products at higher standards. Somehow a symbiotic linkage among these systems was created towards ensuring chemical produced does not cause detrimental impact to human and environment. Today's model of symbiosis should be made known to stake holders with hope it's benefits will be optimized as a continuous effort in minimizing the risks of using hazardous chemical, ultimately towards sustainable future.

## P129-A2

### Optimization of the Experimental Conditions in High Performance Liquid Chromatography by Using Response Surface Methodology for Macrolide Antibiotics

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Macrolides are a group of antibacterial compounds that are active against Gram-positive and some Gram-negative bacteria. They are basic and lipophilic molecules that consist of macrocyclic lacton rings containing 14–18 atoms with sugars linked via glycosidic bonds. They have been widely utilized in medical and veterinary practice to prevent microbial infections. Chromatographic methods, which allow multiresidue analysis, are appropriate techniques and high performance liquid chromatography (HPLC) has been widely applied to the determination of macrolides, its related substances and degradation products in bulk samples and preparations.

In this study, response surface methodology (RSM) was used as an optimization method for determination of five macrolide antibiotics (erythromycin, tylosin, tilmicosin, josamycin, roxithromycin) by HPLC. An important aspect of RSM is design of the experiment. Central composite design was used for method optimization. The investigated response is resolution and capacity factors for each macrolide antibiotics. Acetonitrile percentage, pH of mobile phase and column temperature were investigated as factors. X Terra RP-18 column was used as stationary phase. This column was specially designed for the analysis of basic compounds in LC and used successfully as a stationary phase for the macrolide analysis [1-3]. X Terra has an extended pH stability, to be thermally more stable, and to be more efficient than classical silica-based packings. Thereupon, pH 2.5 as mobile phase pH, 35% (v/v) of acetonitrile content and 30 °C column temperature were finally chosen for the optimal separation of selected macrolide antibiotics. Good selectivity, sensitivity and peak symmetry were obtained in the study.

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## P130-A2

### Persistence, Bioaccumulation and Toxicity (PBT) Screening for REACH (Registration, Evaluation and Authorisation and Restriction of Chemicals) Using a Quantitative Structure Activity Relationship (Q)SAR Approach

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Small to medium size enterprises (SMEs) in the EU are facing challenges due to the introduction of new legislation designed to protect consumers and the environment, REACH (Registration Evaluation and Authorisation of Chemicals). There can be high costs associated with implementing REACH because data on mammalian toxicity, environmental toxicity and environmental fate properties is required and if it is obtained experimentally the cost is significant. These costs can be reduced if reliable (Q)SAR models can be used instead (Lahl 2008 and Tyle 2001). This project which is part of a bigger EU project (TESS) aims to provide support for SMEs on the use of (Q)SAR for REACH registration since they lack the experience in this field.

In this poster a user friendly (Q)SAR-based methodology to identify the PBT properties of chemicals of interest to SME is presented. The method is based on a stepwise approach (Figure 1). This method was then applied to screen 15 chemicals of interest to the SMEs involved in this project. Freely available (Q)SAR software packages and databases were used for the assessment. These were the EPIWIN computer programme, the Danish (Q)SAR database and the PBT profiler tool. Using the EU screening criteria two of the substances were found to be PBT (t-dodecanethiol and cyclododecane). Using this approach the toxicity of four of the chemicals could not be determined as they were outside the model domain (Figure 2) and further experimental data was needed.

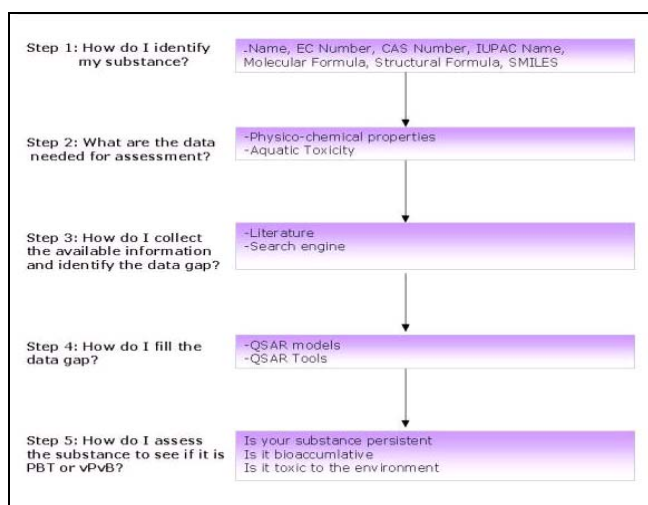


Figure 1: PBT screening methodology based on (Q)SAR

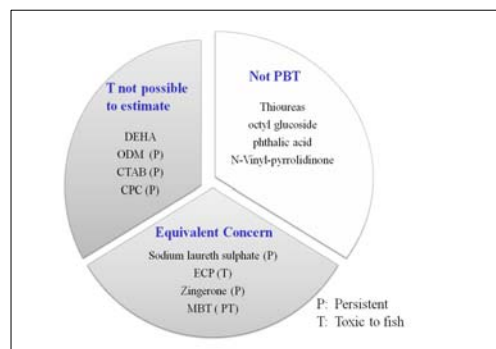


Figure 2: PBT

In this study problems were also identified when using the (Q)SAR databases such as the Danish data base and PBT profiler and it is important to highlight these and explain how these problems can be both identified and overcome such that SMEs can be confident about the results they obtain (Zachary and Greenway 2009)

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#### Acknowledgements

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## P131-A2

### Evaluation of the Effect of E-Beam Irradiation in Ready to Eat (RTE) Food by Principal Component Analysis (PCA)

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The deep change of the dietary population habits has led to manufacture a great variety of ready-to-eat (RTE) foods which preparation involves processing operations increasing contamination risks by a wide variety of pathogens. To control food contamination, E-beam irradiation is an effective way for sanitation purposes. However, it may produce changes in sensory properties and nutritional food quality.

The aim of the present study was to evaluate the effect of E-beam irradiation on major components of different RTE foods, including cooked and dry cured Spanish ham, smoked salmon, minced meat and soft cheese.

Several parameters such as protein<sup>1</sup>, fat<sup>2</sup>, water<sup>3</sup>, nitrate and nitrite content<sup>4</sup> as well as free amino acids<sup>5</sup>, produced by the radiolysis effect, were determined in both non irradiated and irradiated samples at doses up to 8 kGy employing official and/or validated analytical methods. The data obtained were evaluated by means of analysis of variance (ANOVA). The one-way ANOVA test showed that, in the most cases, there were statistically significant differences between the parameters studied from one level of radiation to another at 95.0% confidence level.

Because of the radiation effect on food composition was different depending on food nature, principal component analysis (PCA) was applied. This technique showed correlations between experimental results obtained as well as some relationships between the studied parameters. In all cases, the two first principal components (PCs) described more than 70.63% of the total data variance. The plots of the second PC versus the first one showed that protein and fat content were the parameters most affected at high level irradiation doses in all the studied foods. On the other hand, changes in composition were observed in minced meat and in soft cheese at irradiation levels higher than 6 kGy while for the other RTE foods, these changes were observed from 2 kGy radiation dose.

<sup>1</sup> AOAC Official Method 981.10

<sup>2</sup> Dionex Technical Note no. 334, no. 345 (accelerate solvent extraction, gravimetric method)

<sup>3</sup> AOAC Official Method 930.15

<sup>4</sup> AOAC Official Method 973.31 and 993.03, ISO 14673-1:2004

<sup>5</sup> HPLC-UV method (C<sub>18</sub> column, isocratic separation with methanol - 20 mM pH 6 ammonium acetate buffer (6:94 v/v), 260 nm)

## P132-A2

### Infrared Spectrometric Purity Control of Chemical and Pharmaceutical Substances in Process Environments

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All organic and most inorganic compounds show characteristic Infrared Spectra. Therefore IR Spectroscopy can be used as an effective tool for quality control. With modern instrumentation and accessories like Diamond ATR Units the spectra obtained are highly reproducible making it possible to detect even minor spectral deviations caused by impurities. In this presentation we focus on the quality control in chemical or pharmaceutical process environments where the purity of materials has to be determined more or less automatically.

The correlation between the spectrum of a potentially contaminated sample and the reference spectrum of the corresponding pure compound can be used as measure for purity in terms of the correlation coefficient R [1]. R is obtained by regressing the sample spectrum on the reference spectrum [2]. A simulation study showed that it is advantageous to transform R to Fisher's Z coefficient [3] because Z is much better suited than R for detecting small spectral deviations caused by impurities.

As a second way for discovering impurities we developed a method of dynamic difference spectroscopy, by which the difference between the spectrum of the sample and the reference and the corresponding difference factor are calculated automatically. As spectral purity grades the difference factor itself or alternatively the integral of the difference spectrum were used.

For the purity control of liquid materials we used the phthalate plasticizer Palatinol N and for solids acetyl salicylic acid as examples. The above described different kinds of spectral purity parameters were determined and discussed with respect to their suitability for the purpose of purity control in a process environment.

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## P133-A2

### **The Use of an On-Line Questionnaire as a Quality Control Tool for the Lab Work of Subjects. Preliminary Results**

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The present work has been developed by the members of INDONUTyB. This is a new network created at the University of Alicante and devoted to the research, development and application of new teaching methodologies to subjects of the Analytical Chemistry, Nutrition and Food Sciences Department. At the present these subjects have a relatively high number of hours of practical type (i.e., more than 50 % of the hours). This practical work is carried out by performing some experiments at the laboratory of the Department. The diversity of students with very different lab skills and background knowledge (about chemistry, nutrition and food chemistry), the idiosyncrasy of the different teachers (mainly due to their part time dedication to teaching at the University) and the heavy load of work (practical) at the end of the semester are factors that can profoundly modulate the quality of the teaching process.

In order to improve the results afforded by the students, a tool for the evaluation of the lab-work was proposed. This tool consisted of an on line questionnaire that the students must fill in via the web of the University (Campus Virtual) after the lab work has been finished. Using the information obtained an insight on the perception of the students about the work developed could be obtained and subsequent actions could be taken.

The first subject evaluated was Toxicology. The main results obtained showed that more than 50 % of the students have a positive global perception of the lab work. On the contrary, a 17 % ranged it as bad or very bad. The most appreciated lab experiments were those less sophisticated. Students valued more positively working in groups than individually. In addition the lack of more instrumentation was quoted as an important negative aspect because students had to wait a lot of time to use one of the instruments. All these conclusions will be integrated for the edition of both teachers' guidebook and students' notebook that will be used in the forthcoming academic year (i.e., 2009-2010).

## P134-A2

### Development Determination Method of Composition Modified Atmosphere by Volatile Corrosion Inhibitors

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The action of Volatile Corrosion Inhibitors (VCI) is based on the property of compounds vaporizing at room temperature (such as some amines), generating an atmosphere of VCI then condensing on a metal surface. This process makes the metal less susceptible to corrosion. Plastic films impregnated with VCI or sachets impregnated with VCI are the most common ways for utilization. However, the quality valuation of these products is not well established and is currently held by long electrochemical tests. In this work we suggest an analytical method to evaluate the efficiency of the VCI to generate atmospheric vapor in stationary state. A simple method to collect vapor of VCI using a hanging drop was implemented. Preliminary experiments were carried out in order to optimize the droplet sampler. Solution acidified with hydrochloric acid, at a pH of 2.0 was used to make the drop. For determination of composition of VCI vapor, the capillary electrophoresis (CE) was used because this technique uses small volumes of the sampler. For CE, amines were separated from their cationic forms and were indirectly determined by imidazole addition in the running electrolyte, with reference absorption at 214 nm. The analyses were performed in a 75  $\mu\text{m}$  i.d. uncoated fused-silica capillary with 53.5 cm length (effective length of 45 cm) using a running buffer consisting of 0.010 molL<sup>-1</sup> hydroxyisobutyric acid (HIBA), 0.010 molL<sup>-1</sup> 18-crown-6-ether and 0.010 molL<sup>-1</sup> imidazole, pH 4.3. Amines were separated with a voltage of 18 kV. Samples were injected hydrodynamically by applying 50 mBar pressure during 3 s. This study demonstrates that the developed method is capable of evaluating the amines vapor concentration of dicyclohexylamine (DCHA), monocyclohexylamine (MCHA) and monoethanolamine (MEA), from anticorrosion trade plastics films. A short time analysis was achieved (8-12 min) from gas collecting to the CE separation.

## P135-A2

### Sea in a Drop of Water - Teaching Water Chemistry by Using MathCAD

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Calculation of concentrations of the various species present in the case of many simultaneous equilibria is a laborious operation. These kinds of calculations are usually included in some forms in the curricula of *Analytical chemists* for a better understanding or developing of new analytical techniques, *Environmental engineers* and *scientists* examining drinking water quality, designing water treatment technologies, studying the fate and transport of chemicals in surface and ground waters, *Geologists* looking at solute interactions with minerals, *Toxicologists* interested in the interactions of chemicals with organisms and *Ecologists* looking at the dynamics of chemicals in the food chain. Speciation calculations have therefore a well defined place in the education of natural scientists and chemical engineers. When the subject of speciation is introduced in their curricula there are at least two confusing issues that frustrate students: 1. what is the minimum number of required information to set up an adequate algebraic model of the chemical system, 2. how to solve the generated nonlinear system of equations in a convenient way.

Conference contribution will illustrate how the Analytical, Stoichiometric and Thermodynamic Information can be organized in a consistent way in the form of the so called ASTI matrix. This matrix provides a useful help to set up the non-linear system of equations (NLSE) required to solve a speciation problem. Solution of the NLSE is handled by the MathCAD program which is a great help to non-programing users and takes away the burden of numerical calculations. Contribution will demonstrate how to use the ASTI matrix to solve speciation problems including acid-base, precipitation, complexation, redox and interfacial equilibria occurring in aquatic systems. The reliability of the ASTI matrix approach and the elegant way of solving the NLSE are highly appreciated by the students especially when they are asked to model "what if" scenarios occurring in aquatic chemistry.

## P136-A2

### Precipitation Liquid Chromatography as a Tool in Polyolefin Analysis

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Polypropylene takes a key position in various sectors of industry such as packaging, automotive, mechanical engineering and electronics, thanks to its excellent material properties and superior cost-performance ratio. Progress in catalyst development and innovative processing technologies promise that polypropylene is going to gain even more importance in the future.

However, a profound analysis is crucial in order to understand the relationship between catalysis mechanisms and processing conditions on the one hand and the material properties of the generated polypropylene on the other hand. Therefore, properties such as chemical composition, molecular weight distribution, rheologic behavior, branching, cross-linking and tacticity have to be examined. The tacticity of polypropylene is usually determined by performing NMR experiments. Some information on the tacticity, the presence of a rubber phase or the degree of cross-linking can also be gained by performing fractionation experiments with different solvents. Although basic relationships are already known [1, 2, 3], the substances contained in the different fractions have not yet been thoroughly characterized.

In course of this work we evaluated the potential of precipitation liquid chromatography for the characterization of fractions extracted from polyolefins. In precipitation liquid chromatography all or a part of the sample is immobilized on the top of the column at the conditions during the injection. The immobilized fraction is subsequently eluted from the column by changing the mobile phase. By coupling this separation mechanism to size-exclusion chromatography (SEC), a deeper insight in the composition of different fractions of polypropylene was obtained.

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## P137-A2

### Delivering Analytical Science E-Learning in the Workplace

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The Open University (OU) is well known for the delivery of world class distance education. From 2010, the OU will offer a distance learning course to train analytical scientists in their workplace. The course *Analytical Science in Practice* is delivered primarily over the web with supporting text material. This work-based course, developed in collaboration with the UK water industry, complements existing practical skills, developing understanding of the science underpinning analytical work. Students will learn information technology skills necessary to operate effectively in the modern workplace, and the numerical skills to carry out the calculations required in their job with accuracy and confidence. The course explains the regulations and science for safe working, enabling students to become safer practitioners. Considerable emphasis is given to developing basic laboratory operations and techniques using video clips and interactive assessment. Effective teamwork and communication are vital in an analytical laboratory and the course will help improve an individual's proficiency in these areas. Finally, problem-solving skills are developed, alongside learning how to make evidence-based decisions.

The course forms part of a new Foundation Degree in Analytical Sciences, developed to meet needs of staff already employed in analytical laboratories and to enhance the skills base of the workforce. The degree is interdisciplinary in approach with opportunities for specialisation in chemistry or biology. The Foundation Degree combines work-based and supported open learning. Responsibility for learning is shared between the employer and The OU. The OU provides study materials and tutorial support and manages the assessment procedures, while the employer substantially manages the work-based learning on the programme. The OU is able to offer support in training staff identified as practice assessors. The student remains in employment while they study.



## **P138-A2**

### **Validation of UHPLC Methods: The Application of Efficient Experimental Design and Utilization of Waters Empower™ 2 Method Validation Manager to Expedite Method Validation**

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The validation of analytical methods has traditionally been a time consuming process. Multiple experiments on large numbers of solutions have led to significant lab time and consumption of large quantities of solvents. Innovations in experimental design to make more efficient and combined use of solutions for multiple validation tests, alongside the utility of injection volume variation for linearity has significantly reduced overall lab time and solvent consumption. In addition, this poster builds on the approach with an evaluation of Waters Empower™ 2 Method Validation Manager to bring together the practical elements of experimental design and state of the art data interpretation and processing to expedite UHPLC method validation.

## P139-A2

### The Importance of Magnetic Behaviour of Impurities Present in $\text{CaF}_2$ , $\text{AlF}_3$ and Cryolite for Fluoride Analysis by ss-NMR Spectroscopy

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Process control in solid state is not still highly-developed in the industrial field, nevertheless the advance in instrumentation allows new applications. Solid state nuclear magnetic resonance (ssNMR) spectroscopy has becoming increasingly important and accessible to the non-NMR specialist.

In this work, pulsed and low resolution F-NMR spectroscopy with quantitative purpose has been studied in solid materials from the HF manufacture industry, with the aim of implementation a new, fast, economic and green analytical method for fluoride analysis.

Previous the application of NMR-F in solid state, it is necessary to carry out a magnetic study since the presence of impurities at trace levels can affect the magnetic behaviour of diamagnetic solids. The nature and position of such impurities can conditioned the ability of solid state nuclear magnetic resonance of fluoride (ssNMR-F) to be applied at line process control of the manufacture of aluminium fluoride and cryolite in the HF industry.

Electronic paramagnetic resonance (EPR) and magnetic measurements techniques have been performed in order to study the magnetic behaviour of the impurities present in such materials, as well as in certified reference materials (CRM).

From the experimentation carried out in this work, the requirements for the ss-NMR spectroscopy implementation for fluoride determination have been established. Low resolution and pulsed F-NMR spectroscopy has demonstrated to be a useful tool for the analysis of fluoride in the manufacture of  $\text{AlF}_3$  in the fluorine industry.

**Keywords:** fluorite, aluminium fluoride, cryolite, solid state, low resolution and pulsed ssNMR-F, ferromagnetic and paramagnetic impurities, MINISPEC

## P140-A2

### Analytical Process Control of $\text{Ca}(\text{OH})_2$ Content in Anhydrite by FTIR-ATR Spectroscopy

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Nowadays, environmental concern is an important aspect that governments take into account more and more. So, industrial waste recycling is a way to improve resources, economy and environmental issues. In this sense anhydrite, a subproduct, has different applications such as: building material and road construction.

Anhydrite is formed as a result of the HF production process, when fluorspar mineral ( $\text{CaF}_2$ ) reacts with  $\text{H}_2\text{SO}_4$  (cc). However, before using this subproduct, anhydrite must be neutralized with CaO. The excess of CaO is rapidly hydrated forming  $\text{Ca}(\text{OH})_2$  and the determination is actually carried out using chemical analyses which are time and reactive consuming.

The aim of this research consists on the development of an easy, fast and green analytical methodology, using Fourier Transform Infrared Attenuated Total Reflectance (ATR) (FTIR-ATR) spectroscopy and Partial Least Squares (PLS-1) as the quantitative analysis procedure to quantify  $\text{Ca}(\text{OH})_2$  content in the neutralization process of anhydrite.

The spectral data were derivated and the PLS-1 model obtained by leverage correction algorithm was chosen attending to: accuracy, repetitivity, number of principal component (PC), calibration and prediction error (RMSEC and RMSEP, respectively). The method provided satisfactory quantitative analytical results, taking into account the variability of samples.

**Keywords:** anhydrite,  $\text{Ca}(\text{OH})_2$ , FTIR-ATR spectroscopy, chemometric, PLS-1, analytical process control

### Chemometrics, a Powerful Tool for Optimizing Flow Injection Analytical Methods

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Flow injection (FI) techniques are presented as power tools for handling solutions and subsequent chemical analysis. Due to their potentials in terms of automation, miniaturization, versatility and inexpensiveness, applications of FI techniques have ever increasing. In this issue, up to date, more than 10,000 publications are available in the literature. However, within the extracted results, less than 100 publications exploited chemometrics for optimization. In spite of its limitation, most of FI methodologies utilizing the univariate approach for optimization.

It has been proved that chemometrics potentially develops analytical methodologies. The strategy of chemometrics is to gain the highest efficiency of analytical methods in the shortest way. Chemometrics gains that strategy throughout the following approaches. (i) Examining the effects of the main and the interaction of experimental conditions on the efficiency of analytical methods (ii) Optimizing experimental conditions considering their interactions. (iii) Developing more than one analytical aspect, i.e. sensitivity, rapidity, etc., synchronizingly. (iv) Reducing the large amount of data that can be easily interpreted. (v) Testing the ruggedness. Not least but more, the utilization of chemometrics for optimizing analytical methods provide other potential advantages such as time saving, reduction of chemical consumption and effort minimization.

This communication presents selected applications of chemometrics for optimizing FI analytical methods. In this issue, the communication focuses on the potentials of chemometrics. Furthermore, future perspectives of exploiting chemometrics for developing sequential injection chromatography, as a recent version of SIA, are discussed.

On the other side, this communication also presents, for the first time, a comprehensive chemometric optimization study for developing a new assay method for diclofenac utilizing sequential injection analysis (SIA) technique. All previously chemometrically optimized SIA methods considered only reagent concentration, reaction time and flow rate. In the current study, more additional experimental conditions, including volumes of reagents and samples, were optimized; and the 2<sup>5</sup> full factorial design was adopted. It has been found that the latter experimental conditions significantly effect on the efficiency of the newly proposed SIA method.

#### Acknowledgments

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## P142-A2

### From Micro and Trace Analysis to Nanoanalysis. Critical View

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The aim of this paper is to discuss the position of these techniques in the general analytical process. Quantitative analysis is divided in three steps regarding the quantity of the sample used: macroanalysis –  $10^{-1}$ - $10^{-4}$ g; microanalysis  $10^{-4}$ - $10^{-6}$ g and trace analysis  $10^{-6}$ - $10^{-9}$ g.

Nanoanalysis represents the future of chemical analysis. This technique was developed due to a perfect connection between the operational parameters of the method and the functional parameters of the instrument. A big contribution for the development of nanoanalysis has automatic systems of analysis.

Nanoanalysis can be considered as a branch of microanalysis. Generally, one can include the three steps in a general meaning of quantitative analysis. Microanalysis was initiated by a team of famous researchers as F. Pregl (Nobel Prize), F. Feigl, G. Charlot, R. Belcher, etc.

Trace analysis was imposed as analytical technique due to new technologies and biological studies. These techniques were developed rapidly and were imposed as quantitative microtechniques. An interesting field of trace analysis is image analysis using a lot of "special reagents", as X-ray, electrons, neutrons and synchrotron radiation.

All these techniques were discussed in comparison concerning their performances, with a lot of examples of their use in the analytical control process.

Surface Characterizations of Carbon Multiwall Nanotubes by Plasma Modification

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The effect of variations of plasma treatment parameters, such as power, time, and positions of samples inside and outside the barrel, on the MWCNTs surface morphology and structure was systematically analyzed. Meanwhile, different kinds of gas were introduced to the RF plasma chamber such as Ar, O<sub>2</sub>, and CF<sub>4</sub> to investigate the ion bombard effect on the MWCNT surface. The morphology and structure changes due to these treatments have been investigated by using X-ray photoelectron spectroscopy (XPS), Fourier Transformed Infrared Spectroscopy (FT-IR), scanning electron microscopy (SEM) and Raman spectroscopy. The results showed that Ar gas plasma could not graft any functional groups but somewhat cleaning effect on the MWCNT surface by removing the amorphous carbon and etching the surface. When using O<sub>2</sub> plasma, the direct discharge (outside the barrel) could result in a quick grafting of polar function but also an easy damage of MWCNTs after longer time, particularly under intensive power(seen in Fig.1).

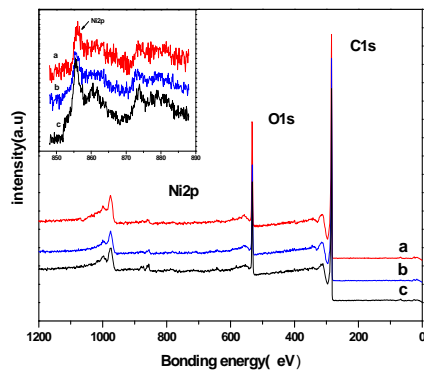


Fig.1. XPS survey spectra of carbon nanotubes film treated by oxygen plasma under the following conditions: (a) 100W, 10min; (b)200W, 5min; (c)250W,5min; Inset: XPS Ni 2p spectrum  
It was also found that the surface of MWCNTs inside the barrel might be changed in three steps during the irradiation, such as, expansion (loosed structure formed), peel off and oxidization. Moreover, a successful fluorination of MWCNT powder with a maximum of fluorine content of 12% could be achieved by using CF<sub>4</sub> plasma (seen in Fig.2).

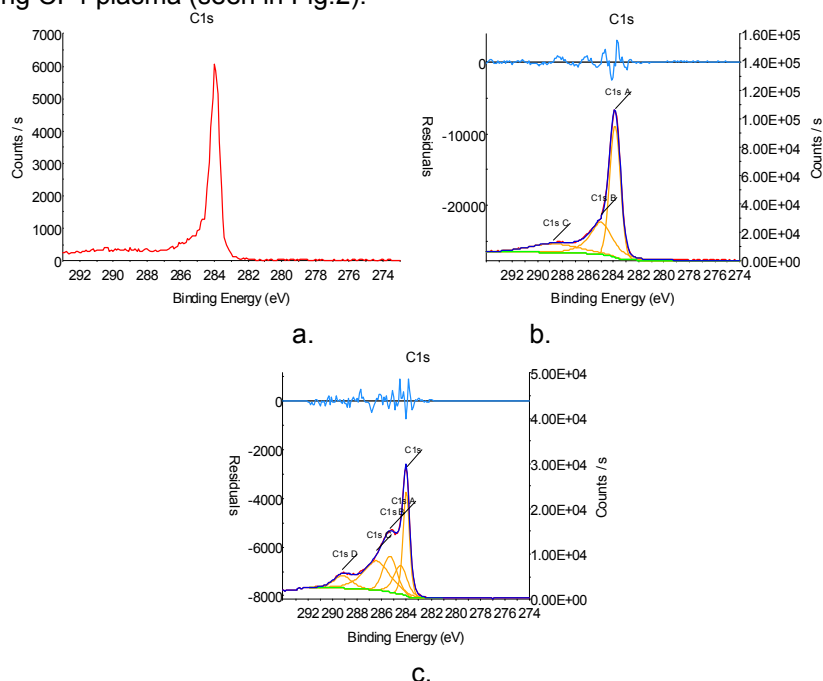


Fig.2. XPS C1s spectrum (a) unfunctionalized MWCNTs; (b) acid oxidized MWCNTs; (c) CF4 plasma treated

#### Acknowledgment

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## P144-A2

### Study of FAIR-FACE Brick Pathologies WITH Grazing Incidence X-Ray Diffraction

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Keywords: Fair-face brick, pathologies, efflorescence, grazing incidence X-ray diffraction (GID)

#### Poster presentation

In order to diagnose fair-face brick pathologies and enable the best repair options to be established, the causes of these pathologies must first be determined. This knowledge can then also help prevent these pathologies in future architectural works. Though so-called minor defects are involved, since they do not affect the building structure but its aesthetic appearance, they need to be appropriately taken into account, since any minor damage could lead to a severe pathology. Fair-face brick pathologies may be due to erosion, efflorescence, or high-pollution environments.

The appearance of efflorescence on fair-face bricks and tiles, stemming from the evaporation of salt solutions and salt depositions, is a problem that principally affects manufacturers and builders, since it diminishes architectural quality and aesthetics. Moreover, these phenomena are difficult to analyse because of the variety of factors influencing the occurrence of these defects or pathologies, such as clay sulphate or sulphide content, sulphate formation during brick drying, evolving sulphurous gases in high-temperature reactions between sulphates and silicates, inappropriate firing processes, etc.

Since this is a complex issue, a methodology to study this type of pathology needs to be developed, using analytical techniques that provide both microstructural and mineralogical information from the brick surface and arising salts, such as grazing incidence X-ray diffraction. Grazing incidence X-ray diffraction (GIXD or GID), typically from a crystalline structure, uses small incident angles for the incoming X-ray beam so that diffraction can be made surface sensitive, providing crystallographic information in thin surface layers of the brick. A Göbel mirror configuration provides between three and twenty-five times higher intensity.

This study addresses one type of pathology in fair-face bricks, namely spalling and degradation of the brick surface a few months after installation. The results obtained by X-ray diffraction and complemented with other techniques (EDX) show that the pathology stems from the migration of soluble salts and subsequent crystallisation of gypsum in the brick surface layers.

This study has been funded by IMPIVA (Generalitat Valenciana) IMIDIC/ 2007/103, through the European Fund for Regional Development (EFRD).

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#### Abstract Topic:

**T4:** Spectrometric Techniques, Mass Spectrometry

**T6:** Industrial Aspects, Material Properties and Analytics, Process Analysis Technologies (PAT)



## Au@Ag Core-Shell Bridged Gold Nanoparticles: Preparation and Characterization

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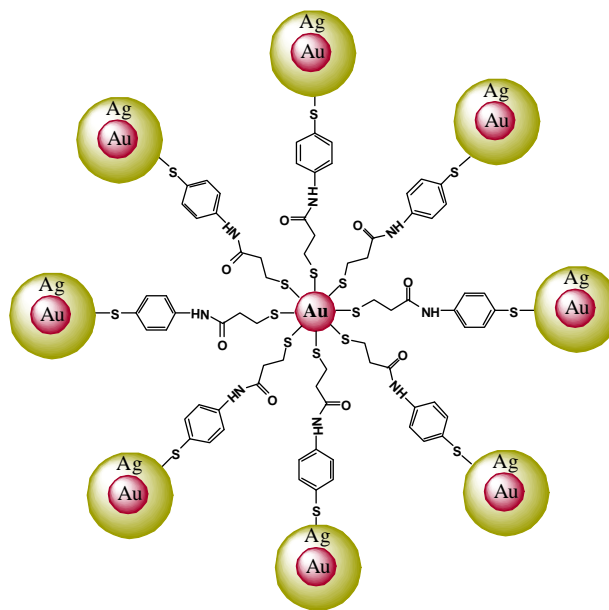
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A metal nanoparticle core surrounded with an organic layer is a specific type of nanoparticle called monolayer protected cluster (MPC). Gold and silver nanoparticles can be functionalized with alkanethiol monolayers which exerts high stability to the colloids against aggregation. MPCs have been investigated by various research groups and exploited as chemical sensors as well as electron transfer model systems.

In this work, we describe the monolayer attachment of mercaptopropionic acid to the gold nanoparticles (AuNPs) to construct mercaptopropionic acid self assembled gold nanoparticles (Au-MPA). Carboxylic acid terminated SAM film surrounding the AuNPs is activated with EDC to bind 4-aminothiophenol to the Au-MPA forming dithiol ended protective organic film. Au@Ag bimetallic nanoparticles are bridged to the AuNPs through the terminal SH groups to construct organic film bridged Au-organic-Au@Ag coreshell dendrimer. We have also investigated the enhancement of the raman signals of the organic film between metal nanoparticles by the accumulation of the dendrimers on the glassy carbon substrate surface. The nanoparticles are contacted by –S-(CH<sub>2</sub>)<sub>2</sub>-CO-NH-Ph-S- linker bridges in which presence of different heteroatoms and functionalities makes it interesting for characterization by XPS, UV, IR and SERS.



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## P146-A2

### Multitask Fluorescence Nanoparticle Sensor for Sensitive Quantification of Proteins, Cells, and Small Molecules

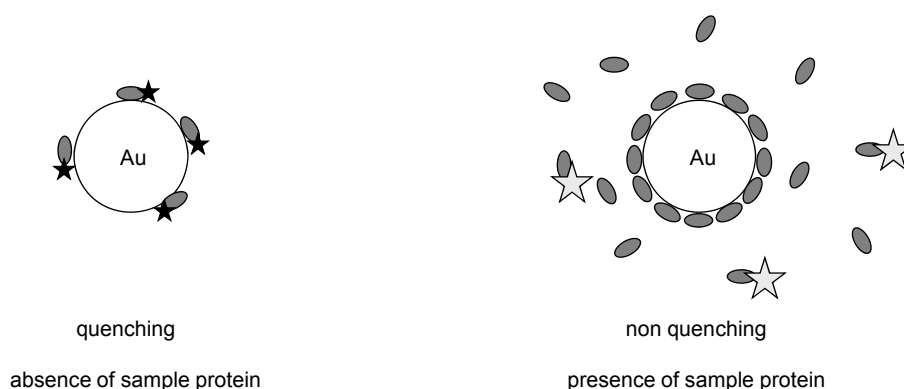
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Highly sensitive analytical methods for quantification of protein concentration in solution are important in biological laboratories. However, available commercial methods for assaying proteins have limitations in sensitivity, protein-to-protein variability, and dynamic range. They are often lengthy and have impractical measurement procedures. The common methods for cell counting, e.g. direct microscopic count or turbidity measurement, are laborious and impracticable for low concentration of cells. Therefore, new inexpensive and user-friendly methods are required.

We developed novel sensitive and easy-to-use methods for total protein<sup>1,2</sup> or cell concentration measurement based on the competitive adsorption of fluorescently labeled protein and sample protein or cells onto nanoparticles. These quantitative homogeneous assays are performed in microtiter wells and the detection is based on time-resolved fluorescence energy transfer (TR-FRET) between Eu(III) nanoparticles and acceptor labeled protein or quenching of fluorescent labeled protein by gold nanoparticles. We reached a sensitivity below 500 pg bovine serum albumin in the sample with an average coefficient of variation 4.5%. The protein-to-protein variability for eleven tested proteins was 15% in the quenching assay. The detection limit for eukaryotic cells was less than ten cells in a microtiter well with both fluorescence energy transfer and quenching assays.

#### Quenching Assay



The developed FRET or quenching particle sensor was applied to measure the size of different molecules or protein aggregation based on the change in adsorption as the size of the molecule changed. The sensor relies on the size dependent adsorption of molecules onto nanoparticles – large molecule covers the particle surface more efficiently than a smaller molecule. The molecular size measurement has been shown to function with different molecules: polyethylene glycol, polyethylene imine and polyamino acid.

Detection of protein aggregation is important due to its underlying cause of many debilitating and often incurable human diseases.<sup>3</sup> The study involves dilute samples and thus requires high sensitivity. The developed method was applied to detect aggregation of bovine serum albumin. We measured size changes at picomolar concentration (500 pM or 30 µg/l) range. This is more than 10 000-fold lower concentration (approximately 1 g/l) range in comparison to well-known methods: absorbance measurement and photon correlation spectroscopy.

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## P147-A2

### In-situ Characterization of Biopolymer Interfaces by Nanoparticle-Enhanced Spectroscopic Imaging

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Biocompatible polymers are a new class of material used for osteosyntheses and for filling of bone defects e.g. in orthopaedics and neurosurgery. Polymer composite of Poly(3-hydroxybutyrate) covered by Poly(vinylamine) exhibits a high mechanical stability and biocompatibility. The analytical challenge is the non-destructive characterization of the chemical bond between the mechanically stable bulk polymer and the biocompatible surface layer.

The application of localized surface plasmon resonance (LSPR) of gold nanoparticles for the non destructive characterization of polymer interfaces was investigated by using attenuated total reflection (ATR) infrared spectroscopic imaging. A thin layer of metallic nanoparticles was placed in the polymer interface of implantation material and coating material.

In a first study, molecular interactions between Polycarbonate and Poly(vinylamine) were investigated, showing a formation of interfacial amide bonds. The spectroscopic method was applied to the polymer composite Poly(3-hydroxybutyrate) - Poly(vinylamine). Gold nanoparticles were deposited on the bulk polymer and afterwards covered with a layer of Poly(vinylamine). A reference sample was prepared without gold nanoparticles. The polymer composites were placed onto an ATR prism. Molecular interactions are initiated by heating, resulting in a chemical bond between the both polymer phases. Infrared spectra of LSPR reveal chemical processes whereas spectra from the reference sample without nanoparticles provide no information about the polymer interface. Moreover, the spectroscopic images show domains with different molecular structure. This experiment demonstrates that LSPR infrared spectroscopic imaging is a promising technique for a sensitive and non destructive characterization of polymer interfaces.

## P148-A2

### Pd-Decorated Carbon Nanotubes as a Catalyst for Hydrodehalogenation Reaction

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Halogenated aromatic compounds exhibit high toxicity and have long been regarded as a major source of environmental pollution. These compounds are environmentally persistent chemicals which accumulate in fatty tissues and show carcinogenic and mutagenic activity. Thus, many researchers have devoted their efforts to develop methods for dehalogenating these aryl halides, including incineration, pyrolysis, hydrolysis, chemical/biodegradation. However, each can lead to the formation of other halogenated toxins and do not constitute the best practicable environmental option. Catalytic hydrodehalogenation is now emerging as a viable alternative non-destructive treatment that can generate compounds of economic value from halogenated waste.

Recently, CNTs are receiving considerable attention as catalyst supports in both heterogeneous catalysis and electrocatalysis due to their high mechanical strength, large surface area, good electrical conductivity, and durability under harsh conditions. Some researchers have reported that CNT supported catalysts (e.g., Pt, Pd, Au, Ru and RuO<sub>2</sub>) exhibited good catalytic behaviors in various chemical reactions, involving methanol electro-oxidation, selective hydrogenation, alcohol oxidation, Suzuki coupling, CO oxidation, and Fischer-Tropsch synthesis, etc. However, few studies have conducted about the CNTs as catalyst supports for hydrodehalogenation reaction which is very important for the reduction of environmental pollution. In the present work, we prepared Pd-decorated CNTs by introducing thiol groups on multiwall carbon nanotube (MWNT) surfaces. The concentration of Pd nanoparticles on the surface of CNTs was about 2.1 atomic % as determined by XPS analysis. Their catalytic performance for hydrodehalogenation reaction was examined, and compared with the reference systems. The morphology and structure of these materials were also examined by transmission electron microscopy, Raman spectroscopy and X-ray diffraction (XRD).

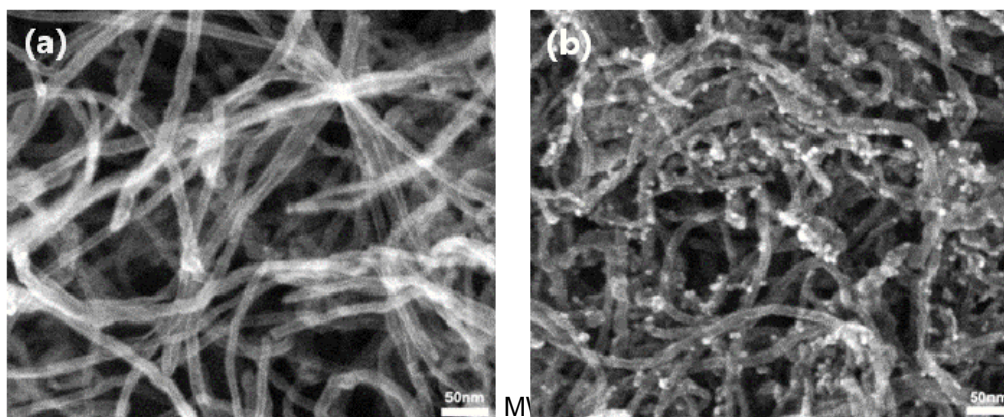
## P149-A2

### Synthesis of Pd-CNT Nanocomposites and Investigation of their Catalytic Properties in the Hydrodehalogenation Reaction

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Since their discovery by Iijima, carbon nanotubes (CNTs) have drawn particular attention on account of both their scientific interest and their potential for technological applications. Recently, CNTs are receiving considerable attention as catalyst supports in both heterogeneous catalysis and electrocatalysis due to their high mechanical strength, large surface area, good electrical conductivity, and durability under harsh conditions. Some researchers have reported that CNT supported catalysts (e.g., Pt, Pd, Au, Ru and RuO<sub>2</sub>) exhibited good catalytic behaviors in various chemical reactions, involving methanol electro-oxidation, selective hydrogenation, alcohol oxidation, Suzuki coupling, CO oxidation, and Fischer-Tropsch synthesis, etc. However, few studies have conducted about the CNTs as catalyst supports for hydrodehalogenation reaction which is very important for the reduction of environmental pollution. In the present work, we prepared highly dispersed CNT-supported Pd nanoparticles by introducing thiol groups on multiwall carbon nanotube (MWNT) surfaces. Their catalytic performance for hydrodehalogenation reaction was examined, and compared with the reference systems.



## P150-A2

### Rapid Optical Emission Spectrochemical Analysis of Chlorine, Sulphur and Carbon in Concrete

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Durability is a basic concern for concrete structures exposed to aggressive environments. A lot of environmental phenomena are known to significantly influence the durability of reinforced concrete structures. Carbonation, sulphatization and chlorine corrosion are the major factors to cause structure deterioration.

The combined laser-spark instrumentation is maximally suitable for a routine practice and relies on the use of multichannel detection for recording the space-, time-, and spectrally resolved emission. The increase of spectra intensity was carried out at imposing on laser ablation plasma of the pulsed electrical discharge. The fundamental (1.06  $\mu\text{m}$ ) harmonic of a Q-switched Nd:YAG laser with a pulse duration of 6-8 ns is employed. The laser beam, with an aperture of 6 mm and a beam divergence of 0.8 mrad, is focused on the sample surface by a 50 mm focal length lens. The laser pulse energy can be varied from 10 to 270 mJ. Tungsten wire electrodes with 3 mm gap have been set at a distance of 1.5 mm from the sample surface. The laser ablation plume seals-in the electric circuit containing a low-inductive 1  $\mu\text{F}$  capacitor charged to 4 kV, which gives rise to a 4  $\mu\text{s}$  damping electric discharge. In this mode significant energy (in our case up to 8 J) deposition in the volume containing the analyte can be realised. The light emitted from the expanding plasma plume is collected by a plano-convex quartz lens and imaged (1:1) onto the horizontal entrance slit of the 0.38 m spectrograph. The spectrum is recorded by the optical multichannel analyzer. Layer-by-layer and surface analysis as well as microanalysis are possible. The IR spectral lines have been chosen to avoid interferences of tungsten lines. In the laser-spark approach utilization of low-cost and portable laser instrumentation as well as micro influence on the target surface are possible.

## P151-A2

### Nanoparticle-Embedded Extraction Tips for Selective Enrichment of Phosphorylated Peptides from Tryptic Digests

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In this study the potential of an organic monolith with TiO<sub>2</sub> and ZrO<sub>2</sub> nanoparticles incorporated into the polymeric structure was evaluated for the selective enrichment of phosphorylated peptides from tryptic digests. A complete automated process was developed for the production of the extraction tips as well as for the enrichment of phosphopeptides.

For the preparation of TiO<sub>2</sub> and ZrO<sub>2</sub> doped poly(DVB) pipette tips, a mixture comprising DVB, nanopowders and porogens was prepared and polymerized inside of tips.

After the preparation of tips, a phosphopeptide enrichment was performed using several buffer solutions and washing steps. Finally, all retained phosphopeptides were eluted and spotted on a stainless steel target covered with a layer of dihydroxybenzoic acid (DHB) matrix. Measurements were recorded by a MALDI-TOF/TOF MS.

Poly(DVB)TiO<sub>2</sub>/ZrO<sub>2</sub>-Tip fabrication and phosphopeptide enrichment was completely automated using a MEA™ Personal Purification and Enrichment System.

Enrichment of phosphopeptides was performed using α-casein, β-casein and in vitro phosphorylated extracellular signal-regulated kinase 1 (ERK1) digests. About 20 phosphopeptides could be retained from α-casein and five from β-casein digests. Further selectivity for phosphopeptides was demonstrated by enriching a digest of *in-vitro* phosphorylated extracellular signal regulated kinase 1 (ERK1). Two phosphorylated peptides of ERK1 could be identified by MALDI-MS/MS measurements and a following Mascot database search.

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## P152-A2

### Application of Chromatographic Methods for Separation of Selected 1-alkyl-3-ethylimidazolium Cations and Assessment of [BuMelm][Tf<sub>2</sub>N] Room-Temperature Ionic Liquids Stability under Gamma Irradiation for Nuclear Applications

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Room-Temperature Ionic Liquids (RTILs) are a family of salts that are liquid below 100°C. Physico-chemical properties of RTILs are tunable depending on cation and anion structure. Interest in RTILs for their potential in different chemical processes is increasing, due to their non volatility, high thermal stability and wide electrochemical window making them suitable for a wide range of applications [1]. In particular, they are envisioned as potential media in the frame of nuclear fuel reprocessing for actinid/lanthanid media partitioning [2]. One of the most commonly used RTIL is formed by the 1-butyl-3-methylimidazolium cation ([BuMelm]<sup>+</sup>) associated with weakly coordinating bis-(trifluoromethanesulfonyl)imide anion ([Tf<sub>2</sub>N]<sup>-</sup>). Nevertheless, one should assess RTILs behaviour under ionizing radiation, both by quantifying [BuMelm]<sup>+</sup> degradation and characterizing radiolysis products formed along the radiolysis. In these ways, applications of chromatographic methods for analysis of imidazolium cations in biological or environmental samples have been extensively described, essentially by the use of reversed-phase chromatography and ion chromatography [3]. Due to the nature of polar and apolar molecular interactions of imidazolium cations with stationary phases, various HPLC bonding phases can be envisioned for these studies to optimize selectivity and resolution of separations and to develop sensitive method for cations characterization in irradiated media.

Firstly, we have focused on HPLC separation processes to assess optimal selectivity and resolution for the separation of selected 1-alkyl-3-methylimidazolium. Various HPLC bonding phases have been used, including polar-reversed phase (naked silica or functionalized silica with –OH and –NH<sub>2</sub> bondings) and mixed-mode phases (functionalized silica with alkyl bondings and strong/weak ion exchange bondings) in order to use both hydrophilic/hydrophobic partition mechanisms and ion exchange mechanisms of RTILs cations with stationary phases. Secondly, the development of a suitable method has been applied to assess [BuMelm][Tf<sub>2</sub>N] RTIL stability under gamma irradiation: (1) by tracking cation degradation along the irradiation and (2) by characterizing radiolysis products by hyphenated HPLC/ESI-MS technique.

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## P153-A2

### Determination of Dissociation Constants of Folinic Acid, 5-Fluorouracil and Irinotecan in Hydroorganic Media by Spectroscopic Method

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Colorectal cancer, also called colon cancer or large bowel cancer, includes cancerous growths in the colon, rectum and appendix. With 639,000 deaths worldwide per year, it is the third most common form of cancer and the second leading cause of cancer-related death in the Western world [1]. Folinic acid (leucovorin) , 5-fluorouracil and irinotecan are the drugs used for treatment of colorectal cancer and FOLFIRI is the chemotherapy regimen that agents used in combination.

Dissociation constants are useful physicochemical properties by which we can describe the amount of ionization of functional groups with respect to pH. These important parameters have a lot of applications in research areas such as pharmaceutical drug development, solvent extraction, acid-base titration and ion transport. The toxicity, chromatography retention behavior and pharmaceutical properties of organic acid and bases are affected by acid-base properties [2].

Very often, the main difficulty in the determination of dissociation constants of drugs is their aqueous insolubility that forces the use of spectroscopic technique. UV spectroscopy is an excellent method for pK<sub>a</sub> determination. This technique requires very low analyte concentration and allows suitable absorbance measurement in aqueous solution even for products with low aqueous solubility.

In this study, UV spectrometric method was used for the determination of the dissociation constants of folinic acid (leucovorin) , 5-fluorouracil and irinotecan. Approximately  $1.10^{-5}$  M solutions in water and acetonitrile-water binary mixtures were titrated with NaOH for determination of dissociation constants [3]. The absorbance/pH profiles were assessed and found to conform to those of polyprotic acids. The data evaluation was performed by using STAR software program which calculate stability constants and molar absorbances of the pure species by multi linear regression analysis [4].

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## P154-A2

### **Studies on Gadolinium Nitrate in Heavy Water as well as in Light Water in Connection with its Use as Soluble Neutron Poison in 540 MWe Indian PHWRs**

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In Indian Pressurized Heavy Water Reactors (540 MWe PHWRs), soluble gadolinium nitrate in the heavy water moderator system is first time used as neutron poison in reactivity shim and in the first acting secondary shut down system. It is well known that the use of gadolinium nitrate has many advantages as compared to the use of soluble boron compounds but the only disadvantage is that it has a precipitating tendency. Hence, in order to make stringent chemistry control of moderator system, the present work which encompasses determination of true equivalent conductance of gadolinium nitrate in light water by suppressing the hydrolysis of Gd by adjusting the acidity, kinetics of precipitation of Gd (13-20mg/L) in heavy water at different pD, the effect of concentration of bicarbonate and silica on the precipitation of Gd in light water as well as in heavy water has been carried out. The effect of temperature on the precipitation of Gd and its redissolution kinetics with lowering of pH has also been studied in this work.

## P155-A2

### Gas Chromatographic-Mass Spectrometric Analysis of High-Purity Isotopically Enriched Volatile Substances

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At present time there is a great interest to simple substances, including isotopically enriched ones, with the ultimately low content of impurities. A suitable method for production and ultra purification of the initial substances is their use in the form of volatile hydrides and fluorides. A particular feature of high-purity substances is the presence of compounds absent in the individual state. Gas chromatography-mass spectrometry is the most promising method of analysis of high-purity substances which make it possible to reliably detect the impurities with high sensitivity. It is for the first time that the method of gas chromatography-mass spectrometry is used to determine the impurity composition of isotopically enriched silane  $^{28}\text{SiH}_4$ ,  $^{29}\text{SiH}_4$ ,  $^{30}\text{SiH}_4$  and germane  $^{74}\text{GeH}_4$ . The Agilent 6890/MSD 5973N gas chromatograph-mass spectrometer with quadrupole mass analyzers was used. For separation of impurities in hydrides the application of adsorption capillary PLOT columns GS-Q 30 m x 0.316 mm, GS-GasPro 60 m x 0.32 mm (Porapak Q and modified silica gel) and 25 m x 0.22 mm column with polymethylsilylpropine fs adsorbent were investigated. Introducing of gaseous samples is carried out by automatic two-position valve "Valco EH2C6WEZPH-CER5" operating in the atmosphere of helium as a protective gas, connected with the developed sampling vacuum system. Using the electron impact ionization the 56 impurity components are determined including the permanent gases, arsine, phosphine, the homologs of silane, disiloxane, sulfur hexafluoride, hydrocarbons, chloro-fluoroorganic substances. With the use of positive chemical ionization the impurities of fluorodisiloxane, trisiloxane and fluorotrisiloxane are identified absent in the individual state and thus not included into the libraries of mass spectra. The quantitative analysis was conducted by the method of absolute calibration. The determination of substances absent in the individual state was based upon the dependence of analysis sensitivity with their ionization cross section. The limits of detection for impurities are  $2 \cdot 10^{-6}$  -  $1 \cdot 10^{-9}$  mol.% which are by 8-20 lower than those given in literature.

## P156-A2

### Study by FT-IR Spectroscopy of Adsorption of Acid Dyes from Aqueous Solutions onto Activated Carbon Prepared from Seawater Alga

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The adsorption of acid dyes from aqueous solutions onto activated carbon prepared from seawater algae was investigated, with respect to contact time, pH and temperature. The surface characterisation of activated carbon was performed using FT-IR spectroscopy. The use of biomaterials [1, 2] for the removal of colour from its aqueous solution will provide a potential alternate to the conventional treatment techniques. Methylene blue was selected as a model compound as an attempt to use "*Ulva lactuca*" and "*Cystoseira stricta*" modified chemically and physically as adsorbents for the removal of dye from wastewaters. The results show that a maximum adsorption capacity of 158 mg /g was obtained for the *ulva lactuca* physically activated and 172 mg /g for the *Cystoseira stricta*. The Langmuir and Freundlich models fit well the adsorption isotherms of methylene blue. The determined iodine number shows a higher microporosity for the two adsorbents used in this study [3]

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## P157-A2

### Novel Application of GS-AED for the Study of the Sulfidation Behavior of the Co(Ni)Mo(W) Hydrotreating Catalysts

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The gas chromatography with atomic emission detection (GC-AED) is an useful tool for the analysis of heteroatom in the petroleum feed and finds application for tracking of the S- and N-containing molecules conversion in diesel feeds during the improving of catalyst formulation and optimizing hydrotreating processes condition. The present topic deals with the novel GC-AED application for the study of the sulfidation behavior of Co(Ni)Mo(W) hydrotreating catalysts.

It is generally accepted that the last generation catalysts with the high HDS and HDN activity contains well dispersed sulfide Co(Ni)Mo(W) phase. Hydrotreating catalysts are supplied in the oxide form that is converted into the catalytically active sulfide phase during the sulfidation by the SRGO spiked with dimethyldisulfide (DMDS). Increasing developing of the new methods of the oxide precursor's preparation demands the detailed study of their sulfidation behavior.

In the present topic the comprehensive approach based on the on-line analysis of products formed by the DMDS decomposition is proposed for these purposes. The samples of liquid and gases product were characterized using GC with atomic-emission and with thermal conductivity detectors respectively.

The analysis of intermediate sulfur compounds let us to reveal the peculiarities of the catalyst's sulfidation depending on the support, active metal (Mo or W) and promoter (Co or Ni) nature as well as the thermal treatment conditions. The examples of the application of this approach for the study of the sulfidation of Co(Ni)Mo/Al<sub>2</sub>O<sub>3</sub> hydrodesulphurization and Ni(Mo)W/(Al<sub>2</sub>O<sub>3</sub>-zeolyte) hydrocracking catalysts is considered. It is shown, that the comparative analysis of DMDS, intermediate S-containing products and H<sub>2</sub>S during the sulfidation allow us to correct the temperature and duration of each sulfidation steps to reach the proper sulfidation and to obtain high activity catalysts. The approach proposed is a useful tool for the optimization of the sulfidation procedure of the newly developed hydrodesulfurisation and hydrocracking catalysts.

## P158-A2

### Synthesis and Spectral Characterization of New Schiff Bases Derived from 4-Chloro-2-Aminophenol and Various Dimethoxybenzaldehydes

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Compounds with the structure of -C=N- (azomethine group) are known as Schiff bases, which are usually synthesized from the condensation of primary amines and active carbonyl groups. Schiff bases have been extensively studied as they possess many interesting features, including photochromic and thermochromic properties [1], proton transfer tautomeric equilibria [2], biological and pharmacological activities [3,4] as well as suitability for analytical applications [5].

In this study five new Schiff bases (Fig. 1), derived from 4-chloro-2-aminophenol and 2,3-; 2,4-; 2,5-; 3,4- and 3,5-dimethoxy benzaldehydes (the compounds I - V), were synthesized in ethanol under reflux after 2 h reaction. They were characterized by elemental analysis, FT-IR,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR, Mass, UV-visible spectroscopies.

In the  $^1\text{H}$ -NMR spectra of the compound, OH proton appears above 9.0 ppm as a singlet; -CH=N- imine proton is observed between 8.5 and 9.0 ppm. In the  $^{13}\text{C}$ -NMR spectra imine carbon atom is observed near 160.0 ppm. A strong band at the 1620 - 1650  $\text{cm}^{-1}$  range is attributed to  $\nu(\text{CH}=\text{N})$  in the IR spectra of the compounds. The  $\nu(\text{OH})$  appears between 3300 and 3500  $\text{cm}^{-1}$ . The mole peaks (m/z) of the compounds are determined in their ESI-MS spectra.

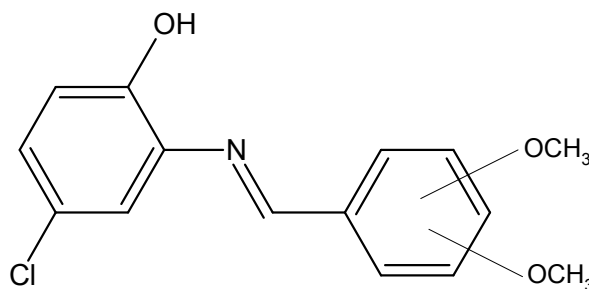


Fig.1. Schematic view of Schiff bases under the study.

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## P159-A2

### Determination of Agglomerates Size in the SDS/water System and the SDS/water/pentanol/vitamin E Microemulsion by the Dynamic Light Scattering Method

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The dynamic light scattering (DLS) method was used for characterization of the aggregation process in the aqueous sodium dodecyl sulfate (SDS) solution. The surfactant concentration in the series of samples ranged from 1mM to 900 mM. The results of DLS measurements were compared to those obtained from the theoretical investigation and literature data. In the next stage of research, the oil-in-water (o/w), water-in-oil (w/o) and bicontinuous (bc) microemulsions composed of pentanol, water and SDS were prepared and characterized by means of the phase diagram analysis as well as the conductivity and viscosity measurements. The undertaken investigation was focused on this three-compound system as the potentially useful model carrier of bioactive molecules (e.g. vitamin E) in pharmaceuticals and cosmetics. The polydispersity index and the distribution of hydrodynamic diameter for microemulsions were obtained by the DLS method in back scattering mode. Measurements were made by means of Zetasizer Nano ZS with He-Ne laser ( $\lambda=633\text{nm}$ ) at a temperature of 293 K. Analyses were made directly after samples preparation and also during their 1-month storage. Reproducibility of the obtained results was very high, which confirms stability of the microemulsion in time. It was observed that the increase of pentanol concentration in the sample caused insignificant enlargement of aggregates in oil/water systems. The size distribution in the bicontinuous system corresponded to the size of two-molecular layers created by SDS. The influence of vitamin E on the size of agglomerates of dispersed phase was observed only for the water/oil system.

## P160-A2

### Studies on Characterization and Thermal Behavior of Amine Salts of 3-Nitro-1,2,4-triazol-5-one (NTO)

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Four new amine salts of 3-nitro-1,2,4-triazol-5-one (NTO) were prepared by the reaction of NTO and amines such as , N,N'-dimethyleguanidine, cyclohexamethylenetetramine, semicarbazide and melamine. Their structures were characterized by FT-IR, <sup>13</sup>CNMR spectroscopy. The results showed that the formation of amine salts of NTO is achieved by proton transfer of N(4) atom, and it makes a higher nitrogen content and lower acidity. Their thermal decomposition and the non-isothermal kinetics of the dehydration reaction were studied under the non-isothermal condition by DSC and TG methods. The kinetic parameters were obtained from analysis of the DSC and TG curves by Kissinger method. The thermodynamic parameters of the amine salts of NTO such as activation energy and enthalpy of vaporization were calculated. The results showed that melamine salt of pure NTO is thermally stable than NTO when compared in terms of activation energy.

Keywords: 3-Nitro-1,2,4-triazole-5-one (NTO); Amine salts; Thermal decomposition



## P161-A2

### A Systematic Study of the Chemiluminescence Reactions of Acidified Potassium Permanganate with Inorganic Ions

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This paper reports a systematic study into the chemiluminescence from acidic solutions of potassium permanganate in the presence of twenty-two different inorganic analytes. The reactions were undertaken using one of formaldehyde, formic acid, glyoxal or sodium hexametaphosphate and employed a variety of acid concentrations (0.001M to 1.00M). The resultant chemiluminescence is discussed with respect to the analytes oxidation state, speciation and the enhancer employed. Iron(II) was observed to yield analytically useful chemiluminescence when reacted with acidic permanganate in the presence of (one of) glyoxal, formaldehyde or formic acid. In each case, the resultant signal for Fe(II) was maximum when the reaction was acidified with 1M hydrochloric acid. Surprisingly, copper(II) yielded weak emission when reacted with acidic permanganate in the presence of glyoxal. As with Fe(II), the greatest intensity was observed when the reaction was acidified with 1M hydrochloric acid. Antimony(III) exhibited chemiluminescence when reacted with acidic permanganate in the presence of either formic acid or glyoxal. In contrast to results obtained for Cu(II) and Fe(II) the maximum response for antimony(III) was observed when the hydrochloric acid concentration was reduced to 0.01M. This is the first report of chemiluminescence from the reaction of acidic permanganate with Sb(III). Under optimised conditions, Fe(II) and Sb(III) methods exhibited a linear range between 10-800  $\mu\text{g L}^{-1}$  and 100-1000  $\mu\text{g L}^{-1}$ , with detection limits of 1.6  $\mu\text{g L}^{-1}$  and 15  $\mu\text{g L}^{-1}$ , respectively.

**Comparison of Essential Oil Composition of *Eucalyptus Procera* Obtained with Supercritical Carbon Dioxide Extraction and Hydrodistillation Methods**

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Essential oil of *Eucalyptus procera* Dehnh., cultivated in Iran, was obtained by hydrodistillation and supercritical (carbon dioxide) extraction methods. The oils were analysed by capillary gas chromatography using flame ionization and mass spectrometric detections. The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). An orthogonal array design OA9 was applied to select the optimum extraction condition in supercritical extraction. The effects of pressure, temperature and modifier volume on the extraction efficiency were investigated by the three-level orthogonal array design. The extract obtained from *Eucalyptus procera* by using supercritical fluid extraction was compared with the essential oil obtained by hydrodistillation. SFE products were found to be of markedly different composition, compared with the corresponding hydrodistilled oil.

Keywords: *Eucalyptus procera*, Essential oil, supercritical extraction

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## P163-A2

### Rapid and Highly Sensitive Detection of *Flavobacterium Psychrophilum* Using High-Gradient Immunomagnetic Separation

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The bacterial cold water disease caused by *Flavobacterium psychrophilum* is a concern not only for the aquaculture industry, but also the environment. We have developed several methods for detecting *F. psychrophilum* using immunomagnetic separation (IMS), performed with immunomagnetic beads composed of oxide iron and flow cytometry (FCM). The magnetism of oxide iron, however, is not very strong. Therefore, to increase the magnetic field, we developed a novel method using high-gradient magnetic separation (HGMS). HGMS is a magnetic separation method in which the magnetic force is strengthened by introducing a magnetic gradient between the magnetic filter and the nearby column. *F. psychrophilum* cells that had reacted with immunomagnetic beads were loaded onto the column containing the magnetic filters. When the column was placed inside a neodymium ring magnet, the cells became stuck to the magnetic filters. The collected cells were easily removed from the filters by turning off the magnetic field, and then applied to FCM assay. This detection sensitivity was 3 orders of magnitude higher than that of a conventional FCM assay. The cell numbers determined by FCM correlated with those obtained using the colony-counting method in the range of approximately 10–10<sup>4</sup> colony-forming units per milliliter. One FCM assay could be completed within 60 s and the total assay time, including sample preparation, was less than 3.5 h. The proposed method using HGMS with FCM allows for rapid and highly sensitive detection of *F. psychrophilum*.

## P164-A2

### Catalytic Activity of Metalloporphyrin-Titanium(IV) Mixtures in Microemulsions

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Water-in-oil (W/O) and oil-in-water (O/W) microemulsions containing Ti(IV)-citrate complex (polar) and Zn-porphyrin (non-polar) were irradiated with UV and visible light. The interface between water and oil contains the surfactant, whose polar head will stand facing the water, interacting with the titanium complex. Meanwhile, the surfactant tail will join the oil phase interacting with the Zn-porphyrin. Some of the experiments were developed to study the 4-chlorophenol photodegradation. A study on the photocurrent generated by irradiation of an electrode casted with a liquid crystal-like microemulsion was also included. UV-Vis spectrometry, voltammetry, conductimetry and GC/MS techniques were used to characterize the systems.

GC/MS analysis showed that degradation of 4-chlorophenol was a fact in both UV and visible light. However, in O/W microemulsion, phenol was produced as the first major component, and methylated benzenes, such as 1,2,4-dimethyl benzene, 1,3,5-dimethyl benzene, 1,2,3-trimethylbenzene were produced instead of the hydroxybenzene compounds currently reported for such degradation. When W/O was used, the methylated benzenes were also present but phenol was not the major component. On the other hand, when TiO<sub>2</sub> particles were used instead of the Ti complex (in the microemulsions), the photodegradation under UV irradiation appeared to be slowly and phenol was the only product obtained. The irradiation of the particles with visible light did not show any photodegradation of the organic compound. The photocurrent studies were developed by irradiation of a working electrode casted with liquid crystal-like microemulsions containing the photoactive compounds. The results on those experiments will be discussed.

## P165-A2

### Quantum Mechanical Calculations of Cytosine, Thiocytosine and their Radicals

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It is well known that Cytosine is the smallest molecule, which is present in the both nucleic acids RNA and DNA. Since the genetic information is based on the sequence of the nucleic acid bases, this is the main reasons to investigate the nucleic acid bases at the quantum mechanical level. 2-Thiocytosine is the analogue of the nucleic acid bases cytosine whose oxygen atom is substituted by S atom. The energies, geometries, charges and vibrational characteristics of the cytosine and thiocytosine and their corresponding radicals were carried out by using DFT method with b3lyp/6-311++g\*\* basis set. The comparison between the calculated vibrational wave numbers of cytosine and thiocytosine which are affected by the presences of the S atom. The calculation for anion of the thiocytosine is found to be unstable at b3lyp/6-311++g\*\* level. Removal of one electron is leads to decreases the electronic charges mainly from the O atom for the cationic cytosine radical. The C=O/S bonds are slightly lengthened for the radicals of the cytosine and cationic radical of thiocytosine than their neutral parent molecules. The C=C bond of the ring is increases for the anionic radicals of cytosine and thiocytosine and the double bond character is loses by the cationic radical of cytosine. The observed IR and Raman frequencies for cytosine and thiocytosine had been reported by many workers [1-3]. The calculated N-H stretching frequency decreases in all cationic radicals with respect to the neutral molecules. The radicalization leads to significant changes in the magnitudes and intensities corresponding some of the normal modes for all the two cases.

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## P001-B1

### Analysis of Arsenicals in Bio-Samples from the Human Gastrointestinal Tract Using HPLC-ICP-MS

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Inorganic arsenic (iAs) has been classified as a Type 1 carcinogen and has also been linked to several noncancerous health effects. In humans iAs produces dimethylarsinic acid (DMA<sup>V</sup>) as primary urinary metabolite. [1] Monomethylarsinic acid (MMA<sup>V</sup>) and iAs are also excreted to a lesser extent in the urine while reactive metabolite intermediates, monomethylarsinous acid (MMA<sup>III</sup>) and dimethylarsinous acid (DMA<sup>III</sup>), have also been reported. Prior to 1995, the As<sup>V</sup> methylation pathway was generally considered to be a detoxification pathway, but cellular and animal studies involving MMA<sup>III</sup> and DMA<sup>III</sup> [2, 3] have indicated that their toxicities meet or exceed that of iAs, suggesting an activation process. We examined the biotransformation of iAs using a *in vitro* technique, i.e., the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) [4] which utilizes microbes of the human colon by culturing bacteria from harvested human faeces. To analyze the arsenicals resulting from biotransformation in this system, we developed a method using HPLC-ICP-MS starting from Wallschlagers method [5]. Here we used Hamilton PRP anion exchange column and were able to separate, identify and quantify As<sup>III</sup>, As<sup>V</sup>, DMA<sup>V</sup>, MMA<sup>III</sup>, MMA<sup>V</sup>, MMTA at 2 ppm concentration levels till date. Currently the resolution between As<sup>III</sup> and DMA<sup>V</sup> is under optimization. Moreover, we are also introducing DMA<sup>III</sup> in the same analytical procedure to have a single method which characterizes all important arsenic species. Going to use the fully developed method to characterize the arsenic species from bio-samples that are incubated in SHIME reactor (an in-vivo model of human gastro intestinal tract).

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## P002-B1

### Evaluation of PAGE Separation Conditions for the Detection of Metals Bound to Proteins by LA-ICP-MS

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Laser Ablation (LA) Inductively Coupled Plasma (ICP) Mass Spectrometry (MS) has been increasingly used as a system to detect metals bound to proteins after separation by Polyacrylamide Gel Electrophoresis (PAGE). Because of this, it is of great importance to keep the integrity of the metal-protein bindings during the procedure. When heteroatom-containing proteins are been investigated, the interactions metal-protein are strongly enough to remain intact during electrophoresis. Nevertheless when the object of the study are metal-binding proteins with weaker interactions it can occur that the metals are lost during the process and they cannot be detected bound to the proteins subsequently by LA-ICP-MS. This fact makes necessary the development of new procedures to separate proteins preventing the loss of metals bound to these proteins.

Starting from a previous paper [1], we have carried out some experiences to determine the influence of some factors concerning the electrophoretic procedure in the detection of metals by La-ICP-MS of two proteins ,superoxide dismutase (SOD), containing Zn and Cu, and alcohol dehydrogenase (ADH), containing Zn. Not only the influence of the nature of the electrophoretic method has been studied, but also the effect of other aspects such as intensity applied, trailing ion chosen and post-separation gel treatment. For a denaturing PAGE of SOD, we recommended tricine as trailing ion . While non denaturing PAGE based on tricine is better for ADH in order to maintain the integrity of metal-protein binding.

Regarding to the intensity applied, as higher it is the possibilities of metal-protein binding losses are higher. All of our results have demonstrated that it is preferred to avoid staining steps because they can alter the stability of the metal-binding proteins and prevent detecting metals bound to them.

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## P003-B1

### Determination of Thorium in Biological Materials by NAA and ICP MS

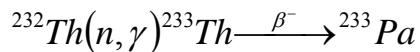
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Determination of thorium in biological system is important for a number of reasons. Thorium is widely distributed in the environment at low concentrations of the order of ten parts per million. The geochemistry of thorium is of particular interest for radioecological studies and little is known about phytotoxicity of thorium. It should be noted that toxicity of thorium can be similar to that of heavy metals but also it can cause additional effects on a plant due to its radioactive decay.

Data on the low level of thorium present in biological samples are variable. Due to this fact an attempt was made to evaluate a reliable method for thorium determination in plant materials. The devised method is based on a combination of quantitative and selective separation of thorium by ion-exchange chromatography, followed by gamma-ray spectrometric measurement of  $^{233}\text{Pa}$  or mass-spectroscopy measurement of  $^{232}\text{Th}$ .

Thorium content was determined by NAA via 311 keV line of  $^{233}\text{Pa}$  due to the fact that after irradiation in nuclear reactor there is a subsequent decay of the short lived  $^{233}\text{Th}$  ( $T_{1/2}=22.3$  min) product to  $^{233}\text{Pa}$  ( $T_{1/2}=27$  d).  $^{233}\text{Th}$  determination offers short half-life and lower sensitivity.



Gamma-ray spectrum of thorium fraction practically did not show any other activities except background peaks and small amount of impurities from chemicals used during pre-irradiation separation of thorium from the matrix.

Isolated thorium fraction was also measured by ICP MS. During calibration four internal standards were tested:  $^{187}\text{Re}$ ,  $^{103}\text{Rh}$ ,  $^{115}\text{In}$  and  $^{209}\text{Bi}$ . Sensitiveness of the detector was satisfactory for  $^{115}\text{In}$  and  $^{209}\text{Bi}$ . In case the of real samples  $^{209}\text{Bi}$  was used as an internal standard.

Accuracy of the method was demonstrated by analyzing several plant matrix CRM.



## **P004-B1**

### **Evaluation of Fast Sequential Determination of Cadmium, Copper, Lead and Zinc in Biological Samples by Thermospray Flame Furnace Atomic Absorption Spectrometry (TS-FF-AAS)**

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Fast sequential flame atomic absorption spectrometry (FS-FAAS) is a sequential multi element technique which holds the advantages of FAAS, being an ideal tool for routine determination of elements in the  $\text{mg L}^{-1}$  range. With the development of Thermospray Flame Furnace AAS (TS-FF-AAS) in 2000 it was possible to improve the power of detection of FAAS for volatile elements. Despite these advantages, TS-FF-AAS is a slow monoelemental technique comparing to conventional FAAS. In order to exceed the analysis speed of Cd, Cu, Pb and Zn by TS-FF-AAS, this study evaluates the combination of FS-FAAS and TS-FF-AAS. All experiments were performed in a flame atomic absorption spectrometer working in fast sequential mode (SpectrAA 240 FS; Varian) fitted with a deuterium background corrector. A Ni tube was placed on the burner head and the sample to be nebulized was directly introduced into the tube *via* a ceramic capillary. Nickel tubes with inner diameters of 8 and 10 mm having 6, 10 and 16 holes were evaluated for the sequential determination of Cd, Cu, Pb and Zn observing the necessary delay time to obtain maximum absorbance and to avoid memory effects. The fast sequential TS-FF-AAS system has been optimized for Cd, Cu, Pb and Zn whereby it was found that a Ni tube with an inner diameter of 8 mm with 10 holes combined with delay time of 55 seconds gave the best results (high absorbance values and no memory effects). After 55 seconds delay, Cd, Cu, Pb and Zn could be determined in four seconds (one second for each element). Limits of detection obtained for Cd, Cu, Pb and Zn were 0.25, 7.5, 4.4 and  $0.3 \mu\text{g L}^{-1}$ , respectively. The proposed method was applied for Cd and Pb determination in two certified materials and the obtained recoveries were 115 and 94%, respectively.

## P005-B1

### LC-MS/MS for the Determination of Ethylglucuronide in Hair and Meconium

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Ethylglucuronide (EtG), a minor metabolite of alcohol, is proven to accumulate in different human matrices and can be used to determine alcohol use over a longer period of time than the parent compound. In this study, we optimised and validated an LC-MS/MS method for the determination of EtG in i) hair and ii) meconium (the first fecal matter passed by a neonate). Different solid-phase extraction (SPE) cartridges (Oasis WAX, Oasis MAX, Bond Elut-NH<sub>2</sub>) were tested for the sample preparation of each matrix. The LC-MS/MS analysis was based on hydrophilic interaction liquid chromatography (HILIC) because of the highly polar nature of EtG. SPE on Bond Elut-NH<sub>2</sub> gave the best results for the extraction of EtG from meconium, while the optimal sample preparation from hair consisted of an overnight incubation followed by an evaporation and reconstitution step. For both matrices, the analysis on Phenomenex Luna HILIC column (150 mm x 3 mm x 5 µm) showed good retention coupled to optimal conditions for the mass spectrometric detection. Both developed methods were fully validated by assessing linearity, accuracy, precision and limit of quantification. Both methods showed good linearity ( $r^2 > 0.99$ ), accuracy (80-120%) and precision (< 20%). The limits of quantification were 50 and 20 ng/g for meconium and hair, respectively. Meconium samples (n = 18) collected from Antwerp hospitals showed concentrations ranging from < 50 to 956 ng/g. The detection frequency was 6 out of 18 (33%). Until now it is unknown what the LOQ value (50 ng/g) means in terms of alcohol use during pregnancy. Hair samples (n = 15) showed concentrations of EtG ranging from < 20 to 956 ng/g with a frequency rate of 23%. The LOQ value for hair analysis (20 ng/g) is corresponding with the cut-off value for heavy alcohol consumption.

## P006-B1

### Modified Cerium(IV)-Based Spectrophotometric Antioxidant Capacity Measurement in Sulfuric Acid-Sodium Sulfate Medium with Selectivity over Citric Acid and Simple Sugars

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Antioxidants are health beneficial compounds through their combat with reactive oxygen species and free radicals that may eventually give rise to various diseases. It is important to measure the total antioxidant capacity (TAC) of food material and human plasma for food quality estimation and for diagnosis and treatment of diseases, respectively. The authors have recently developed a Ce(IV)-based reducing capacity assay for this purpose. The aim of this work is to modify the existing cerium-based spectrophotometric assay so that Ce(IV) would selectively oxidize antioxidant compounds but not citric acid and reducing sugars. The redox potential of the Ce(IV) oxidant was fine tuned in 0.3 M H<sub>2</sub>SO<sub>4</sub> + 0.7 M Na<sub>2</sub>SO<sub>4</sub> aqueous medium so as to selectively oxidize true antioxidants but not citric acid, simple sugars, and other pharmaceutical ingredients. The trolox equivalent antioxidant capacity (TEAC) values in the order of quercetin > rutin > gallic acid > catechin > caffeic acid ≥ ferulic acid > naringenin ≥ naringin > trolox ≥ ascorbic acid were established with the proposed method, and were found to be compatible to those found with other antioxidant assays. It is noteworthy that naringin and rutin were also hydrolyzed in the acidic medium of the method so as to exert their full antioxidant capacity not measured by other TAC assays. The modified Ce(IV)-based method tolerated citric acid and reducing sugars without affecting the TAC measurement of quercetin. The simultaneous hydrolysis and oxidation of naringin is another advantage over other similar assays. The proposed TAC assay with Ce(IV) is simple, low-cost, rapid, and can be easily applied to modestly equipped conventional laboratories.

## P007-B1

### Determination of Total Antioxidant Capacity by Using Ferric Ferrozine Method and its Applications

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Antioxidants are health beneficial compounds, either uptaken through diet or synthesized within the cells, that help humans live a long and healthy life, and extend the shelf-life of foodstuffs. Antioxidant molecules quench excessive reactive species such as oxygen or nitrogen radicals under 'oxidative stress' conditions. The magenta-colored iron(II)-ferrozine (Fe(II)-FZ) complex showing an absorbance maximum at 562 nm has previously been used for iron-binding assays, but has not been utilized for antioxidant determination. Ferrozine is a highly ferrous-stabilizing ligand such that the ferric ion in the presence of ferrozine easily oxidizes antioxidants and is itself reduced to Fe(II)-FZ, yielding a high molar absorptivity for most antioxidants. The molar absorptivities achieved for trolox, quercetin, caffeic acid, ferulic acid, rutin, ascorbic acid, catechin, gallic acid, rosmarinic acid, and ellagic acid were  $6.01 \times 10^4$ ,  $2.19 \times 10^5$ ,  $7.47 \times 10^4$ ,  $5.52 \times 10^4$ ,  $9.02 \times 10^4$ ,  $6.07 \times 10^4$ ,  $7.95 \times 10^4$ ,  $1.58 \times 10^5$ ,  $1.59 \times 10^5$ ,  $1.47 \times 10^5$  L mol<sup>-1</sup> cm<sup>-1</sup>, respectively. Compared to other electron-transfer based antioxidant assays, Fe(III)-FZ thus provides higher sensitivity. The order of antioxidant power measured with the proposed method was in accord with theoretical considerations expected from structure-activity relationships of antioxidants. The Fe(III)-FZ assay was applied to synthetic antioxidant mixtures to yield additive absorbance values, which is a prerequisite for precise determination of antioxidant capacity of complex mixtures. Synthetic solutions of antioxidant mixtures gave the theoretically expected (additive) results. Ferrozine assay was used in real sample solutions such as green tea and nettle infusions by using the method of standard additions of a reference compound (trolox). The calibration curves (lines) of trolox individually and in herbal infusions were parallel, confirming that the herbal antioxidants and trolox did not chemically interact among each other so as to cause apparent deviations from Beer's law in the Fe(III)-FZ assay. The proposed method was applied to medicinal plant infusions for total antioxidant capacity assay as trolox-equivalents, and the results were compared to those found with CUPRAC (cupric reducing antioxidant capacity), FRAP (ferric reducing antioxidant power) and Folin total phenols assays.

## Micro Mixers Used for Light Induced Time Resolved Flow-Flash Experiments on Proteins

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We report a novel time-resolved step-scan Fourier transform infrared (S<sup>2</sup>-FTIR) experiment using a micro-fluidic mixing chamber. Combining specially designed micro mixing devices with a step-scan FTIR spectrometer allows label-free monitoring, even for non-cyclic reactions, such as protein folding and bio-ligand interactions, in the microsecond time domain for the first time.

The micro-fluidic mixing cell had a path length of 10  $\mu\text{m}$  [1, 2]. The mixer was mounted in an experimental setup featuring a Bruker IFS 66v/S spectrometer with a dedicated beam condenser focusing IR radiation onto the micro mixer from beneath. The photoactivation of the time-dependent processes is achieved by a laser beam which is focused onto the micro mixer from above.

To demonstrate the possibilities and potential of this method, photolysis of the CO-complex of myoglobin was chosen. Myoglobin in solution was reduced and then saturated with CO. This carboxy-Mb solution was pumped continuously through both channels of the micro mixer during the S<sup>2</sup>-FTIR experiment. The reaction was initiated by a laser pulse at 540 nm, corresponding to the absorption maximum of the  $\beta$ -band of the Mb-CO complex. A continuous liquid flow ensured that for every repetitive measurement new, freshly mixed solution was present in the cell.

The laser pulses triggered the immediate dissociation of CO molecules, resulting in a negative band in the recorded FT-IR difference spectra. The rebinding of CO was then tracked with a time resolution of 5  $\mu\text{s}$ . Simultaneously, changes in the protein absorption, which are indicative for changes in the protein structure due to the laser-induced dissociation, were recorded. Figure 1 shows exemplarily the relaxation of a band at  $1658\text{cm}^{-1}$  assigned to a shift of an  $\alpha$ -helix. To eliminate drifts in the baseline the difference to a nearby isobestic point is plotted. The kinetics of the structural changes of the protein do not follow a simple mono-exponential behavior therefore a bi-exponential fit was used, yielding time constants of 17  $\mu\text{s}$  and 1,2 ms respectively, which are corresponding to different relaxation processes. The results prove that the principle of flow through light-induced S<sup>2</sup>-FTIR using micro mixers works and will permit the monitoring of reactions, comprising two components, in the microsecond time range. This will allow the examination of the interaction mechanism of proteins with ligands as well as, for example, the study of non-cyclic reactions, which were not accessible by S<sup>2</sup>-FTIR before.

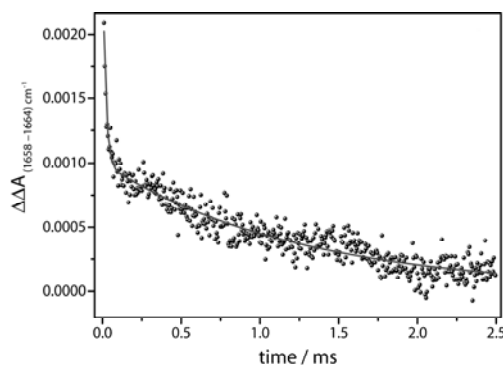


Fig. 1: Time trace of the difference band at  $1658\text{-minus-}1664\text{cm}^{-1}$  including a bi-exponential fit

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## P009-B1

### Profiling of Hepatocyte-Generated Volatile Degradation Products of $\beta$ -Carotene by GC-MS Subsequent to SPE

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Dietary supplementation with  $\beta$ -carotene (BC), a precursor of vitamin A, has been considered a cancer preventive means and was therefore applied not only as nutrition supplement but also in therapy due to its radical scavenging and anti-genotoxic properties. In contrast, under conditions of oxidative stress adverse consequences, *i.e.* prooxidant and cocarcinogenic effects, can be induced as shown by recent comprehensive studies. These detrimental impacts mainly affect smokers and asbestos workers who are at a higher risk to develop lung cancer as a result of BC supplementation due to the formation of oxidative cleavage products (CPs) of BC. Research focuses on two major CP classes in the elucidation of responsible cancerogen(s): (i) apo-carotenals, *i.e.* long-chain CPs and (ii) volatile low molecular weight CPs, whereby the identification and quantification of analytes belonging to the latter class have only marginally been addressed up to now.

Current results have revealed several prominent volatile CPs ( $\beta$ -ionone, cyclocitral, dihydroactinidiolide, 1,1,6-trimethyltetraline, epoxy- $\beta$ -ionone), which have been formed in hepatocyte cultures subjected to oxidative stress either by chemical induction or a change in the oxygen supply. Optimized solid phase extraction allows for a recovery >60% in either case which is considerably higher than for liquid-liquid extraction protocols applied previously. GC separation is performed with a DB-20 WAX column applying linalool as an internal standard due to the absence of commercially deuterated target compounds. The validation of the preparation method covers repeatability, intermediate precision, linearity, LOD and LOQ, recovery and the optimization of solutions employed for the application of BC to cell cultures as well as for elution of CPs from SPE columns and their injection into GC-MS.

## P010-B1

### Determination of Zinc by Flame Atomic Absorption Spectrometry after on Line Preconcentration with Zincon

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Zinc is an essential trace element to the human organism considering this is a constituent of several enzymes and has an important role in the metabolic process of carbohydrates, lipids and proteins. In this case the development of sensitive methods for its determination is advisable. On-line preconcentration procedures show advantages such as an increase in the detectability, high sample throughput, simplicity of automation and low contamination risk.

Liquid-solid extraction with a flow injection flame atomic absorption spectrometric method is described for the determination of zinc. The experimental procedure used a column (35 x 3 mm) containing about 74 mg of Dowex 1X8-200 resin modified with 1% (w/w) zincon, through which a solution containing zinc is passing. The metal is retained in the column, in the form of Zn-zincon complex. During extraction procedure it was verified that the metal reacts completely with the complexing reagent in the pH range from 9.0 to 10.0.

After the optimization of parameters such as: type and concentration of the eluent, time of preconcentration and elution, flow rate of sample, buffer and eluent, reaction time (length of coil reaction) and the effect of foreign ions, the system showed a sampling frequency of 26 determinations/hour. The efficiency of the minicolumn packed with modified resin was not affected even after 200 preconcentration runs. A preconcentration factor of 10 and limit of detection of  $0.6 \mu\text{g L}^{-1}$  was obtained with a relative standard deviation of 5 %. The accuracy was evaluated by using certified reference material allowing to conclude that the proposed method may be used for the analysis of food samples.

CNPq, FAPESP

## **P011-B1**

### **HPLC Determination of Carbamazepine in Plasma of Epileptic Patients**

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Therapeutic drug monitoring of anti-epileptic drugs has been found to be very useful in optimizing seizure control and in avoiding and detecting drug toxicity. Carbamazepine is a first line drug used in a variety of seizure disorders including partial seizures and generalized tonic clonic seizures.

The aim of our study was to establish a rapid, reliable and precise method for solid - phase extraction (SPE) followed by RP-HPLC for determination of carbamazepine in plasma of epileptic patients.

The extraction of carbamazepine and the internal standard nitrazepam from plasma samples was performed by means of an original solid - phase extraction procedure using Oasis HLB cartridges.

Excellent recovery (95,9 %) and cleanup efficiency (negligible matrix effect 0,24 %) were achieved.

After solid - phase extraction, separation was carried out on a reversed-phase column (Zorbax Extend C18, 150 x 4,6 mm i.d.) using isocratic elution with acetonitrile and water (35:65) as a mobile phase.

The temperature was 30°C and UV detection was set at 220 nm. The method was validated according to the ICH guidelines.

In conclusion, the validation data demonstrate that the proposed method is appropriate for determining carbamazepine plasma concentrations for therapeutic monitoring.



## P012-B1

### HPLC Measurement of Thiamine and its Phosphorylated Forms in Whole Blood and Plasma: Thiamine Status Assessment

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Thiamine diphosphate is a biologically active form of thiamine playing a vital role in carbohydrate metabolism. Processing of large amount of glucose automatically increases demand for dietary thiamine. The assessment of thiamine status is best achieved by the direct measurement of thiamine and its esters in blood using HPLC or by the functional erythrocyte transketolase activity measurement.

We report here a new gradient ion-paired HPLC utilising fluorescence detection for the simultaneous determination of thiamine and its mono- and diphosphate esters in human whole blood and plasma. First, the samples were deproteinized and thiamines were derivatized via oxidation to thiochromes, which were then separated on a reversed-phase column Luna C18 in a gradient mode using a mobile phase of pH 7.0 consisting of phosphate buffer, methanol and tetrabutylammonium hydroxide as an ion-pairing reagent, at a flow-rate of 1 mL/min and temperature of 30 °C, and detected fluorimetrically at an excitation wavelength of 365 nm and an emission wavelength of 435 nm. The method exhibited linearity in the range up to 100, 20 and 200 nmol/L for thiamine, thiamine mono- and diphosphate, respectively. The limits of detection were from 0.2 to 0.4 nmol/L. The intra- and inter-assay precisions were within 4 and 8%, respectively, for thiamine, thiamine mono- and diphosphate concentrations of 12.5, 1.25, and 25 nmol/L, respectively.

The method has been sufficient for the accurate, reliable and reproducible determination of thiamine status in healthy persons and also patients with diabetic nephropathy. Moreover, the method strongly correlated with the activity transketolase measured by kinetic modification of the NADH-dependent method utilising triosephosphate isomerase and glycerol-3-phosphate dehydrogenase.

Supported by the grants LC06023 of the Ministry of Education and NR9443-3 of the Ministry of Health of Czech Republic.

## P013-B1

### **A Micro-Coated Wire Ion-Selective Electrode for Flow-Injection Analysis of Trazodone in Pharmaceuticals, Human Urine and Serum**

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Trazodone (TRZ), 2-[3-[4-(m-chlorophenyl)-1-piperazinyl]propyl]-1,2,4-triazolo [4,3-a]pyridine-3(2H) one hydrochloride, is a triazolopyridine antidepressant chemically unrelated to any other class of antidepressant. It blocks the reuptake of serotonin in the presynaptic neurones and also acts at the 5-HT<sub>1</sub> receptors. Trazodone is used in the treatment of depressions, trembling states, dyskinesia and emotional disorders, among others. The therapeutic importance of this drug requires selective, rapid and accessible analytical methods for its determination.

Flow-injection analysis (FIA) is a simple, rapid and versatile technique with widespread applications in quantitative analysis. ISEs in flow-injection potentiometry (FIP) have several advantages: rapid and easy procedures, simple instrumentation, fast sample throughput, wide dynamic range, high precision, small sample volumes and the economical use of reagents.

A novel micro-coated wire trazodone-selective electrode placed in an FIP system is characterized and used to determine trazodone levels in various samples. The electrode is based on the immobilization of a PVC-sensing membrane, composed of a trazodone-tetraphenylborate ion-exchanger, on a platinum wire and incorporated in a novel miniaturized FIA-cell. The method provided a fast, stable and near-Nernstian response over a wide trazodone concentration range and with a low detection limit. The electrode also showed good repeatability, reproducibility and selectivity with respect to some inorganic and organic compounds, including the main trazodone metabolite.

The electrode provided good analytical results in the determination of trazodone in pharmaceuticals and spiked urine and serum samples.

## P014-B1

### Detection and Identification of Urine Omeprazol Metabolites by CE- MS

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Omeprazole is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal reflux disease and Zollinger-Ellison syndrome. Omeprazole is a racemate. It contains a tricoordinated sulfur atom in a pyramidal structure and therefore can exist in equal amounts of both the S and R enantiomers. In the acidic conditions of the stomach react with a cysteine group in H<sup>+</sup>/K<sup>+</sup> ATPase, thereby inhibiting the ability of the parietal cells to produce gastric acid. Omeprazole is completely metabolized by the cytochrome P450 system, mainly in the liver. The sulphone, the sulphide and hydroxy-omeprazole are the most important metabolites, which exert no significant effect on the acid secretion. About 80% of an orally given dose is excreted as metabolites in the urine and the remainder is found in the feces, primarily originating from bile secretion.

In a previous work, we are developed a new method for the determination of omeprazole and its main metabolites in urine by using UV-Vis detection. Due to the presence of some unknown peaks, probably others metabolites, a new EC-ESI-MS method have been point out in order to confirm and identifier these metabolites. Different chemical and instrumental parameters were studied to obtain the optima values for the electrophoretic separation.

A 10 mM ammonium acetate buffer solution at pH 9 was used as separation electrolyte, hydrodynamic injection (10 s, 0.5 psi) and a separation voltage of 30 KV. The sheath liquid used for detection in the ESI-negative mode was a solution consisting of 70 % 2-propanol and 5 mM running buffer (ammonium acetate, pH 9), with a flow rate of 6 µL/min, the electrospray voltage selected was 4 KV and the sheath gas flow rate 50 (arbitrary units).

Under these conditions the analytes were separated in less of 7 min. The method was applied to real samples (different human urines of volunteers undergoing medical treatment). In order to confirm the identity of the omeprazole metabolites a selective fragmentation using MS<sup>2</sup> experiment was carried out.

As result two metabolites have been identified in the urine as 5-hydroxy sulphide and 5-hydroxy omeprazole.

## **P015-B1**

### **Molecular Imprinting of X-Ray Contrast Agents**

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X-ray contrast agents have become indispensable in modern medical imaging for soft tissues of the human body. Especially during coronary angiographies x-ray contrast agents enable detailed insight on the branching of coronary vessels, thereby improving the recognition of diseased tissue. Launched in 1996, iodixanol has soon been established among the most widely applied contrast agents in the western world. Although showing less nephrotoxic effects in comparison to other x-ray contrast agents such as e.g., iohexol, ioxithalamate or diatrizoate the usual dose of a highly concentrated iodixanol injection with a concentration of up to 652 mg/ml still poses a severe stress to the renal system, which is of particular importance for patients who already suffer from renal dysfunction.

Eliminating or at least reducing the concentration of iodixanol in blood prior to reaching the kidneys is a potential path toward diminishing adverse effects on the renal system. Sorbents based on molecularly imprinted materials represent a strategy for developing selective scavenging materials for removing iodixanol from blood ideally without changing its composition. However, the synthesis of molecularly imprinted polymers for water-soluble templates such as e.g., iodixanol remains a challenging task.

In this study, we present first results on molecularly imprinted matrices selective for iodixanol based on conventional bulk-imprinting. Besides the molecular imprinting approach, various non-imprinted adsorbents were tested including e.g., cellulose, silica gels, self-assembled monolayers, and charcoal according to their capability for binding iodixanol. Furthermore, an outlook toward more sophisticated imprinting strategies resulting in high-capacity scavenging matrices for iodixanol is discussed.

## P016-B1

### Determination of 2-Methylthiazolidine-4-Carboxylic Acid, a Condensation Product of Acetaldehyde and Cysteine, Found in Human Blood and Urine by Liquid Chromatography - Electrospray Ionisation - Tandem Mass Spectrometry

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Alcohol consumption has a tremendous influence on social, cultural, economical and health affairs all over the world. In this framework the determination of ethanol intake is of importance for various fields such as clinics, forensics, workplace monitoring, etc. Acetaldehyde is the main oxidative metabolite of ethanol. The non enzymatic formation of adducts between this highly reactive compound and nucleophilic groups of proteins such as -NH<sub>2</sub> and -SH are well known. In this context, cysteine has been recognized as a scavenger for this potentially harmful and cancerogenous substance via a condensation reaction<sup>1,2,3</sup>. Herein, we demonstrate the evaluation of the resulting 2-methylthiazolidine-4-carboxylic acid (MTCA) as a potential marker for recent alcohol consumption. The back reaction of MTCA to the educts could be hindered through N-derivatization by using acetic anhydride. The stable isotope internal standard D<sub>4</sub>-MTCA was synthesized and used for optimization of sample workup and quantitative analysis. A procedure to determine MTCA in human blood and in urine by liquid chromatography - electrospray ionisation - tandem mass spectrometry in selected reaction monitoring mode will be presented.

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## P017-B1

### CZE Separation of Cellular Energetically Important Metabolites

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Nucleotides are essential for viability and growth of all living cells. They have at least four important functions; storage and transport of cellular metabolic energy; activation and transfer of precursors for cellular biosynthesis; synthesis of DNA and RNA; control and regulation of cellular metabolism. Their total concentration and distribution of individual species is highly dynamic with changes in environmental conditions. Monitoring the nucleotides pool is able to verify the physiological state of the cell and predict this state due to the presence or ratios to each other.

Due to the importance of nucleotides for metabolism, numerous methods for their determination in biological samples have been reported, mainly HPLC, CE or TLC.

The main aim of this work was to find appropriate conditions for the selective and rapid determination of purine (AMP, ADP, ATP, GMP, GDP, GTP), pyrimidine (CMP, CDP, CTP, UMP, UDP, UTP) nucleotides, adenine coenzymes (NAD<sup>+</sup>, NADH, NADP<sup>+</sup>, NADPH) and Acetyl CoA using capillary electrophoresis (CE). Because of low intra- and extracellular concentration of these metabolites the capillary zone electrophoresis was combined with the on-line preconcentration technique -field enhanced sample stacking- to improve the concentration sensitivities.

The determination was performed in a bare fused silica capillary using separation voltage 20 kV (positive polarity) and direct detection at 260, 280 and 340 nm. For method optimization different concentrations of phosphate buffer (50 – 80 mM), pH range from 5 to 7 and temperature of capillary from 16 to 25 °C were tested. The best resolution was found in the pH range 5.5 – 6 for each buffer concentration; hence this pH range was examined in detail. Metabolite samples dissolved in deionised water were injected into the capillary hydrodynamically (50 mbar, 14 s).

#### **Acknowledgements**

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## P018-B1

### Novel Zwitterionic Chiral Ion Exchangers for HPLC-Enantioseparation of Chiral Acids, Amino Acids and Peptides

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Well established chiral weak anion exchangers (WAX) based on cinchona alkaloid derivatives (tert-butyl carbamoyl-quinine and –quinidine) represent a powerful tool for HPLC enantioseparation of chiral acidic analytes both on analytical and preparative scale [1]. By fusion of complementary strong cation exchange moieties (SCX) with the quinine based anion exchanger a novel, now zwitterionic, chiral ion exchanger is created.

When bound to silica, the corresponding brush-type chiral stationary phase (CSP) are capable to separate not only chiral acids, but also a broad range of amphoteric compounds like amino acids and peptides [2].

Within this contribution, a set of zwitterionic chiral selectors is presented with special focus on variations of the sulfonic acid SCX moieties. The impact of an increasing aliphatic side chain (C<sub>1</sub> to C<sub>4</sub>) on enantioselectivity towards amino acids and peptides is shown. Moreover, the introduction of an aromatic- and the sterically demanding camphor scaffold in vicinity to the sulfonic acid group will be presented and the effect on the overall enantioseparation ability is investigated.

Based on mobile phase optimizations by recent studies [3] the main chromatographic parameters as retention, enantioselectivity, resolution, and efficiency are discussed on a distinctive set of amino acid and peptide analytes.

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## P019-B1

### A Novel Capillary Liquid Chromatography-Evaporating Light Scattering Method for the Quantification of Heterocyclic Amines in Human Urine Samples

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Heterocyclic aromatic amines (HAAs) are formed in the surface layer of meat during cooking and are carcinogenic in animal models, but the effect in human beings remains to be elucidated. It has been observed that the rising cancer incidences in humans in the developed countries, especially diseases of colon, large intestine, prostate, liver and kidneys are related to the greater consumption of meat as well as preserved meat [1]. Thus, methods have been developed to determine these compounds in biological samples (mainly urine samples) to reflect daily intake and recent HAAs exposure [2].

A rapid and simple method for separation and detection of six heterocyclic aromatic amines (2-amino-1-methyl-6-phenylimidazo [4,5-b]-pyridine, 2-amino-1-methyl-imidazo [4,5-f]-quinoline, 2-amino-3,8-dimethyl-imidazo [4,5-f]-quinoxaline, 2-amino-3,7,8-trimethyl-imidazo [4,5-f]-quinoxaline, 2-amino-3,4,8-trimethyl-imidazo [4,5-f]-quinoxaline and 2-amino-3,4-dimethylimidazo [4,5-f]-quinoline) in human urine samples is proposed. This method comprises previous clean-up and pre-concentration of the analytes on Strata-X reversed phase extraction cartridges followed by capillary liquid chromatography and light scattering detection (ELSD). A mobile phase of acetonitrile and ammonium acetate 35mM at pH 5.15 through a gradient of composition and a flow rate of 15  $\mu\text{L min}^{-1}$  resulted in good separations between the analytes. Temperature and gas pressure were optimized for detection. This method allows determining the HAAs in a range between 5 and 100  $\text{ng mL}^{-1}$ , with relative standard deviations lower than 4.8% in all cases. Good recoveries were obtained in the analysis of urine samples spiked with the HAAs. The usefulness of the proposed method was demonstrated by the analysis of spiked and natural human urine samples.

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## P020-B1

### Magnetic Beads-Based Extraction and Liquid Chromatography-Electrospray-Linear Ion Trap Mass Spectrometry for a Sensitive Analysis of Estrogens in Children Serum

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The evaluation of estrogenic status is necessary for many physiological and pathological conditions in pediatric as well as adult endocrinology. Current immunoassays exclusively measure estradiol with an insufficient sensitivity for prepubertal children [1]. The diagnosis of pre-pubertal disorders also requires an accurate estrogen assay method. Consequently, a re-evaluation of these plasmatic hormonal levels using an adequately sensitive and specific confirmatory measurement technique is demanding and is a pre-requisite for any risk assessment study devoted to these compounds.

We developed an innovative magnetic bead-based extraction method followed by liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS/MS) determination of serum estrone and estradiol at the concentration levels encountered in prepubertal children. This assay is based on the extraction of estrogens with C18 magnetic beads from 7  $\mu$ L of serum. Instrumental analysis was performed on an linear ion trap tandem mass spectrometer in the multiple-reaction monitoring mode after reversed-phase LC. The short chromatographic run time (<3.5 min) makes the method suitable for high-throughput analysis of estrogens. The calibration curves exhibited linearity and repeatability in the 10-200 pg/mL range. Interassay CVs were 4-8 % at mean concentrations of estrogens of 30 pg/mL. Limit of detection was 5 pg/mL and the overall method recovery of estrone and estradiol was >70 %.

Sensitivity and specificity of the method was tested on children serum samples.

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## **P021-B1**

### **Determination of Lead in Urine Samples by Flame Atomic Absorption Spectrometry (FAAS) by Formation of its Hydride**

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Optimization of experimental conditions for lead hydride formation and determination of lead in urine samples of exposed donors is described. Best sample treatment was accomplished by microwave assisted acid digestion using a mixture of  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$ , which produced clear sample solutions, after 5 minutes heating, ready for subsequent plumbane generation after reaction with sodium borohydride. Plumbane generation found to be critically dependent on type of acid used, final acid concentration of the sample solution (pH), type and amount of oxidant employed for transformation of Pb(II) into Pb(IV), and amount of  $\text{NaBH}_4$  added as a reductant.

The flow of Ar used to transfer the hydride from MHS10 reaction vessel to the quartz T cell and the absorption were also highly influential on the sensitivity and precision of the measurements carried out.

Under conditions of 3.0 M nitric acid (pH<0,5), 6.0 %v/v  $\text{H}_2\text{O}_2$ , 3.0 mL of digested urine sample taken to a final volume of 5.0 mL with deionized water were prepared before the reaction with the reductant agent, and measuring at the 217.0 nm wavelength, the following figures of merit were obtained: limited detection (3s, n=10) 2.85  $\mu\text{g/L}$ ; precision as %RSD (n=10) was 6.5%, accuracy as analyte recovery percentage was in the range of 96-103%, correlation coefficients for aqueous standard calibration graphs were 0,999 or better.

## P022-B1

### Atomic Spectroscopy Applied to Neonatal Cystic Fibrosis Screening

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Cystic fibrosis (CF) is a common autosomal recessive hereditary disease. Due to initiation of pathological effects at a young age, and high mortality rate, the disease has received considerable attention in public health policies, early diagnosis being vital to improve chances of survival. The objective of this work was to investigate levels of trace elements in the saliva of individuals diagnosed with cystic fibrosis, as well as of healthy individuals.

The study population comprised 35 persons with CF and 39 healthy persons (control group). Saliva samples were obtained by stimulation, and digested in a closed system with microwave assistance, using an acid medium (double-distilled HNO<sub>3</sub>). The elements Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Si, Sr, Ti, V and Zn were quantified using mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS). Concentrations of As and Se were determined by hydride generation coupled with optical emission spectroscopy (HG-ICP-OES).

Al, Ba, Cd, Cu, Fe, Mg, Mn, Ni, Ti, Sr and Zn showed no significant differences between study groups. Statistical analysis indicated that the elements Na ( $167.72 \pm 101.61 \text{ mg L}^{-1}$ ) and K ( $1249.01 \pm 446.95 \text{ mg L}^{-1}$ ) were present at higher concentrations in the saliva of individuals with CF, while the trace elements V ( $0.89 \pm 2.88 \text{ mg L}^{-1}$ ), Cr ( $4.55 \pm 18.43 \text{ mg L}^{-1}$ ) and Se ( $10.13 \pm 27.93 \text{ mg L}^{-1}$ ) were found at lower concentrations, compared to the control group. The levels of As in CF group samples were below the limit of quantification of the technique, while samples from the control group gave an average value of  $8.74 \pm 11.36 \text{ mg L}^{-1}$ . Glandular alterations caused by CF probably modify the saliva elemental composition, permitting distinction between the two groups using statistical procedures. Atomic spectroscopy techniques therefore show considerable potential in the development of methodologies designed to detect neonatal cystic fibrosis.

## P023-B1

### Detection of DNA Reactive Photodegradation Products of benzo(a)pyrene

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants that can enter the environment via different sources. PAHs are classified as carcinogenic in different classes according to their chemical structure. PAHs toxicity is especially exerted when they undergo metabolic, chemical or photochemical oxidation because of the formation of reactive compounds able to form stable adducts with DNA especially by covalent binding to guanine and adenine base. In this study benzo(a)pyrene (BaP) was used as a model analyte due to the ability to form reactive species under metabolic chemical or photochemical activation and to its well known toxicity. A photo-degradation protocol was used to evaluate the degradation kinetic parameters using aqueous solutions of BaP at different pH (7.0, 4.7). The disappearance of BaP was measured via LC-FL. Once the photo-degradation protocol was optimised, different analytical approaches were developed to: 1) detect the species that are formed during the degradation protocol, 2) to assess the toxicity of these species via the formation of stable adduct with guanine and adenine base or with oligonucleotidic sequences. The characterization of reactive species formed during the degradation protocol was performed by GC-MS and LC-MS, whereas the formation of stable adducts, when activated BaP was exposed to guanine or adenine bases was monitored via LC-MS. Finally an electrochemical DNA biosensor was used to evaluate the possibility to use this simple devices as a toxicity screening test of samples possibly contaminated with PAHs. The results confirm that BaP photo-activation, especially in the UV-A range, induces the formation of reactive compounds, that these compounds are able, under certain conditions, to form stable adducts with guanine bases, and that, it is possible to detect the reactive species via a DNA electrochemical biosensor.

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## P024-B1

### **μSPE-HPLC-MS/MS for the Determination of Illicit Drugs in Oral Fluid**

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The use of illicit drugs is a social threat constantly under discussion and it has been demonstrated to be a risk factor for road accidents and industrial injuries. Oral fluid have been recently considered a promising alternative matrix for the assay, due to several advantages vs. blood and urine: a non invasive sample collection, the respect of patient dignity, sampling procedures carried out by untrained staff, good correlation with plasma concentrations of analytes.

Confirmatory analysis for drugs of abuse in biological fluids are conventionally performed by gaschromatography-mass spectrometry (GC-MS), which has been widely applied in forensic as well as toxicology field; however, this technique is not suitable to perform multiclass analysis at very low concentration levels. Furthermore, the high viscosity of oral fluids, due to the presence of mucine, could cause problems in terms of matrix effect, so a clean-up step is necessary prior to analysis.

The aim of this study was the development of a confirmatory method based on ESI-LC-MS-MS for the determination of illicit drugs belonging to different chemical classes, such as amphetamine, methamphetamine, MDA, MDEA, MDMA, cocaine, BEG, ketamine, mescaline, fenciclidine and psilocibine.

A very simple and reliable clean-up has been developed by means of micro solid phase extraction with modified tips, made of a functionalized fiberglass with apolar chains of octadecylsilane into a monolithic structure. The extraction procedure requires small samples volumes (100μL) and it is very low time and solvent consuming.

For the LC-MS/MS analysis the drugs were separated using a reversed phase C18 column (10 cm x 2.1 mm ID) packed with particles of average diameter of 3μm with acetonitrile and water, both at a concentration 5 mM of formic acid as the mobile phases, with a gradient elution. The complete separation of all compounds occurs in 12 minutes.

## P025-B1

### Antibody-Based Enrichment by Immunoprecipitation of Carbonic Anhydrase II on Magnetic Beads for LC-MS/MS Quantification in Human Serum

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One of the main challenges facing proteomics research is the development of reproducible, standardized and comprehensive high-throughput methods to quantify a protein from a complex matrix such as serum. The development of highly sensitive and specific assays for quantifying proteins is still today the major bottleneck for validation of new clinical diagnostics. Thus, selective sample enrichment strategies are particularly useful to analyze proteins present in low abundance in biological fluids.

In this study a strategy based on the use of antibody magnetic-bead-based platform to isolate a protein of interest from biological matrixes, such as serum, followed by proteolytic digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection, was presented. In this approach, the key steps involved immunoaffinity purification of carbonic anhydrase II (CA II) from human serum followed by on-bead digestion with trypsin to release a surrogate peptide [1].

This tryptic peptide was quantified using a synthetic peptide standard and a structural analogue free-labeled internal standard. Using this strategy, a sensitive and selective LC-MS/MS assay was developed to measure CA II in serum. In fact, basing on quantitative equivalence between the protein and a selected peptide coming from protein tryptic digestion, the result was stoichiometrically converted to CA II concentration in serum.

We demonstrated that antibody capture followed by MS analysis can be useful particularly to achieve selective sample enrichment and to analyze low abundance target proteins in complex biological samples. The precision (<10%) and accuracy (c.a. 20%) of the present method are suitable for quantifying biomarkers in the physiologically relevant nmol range. Therefore, this strategy hold great potential for providing a needed bridging technology between biomarker discovery and clinical application.

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## P026-B1

### Structural Features of Human Tooth Tissues Affected by High Dose of External Ionizing Radiation

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Raman spectroscopy analysis has been carried out to study the effects of high doses (0.5-1.7 Gy) of ionizing radiation on human tooth tissues for persons exposed to external irradiation during their work in the Chernobyl zone after the accident. For comparison the tooth tissues of men who had not been exposed to radiation (control group) were investigated too. This analysis is a continuation of previous investigation of changes of tooth tissues performed with Infrared spectroscopy.

By diamond disk the teeth` samples were cut in slices with thickness of 1-1.5 mm. Determination of the tooth tissues composition was performed by a Renishaw InVia micro-Raman spectrometer with laser excitation at 785 nm. The micro-Raman spectrometry allows to study changes of tissues along an enamel layer and on the board of enamel/dentin by a focused laser beam with a diameter of several micrometers.

The mineral part of teeth tissue is represented by apatite material containing also carbonate components - carbonate hydroxyl-apatite. The  $\nu_1$  phosphate peak at  $961\text{ cm}^{-1}$  is a good marker for the bone mineral matrix of the tissues. The bands of amide I at  $1680\text{ cm}^{-1}$  and amide III at  $1260\text{ cm}^{-1}$  characterize organic components in the dental tissues. Bands of  $\nu_1\text{ PO}_4$  and amide I are commonly used for the determination of the mineral and organic composition of the tissue.

For dentin and enamel of irradiated patients we noted a decreasing of phosphate component content (by detection of the decreasing intensity of  $\nu_1\text{ PO}_4$  band at  $960\text{ cm}^{-1}$ ) and changes of ratio between amide I and amide III towards a decreasing of Amide I content in dentin tissues. The obtained results demonstrated that high doses of radiation lead to an imbalance between mineral-organic phases level. These changes have an effect on the dental matrix strength.

## P027-B1

### Solid-Phase Microextraction On-Fiber Derivatization for the Analysis of Some Polyphenols in Wine and Grapes Using Gas Chromatography-Mass Spectrometry

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The healthy properties of wine and grapes are basically due to the presence of antioxidants such as polyphenols, which include flavonoids (catechins) and stilbenoids (resveratrol and piceatannol). Polyphenols are generally determined by HPLC, as they are non-volatile; however, the use of GC-MS provides important advantages. Conventional approaches for sample treatment are tedious and time-consuming and because of the importance of developing clean chemistry procedures, emerging methods for food matrices are based on solvent-free procedures. Solid phase microextraction (SPME) is a non-harmful environmentally pre-concentration technique which is clean, selective, rapid, efficient, simple and solvent-free. This study describes a new pretreatment system based on SPME for the sensitive determination of *cis*- and *trans*-resveratrol isomers, piceatannol, catechin and epicatechin. The separation conditions of polyphenols were optimized using a DB-5MS column. The GC temperature was: start at 100 °C, increase to 320 °C at 30 °C/min and hold for 5 min. The sequence of the ions selected as a function of the eluting time was: *cis*-resveratrol (444, 7.67 min), *trans*-resveratrol (444, 9.0), epicatechin (369, 9.54), catechin (368, 9.71) and piceatannol (532, 9.74). A derivatization process was necessary to convert the polar non-volatile compounds into volatile derivatives. Direct immersion (DI-SPME) was used for the adsorption of polyphenols and, then, the fiber was placed in the headspace of the derivatizing reagent, bis-(trimethylsilyl)trifluoroacetamide (BSTFA). Optimal extraction conditions were 25 °C for 10 min under continuous stirring using a PA fiber. After extraction, the fiber was inserted into the headspace of BSTFA (10 µL) and polyphenols were derivatized for 15 min at 50 °C. Desorption was carried out at 280 °C for 5 min. The method was validated for linearity, detection and quantitation limits, selectivity, accuracy and precision. Quantitation was based on the target ion. Detection limits ranged from 0.05 to 0.9 ng/L at a signal to noise ratio of 3. Recoveries obtained for spiked samples were satisfactory for all compounds. Ten different samples of wine (red, rosé, white and sweet) and two samples of grapes (red and white) were analyzed. As expected, higher levels for all polyphenols appeared in red wine and grapes. On the other hand, levels of piceatannol were lower than those of resveratrol.



## P028-B1

### Electrochemical Microfluidic Chip Coupled to Magnetic Immunoassay for Zearalenone Determination in Infant Foods with Simplified Calibration

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A novel analytical strategy coupling external ELISA and microfluidic chip for the determination of zearalenone mycotoxin in infant foods is presented. Using creatively ELISA microwells as microchip reservoirs, this proposal avoids sophisticated integrated immunoassays on microfluidics but taking their inherent advantages: very fast analysis times, extremely low samples and reagent volumes, calibration multiplexed (integrated and simplified) and future parallelization. The immunoassay is developed on the basis of a competition scheme where the mycotoxin zearalenone and an enzyme-labelled derivative (HRP) compete for the binding sites of the specific antibody. Protein G covalently bound to magnetic particles acts as oriented immobilization support for the capture of the anti-mycotoxin antibody. After molecular recognition, the extent of affinity reaction is evaluated by the addition of the enzymatic substrate and electrochemical mediator which subsequent to enzymatic reaction were electrokinetically injected into the microfluidic platform. *End-channel* amperometric detection of the enzymatically oxidized mediator HQ is directly related to the activity of the enzyme tracer.

Analytical cycles for fast analysis of the sample and its sequential calibration was performed in about 200s. An extremely low concentration level of ZEA less than 1 ppb was detected with reliability. This level was 20 times less than the strictest tolerable limit (20ppb) for infant products become microfluidic approach as new anticipated analytical security tool for the future. The reliability of the proposal was demonstrated evaluating the accuracy using a certified reference material and demonstrating their suitability during the control of regulatory limits of ZEA in infant foodstuffs.

## P029-B1

### Fast Detection of Antioxidants Using Eletrokinetic Microfluidic Chips with Carbon Nanotubes Detectors

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Detection is one of the main challenges for microfluidic chips, since very sensitive techniques are needed as a consequence of the ultra-small sample volumes used (pL-nL). Electrochemical detection (ED) is one of the most commonly routes used because apart from its high sensitivity and inherent miniaturization, another added functionality is the opened opportunity to modify these surfaces suitably with nanomaterials. An excellent example of this is carbon nanotubes (CNTs). CNTs are a group of nanomaterials which offers notably favorable possibilities involving the large active surface at electrodes of small dimensions and the enhancement of electronic transfer. These properties have clear influence on their analytical sensitivity which is enhanced by the use of these nanomaterials. There are two main types of carbon nanotubes that can have high structural perfection: single-walled nanotubes (SWCNT) which consist of a single graphite sheet seamlessly wrapped into a cylindrical tube, and multi-walled nanotubes (MWCNT) which comprise an array of such nanotubes that are concentrically nested like rings of a tree trunk.

In this work, the analytical potency of microfluidic chips with multiwalled carbon nanotubes, in two analytical formats-flow injection and separation electrokinetic driven systems- using real samples is explored. Accordingly, two applications of high significance have been chosen to demonstrate the suitability of the electrokinetic's platform integrating nanoelectrochemical detectors: the determination of total isoflavones with integrated calibration and the fast detection of the major natural antioxidants where microfluidic chips were used as flow injection and separation formats, respectively.

[2] A.G. Crevillén, M. Pumera, M.C. González, A. Escarpa *Lab Chip* **9** (2009) 346-353

## P030-B1

### Mid-IR Wine Analysis with High Performance Liquid Chromatography Using Two QCLs Simultaneously

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Quantum cascade lasers (QCLs) are a new generation of powerful light sources for mid-IR spectroscopy. Due to their high spectral power density, longer optical paths can be realized in transmission measurements as compared when using standard FTIR spectrometers. Therefore, the use of these lasers holds great promise to increase sensitivity in on-line IR detection in high performance liquid chromatography (HPLC), based on transmission measurements. However, due to the general small tuning range of standard QCLs, spectral information is lost when replacing the FTIR spectrometer by a single quantum cascade laser [1]. Here, we report our results obtained by interlacing two different Fabry P erot QCLs for quasi simultaneous absorption measurements at two different wavelength regions (one centered at  $1393\text{ cm}^{-1}$  for organic acids and the other at  $1080\text{ cm}^{-1}$  for sugar).

Our set-up, based on three gold mirrors and a ZnSe beam splitter, is used to direct the emitted laser light through a liquid flow cell with an optical path length of  $100\text{ }\mu\text{m}$ , onto a mercury-cadmium-telluride (MCT) detector.

A gate delay of 300 ns for the first QCL and 200 ns for the second one was selected. For both lasers the pulse repetition rate was set to  $25\text{ }\mu\text{s}$  and the pulse length was adjusted to 50 ns.

The voltage output signal of the detector was split and processed by two box car averagers. The QC-laser signals were evaluated separately, setting the gates of each box car averager on one laser pulse. The averaged output signal was connected to an AD-Converter. The digital signal was processed via LabVIEW SignalExpress.

The use of a dual QC-laser system leads to more selective and robust measurement systems as more structural information on the investigated system is obtained. To show the usefulness of the proposed detection system, we employed it for the analysis of eight different components of wine and grape juice samples. On-line dual QC laser detection in HPLC provides detection limits, for example of  $0.19\text{ mg/mL}$  for acetic acid, successfully showing detection in the low  $\text{mg/mL}$  region for the first time.

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## **P031-B1**

### **Analysis of Anthocyanins by Desorption Electrospray Ionization and Matrix-Assisted Laser Desorption/Ionization**

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Anthocyanins are natural pigments. They cover a colour spectrum from orange to blue. They occur in hay, flowers, roots, stems and leaves. They give colour to parts of plants. Anthocyanins have antioxidant function, demonstrate antibacterial effects, etc. They are studied using many analytical techniques including mass spectrometry.

In this case, anthocyanins were analyzed in red wines by two ion sources - desorption electrospray ionization (DESI) and matrix-assisted laser desorption/ionization (MALDI). Six kinds of wine, two vintages of three cultivars, were measured.

DESI is very fast and simple technique for solid sample and surface analysis. Experiments were performed by an ion trap mass spectrometer (Thermo Finnigan, San Jose, USA) without any sample purification. Composition of spraying liquid was essential for successful analysis and after optimization mixture of methanol : water 75 : 25 (v/v) with 0.2% HCOOH was used.

MALDI is also suitable for identification of anthocyanins. Experiments were performed by a Q-TOF Premier mass spectrometer (Waters, Milford, USA). The wine samples were acidified by formic acid. Four matrixes (□-cyano-4-hydroxycinnamic acid, 2,4,6-trihydroxyacetophenone, □-indoleacrylic acid and 2,5-dihydroxybenzoic acid) were tested. 2,4,6-trihydroxyacetophenone gave the best sensitivity and the lowest chemical noise.

It was demonstrated that the wine samples differ in type of anthocyanins and/or in their amount. These differences can be used in wine characterization. Usefulness of DESI in surface analysis was evident from identification of anthocyanins in berries of wine, elder and crane black as well as identification of a kind of wine in a spot on cotton fabric. Further, MALDI Q-TOF is useful for analysis of analyte of low molecular weight, high resolution and mass accuracy support positive identification. Both studied techniques are promising for fast wine analysis.

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## P032-B1

### Analytical Method for the Determination of Some of the Most Studied Pharmaceutically Active Compounds as Wastewater and Surface Water Pollutants

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Tons of pharmaceutically active compounds are yearly consumed all over the world, discharged to the sewer systems and, afterwards, continuously discharged to the aquatic environment [1]. In the last years, the presence of pharmaceutically active compounds in wastewater and superficial waters has been described in several developed countries [2]; however, scarce information about their presence in other geographic localizations has been found in literature. This fact could be due to the complexity of the methods reported for the determination of these pollutants in aqueous environmental samples. Most of them are based on chromatographic determination with mass spectrometry detectors. These methods are advantageous in terms of rapidity, sensitivity and selectivity but present several disadvantages as their high cost and the necessity of high qualified operators. As consequence, they are not available in developing countries with emerging and developing economies where detection of these compounds is expected to occur.

In this work an analytical method for the simultaneous determination of seventeen pharmaceutical active compounds in wastewater and superficial waters has been developed using a conventional high performance liquid chromatography with diode array and fluorescence detectors. Pharmaceutical compounds selected were six anti-inflammatory drugs, a psychoestimulant, two antibiotics, two lipid regulators, an antiepileptic drug, a  $\beta$ -blocker and four hormones. Sample treatment is based on extraction and preconcentration by solid-phase extraction. Recoveries were in the range 61-116%. Chromatographic separation was carried out on a Zorbax Eclipse XDB-C18 (150 mm x 4.6 mm, 5  $\mu$ m) column by gradient elution with acetonitrile and a 25 mM potassium dihydrogen phosphate solution. Limits of detection were ranged between 0.003-0.30  $\mu$ g L<sup>-1</sup> for ultraviolet detector and 0.001-0.137  $\mu$ g L<sup>-1</sup> for fluorescence detector.

The authors wish to thank the financial support of this work from the Ministerio de Educación y Ciencia, Spain (Project nº. CGL2007-62281/HID).

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## P033-B1

### Dispersive Solid Phase Extraction for In-Sorbent Surface Attenuated Total Reflection Infrared Detection

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The first combination of dispersive solid phase extraction (DSPE) and attenuated total reflection (ATR) infrared spectroscopy (FTIR) is presented as a useful tool for analytical purpose. In this case, DSPE is not used only for cleanup purposes but also for the preconcentration of analytes. The sorbent material is dispersed under continuous stirring in the sample allowing the retention of the analytes. Later on, the solid is washed, separated by centrifugation, and dried before the IR measurement. The IR spectrum is recorded on the surface of the solid sorbent not requiring a previous elution with an organic solvent. The proposal, which is schematically described in Fig 1, is presented using the joint determination of sorbic and benzoic acids in fruit juice as a model analytical problem.

The proposed approach has been qualitative and quantitative studied. The hierarchical cluster analysis permits the sample classification according to the relative concentration of the analytes. The precision of the quantitative analysis was better than 6% with analyte recoveries in the range 87-90%. The main advantages and limitations of the new proposal are presented and compared with existing alternatives such as conventional solid phase extraction (SPE). According to the experimental results, the dispersion procedure enhances the extraction of the analytes and therefore the sensitivity of the IR detection since the interaction between the analytes and the sorbent is favored. Although the selectivity of the proposed configuration is lower than that of chromatographic methods or mass spectrometric detection, the binomial DSPE-ATR-FTIR can drive to faster and cheaper analytical determinations.

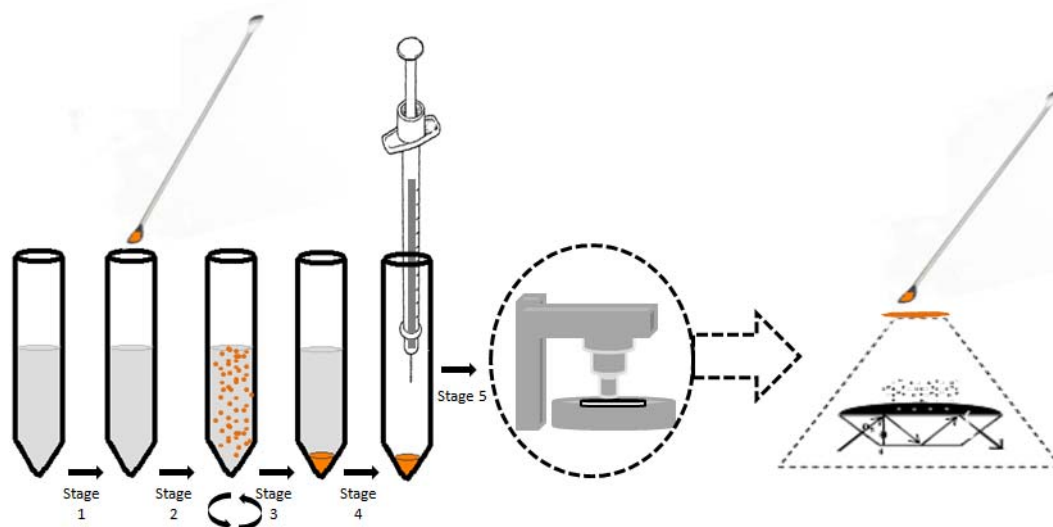


Fig. 1. Schematic procedure of the DSPE-ATR-FTIR combination. Stages: 1) Sorbent addition; 2) Stirring/dispersion; 3) Centrifugation; 4) Phase's separation and 5) In sorbent surface monitoring.

## P034-B1

### FIA Potentiometric System Based on Periodate Polymeric Membrane Sensors for the Assessment of Ascorbic Acid in Commercial Drinks

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Ascorbic acid is consumed worldwide on a large scale as an antioxidant agent in food and beverages and in medicines. It has been used for long at the prevention and treatment of common cold and mental illness [1]. Different analytical methods have been employed to determine ascorbic acid in pharmaceuticals, foods and biological fluids. As a new alternative method, the present work proposes development a new periodate tubular electrode based on tetraphenylporphyrin iron (III) chloride [ $\text{Fe}^{\text{III}}$ -TPPCI] and its use for the flow-injection determination of ascorbic acid in food samples. The sensor that was added of tetraoctylammonium bromide (TOAB) displayed the best analytical behavior in terms of slope ( $73.9 \text{ mV decade}^{-1}$ ) and detection limit ( $0.5 \mu\text{g ml}^{-1}$ ). The potential was unaffected by pH within 2-7 and the electrodes showed good selectivity over many common organic and inorganic anions. The general analytical features for ascorbic acid readings under hydrodynamic mode of operation pointed out a linear response of potential versus concentration. The dynamic linear range was from  $0.8\text{-}16 \mu\text{g ml}^{-1}$ , with a slope of  $2.12 \text{ mV } (\mu\text{g ml}^{-1})$  ( $r^2= 0.995$ ) and a sampling rate of  $\sim 50$  sample  $\text{h}^{-1}$  (Fig. 5). The method was successfully applied to the analysis of commercial flavored-waters and soft drinks.

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## **P035-B1**

### **On-Line Analysis of Airborne Toxic Chemicals at Trace Levels Using Thermal Desorption and a New Time of Flight Mass Spectrometer**

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The acute toxicity of certain compounds (eg CWA) requires continuous on-line (air) monitoring at military and important civilian locations/buildings. The former is an occupational health requirement, the latter to monitor/respond to terrorist attack. A hyphenated TD-GC-TOF system is described comprising on-line, near real time (NRT) analysis of trace level (airborne) chemical warfare agents using a continuous sampling twin trap thermal desorption (TD) system (TT24-7<sup>tm</sup>) and a new bench top Time of Flight (TOF) mass spectrometer (BenchTOF-dx<sup>tm</sup>).

Pre-concentrated air samples are desorbed into a GC for separation, and analytes are detected using a new reflectron TOF detector. Compound spectra are evaluated using a deconvolution/chemometric based software to identify target compounds within the sample.

The analytical system will be described with relevant performance characteristics and its application to either fixed or mobile laboratory reviewed.

For proof of performance/concept thermal desorption sample tubes containing Tenax TA sorbent were spiked with the CW simulants methyl salicylate (MS), tributyl phthalate (TBP), and triethylphthalate (TEP). After tube desorption and GC-TOF analysis the data was processed using TargetView software. This is based on a multivariate data analysis (PCA) program of deconvoluted spectra and ultimately pattern recognition for compound identification.

The high (full scan) sensitivity of the TOF make it an ideal detector for trace level analysis especially in combination with sample preconcentration, and the fast spectral acquisition complimentary to the deconvolution/chemometric approach and the requirement for NRT analysis. The analysis of tube based CW simulant samples enables characterization of the online continuous sampling system.



## **P036-B1**

### **Determination of BTEX by Dynamic Headspace–Gas Chromatography–Mass Spectrometry**

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Benzene, toluene, ethylbenzene and xylenes are usually referred to by the acronym BTEX. They are frequently used compounds in the chemical industry.

BTEX are common contaminants of soil and groundwater. This kind of pollution typically occurs near petroleum and natural gas production sites, petrol stations and other areas with storage tanks containing gasoline or other petroleum-related products. 'Total BTEX' is the sum of the concentrations of BTEX compounds. It can be used to assess the relative risk and seriousness of contamination.

Headspace–gas chromatography–mass spectrometry is a well established technique in the determination of BTEX in water and soil. Lowering detection limit is ever existing challenge. In case of volatile compounds dynamic headspace (DHS) is the most straightforward technique to meet this challenge. The samples are placed in standard headspace vials. The headspace of the heated and agitated sample is purged with inert gas, e.g. helium. Analytes are swept from the sample headspace and concentrated on a sorbent bed filled in a glass tube. After this the tube is removed and placed in a thermal desorption unit. The thermal desorption unit is coupled to the programmable temperature vaporizing inlet of the gas chromatograph where the target compounds are retrapped. The thus focused compounds are transferred to the column by a thermal shock (720 °C/min). The analytes are identified and quantified by selected ion monitoring (SIM).

Exploiting the capabilities of automatizable DHS in achieving lower limits of detection and quantification results in an outstandingly effective and modern analytical method.

## **P037-B1**

### **Chemosorption of Various Substances on Polyurethane Foam and Potentials of its Application in Analysis**

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Analytical potential of polyurethane foam (PUF) functional groups has been systematically studied. Heterogeneous chemical reactions of terminal toluidine groups result in formation of intensely colored products. Those are diazotization with sodium nitrite, azo-coupling with 4-nitrophenyldiazonium tetrafluoroborate and diazotized aromatic amines, condensation with aromatic aldehydes and azo-coupling of diazotized PUF with different compounds. It has been shown that terminal toluidine groups have chemical properties similar to those of monomeric aromatic amines. The main factors influencing on chemosorption of the substances on PUF have been revealed. These are pH and composition of aqueous phase, phase contact time, structure of the PUF polymeric chain and concentrations of the reagents. The conditions of maximum yield of diazotized polyurethane, polymeric azo-compounds and Schiff bases have been determined. Using diffuse reflectance and IR spectroscopy, the new absorbance bands have been found and interpreted that proves the chemical modifying of PUF. Schemes of the chemosorption of different substances on PUF have been suggested. The area of application of PUFs as polymeric chromogenic reagents for diffuse reflectance spectroscopy and test-methods has been proved. The role of functional groups in such reagents is played by terminal toluidine groups or different groups of compounds covalently linked with PUF. It has been suggested to use office scanner, digital camera and computer data processing as an alternative approach to numerical evaluation of color intensity of the reaction products of unmodified as well as modified PUF.

The examples showing the advantages of PUF as a polymeric chromogenic reagent have been given. It has been shown that there is an opportunity of its application for determination of nitrites, phenols, aromatic amines and aldehydes. The metrological characteristics of the methods and the results of samples analysis have been estimated.

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## P038-B1

### Synthesis and Sorption Properties of Polymers with Molecular Imprints of Chlorine-Containing Pesticides

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In recent years, molecularly imprinted polymers (MIPs) have been used increasingly extensively for the selective separation of organic compounds by solid-phase extraction. MIPs are largely synthesized by the noncovalent imprinting method. The synthesis scheme includes several stages, such as the formation of an intermolecular prepolymerization complex between functional monomer (FM) and template (T) molecules, the polymerization of this complex in the presence of large amounts of a cross-linking agent providing the production of a polymer with a rigid structure, polymer grinding and sieving to obtain particles with the desired size, and multiple washing by organic solvents to remove the template from the polymer.

In this work, by the noncovalent imprinting technique, we synthesized new MIPs with molecular imprints of 2,4-dichlorophenoxyacetic acid (2,4-D), 3,6-dichloro-2-methoxybenzoic acid (3,6-DMB, dicamba) and (RS)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol (RS). The synthesis was performed by the technique of bulk radical polymerization. Acrylamide was used as a functional monomer, and ethylene glycol dimethacrylate as a cross-linking agent. Polymerization was initiated by 2,2'-azobisisobutyronitrile. The goal of this work was to study the factors varied at the stage of MIP synthesis (the FM: T ratio in the prepolymerization mixture, the nature of template and solvent molecules) and influencing the specific surface area of polymers and their sorption properties. The sorption isotherms were plotted and the values of imprinting factors (IF) were calculated for the chlorine-containing pesticides in order to compare their binding properties.

It was found that specific surface area and sorption characteristics of the polymers with molecular imprints of chlorine-containing pesticides depended on the nature of template molecules, functional monomer: template ratio in the polymerization mixture, and nature and content of solvents varied at the synthesis stage. The difference in the sorption behavior of molecularly imprinted and reference polymers was observed over a wide range of chlorine-containing pesticide concentrations. The selectivity of the adsorbent with 2,4-D imprints was estimated for the example of structurally related compounds (3,4-dichlorophenoxyacetic acid, 2,4-dichlorophenol, 2-, 3- and 4-chlorophenols). The synthesized polymers were used for solid-phase extraction of 2,4-D, dicamba and 2,4-dichlorophenol.

## P039-B1

### Preconcentration and Determination of Cu(II) in Natural Water Sample by a Modified Silica Gel as a Solid Phase Extraction Adsorbent

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Due to low concentration in which, usually, some metal species are found in the environment, the quantification procedure may involve multiple steps or the use of sophisticated techniques. In this context, in this work a silica gel matrix was modified through the attachment of the 4-amino-2-mercaptopyrimidine molecule ligand, which was used in the sorption and preconcentration of Cu (II) in aqueous medium. The characterization of the product obtained, Si-AMP, was done by the Fourier Transform Infrared Spectroscopy (FT-IR), Nuclear Magnetic Resonance (NMR), measurement of specific surface area and nitrogen elemental analysis. The FT-IR spectra presented bands in 3347  $\text{cm}^{-1}$  and 1541  $\text{cm}^{-1}$  region, which were attributed to amino groups vibrations in the ligand molecule and the NMR spectra of the  $^{13}\text{C}$  and  $^{29}\text{Si}$  obtained between 0 and 50 ppm and -65,560 ppm respectively, confirmed that the organic group is covalently attached to the silica matrix surface. The anchoring of the ligand in the matrix also resulted in changes in area of the material surface, which had reduced of 331,70 to 289,74  $\text{m}^2\text{g}^{-1}$ . The elemental analysis indicated the presence of 0,245 mmols of ligand per gram of Si-AMP. The functionalized material was applied to sorption experiments by the batch method and the sorption isotherms were adjusted to the modified Langmuir equation and the  $N_s$  values (maximum sorption capacity) was 0,447  $\text{mmol g}^{-1}$  for Cu (II). The preconcentration experiments, using a mini-column packed with 5mg of Si-AMP, showed an enrichment factor of 20 times, which is in agreement with the results obtained by natural water sample and SRM material. The metal quantification was performed by atomic absorption spectrometry using flame and electrothermal atomization in graphite furnace.

## P040-B1

### Hybrid Organic-Mineral Adsorbents for Selective Preconcentration of Organic Pollutants

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The estimation of environmental pollution monitoring demands selective and sensitive analytical methods for determination of organic pollutants. The content of organic pollutants is often lower than the response limit of modern analytical devices. Therefore pre-concentration step is needed for determination of organic pollutants. Existing pre-concentration cartridges containing polymeric, carbonic materials and organic polymeric composites are not selective at the extraction and enrichment step. Hybrid organic-mineral adsorbents (HAs) can be alternative to existing materials such they can display selectivity to definite classes of dangerous pollutants. It was proposed three approaches of preparation such HAs for analysis of phenolic compounds (their ability to react with aryldiazonium salts producing intensively colored products was used), pollutants with acidic nature (their ability to form ionic associates with cationic surfactants was used), chemical inert pollutant containing nitro groups (their ability to form charge transfer complexes was used). Proposed principles were realized at the preparation of these HAs for pre-concentration and quantitative determination of organic pollutants such as phenolic compounds, their nitro-derivatives and chlorophenoxyacetic acids. In this report the results of using of HAs is represented for analysis of 2,4-dichlorophenoxyacetic acids, phenol, 1-naphtole and picric acid. Three types of HAs were proposed: silica with immobilized *m*-aminophenylarsonic acid (type 1), silica with immobilized polyethoxylated isoctylphenolic groups (type 2), silica with immobilized 2,3,5-triphenyltetrazole (type 3). All the proposed approaches for determination of phenolic compounds allow to carry out visual monitoring of polluted water at their trace levels. Application of diffuse reflectance spectroscopy and RP HPLC allows determining dangerous pollutants at their micro- and nano-quantitation levels.

## P041-B1

### Selenium and Arsenic Determinations via Hydride Generation Headspace Single Drop Microextraction (HS-SDME) Electrothermal Atomic Absorption Spectrometry

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In this study, headspace single drop microextraction (HS-SDME) method in combination with electrothermal atomic absorption spectrometry (ETAAS) with Zeeman-effect background correction was validated for separate determinations of selenium and arsenic in water samples.

Arsenic and selenium species were converted to arsine and selenide in the presence of NaBH<sub>4</sub> in a closed 20 mL headspace vial and trapped onto 4 µL drop of trapping agent in the tip of a microsyringe. Pd (II) for Se and APDC for As were selected as the acceptor phases. When the equilibrium was reached between the arsine (or selenide) in the headspace and microdrop suspended above the 10 mL solution in the closed HS vial, the drop was retracted back into the syringe and injected manually into graphite furnace for the determination.

The analytical parameters of the method such as microextraction time, sampling temperature, NaBH<sub>4</sub> concentration, trapping agent concentration, pH of the medium, possible interference effects, analytical figures of merit have been investigated. Precision of the method in terms of inter-batch and intra-batch repeatability has been evaluated and method has been validated by analyzing certified reference materials (CRM) such as LGC 6011.

**Keywords:** ETAAS, SDME, As, Se, HS-SDME

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## P042-B1

### Optimization of Programmed-Temperature Vaporizer-Based Large Volume Injection GC/MS/MS for the Determination of Short Chain Chlorinated Paraffins at Trace Level

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GC is still the method of choice for the analysis of complex mixtures of halogenated pollutants for its unsurpassed resolution, its widespread coupling to mass spectrometry, and its solvent-free functioning. In a typical GC analysis, injection of 1-2  $\mu\text{L}$  results in most of the sample being vented. This is yet a significant shortcoming in environmental trace analysis. Injection of larger volumes of sample onto a GC column allows either to enhance the sensitivity or to reduce the sample handling by leaving out the final extract concentration step.

Though programmed-temperature vaporizer (PTV) has been used for over two decades and at an early stage was recognized as a potential large volume injector, the interest in development and applications has only recently arisen.

Typically, the solvent is introduced into the 'cold' PTV injector, which is set at a temperature just below the solvent boiling point. Then, the solvent is removed by heating to above the boiling point and evaporating the solvent by a split flow through the opened split valve. Finally, the split valve is closed (splitless mode) and by rapidly raising the injector temperature, the target pollutants are transferred into the analytical column.

Gas chromatography coupled to tandem mass spectrometry and PTV-based large volume injection (PTV-LVI) were used to increase sensitivity for the analysis of Short Chain Chlorinated Paraffins (SCCPs), a group of emerging halogenated analytes. This talk will present a practical optimization of the PTV-LVI injection settings, that is, vaporizing temperature and time, and purge flow. The suitability of packed and empty liners was also evaluated. The enhancement in sensitivity achieved as compared with that obtained with the conventional 1  $\mu\text{L}$  splitless injection will be highlighted. Furthermore, the presentation will demonstrate the gain of the PTV-LVI GC/MS/MS analysis for compliance checking according to the Water Framework Directive.

## P043-B1

### Liquid Phase Microextraction Combined with Modified Graphite Furnace Atomic Absorption Spectrometry Very Simple, Rapid and Sensitive Method for the Determination of Thallium in Water

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*Summary* – The proposed method is a simple process for the determination of trace amount of thallium in the water samples. The liquid phase microextraction (LPME) was combined with the modified Graphite furnace atomic absorption spectrometry (GF-AAS) for determination of thallium in the water and solid samples. In a preconcentration step, thallium was extracted from a 2 ml of its aqueous sample in the pH =4.5 as thallium-Rhodamine B complex into a 4 µl drop of benzyl alcohol drop of immersed in the solution. After extraction, the micro drop was retracted and directly transferred into a graphite tube modified by [W.Pd.Mg](c). Some effective parameters on extraction and complex formation, such as type and volume of organic solvent, pH, concentration of chelating agent, extraction time, stirring rate and effect of salt were optimized. Under the optimum conditions, the enrichment factor and recovery were 585 and 96%, respectively. The calibration graph was linear in the range of 0.03-13 µg L<sup>-1</sup> with correlation coefficient of 0.9986 under the optimum conditions of the recommended procedure. The detection limit based on the 3S<sub>b</sub> criterion was 0.0064 µg L<sup>-1</sup> and relative standard deviation for (RSD) for eight replicate measurement of 0.1 µg L<sup>-1</sup> and 0.4 µg L<sup>-1</sup> thallium was 4.1 and 3.4% respectively. The characteristic concentration was 0.0042 µg L<sup>-1</sup> equivalent to a characteristic mass of 24 fg. The results for determination of thallium in reference materials, spiked tap water and seawater demonstrated the accuracy, recovery and applicability of the presented method.



## P044-B1

### Optimization and Validation of Amoxicillin Analysis in Waters by Direct Injection-Liquid Chromatography-Tandem Mass Spectrometry

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Pharmaceuticals compounds, including antibiotics, have recently captivated the attention of the scientific community. Recent studies have shown that antibiotics residues are present at ng to  $\mu\text{g.L}^{-1}$  levels in several matrices (surface and drinking waters, groundwaters, wastewaters, soils and sediments). These compounds and their metabolites are introduced into watercourses mainly through excreta, waste effluents of manufacturing processes and discharges from wastewater treatment plants (WWTPs) [1]. Although present at trace levels, antibiotics may promote adverse effects on aquatic and terrestrial ecosystems due to their persistence and bioaccumulation and their ability to cause resistance amongst natural bacterial populations.

A high percentage of antibiotics is excreted unchanged. This is the case of amoxicillin, one of the most prescribed  $\beta$ -lactam antibiotics in Europe and in the USA, from which 80-90% is excreted unmodified [2]. Currently, legal limits for antibiotics in environmental waters have not been established.

In accordance with above concerns, the aim of this work was to develop, optimize and validate a LC-MS/MS analytical method for the determination of amoxicillin in water matrices. The analyses were performed using a Varian 500-MS LC Ion Trap Mass Spectrometer equipped with an electrospray ionization source by direct injection and positive ionization mode. The first step was the optimization of LC-MS conditions (mass, ion source and chromatographic parameters) and after the method was validated. For this purpose, linearity ( $10\text{-}1000 \mu\text{g.L}^{-1}$ ), limit of quantification ( $6 \mu\text{g.L}^{-1}$ , determined from  $S/N=10$ ), precision and accuracy were evaluated. Because real samples (tap and river waters) were used, it was necessary to carry out a study about the possible matrix effects. This was verified in tap water samples, and attending the relatively low recoveries achieved ( $\approx 10\%$ ), matrix-matched calibration was applied. In this study, the global uncertainty was also determined according to EURACHEM (5%).

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## **P045-B1**

### **Programmed Temperature Vaporizer Based Method for the Sensitive Determination of Trihalomethanes and BTEX in Soils**

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A methodology based on the coupling of a headspace autosampler with a GC and a MS detector operating in SIM mode has been developed for the determination of volatile organic compounds (THMs and BTEX) in soils. The GC device used is equipped with a programmable temperature vaporizer (PTV) packed with Tenax-TA<sup>®</sup> to introduce the samples (the injection mode used was solvent vent), and a modular accelerated column heater (MACH<sup>™</sup>) to control column temperature. The proposed measurement procedure reduces the sample pretreatment step to a minimum. Combined use of solvent vent injection mode and mass spectrometry detection allows a highly sensitive method to be proposed, with limits of detection of the order of ng/kg for all the target compounds. Furthermore, the capillary column used allows rapid separations of compounds in less than 4.60 min, affording a very short total analysis cycle time of 9 min.

## P046-B1

### In-Tube Derivatization and SBSE-TD-GC-MS for the Determination of Endocrine Disruptor Compounds in Water Samples. Optimisation and Validation

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Endocrine disruptor compounds (EDCs) such as nonylphenols (NPs), octylphenols (OPs), BPA and steroid hormones have become emerging contaminants due to their presence in environmental waters and the concern about their effects [1]. The inclusion of such compounds in legislations such as the Water Framework directive requires the development of robust analytical procedures to determine these compounds.

Preconcentration is necessary for the determination of EDCs in water samples. Due to the importance that miniaturisation has reached in the analytical chemistry field, extraction techniques such as Stir Bar Sorptive Extraction (SBSE) have attained great importance [2] since this method requires no solvent and minimises analysis time and improves the sensitivity of the global analytical method [3]. SBSE can be coupled to thermal desorption (TD) when coupled to gas chromatography (TD-GC). However, compounds such as pharmaceuticals, hormones or EDCs are highly polar and are not best chromatographed by GC. In this case, a derivatization step is necessary and, in some cases, this derivatization can occur in-tube in the TD unit [4].

In this study, a simple and automated method was developed for the determination of some EDCs in water (4-tert-octylphenol, n-nonylphenol, 4-octylphenol, bisphenol A, diethylstilbestrol, cis-androsterone, estrone, equilin, 17 $\beta$ -estradiol, testosterone, equilenin, mestranol, 19-norethisterone, 17 $\alpha$ -ethynyl estradiol, progesterone, estriol, coprostanol, cholesterol and stigmastanol). Best extraction conditions (360 min extraction time, 20% NaCl, pH=7.5 and without the addition of methanol) were fitted by means of experimental designs. Derivatization and thermal desorption conditions were simultaneously studied using BSTFA as derivatization reagent. Optimum working conditions were fixed [desorption temperature (300 °C), desorption flow (60 mL/min) and BSTFA volume (2  $\mu$ l)]. Under the optimized experimental conditions, the method showed good linearity, sensitivity and repeatability. Finally, the proposed method was successfully applied to the determination of the target compounds in real water samples from a wastewater treatment plant.

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## P047-B1

### New Analytical Strategies for the Determination of Disinfection By-Products in Drinking Water

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In the last few years, chlorine, ozone, chlorine dioxide, and chloramines are the most common chemical disinfectants used to kill harmful microorganisms in water systems. Although the effectiveness demonstrated, this practice has the consequence of the formation of the undesirable chemical disinfection by-products (DBPs), which are produced when disinfectants react with natural organic matter. Among these hazard compounds, carbonyls compounds such as aldehydes and ketones have been recently attracting much attention because of their adverse health effect. However, the maximum contaminant levels for these particular DBPs in drinking water are still not applicable by the International Advisory Committees. These compounds are also acknowledged to be harmful organic pollutants that exist in the atmosphere as a result of discharged from other sources, mainly exhaust gases from motor vehicles, biomass burning, among others.

In this contribution, a novel enrichment technique with *in-situ* derivatization, using pentafluorophenyl hydrazine as derivatising agent, followed by liquid desorption and high performance liquid chromatography with diode array detection (HPLC-DAD) was applied to monitor six short chain carbonyl compounds (formaldehyde, acetaldehyde, propanal, acetone, butanone and 2-hexenal) in drinking water matrices. Assays performed on 30 mL water samples spiked at the  $25.0 \mu\text{gL}^{-1}$  level under optimized experimental conditions yielded recoveries up to  $85.2 \pm 3.8 \%$ . The analytical performance showed good precision (RSD < 13 %), detection limits in between 42 and  $137 \text{ ngL}^{-1}$  and remarkable linear dynamic ranges ( $r^2 > 0.990$ ) from 1.0 to  $80.0 \mu\text{gL}^{-1}$ . By using the standard addition methodology, the application of the present analytical approach to drinking water samples treated with different chemical disinfectants, namely, chloride, ozone and both of disinfectant, allowed very good performance at the trace level.

## P048-B1

### Optimization of Pesticides Extraction from Pine-Needles

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Nature is a key element in the identification of the extent of damage it has been enduring mainly under anthropogenic influence. In fact, several forms of vegetation can act as natural monitors for the occurrence of a wide range of contaminants in the environment and this advantage has been profited and reported since the 1960s [1]. The worldwide presence of some species allows not only a very representative estimation but also comparative data in order to establish bioaccumulation or travelling patterns between different locations.

Pine trees can act as biomonitors for some persistent pollutants such as pesticides, mainly through the retention properties exhibited by the waxy layer of their needles [2]. However, the same properties which allow the capture of such pollutants can also originate difficulties in the extraction and clean-up procedures needed to separate them. Hence, many approaches are continuously being attempted in search of faster, cleaner and reliable analytical methodologies.

The objective of the current work is to test the efficiency of several solid-phase extraction (SPE) clean-up approaches (ENVI 18 and Florisil cartridges and QuEChERS), following ultrasonic extraction (USE) for the determination of 20 pesticides (alachlor, ametryn, atrazine, azinphos-ethyl, azinphos-methyl, chlorpyrifos, diazinon, malathion, metolachlor, molinate, parathion-ethyl, parathion-methyl, pendimethalin, pirimicarb, prometryn, propazine, simazine, terbutylazine, terbutryn, trifluralin) in *Pinus pinea* needles.

One sample mass (5 g) and two pesticides spiking levels (20 ng/g and 100 ng/g) were used for comparison in terms of validation parameters (detection limits, precision, recovery). The levels of pesticides were also assessed in some naturally contaminated needle samples. Analysis and quantification is performed by gas chromatography–mass spectrometry (GC-MS) in Selected Ion Storage (SIS) mode, using triphenyl phosphate (TPP) as internal standard.

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## P049-B1

### **Membrane Probe for Sampling Preparation of Volatile Compounds for HPLC-UV Analysis. Application to the Determination of Ammonia and Aliphatic Amines in Environmental Aqueous Samples**

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In this work an innovative sampling preparation approach for the extraction of volatile compounds was developed. The extraction step is based on a membrane probe and is followed by HPLC-UV analysis of the extracts. The probe consists in a homemade Teflon module of our own design, it has at its bottom a microporous hydrophobic membrane (PTFE) that avoids the diffusion of the aqueous solvent but allows the mass transfer of volatile compounds. Inside the probe a small volume of an acceptor solution is placed. There are several parameters that influence the extraction process, such as the temperature and the time of extraction, type of acceptor solution and others. At the end of extraction procedure the acceptor solution is collected and analyzed.

The probe was used for the determination by HPLC-UV of ammonia and aliphatic amines. The determination of such compounds by HPLC-UV can be achieved through a reaction of derivatization with phenyl isothiocyanate (PTIC), producing phenylthioureas, [1]. Thus, a solution of PITC 1% in acetonitrile with 0,1M carbonate buffer (1:5) is placed inside the probe. The probe is immersed in a 25 mL sample under agitation at a selected temperature and extraction time. After this period of time the acceptor solution, containing the extracted and derivatized amines, is collected and injected in the chromatographic system.

Since it is necessary, for the probe, a membrane with small dimensions, it can be economically throwaway and replaced by a new one with similar characteristics. Therefore, it is easily overcome the membrane fouling problem. The use of the probe for sample preparation is quite simply and versatile, since it can be used with a larger number of compounds and several separation/detection techniques. In fact this work it is a sequence of previous work with the probe such as the determination of vicinal diketones in beer [2].

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## P050-B1

### Concentration and Determination of U(VI) Ions with Polymer Sorbent

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Control of the contents of radioactive metals in environment object is an important ecological problem. So, the need in development of the new ways of concentration of radioactive metal with the following determination by different methods appears.

In the presented work has been synthesized a new chelatoformed sorbent on the basis of styrene-maleic anhydride copolymer, containing fragment as 4-nitroanilin-2-arsenic acid as functional complexformation groups. The composition of the new sorbent has been studied by method of IR-spectroscopy. To determine the constant of ionization of sorbent we studied its total static sorption capacity on  $K^+$  ions ( $SSC_{K^+} = 11,8 \text{ mmol/g}$ ) and the potentiometric titration has been determined by known technique.

In static capacity there was explored the influence of pH and ionic force of fluid phase on sorption U(VI) ions by synthesized sorbent. Dependency the sorption processes on concentration of uranium (VI) and time has been investigated (tabl.).

**Tabl. Optimal conditions of concentration of uranium (VI) ions by polymer sorbent in static conditions**

SC, mg/g	pH <sub>opt</sub>	$\mu^*$ , mol/l	Time, hours	d, mm
413	4	0,6	3,0	0,14

\*- ionic force influencing on sorption

There was investigated influence of the alike volumes and concentration of  $\text{Щ}^{\text{Б}}\text{ЛQ}_4$ ,  $\text{Щ}_2\text{CO}_4$ ,  $\text{ЩHO}_3$ ,  $\text{Щ}^{\text{Б}}\text{J}$  acids on desorbition of sorbent ions of uranium (VI) from polymer sorbent. Experiment has shown, that maximal desorbition of uranium (VI) occurs in perchlorate acid.

The desorption also has been investigated at dynamic conditions. The rate of introduction of an acid and the influence of the concentration has been studied. The optimal conditions of concentration of U(VI) ions by polymer sorbent has been established. The experiment shows that at optimal conditions the concentration of U(VI) ions quantitatively absorbs and desorbs ( $R > 95\%$ )

## P051-B1

### Automatic Flow-Based Methods for Sequential BCR Extraction of Trace Metals in Fly Ash

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Two novel dynamic extraction approaches, the so-called sequential injection microcolumn extraction (SI-MCE) [1] and sequential injection stirred-flow chamber extraction (SI-SFCE) [2] based on the implementation of a sample-containing container as external extraction reactor in a sequential injection network, are for the first time, optimized and critically appraised for fractionation assays. The three steps of the original BCR sequential extraction scheme [3] have been performed in both automated dynamic fractionation systems to evaluate the extractability of Cr, Cu, Ni, Pb and Zn in a standard reference material of coal fly ash (NIST 1633b). In order to find the experimental conditions with the greatest influence on metal leachability in dynamic BCR fractionation, a full factorial design was applied, in which the solid sample weight (100-500 mg) and the extraction flow rate (3.0-6.0 mL min<sup>-1</sup>) were selected as experimental factors. Identical cumulative extractabilities were found in both sequential injection (SI)-based methods for most of assayed trace elements regardless of the extraction conditions selected, revealing that both dynamic fractionation systems, as opposed to conventional steady-state BCR extraction, are not operationally defined within the selected range of experimental conditions. Besides, the proposed automated SI assemblies offer a significant saving of operational time with respect to classical BCR test, that is, 3.3 h versus 48 h, for complete fractionation with minimum analyst involvement.

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## **P052-B1**

### **Development of “Sediment Washing” by Natural Organic Substances of Dredged Sediments of the Venice Lagoon, Italy**

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The contamination of sediments in coastal areas and harbours is due to a wide range of organic pollutants and trace elements; in these areas sediments may be a significant sink and/or source of these pollutants. Remediation and environmental recovery of sediments are extremely important in harbour areas, considered the need to dredge sediments in order to keep channels of navigation open. The main goal of this project is to assess a novel washing procedure for dredged sediments, that is environment friendly and suitable for the variety of pollutants, by exploiting the surface-active and complexing properties of natural organic substances. Dredged sediments from the industrial area of the Venice lagoon were analysed to evaluate the concentrations of POPs and the total concentrations of several trace elements (such as Cr, Zn, Cd, As, Hg, etc.). Furthermore, we used a modified sequential extraction procedure in order to evaluate the concentration of the chemical fractions: the exchangeable, the carbonate bound, the Fe and Mn oxides bound, the sulphur and organic matter bound, the residual bound. In the second phase of this study, the washing process was assessed; different parameters were considered (such as pH, sediments/ washing solution volume ratio, length of washing, etc.). All the batch experiments were run in duplicate, to test the homogeneity and the repeatability of the procedure, by using commercially available natural organic substances (Sigma). After being washed, sediments showed an average decrease in the concentrations of organic and inorganic pollutants (40% and 30% respectively). These results are very promising, due to the holistic approach used for the different classes of pollutants. This study underlines the importance of speciation, since, according to the most recent frameworks on risk assessment, it is essential to know the bioavailability and bioaccessibility of pollutants in order to plan the most suitable remediation project.

## **P053-B1**

### **Evaluation of Polycyclic Aromatic Hydrocarbon Profiles by GC-MS in Aerosol Samples of Extremadura, Spain, and Correlation with other Atmospheric Pollutants and Meteorological Parameters**

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A GC-MS methodology has been optimized for the evaluation of a set of 16 priority polycyclic aromatic hydrocarbons, including benzo-a-pyrene, in PM<sub>10</sub> ambient aerosol samples, obtained at low polluted urban and rural locations of Extremadura (Spain). PAHs were extracted, after high volume 24 hours sampling on 15 cm diameter quartz fiber filters, by reflux heating in HPLC grade toluene during 1h. After filtering and vacuum evaporation, the extract was concentrated to dryness under a gentle nitrogen stream. The final extract was redissolved in toluene and analyzed by gas chromatography-ion trap tandem mass spectrometry. Strict control of the experimental conditions during the sampling, pretreatment and determination steps was proven to be essential for obtaining reliable results at the low concentration ranges encountered for the selected analytes in most of the samples. Detection limits and method uncertainty were derived from repetitive blank measurements, as part of the data quality control procedure, and accuracy was tested by NCS ZC 78002 "PAH in coal fly ash" certified reference samples. The results obtained from a one-year (March 2008 – February 2009) sampling period showed very low PAH levels, with sampling location means ranging from 0.003 to 0.141 ng/m<sup>3</sup>. Benzo-a-pyrene mean values ranging from 0.008 to 0.082 ng/m<sup>3</sup> were well below the annual mean EU target value (1 ng/m<sup>3</sup> annual mean). Significant differences were however found between urban and rural locations. Also, a clear seasonal effect on PAHs was measured during the autumn – winter season with respect to spring – summer. Correlation of PAHs with relevant atmospheric pollutants (ozone, nitrogen dioxide) and meteorological parameters (temperature, solar radiation and humidity) are presented and discussed.

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## P054-B1

### Microwave-Assisted Extraction of Polycyclic Aromatic Hydrocarbons from Polyurethane Foam Adsorbents

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The presence and emission of polycyclic aromatic hydrocarbons (PAHs) to ambient air is of great concern in terms of human health, due to their carcinogenic and mutagenic potentialities. PAHs are distributed between gas and particle phases. Studies concerning sampling, extraction and analysis of particulate PAHs in atmosphere have been reported, however only few of them referred to the distribution of PAHs in the gaseous phase [1]. Polyurethane foam adsorbents (PUFs; 47 mm diameter and 75 mm length, Tecora) are appropriate for sampling the gaseous volatile PAHs (with two or three aromatic rings), due to high collection efficiency, chemical stability, low-cost, and easy handle, storage and transport. Compounds collected on PUFs are mainly extracted by soxhlet that requires large volumes of solvent and long extraction times. Volatile compounds, such as PAHs with low molecular weight, may be partially or totally lost in processes that use long extraction times compromising the analytical results and the adequate environmental and health evaluation. Microwave-assisted extraction (MAE) is a suitable alternative to reduce solvent consumption and time of extraction increasing, simultaneously, accuracy and reproducibility.

In this work, a previously developed methodology for MAE of particulate PAHs [2] was adapted and applied to volatile PAHs collected in PUFs. Comparison with the soxhlet procedure was performed. The overall average recoveries obtained using MAE and soxhlet for spiking levels ranging from 0.00129 to 0.322 µg/g were 77 ± 1% and 64 ± 5%, respectively.

#### Acknowledgements

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## P055-B1

### Gas-Particle Concentration and Distribution of Polycyclic Aromatic Hydrocarbons at an Urban Atmosphere

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Polycyclic aromatic hydrocarbons (PAHs) are harmful to human health because some of these compounds proved to be highly carcinogenic or mutagenic. Traffic emissions are considered one important source of human exposure to PAHs in urban ambiances. Atmospheric PAHs are partitioned between the particulate and the gaseous phases. This partitioning depends mainly on the vapor pressure, particle characteristics, ambient temperature and relative humidity [1]. Most of the studies reported in literature focus the PAHs levels in the particulate matter (PM). The adequate analysis of the PAHs levels in ambient air is achieved by monitoring simultaneously the gaseous and the particulate phases to evaluate the distribution of PAHs on both phases.

In this work, the traffic influence was assessed by the quantification of PAHs on PM and gaseous phases taken at an urban site (Oporto, Portugal). The collected phases were analyzed for 18 PAHs (16 regarded as priority pollutants by EPA, dibenzo(a,l)pyrene and benzo(j)fluoranthene) by microwave-assisted extraction and liquid chromatography with fluorescence detection [2]. The average of total PAHs concentration ( $\Sigma$ PAHs) measured on PM and gas phases were  $20.8 \pm 14.0$  and  $49.1 \pm 28.0$  ng/m<sup>3</sup>, respectively. These values indicated that the average contribution of PAHs in the gas phase to the total PAHs concentration was 70%. The PAHs with 2-4 rings (low molecular weight) were only present in the gas phase while the PAHs with more than 4 rings (high molecular weight) were mainly associated with particulate phase. Source identification based in diagnostic ratios [1] confirmed the strong influence of traffic emissions.

#### Acknowledgements

D.Castro thanks the FCT for her PhD fellowship (SFRH/BD/23605/2005).

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## **P056-B1**

### **Determination of Pesticides in Surface and Ground Waters by Liquid Chromatography-Electrospray-Tandem Mass Spectrometry**

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In this study, a rapid, sensitive and selective multiresidual method for determination and confirmation of pesticide residues in ground and surface waters was developed. Sixteen pesticides that belong to seven different chemical classes were selected for the analysis. The samples were prepared by solid-phase extraction (SPE), and extracts were analyzed by liquid chromatography–electrospray–tandem mass spectrometry (LC–ESI–MS/MS). The selected reaction monitoring (SRM) mode was used for quantification of all pesticides, using the most sensitive transition. Confirmation of residues detected in samples was performed by repeated injection and acquiring additional transitions to that used for quantification. The highest recoveries for all tested pesticides were achieved using OASIS HLB SPE cartridges and methanol-dichloromethane mixture (1:1) as the extraction solvent. The optimal pH-value of the water sample was 6, and the optimal volume was 250 cm<sup>3</sup>. Under optimized conditions good recoveries (65–120 %) and low limits of detection (0.032–5.5 ng dm<sup>-3</sup>) and quantification (0.108–18.2 ng dm<sup>-3</sup>) were achieved for all investigated pesticides. The results showed that developed and optimized analytical method was reliable for detection and confirmation of pesticide residues in water samples. The method was successfully applied to several ground and surface water samples.

## P057-B1

### Determination of Vanadium(V) in the Particulate Matter by Sequential Dissolution and Solid-Phase Extraction

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Vanadium exists in different oxidation states that modulate its toxicity.[1] The pentavalent form is the most stable and toxic form of the element and it is also the major species in air. The pentoxide ( $V_2O_5$ ) is the most common form while various vanadates ( $NaVO_3$ ,  $NH_4VO_3$ ,  $Na_3VO_4$ ) are present in lesser amounts from industrial activities. Inhalation is the prevalent route of human exposure in urban and occupational settings. A method based on selective sequential dissolutions and Solid Phase Extraction on Chelex 100 resin is proposed to determine total V(V) in particulate matter at low concentration values of about  $2 \text{ ng m}^{-3}$ . The vanadium concentration in each fraction is determined by ICP-OES. The described procedure has been tested on atmospheric particulate matter spiked with homogeneous dispersion of the different compounds of vanadium, and on two reference materials, Urban Particulate Matter NIST SRM 1648 and Fly Ash from pulverised coal BCR-038 before and after being spiked. The V(V) recovery is in the range 101% - 103%.

[1] WHO, 2001

## P058-B1

### A Novel Derivatizing Agent for Highly Polar Analytes: 5-Chloro-2,2,3,3,4,4,5,5-octafluoro-1-pentyl Chloroformate

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A novel derivatizing agent, 5-chloro-2,2,3,3,4,4,5,5-octafluoropentyl chloroformate (ClOFPCF), was purposely synthesized and tested as a reagent for direct water derivatization of highly polar and hydrophilic analytes. Its analytical performance was satisfactorily compared with a perfluorinated chloroformate previously described, namely 2,2,3,3,4,4,5,5-octafluoropentyl chloroformate (OFPCF). The chemical properties (reactivity, selectivity, derivatization products, and their chromatographic and spectral features) for ClOFPCF were investigated using a set of highly polar standard analytes, including hydroxylamine, malic and succinic acids, resorcinol, hydroxybenzaldehydes and dihydroxybenzoic acids. Upon derivatization, the analytes were extracted from the aqueous solvent and analyzed by gas chromatography (GC)-mass spectrometry (MS) in the electron capture negative ionization (ECNI) mode. Positive chemical ionization (PCI)-MS was used for confirming the molecular ion information, virtually absent in ECNI mass spectra. ClOFPCF showed good reaction efficiency, good chromatographic and spectroscopic properties (better than with OFPCF), good linearity in calibration curves, low detection limits (1-10 ng/ml). A unique feature of both ClOFPCF and OFPCF derivatization is its effectiveness in reacting with carboxylic, hydroxylic, and aminic groups at once, releasing multiply-substituted derivatives that can be easily extracted and determined by GC-(ECNI)-MS. The entire procedure from raw aqueous sample to ready-to-inject hexane solution of the derivatives requires less than ten min. Another positive aspect of this procedure is that it produces stable derivatives, with optimal volatility for GC separation, and high electron-affinity, which allows their detection as negative ions at trace level. In addition in ClOFPCF derivatives mass spectra, the presence of chlorine isotopic pattern clearly indicates how many polar hydrogen of the analyte undergo derivatization. Lastly, the derivatization with ClOFPCF was tested on ozonated fulvic and humic acids aqueous solutions and real ozonated water samples leading to the identification of twelve unknown highly polar disinfection by-product.

## P059-B1

### AGNES Technique for Zn<sup>2+</sup> Determination in River Waters

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The recent AGNES technique [1] (Absence of Gradients and Nernstian Equilibrium Stripping) was applied to river waters for the determination of free zinc concentration.

Since zinc is both a toxic and an essential trace metal, its speciation in natural samples is of fundamental importance in order to understand its interactions with the environment and to evaluate its toxicity. Many techniques have been developed for speciation: spectroscopy, voltammetry, potentiometry, etc.; AGNES can be complementary or an alternative to these techniques. AGNES had been previously applied for free Zn determination in synthetic solutions [2] and different real samples such as sea water [3] and wines [4]. Here river water was analysed in order to know AGNES behaviour in low ionic strength media.

At first, a calibration was performed in KNO<sub>3</sub> solutions from 0.1 to 0.001 M. With supporting electrolyte KNO<sub>3</sub> 0.001 M, there was a deviation in the calibration plot from linearity for increasing Zn concentrations (larger than 4E-6 M) due to the electroneutrality limitation.

Then, Zn speciation was performed in synthetic solutions at low ionic strength by titration with pyridinedicarboxylic acid (PDCA). All the measurements were well reproducible and comparable with the theoretical curves obtained with MEDUSA program [5].

Because of the complexity of natural samples, AGNES was firstly applied to synthetic river water [6]. Finally, samples collected from Ebro River were analysed. In order to maintain the CO<sub>2</sub> concentration (responsible of the pH control) and, thus, the water equilibria, the solution was purged with a mixture of N<sub>2</sub>/CO<sub>2</sub>.

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## P060-B1

### HILIC-MS/MS for the Simultaneous Determination of Drugs of Abuse and Metabolites in Wastewater

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We developed and validated a method based on hydrophilic interaction liquid chromatography (HILIC) with tandem mass spectrometry (MS/MS) for the determination of nine drugs of abuse (DOAs) and metabolites (cocaine, benzoylecgonine, ecgonine methylester, methadone, ethylenedimethyldiphenylpyrrolidine, 6-monoacetylmorphine, amphetamine, methamphetamine and methylenedioxymethamphetamine) in wastewater. The compounds were isolated from 50 mL wastewater with solid-phase extraction on Oasis MCX cartridges. Analytes were chromatographically separated on a Phenomenex Luna HILIC (150 mm x 3 mm, 5 µm) column with a mobile phase consisting of acetonitrile and ammonium acetate 5 mM, used in gradient. Quantification was performed in positive ionization mode, multiple reaction monitoring acquisition, following two transitions (quantifier and qualifier) for each analyte and one transition for the corresponding deuterated internal standards. The quantification limits were 2 ng/L for ecgonine methylester, 6-monoacetylmorphine and amphetamine, and 1 ng/L for the other analytes. The concentration range of the calibration curves covered the expected values for the real wastewater samples, according to literature. All the curves were linear in the measured range, with good correlation coefficient ( $r^2 > 0.99$ ). The precision and accuracy met the acceptance criteria set for the method ( $\pm 20\%$ ). Recoveries between 60 and 110% were obtained for all analytes, except ecgonine methylester which under these conditions had a lower (36%), but reproducible recovery. Matrix effects were between 10% and 50%. Samples collected from 11 WWTPs across Belgium were extracted and analysed with this new method. For the first time we report on the presence of ecgonine methylester in wastewater samples, with concentrations in the range of those obtained for cocaine (10-220 ng/L). The 6-monoacetylmorphine was the only compound not found in the analysed wastewater samples.

## P061-B1

### Investigation and Determination of Humic Acid by Spectrophotometric and Fluorometric Methods

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Humic substances originating from plants and soil, mixed with natural source waters may change the color of water to yellow or brown, complex with metal ions and produce trihalomethanes reacting with chlorine that is a commonly used substance for the disinfection of water. Humic acids are the subgroup of humic substances that are soluble at greater pH values and precipitates slightly as the solution gets acidic. Beside the benefits of humic acid, the higher amounts of these substances form toxic disinfection by complexing with heavy metals and pesticides in ground and surface water. Therefore, the determination and quantification of this substance reveals a great importance. The determination methods for humic acids found in literature are insufficient for their high maintenance, cost and poor capacity of determining little amounts of analyte.

In this study, the photometric and fluorometric behavior of soluble humic acid was evaluated. The application range of humic acid concentration for photometric method was found as 1-5 ppm ( $\lambda_{\max}=276\text{nm}$ ) and for fluorometric method, 500ppb-5ppm ( $\lambda_{\text{ex}}=630\text{nm}$ ;  $\lambda_{\text{em}}=630\text{nm}$ ). Indirect determination of humic acid with CeIV solution by fluorometric method, in acidic medium was investigated at three different wavelengths letting us to compare the results within a spectrum. The reaction was followed at 360 nm ( $\lambda_{\text{ex}}=256\text{nm}$ ) and 528 nm ( $\lambda_{\text{ex}}=400\text{nm}$ ) which are the maximum emission wavelengths of CeIII, newly formed species, respectively. The application range of concentration for humic acid by indirect determination method was found as 50ppb-1ppm. All spectroscopic data were compared with the aid of molar excitation and emission coefficients. Among three spectroscopic methods; determination of humic acid by UV-Vis spectroscopy, molecular fluorescence spectroscopy and indirect method, the most applicable was found as the determination of humic acid with CeIV inhibiting the interferences of other materials such as chlorine, iron and contamination when analyzing within real samples.

## **P062-B1**

### **Development of a Sensitive Passive Sampler Using Indigotrisulfonate Sorbent for the Determination of Tropospheric Ozone**

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A new sampling and analytical design for measurement of ambient ozone is presented. The procedure is based on ozone absorption and decoloration (at 600 nm) of indigotrisulfonate dye, where ozone adds itself across the carbon-carbon double bond of the indigo. A mean relative standard deviation of 8.6 % was obtained using samplers exposed in triplicate, and a correlation coefficient ( $r$ ) of 0.957 was achieved in parallel measurements using a commercial UV ozone instrument. The devices were evaluated in a measurement campaign, mapping spatial and temporal trends of ozone concentrations in a region of southeast Brazil strongly influenced by seasonal agricultural biomass burning, with associated emissions of ozone precursors. Ozone concentrations showed strong seasonal trends, due to the influences of precursor emissions, relative humidity and solar radiation intensity. Advantages of the technique include ease and speed of use, the ready availability of components, and excellent sensitivity. Achievable temporal resolution of ozone concentrations is 8 hours at an ambient ozone concentration of 3.8 ppb, or 2 hours at a concentration of 15.2 ppb.

## P063-B1

### Simultaneous Determination of Linear Alkylbenzene Sulphonates, Nonylphenols and Di-(2-Ethylhexyl)Phthalate in Sewage-Sludge Amended Soil

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The main disposal route for sewage sludge generated in wastewater treatment plants is the application to agricultural lands as soil conditioner and fertiliser. However, potential risks are associated to urban waste recycling. For instance, organic contaminants, like plasticizers and surfactants, may accumulate in sludge especially during anaerobic treatments, causing diverse effects to the ecosystems [1]. Seven organic-compound families will have to be monitored before sludge application to soils according to a European Directive draft [2]. Among them, the pollutants that have been reported to be at the highest concentration levels in sludge are linear alkylbenzene sulphonates (LAS), nonylphenols (NPEs; sum of nonylphenol and nonylphenol mono and di-ethoxylates) and di-(2-ethylhexyl)phthalate (DEHP) [3].

No method has been found in literature for the simultaneous determination of LAS, NPEs and DEHP in sludge-amended soils. In this work, a method for their simultaneous determination in sludge-amended soils is presented. The method is based on analytical determination by high performance liquid chromatography with diode array and fluorescence detectors after sample treatment by ultrasonication and clean-up by solid phase extraction. Recoveries were in the range between 63% and 99%. The detection limits were between 0.3 and 1.1 mg kg<sup>-1</sup> dry matter (dm) for ultraviolet detection and between 0.2 and 0.6 mg kg<sup>-1</sup> dm for fluorescence detection. The quantification limits were between 0.6 and 3.4 mg kg<sup>-1</sup> dm for ultraviolet detection and between 0.3 and 1.5 mg kg<sup>-1</sup> dm for fluorescence detection.

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## P064-B1

### Modification of the QueChERS Method Applied to Soils with Halogenated Pollutants Used as Target Compounds

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QuEChERS (quick, easy, cheap, effective, rugged and safe) procedure was introduced by Anastassiades et al. in 2003 as a new approach to extract a wide range of pesticides from different food matrices with high water content [1]. This basic procedure is based on extraction with an organic solvent, addition of salts to induce or improve liquid-liquid partitioning and a dispersive solid phase extraction clean-up step. The method has received worldwide acceptance due to its several advantages.

Although QuEChERS has been mainly used for the determination of pesticides in food matrices, some other determinations such as those of pharmaceuticals,  $\beta$ -lactam antibiotics or veterinary drugs have been developed. To the best of our knowledge, the use of QuEChERS in soils is very limited [2, 3] but it provides very good results.

This work proposes to amend the QuEChERS method for the determination of halogenated compounds in soils by fast gas chromatography. The main change is related to the elimination of the cleanup step after the extraction, thus simplifying the original method.

In order to prove the suitability of this approach, halogenated pollutants of different characteristics in terms of their volatility and polarity have been chosen, among them chloroform, bromodichloromethane, hexachloroethane, 1,2,3-trichlorobenzene and hexachlorobenzene. Different solvents for extraction as well as different modes of injection have been studied. In the best conditions, good recoveries have been obtained for halogenated compounds. Use of a micro-electron capture detector ( $\mu$ -ECD) is proposed to improve the sensitivity of the method, which does not include any previous preconcentration step.

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## **P065-B1**

### **A Modeling Tool for the Evaluation of Tropospheric Ozone Time Series in Air Quality Monitoring Networks**

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Tropospheric ozone is an atmospheric pollutant of great concern, with demonstrated negative effects on human health and the environment. As a secondary pollutant, ozone is produced by complex processes involving reactions of primary emissions with sunlight. Evolution of local atmospheric conditions combined with variable anthropogenic activities at the emission sources determine production and transport of ozone, resulting in complex, time-dependent concentration profiles. Chemometric and modeling tools are thus essential for proper interpretation of these profiles. In this work, a mathematical tool based on Fourier series has been developed for the evaluation of tropospheric ozone time series routinely measured at air quality monitoring networks. Long term ozone data from the air quality monitoring network of Extremadura, Spain (low pollution urban areas and baseline rural stations), and Santiago de Chile (heavy pollution urban area), have been used to develop and test mathematical models for ozone data evaluation. Well defined temporal structures can be derived by use of time-series statistical techniques. The model closely represents the general trend of the data, by a simple sinusoidal function. The seasonal part of the model is also capable of analyzing diurnal variability of the ozone data with great precision, showing a night minimum and two daily maxima with several hours interval. Diurnal variability is dependent of the season and to a less extent of the monitoring station. A quality analysis of the model has been derived, to estimate its usefulness to detect outliers and predict future ozone values.

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## **P066-B1**

### **Mercury/Selenium Interaction in Terrestrial Wild Animals from the Almadén Mining Area (Spain)**

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Due to toxicity, persistence in biogeochemical cycles, widespread distribution and tendency to bioaccumulate, it is important to focus on mercury accumulation and the transfer of its compounds in terrestrial food chains, especially since major difference exists compared to aquatic or marine food chains. Additionally the interactions between heavy metals are also important since the accumulation of certain essential elements can be affected by overexposure to others. A special case of interest is the not well established mechanisms of protection and/or detoxification between mercury and selenium.

The Almadén mercury district in the mining area of Sierra Madrona-Valle de Alcudia (Southern Spain) has been selected for this study because it is one of the world's largest Hg-contaminated sites. Currently this area is largely devoted to and occupied by hunting estates, where several hundred red deer and wild boar are bagged every year, and are destined for human consumption. The key aim of our research was therefore to monitor the fate of mercury in this terrestrial ecosystem using red deer and wild board tissues to prevent possible harmful effects on wildlife and humans and to provide valuable evidence on its transfer, transformations and detoxification mechanisms.

Nineteen hunting estates were selected. Liver, kidney, muscle, bones (metacarpus) and testis samples were obtained from hunters during the autumn and winter of 2004-2005 (74 red deer) and 2005-2006 (94 red deer and 58 wild boar). Tissues were freeze-dried and dry samples were digested with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> for total mercury and selenium analysis by ICP-MS. Mercury speciation analysis was carried out in liver samples by CG-pyro-AFS after TMAH extraction.

Differences in tissue concentrations of the studied elements between control and mining areas or between species, the effects of gender and age and the relationships between tissue concentrations of the different elements are under study.

**Solid Phase Extraction-Enrichment of Cu(II) Ions as Schiff Base Complexes Adsorbed on Polyurethane Foam**

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Measuring and controlling the concentration of heavy metals is an important subject from environmental point of view [1]. Because the limitations of many conventional analytical methods, a pre-concentration step is sometimes required for the analysis of the samples containing trace amounts of heavy metals.

Solid phase extraction (SPE) methods are known as powerful trace enrichment techniques because they offer many advantages over liquid-liquid extraction (LLE) and overcome most of the drawbacks of this traditional method. Different types of solids such as carbon black, chemically bonded silica with C8 and C18 groups and polymeric resins have been used in SPE techniques [2].

The use of polyurethane foam sorbents (PUF) in solid phase extraction processes has grown up since the report of Bowen [3]. Various research papers were published describing the use of unloaded foams for analytical pre-concentration and separation of organic and inorganic species in aqueous medium. Additionally, the polyurethane foams have been used as solid supports for specific reagents leading to SPE procedures with high selectivity and analytical throughput [4].

Considering the well documented efficiency of nitrogen/oxygen donor ligands towards copper ions and in continuation to our recent studies on the complexing, extractive and analytical applications of Schiff base ligands [5], in the present communication we report a rapid, highly sensitive and efficient method for the extraction and concentration of trace amounts of Cu(II) ions from aqueous media using polyurethane foam modified with a N<sub>2</sub>O<sub>2</sub>-type Schiff base ligand and its determination by atomic absorption spectrometry.

By passing the sample solutions through a column packed with PUF modified by bis(2-hydroxypropiophenone)-3,3'-diiminodipropylamine, Cu<sup>2+</sup> ions are adsorbed quantitatively while almost all interfering ions pass through the column. The influences of pH, amount of the PUF packed in the column, type and volume of stripping reagent and also sample and stripping reagent flow rates were evaluated and optimized. The proposed method permits a concentration factor of higher than 133.3 and a detection limit of 10<sup>-3</sup> ng ml<sup>-1</sup>. Under optimum experimental conditions the capacity of the column packed by 3 cm of the modified PUF was found to be 203.97(±1.20) µg of copper. The relative standard deviation of the method was found to be less than 1.5%. The presented procedure was successfully applied for determination of copper ions in real samples.

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## P068-B1

### Development of a New Analysis Method to Quantify Low Concentrations of Biogenic and Secondary VOCs in Remote Areas

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Many prospective studies leded especially in remote areas have shown that an important part of atmospheric photochemical reactions in those areas are underestimated. Further studies revealed that biogenic compounds and oxygenated compounds, such as monoterpens or long chain carbonyl compounds, could contribute to these unexplained photochemical reactions. The study of this kind of compounds is therefore a priority. Still, no technique allows an efficient and simultaneous measurement for both of them. In order to complete this lack, an original analysis method has been developed. Based on active sampling / thermodesorption / GC analysis / MS-FID detection, it allows the measurement and quantification of 21 compounds; monoterpenes (such as limonene, camphene or  $\alpha$ -pinene), long chain carbonyl compounds (from n-hex anal to n-undecanal) and long chain alkans (from n-octane to n-hexadecane).

Air is actively sampled onto a multi-adsorbent cartridge using the automatic sampler SyPAC, provided by the society TERA-Environnement. Various tests conducted in the lab have permitted to define the better sampling parameters, which include breakthrough volume, and impact of ozone and humidity.

Various tests have been leded to define the best way to analyse chromatographically the targeted compound. The thermodesorption conditions (temperature, flow, split ratio, nature and temperature of the cryogenic trap), and the chromatographic temperature program were so optimised in order to prevent any depletion of the targeted compounds, and allow a good separation, with a high repeatability. A precise quantifying method has then been developed. It allows the quantification of each compound. The detection limits estimated for each one of them ranges between 5 and 10ppt. The double detection system used, including a FID and a MS detector, insure a correct identification of every compound.

This method has proved its efficiency during two measurement campaign of one month lenght, in winter and summer 2009.

## P069-B1

### Analysis of Oils and Greases in Portuguese Bathing Waters. Implementation and Validation of a FT-IR Method

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The term “Oils and Greases” (OG) encompasses a broad family of chemical compounds such as fatty material of biogenic origin or petroleum hydrocarbon constituents that are used widely in a range of domestic and industrial applications. These compounds can cause environmental degradation and induce related public health risks when discharged in surface waters.

As Portugal has beautiful beaches and pleasant weather, motorized water sports have gained importance in the last years and there are increasingly more boats and personal water crafts for tourism, recreation and sport use that, fueled by gasoline and diesel, are important sources of pollution with still unknown environmental impact.

OG concentration data cannot be used to quantitatively estimate human health risk, as they may comprise very different compositions. Even so, it is an important tool that can be used for three purposes: determining if there is a problem, assessing the severity of contamination and following the progress of a remediation effort.

Two methods have been specified for the determination of OG in waters: a gravimetric method and an infrared (FT-IR) method. Although gravimetric methods are simple, quick and inexpensive, they present the disadvantages of low sensitivity, loss of volatile constituents and inclusion of compounds which are not OG but are extracted by the solvent and therefore contribute to the final weight.

The aim of this study was the validation of a FT-IR methodology for the analysis of OG in bathing waters and then, correlates the results with the two bathing water quality parameters – *Escherichia coli* and intestinal enterococci, specified by the new EU Directive 2006/7/EC.

EPA 413.2 and 418.1 methods for OG and hydrocarbons described absorbance measurements only at  $2930\text{ cm}^{-1}$ , related to the stretching of aliphatic  $\text{CH}_2$  groups. However, IR-based methods using a single  $2930\text{ cm}^{-1}$  frequency do not adequately measure OG, as some bathing waters shows a significant absorbance band at  $2960\text{ cm}^{-1}$ , spectral zone of  $\text{CH}_3$  groups, characteristic of gasoline, as was confirmed after the preparation of a spectra library using some animal, vegetable and mineral oils. Compliance with EU Directive only requires the condition of no visible oil films being present at the water surfaces. However, while films were almost never been detected throughout the bathing season, the values for OG were, in many samples, greater than the old recommended guideline value of  $0.3\text{mg/l}$ , demonstrating that this Directive does not efficiently safeguard bathers against the possibility of chemical hazards exposition.

## P070-B1

### **Pressurised Matrix Solid-Phase Dispersion and Gas Chromatography and Mass Spectrometry in Tandem Method to Determine Flame-Retardants in Indoor Dust**

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This communication presents a fast, simplified and automated method, which integrates extraction and clean-up steps, for determining polybrominated diphenyl ethers (PBDEs) in indoor dust by gas chromatography and mass spectrometry in tandem.

PBDEs are organobromine compounds that are used as flame retardants in a wide array of products, including building materials, electronics, furnishings, motor vehicles, airplanes, plastics, polyurethane foams and textiles. The health hazards of these chemicals have attracted increasing scrutiny.

Published studies express concern because exposure to PBDEs impairs nervous system development, and PDBEs has also been shown to have hormone disrupting effects, particularly on estrogen and thyroid hormones. They can also accumulate in indoor dust. Children are more prone to exposure to indoor dust; in fact, studies show that children in the United States, Norway and Australia have higher levels of PBDEs than adults.

Indoor dust samples were dispersed in 4 g of Florisil and extracted with 1:1 n-hexane:dichloromethane at 40°C and at 1500 psi for 2min. This matrix solid-phase dispersion procedure provided cleaner extracts than simple PLE because an in-cell clean-up was performed.

The method enabled PBDEs to be determined in dust below 2 ng g<sup>-1</sup> in all cases, with good intermediate precision (relative standard deviation values less than 10%) and with recovery values ranging from 82 to 101%. Accuracy was also checked by analysing a standard reference material.

**P071-B1**

**In-Cell Clean-Up Pressurised Liquid Extraction Method to Determine Alkylphenols and Bisphenol A in Sewage Sludge by Gas Chromatography-Mass Spectrometry**

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This communication presents a method to determine alkylphenols and bisphenol A in sewage sludge. Bisphenol A (BPA) is used primarily to make plastics and can be found in many household goods. Alkylphenols (APs) are used extensively in detergent and polymer manufacture. BPA and APs are endocrine disruptors. They can mimic the body's own hormones and cause negative health effects. Thus, there is concern that long-term exposure to BPA and APs may induce chronic toxicity in humans.

The proposed method is based on pressurised liquid extraction (PLE) for extracting analytes from sewage sludge and gas chromatography analysis with mass spectrometry detection. Since PLE provided very dirty extracts, a clean-up step was necessary before GC injection. In order to simplify and automate the whole method, an in-cell clean-up strategy was assayed. Different types of sorbents were mixed with the sample prior to PLE. Different temperatures and solvents were also studied using a multicategorical factorial design. This study showed that Florisil, dichloromethane and high temperature provided the best results in terms of recovery and clean-up efficiency. Lastly, the method was characterised in terms of detection limits, intermediate precision and accuracy.

## P072-B1

### **A Novel Solid Phase Extraction Method for Preconcentration of Gadolinium Based MRI Contrast Agents from the Environment**

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Magnetic resonance imaging (MRI) has become one of the most powerful techniques in early medical diagnostics. Gadolinium based contrasting agents (GBCA) are commonly used to improve the visibility of abnormal structures in the body. Because gadolinium is highly toxic, it is reversibly chelated in complex structures in the contrast agent solution. Among various GBCA used, Magnevist (Gd-DTPA), Omniscan (Gd-DTPA-BMA), Dotarem (Gd-DOTA), Multihance (Gd-BOPTA) and Gadovist (Gd-BT-DO3A) are found to be widely used in MRI examinations. GBCA are known to be very stable and completely bind the free gadolinium. But, the recent studies have confirmed that GBCA are responsible for a rare and serious disease called Nephrogenic Systemic Fibrosis (NSF) especially with patients with renal inefficiency. With each application, the concentration of GBCA is increasing in the environment resulting gadolinium anomaly.

In environmental samples the determination of GBCA is a challenging task due to low concentrations (ppt level). Among various preconcentration methods available, solid phase extraction (SPE) technique became vital and mostly used method in a wide variety of analytical areas. Until now, the reported SPE methods were developed only for preconcentration of  $Gd^{3+}$ . The present work was performed with three different SPE materials namely, Chromabond SA (strong cation exchanger), Chelex-100 (weak cation exchange resin) and Di-(2-ethylhexyl) phosphate (HDEHP) coated reverse phase column and subsequently analysed using ICP-MS. Our work demonstrate that a preconcentration of Gd and its complexes using SPE is suitable for the environmental samples. The results based on the preconcentration studies on Gd and its contrast agents using the above mentioned SPE materials will be presented in the poster. Among the studied SPE materials, especially the results with a HDEHP coated reverse phase column were promising is found to be the best suitable for preconcentration of GBCA. The presentation will provide first data from screening of samples from surface water and waste water treatment plant.

## P073-B1

### Ultrasonic Energy as a Powerful Tool for the Evaluation of Trace Elements Profile in Ambient Aerosol Samples by ICP-MS

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The major and trace elemental profiles of ambient aerosol samples supplies valuable data for air quality evaluation (toxic elements and compounds included in human and environmental protection regulations) and also for environmental assessment (environmental impact of particulate matter deposition and source apportionment). Standard methodologies for inorganic analysis of aerosol samples, mostly based on wet digestion followed by GF-AAS, ICP-AES or ICP-MS, are routinely used in air quality networks and monitoring campaigns. Most common pretreatment procedures include acid treatment in closed vessels on a hot plate and microwave assisted acid heating (1). Ultrasonic solid-liquid extraction, a promising but still scarcely used pretreatment tool in ambient aerosol samples (2), has been proposed by some authors as a valid alternative for activation of aerosol digestion with short digestion times of less than 30 min (3). Whereas published results are based on ultrasonic bath digestion of aerosol filters, we aim here to demonstrate the applicability of a miniaturized ultrasonic probe for the fast digestion of small aerosol samples, followed by ICP-MS determination. Relevant experimental variables as acid medium composition and concentration, ultrasonic amplitude and extraction time have been tested with the goal of obtaining quantitative recoveries of Pb, As, Cd and Hg from standard reference materials and real aerosol samples.

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## **P074-B1**

### **Analysis of Sorptive Properties of Bentonite in Relation to PAHs Accumulation in the Sediments of the Dobczyce Water Reservoir, Krakow, Poland**

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The Dobczyce water reservoir is the source of drinking water for Krakow's agglomeration (over 1000 000 inhabitants). It is constructed on a mountainous Raba river. Thus, with water a substantial amount of contaminants is delivered to the reservoir. Due to the geology of the Raba river catchment, high percentage of clay minerals is present in dragged material and deposited as sediments in the reservoir.

Among other toxic compounds accumulated in the sediments (as a result of sorption, co-precipitation and other physical and chemical processes) PAHs belong to the group of most persistent and dangerous for living organisms. Although the concentration of PAHs in water of the reservoir is low, they tend to accumulate up to 10-fold in the sediments on organic matter and clay minerals.

The goal of the presented paper was to establish the effectiveness of sorption of the selected PAHs on bentonite – mineral belonging to the group of prevailing in the sediments clay minerals of the reservoir.

The mineral composition of the Dobczyce reservoir sediments was analyzed by means of X-ray diffraction method.

Using the HPLC separation method with spectrophotometric detection PAHs were quantitatively determined in the sediments.

Sorptive properties were examined as follows: to dry sample of PAHs free bentonite a water solution containing 10ng of anthracene, pyrene and benzo-a-pyrene was added, mixed and left for 24 hours.

The influence of pH, sorption time and reciprocal influence of PAHs on their accumulation effectiveness and the process kinetics were measured.

It was found that the balance of PAHs sorption is acquired after 24 hours at pH value in the range 9-10. In these conditions up to 90 % of PAHs present in the bathing solution is adsorbed on bentonite.

## P075-B1

### Application of Sequential Injection Chromatography for Separation and Quantification of Heavy Metals in Water Environmental Samples

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Flow injection (FI) techniques are well-suited tools for handling solutions and carrying out wet chemical analysis. The techniques enjoy outstanding features in terms of miniaturization, automation, versatility and inexpensiveness. However, FI techniques had suffered from the limitation of separation and multicomponent determination. Recently, sequential injection chromatography (SIC) has been proposed to overcome that limitation.

SIC has been designed to compete high performance liquid chromatography regarding consumption of solvents, buffers and reagents, besides instrumentation ease of use, instrumentation maintenance requirement and instrumentation cost. Furthermore, SIC is instrumentally friendly since many analytical devices could be easily coupled to the system. This feature allows for developing on-line procedures including such analytical processes as sample preparation and developing reactions. This communication proposes the development of some on-line analytical methods for separation and quantification of Fe, Co, Ni, Cu and Zn in environmental water samples. In these methods, the following on-line procedures will be adopted in the following order: (a) solid-phase extraction procedures. They will be adapted to SIC and conducted into monolithic C18 columns for preconcentration purpose. (b) kinetic complexation reactions with such chelates as 2-(2-quinolinyloxy)-5-diethylaminophenol and tetra(*m*-aminophenyl) porphyrin. It will be conducted into a miniaturized reaction coil installed in the SIC system with applying a critical programmable flow. (c) Separation of complexes into a monolithic C18 column. (d) Spectrophotometric measurement using miniaturized optical devices.

The newly proposed SIC methods will be rapid, selective, sensitive, cost effective, safe for handling chemicals and safe for the environment. The SIC methods also offers possibility of on-site test, which is desirable in environmental analysis.

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## P077-B1

### Analysis of Metals in Natural Waters by High-Resolution Continuum Source Atomic Absorption Spectrometry

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Geomedicine and Thermal Water Centers use waters and minerals as therapeutic choices in certain pathologies, as can be confirmed by the ever increasing numbers of centers of Thalassotherapy, SPA's and modernized Thermal Water Clinics. Portuguese natural waters are known by their mineral quality and thermal health treatments. Although some studies have been published in the last few years trying to link the physical-chemical properties of the natural mineral waters from Thermal Waters Clinics with their therapeutic potential [1, 2], the discussion of the results has been very careful, considering the difficulties to connect the benefits of the treatments exclusively to the physical-chemical characteristics of the waters.

The atomic absorption spectrometry is the standard technique to quantify metals. In this work there were analyzed some metals in Portuguese natural waters (spring, minerals and thermal) with nutritional and therapeutic importance using the very new technology of atomic absorption spectrometry based on a continuous source (Xenon short-arc lamp) from AnalytikJena, the ContrAA 700. With this equipment it was obtained very high resolution, reduced wavelength dispersion and very good background correction. Other advantage of the equipment is the simultaneous determination of several elements. The measurement of absorbance over time is now supplemented by a third dimension, the wavelength. The spectral environment became visible and thus noise or interference are easily corrected. The metals studied by flame atomization were Sodium, Calcium, Magnesium, Copper, Zinc and Manganese; electrothermal atomization was performed for Zinc, Lead, Manganese, Copper and Chromium. The results obtained are in agreement with those obtained with conventional equipment regarding the concentration range, limits of detection, repeatability and reproducibility. Nevertheless, with this equipment it was possible a faster sequential multi-element analysis.

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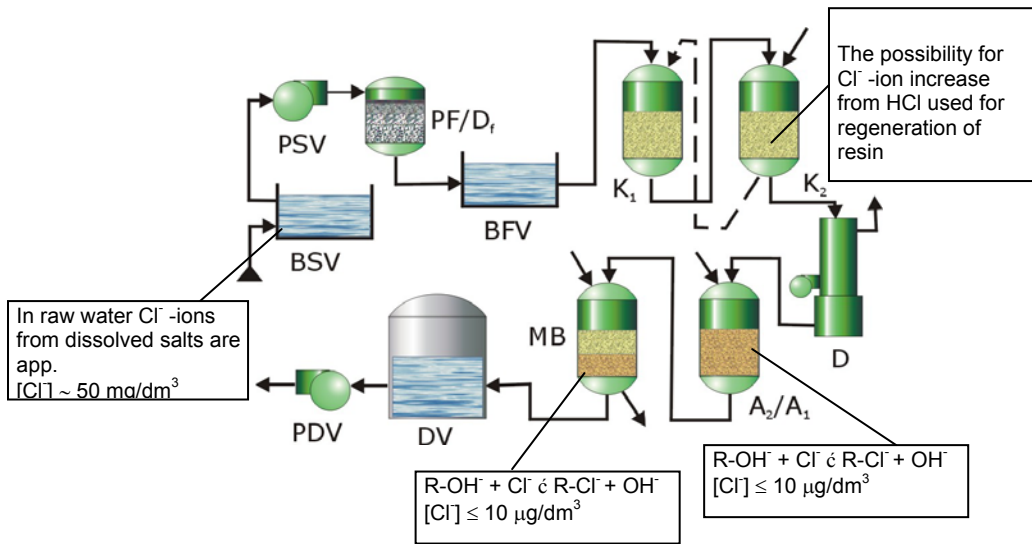
Corrosion in the Water-Steam Cycle in Thermal-Power Plants

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In this paper theoretical and practical aspects of corrosion in thermal-power plants (TPP) in Electrical Power Industry of Serbia are considered. The basic concept for classification of various types of corrosion is explained. The main types of corrosion processes which exist in thermal-power plants are: oxygen corrosion, caustic gouging, ammonia corrosion, chloride corrosion, corrosion fatigue, hydrogen embrittlement, pitting, cavitation corrosion, atmospheric corrosion and crevice corrosion. In Figure below the line for chemical preparation of water in TPP TENT B is shown. The accent is on the processes for chloride-ion concentration removal and/or minimization improvement.



Legend:

BSV- raw water tank	PSV- pump of raw water	PF-sand filter	
D-iron removal reactor	BFV- tank of filtered water		K <sub>1</sub> -weak acid
K <sub>2</sub> -very acid cationic exchanger		D-degassing reactor	A <sub>1</sub> /A <sub>2</sub> -anionic exchanger
MB-mixed bed exchanger	DV-reservoir of demi water		PDV-pump of demi water

The modernization of chemical preparation of water, from the aspect of improvement of lines for demi water and conditioning of water with implementation of new solutions would result in better working media quality and decrease of corrosion processes in TPP. The economic importance and influence of corrosion on the safety and long term reliability indices of thermal-power plants are also taken into consideration.

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**P079-B1**

## **Characterisation of Oil Shale Kerogen by Thermogravimetry and IR Spectroscopy**

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Macromolecular nature and consequent insolubility of oil shale kerogen eliminate many of modern instrumental methods and make the study of its structure difficult. However, that is not the case with thermogravimetric analysis. It was used for studying of the kerogen of oil shale and significant differences were observed in thermal stability and the shape of DTG curves. More detailed information on thermal behaviour of kerogen was obtained by combination of thermogravimetric analysis with IR spectroscopy. The results of these microscale experiments are important for the development of industrial process for retorting of oil shales. The thermogravimetric method for determination of the content of organic matter in oil shales was also developed. Very good correlation of results with values obtained by other methods (IR spectroscopy and chemical analysis) suggested possible use of thermogravimetric analysis for prospection studies of oil shale deposits. However, the quality of the correlation should be verified on the large set of oil shale samples.

**P080-B1**

**Factors Influencing the Rapid Bulk Degradation Method for Characterisation of Oil Shale Kerogen**

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Oil shales are important as possible alternative source of energy or raw material. Oil shale layers and deposits varies substantially in quality and quantity and therefore fast characterisation is of particular interest in prospection studies. Rapid bulk degradation method based on the consumption of alkaline permanganate under standard conditions was used for structural characterisation of kerogen. Kerogen represents the main portion of organic matter in oil shales. The method is sensitive to various factors such as the content and structure of kerogen, particle size and content of pyrite in oil shales. The influence of various factors was investigated in this paper. In the series of oil shale samples with different particle size the consumption of permanganate was correlated with the content of pyrite, the content of kerogen and H/C ratio of kerogen. In order to evaluate the effect of pyrite the method was applied to original oil shales as well as on oil shales with partially or totally removed pyrite. The consumption of permanganate was also determined for some kerogen degradation products which were more homogeneous than kerogen. In some cases additional data were provided from IR spectra before and after the treatment of samples by alkaline permanganate.

## P081-B1

### Sensors and Biosensors Array to Evaluate the Content and the Antioxidant Efficacy of Secondary Metabolites Used as Nutraceuticals

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There is a lot of reported information regarding the efficacy of phytochemicals rich diet against a variety of diseases that have as start point a free radical attack (cancer, atherosclerosis etc). A proper administration of those phytochemicals involves a proper analytical characterisation of the used product from the point of view of active principles content and efficacy. It should be started with the antioxidants quantification and potential identification and then should be performed the evaluation of the radical-scavenger features against significant oxidative markers.

The aim of our work was to provide evidence on the suitability of electrochemical devices-sensors and biosensors- use in evaluation of: 1. the phenolic/polyphenolic secondary metabolites content; 2.the polyphenols radical scavenging effects and 3. the metabolites antioxidant properties against lipo-peroxidation.

A versatile biosensor, using polyphenol-oxidases, was developed to determine the polyphenolic secondary metabolites content: a. Using an optimised biosensor (reproducibility RSD 10 %; 360 days stability), a good sensitivity was obtained  $510\mu\text{A}/\text{mM}$ ; in artificial mixtures and real samples biosensor proved to be free of interferences. When the biosensor response was compared with the LC-DAD-ESI-MS results for real samples (extracts of *Salvia officinalis*, *Mintha piperita* and *Bassilicum*) a good recovery was reached.

A bio-mimetic system based on human lipoproteins (low-density and very low-density lipoproteins) shell deposition on a conductive support was developed as sensor to assess the secondary metabolites efficacy against lipoperoxidation. Free radicals -  $\text{HO}^\cdot$  (thermal generated via  $10\text{ mmolL}^{-1}\text{AAPH}$ ) and superoxide- induce a structural modifications of the deposited lipoprotein shell, initiating lipo-peroxidation ( $\text{LOO}^\cdot$ ) which is the main damaging compound generated by FR excess. Those lipoprotein structural modifications raise an electrical measurable signal, the degree of peroxidation correlating with the free radical concentration and damaging effect. The information is quantified as difference on the signal registered in the absence of free radicals as reference (normal status, used as blank signal) and that registered in the presence of free radicals as indicator (biological hazardous status). The developed sensor was calibrated on a FR concentration range of  $10^{-9} - 10^{-6}\text{ molL}^{-1}$ , then applied to real samples. An index of secondary metabolites efficacy was established as efficiency against lipoperoxides formation **Caffeic > Rosmarinic > Chlorogenic > Gallic.**

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## P082-B1

### Chiral Biosensors Using Chiral Nanocomposite Materials

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Chiral lactate biosensors based on sol-gel, collagen and collagen/sol-gel organic-inorganic hybrid composite materials were developed. These matrixes were used to immobilize L-lactate oxidase (L-LOx) on the surface of glassy carbon electrode (GCE). Interesting results were obtained with collagen matrix and collagen/sol-gel hybrid complex. Chiral recognition of lactate was reversed due to the effect of collagen. Predominant current response was obtained with D-lactate. The chiral selectivity also seems to further enhance by collagen/sol-gel complex that shows an enhanced linear range of lactate concentrations up to 5 mM. The higher sensitivity of this modified electrode could be due to the good porosity degree of the hybrid complex that provide microenvironment to the entrapped L-LOx to retain their activity and thus, effectively catalyzed the oxidation of lactate to pyruvate.

Effects from the toxic ethylene glycol metabolites on the performance of the lactate biosensor were also investigated. The current ratios of the biosensor with and without the interference show a false result due to the cross react between the ethylene glycol metabolites with L-LOx<sup>1</sup>.

Electrochemical characteristics of the biosensors with and without carbon nanotubes (CNTs) in the composite film were also compared. The results showed that the sensitivity of the biosensors could be improved greatly after introduction of the CNTs. Circular Dichroism and Environmental Scanning Electron Microscopy techniques were then used to further characterize these biocomposite electrodes.

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## **P083-B1**

### **A New Voltammetric Sensor Based on a Glassy Carbon Electrode Modified with 8-Hydroxyquinoline-5-Sulfonic Acid**

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This contribution deals with the preparation, characterization and electroanalytical application of a new modified glassy carbon electrode obtained by cycling the potential in 8-hydroxyquinoline-5-sulfonic acid (HQSA) solution. The influence of the deposition parameters (HQSA concentration, negative and positive potential limits, number of cycles, nature and pH of the supporting electrolyte) on analytical performances was evaluated by mean of cyclic voltammetry experiments in a dopamine test solution. According to literature information, p-toluene sulfonic derivatives undergo electropolymerization in similar conditions. However, in such wide potential ranges, even the glassy carbon surface itself can undergo complex redox processes leading to surface functionalisation.

Therefore, at present, the actual surface status of the electrode is not fully understood. X-ray Photoelectron Spectroscopy and Scanning Electron Microscopy characterizations are planned to this aim.

Nevertheless, the modification by HQSA leads to significantly improved electrochemical performances with respect to those of bare glassy carbon electrodes. This was shown by applying the modified electrode to the determination of some species of alimentary and pharmaceutical interest such as dopamine, methylxanthines, food colorants and ascorbic acid.

Perspectives and limitations of the use of the proposed electrode are critically evaluated and discussed in the light of existing literature.

## P084-B1

### Highly Selective Fluorescent Chemosensors for Cu(II) ions Based on Phenylethylidene-3,4-dihydro-1H-quinoxalin-2-one Derivatives

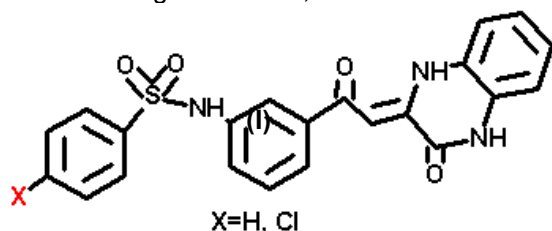
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The development of sensitive and selective fluorescent chemosensors for detection of Cu(II) continues to attract considerable attention due to its significant environmental pollution and being as an essential trace element in biological systems.

Recently new quinoxaline-2-one derivatives that integrate both ionophore and fluorophore into one unit were designed and synthesized as potential HIV integrase inhibitors [1]. In the present study, this new class of organic fluorescent compounds, based on phenylethylidene-3,4-dihydro-1H-quinoxalin-2-one (I) as a representative example, has been examined as a potential chemosensor for various metal ions of biological interest, in both ethanol and acetonitrile.



In ethanol, a solution of I in the presence of Cu(II) induced a reversible formation of a 1:1 metal-ligand complex, which resulted in a red shift of 24 nm in its UV-Vis absorption spectrum and exhibited a selective fluorescence quenching. The three peaks of free **1** at ~395, 418 and 441 nm shifted to longer wavelengths, 418, 441 and 465 nm, respectively, upon formation of the complex, and could be used to determine Cu(II) concentration in ethanol. In addition, in the range of 0-20  $\mu$ M of Cu(II), the intensity of the fluorescence emission decreased almost linearly with increasing concentrations of Cu(II). Interestingly, other investigated metal ions, such as Zn(II), Mg(II), Co(II), Ni(II), Mn(II), Ca(II) and Ag(I) did not show any complexation in their UV-vis spectra.

A selective response of the binding between compound **I** and Cu(II) was also observed by replacing ethanol with acetonitrile. However, in this case, it was irreversible due to the potential oxidation-decomposition of **I** by Cu(II) in acetonitrile [2]. A plausible mechanism for the binding between **I** and Cu(II) will be presented.

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## P085-B1

### Protein Biosensor Based on Electrochemical Impedance Spectroscopy as Detection Method

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The design of biosensor has to include: (i) the transducer for conversion and amplification of the biochemical reaction product into a recognizable, (ii) the matrix for the immobilization of a biomolecule and (iii) the bio-recognition element for analyte recognition. From technological point of view is important to control sensing layer features leading to feasible devices, with good reproducibility and increased sensitivity and specificity of detection. In this context, utilization of the self-assembled monolayers (SAMs) as matrices provides a simple route to functionalize metallic surfaces by organic molecules (thiols with free anchor groups) and allows oriented immobilization of signal biomolecules on a transducer surface.

As detection method, electrochemical impedance spectroscopy (EIS) is a promising technique for biosensor applications due to its capability for both interface characterization with maximum sensitivity by selection of the optimum frequency and real-time monitoring of the sensor signal that can give rise to kinetic aspects of the ligand - analyte interaction. We have used these analyses to study of the level of defects present in 11-mercaptoundecanoic acid (11-MUA) monolayer on gold thin film / nanoparticles and also to detect the various proteins. When a target protein binds to the pre-functionalized probe surface, the impedance of the electrode-solution interface changes and this change is detected electrically over a range of frequencies. The measurement have been performed using a PAR2273 spectrometer, in a three electrodes electrochemical cell configuration with 0,1M KCl electrolyte with / without  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  redox probe, using the following parameters: amplitude of 5 mV rms AC with excitation frequencies ranging from 100 kHz to 0.05 Hz at open circuit potential (OCP) and also with an applied DC potential; the experimental data were fitted to equivalent circuit models of the electrochemical interface using Zsim3.21 software and impedance changes were monitored. The results were corroborated with atomic force microscopy images, cyclic voltammetry and fluorescence spectroscopy data.

## P086-B1

### Carbon Black as Nanostructured Electrode Material for the Construction of Sensors and Biosensors

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In this work, the electrochemical behavior of carbon black (CB) was investigated. Firstly, a paste electrode (CBPE) was prepared using a nanostructured commercial CB (N220) and tested using cyclic voltammetry technique with several potentially interesting analytes. The results were compared with graphite carbon paste electrodes (CPEs). Shifting in the peak potential and/or increase in the peak current for some analytes such as ferricyanide, ascorbic acid, and acetoaminophen were observed. In addition, the carbon black paste was mixed with tyrosinase to construct a biosensor which was challenged in amperometric mode with catechol showing a high sensitivity (625 nA/microM) and low detection limit (8 nM).

In order to construct a sensor able to be mass produced, we have also modified screen printed electrodes with carbon black. A stable dispersion of carbon black was prepared and characterised by the TEM technique. The dispersion was then used to modify the screen printed electrodes (CB-SPEs). The CB-SPEs showed an enhanced oxidation current for several analytes such as NADH, cysteine, thiocholine and, in the case of epinephrine, norepinephrine and benzoquinone also the reduction of the peak-to-peak separation, compared with the bare SPE, was evidenced. The CB-SPE was also challenged in an amperometric batch system with some analytes (for thiocholine a low detection limit=30 nM was obtained). The obtained high sensitivity towards thiols obtained allowed the development of a novel analytical method for mercury detection based on the effect of mercury ions on the thiocholine oxidation current. A decrease of thiocholine amperometric signal was in fact observed due to the formation of a non-electroactive complex between thiocholine and Hg<sup>2+</sup>. This method allows the detection of mercury at concentration of 1.5 ppb.

In conclusion, the results obtained showed that the carbon black is a useful nanostructured carbon material which can be obtained at low cost with interesting electroanalytical properties.

## P087-B1

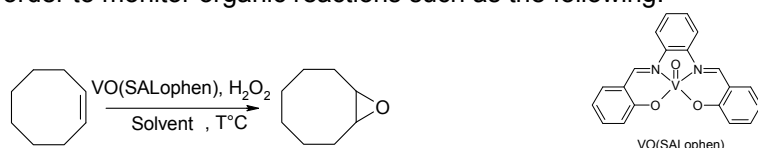
### A Simple Electrochemical Biphasic Method Based on Ionic Liquid to Monitor the Organic Oxidation Reactions

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In this research work, a new analysis system involving a biphasic reaction has been developed in order to monitor organic reactions such as the following:



This reaction, which involves the use of vanadium complex as catalyst and hydrogen peroxide, has been monitored by the amperometric measurement of the decrease of the latter compound. A screen printed electrode chemically modified with Prussian Blue has been used in order to obtain a sensitive, stable and cost-effective sensor for hydrogen peroxide measurement. The reaction was initially carried out in acetonitrile and the  $\text{H}_2\text{O}_2$  consumed was measured off-line in amperometric batch analysis. In the perspective of most sustainable procedures, the reduction of the amount of volatile organic compounds (VOCs) used as solvent is of great importance. In fact, recently many research groups have proposed new reaction media such as ionic liquids. Therefore, a novel biphasic method of analysis has been developed. The biphasic system is characterised by a hydrophobic phase (ionic liquid  $\text{bminPF}_6$ ), in which the organic molecules and the catalyst are present, and a hydrophilic phase (phosphate buffer + KCl) in which the hydrogen peroxide and sensor are present. The oxidation reaction occurs at the interface and is continuously monitored. Good results were obtained in terms of low detection limit (1  $\mu\text{M}$ ) and working stability of sensor (over 6 hours) demonstrating the possibility of continuous monitoring of an organic oxidation process with a simple and economic system.

## P088-B1

### Voltammetric and Amperometric Determination of Nitrated Polyaromatic Compounds Used as Markers of Incomplete Combustion at Amalgam Electrodes

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Nitrated polycyclic aromatic hydrocarbons (NPAHs) are of concern due to their direct acting mutagenicity and/or carcinogenicity. Among others, they are formed during incomplete combustion processes by reaction of PAHs with atmospheric nitrogen oxides. 1-nitropyrene, 2-nitrofluorene, and 3-nitrofluoranthene as the dominating substances are therefore used as markers of NPAHs formation by these processes. In presented study, voltammetric properties and possibility of the determination of these compounds and 5-nitroquinoline have been investigated by means of silver solid amalgam electrodes (AgSAE), which represent a non-toxic alternative to traditional mercury electrodes<sup>1</sup>. Linear calibration curves over three orders of magnitude and limits of determination in the  $10^{-7}$  mol L<sup>-1</sup> concentration range were obtained using direct current and differential pulse voltammetry. Further, satisfactory separation of studied analytes in fifteen minutes was achieved in RP-HPLC using 0.01 mol L<sup>-1</sup> phosphate buffer, pH 7.0 : methanol (15:85, v/v) mobile phase. Limits of detection of cca  $5 \cdot 10^{-6}$  mol L<sup>-1</sup> were achieved using amperometric detection at AgSAE in wall-jet arrangement.

#### *Acknowledgement:*

K. P. thanks to the Grant Agency of the Czech Republic (project 203/07/P261), J. L. thanks to the Grant Agency VEGA (project 1/0852/08) of the Ministry of Education of Slovakia. The project was further supported by the Czech Ministry of Education, Youth and Sports (projects LC 06035 and MSM 0021620857).

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## P089-B1

### A Copper(II) Selective Potentiometric Sensor Based on N,N',N'',N'''-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane in PVC Matrix

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A synthesised N,N',N'',N'''-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane has been used as an ionophore for the preparation a new copper(II) ion-selective electrode. The simple PVC-based membrane containing *tpmc* as ionophore and dibutyl phthalate as plasticizer, was directly coated on the surface of a glassy carbon electrode. The potential response is linear to Cu<sup>2+</sup> ions in the concentration range of  $1.0 \cdot 10^{-1}$  -  $1.0 \cdot 10^{-6}$  M with near-Nernstian slope of  $28 \pm 3$  mV/decade of activity and detection limit is  $\approx 5.0 \cdot 10^{-7}$ . The electrode is suitable for use in aqueous solutions over a wide pH range (1.3-6). The response time of the sensor is 10-50 s. The sensor has a lifetime of about 2 months and exhibits excellent selectivity over a number of many cations including alkali, alkaline earth metal, heavy and transition metal ions. It can be used as an indicator electrode for the end point determination in the potentiometric titration of copper ions against EDTA as well as for the determination of copper ion concentration in real samples.

## **P090-B1**

### **Development of Non-Invasive Detection of Cholesterol by Using Molecularly Imprinted Self-Assembled Monolayer Electrode**

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Control of the total cholesterol level in the body plays an important role for preventing life-style related diseases. The conventional method is troublesome due to the necessity of going to a hospital for invasive blood collection and using the enzyme reaction through many procedures. On the contrary, about 11 percent of the body's cholesterol is found in the skin at the same rate as in the blood, according to the FDA. Therefore, we focus on a simple and non-invasive measurement for cholesterol using a molecularly imprinted self-assembled monolayer (SAM).

A gold electrode was immersed in an ethanol solution containing cholesterol and stearylmercaptan, and then washed in ethanol in order to extract the cholesterol as a template molecule. The extraction of cholesterol molecules creates shape-complementary cavities on the SAM, and the detection of electro-inactive cholesterol is achieved using an electrochemical method with potassium ferrocyanide as the redox marker. The change in the oxidation peak current ( $I$ ) shows a linear relationship with the cholesterol concentration. The change of  $I$  is related to the cavity concentration for the mass-transport of the redox marker on the molecularly imprinted SAM. When the cholesterol-sensitive SAM recognizes cholesterol,  $I$  decreases due to marker diffusion rejection to the gold electrode surface. On the contrary, when the SAM extracts cholesterol, the marker diffuses to the electrode surface and  $I$  increases. The sensing properties of the molecularly imprinted SAM, such as sensitivity, selectivity, and reproducibility, have been examined, and it has been applied for simple and speedy electrochemical sensor development.

## P091-B1

### The Effect of Ring C on Electrochemical Properties of 3-Hydroxyflavone Using Voltammetric Methods

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3-Hydroxyflavone (3-hydroxy-2-phenylchromen-4-one) is a member of flavonoids, the main skeleton of which is 2-phenyl-1,4-benzopyrone. Flavonoids are a large class of natural products of vegetable kingdom and pharmaceutically interesting compounds due to their commonly known antioxidant activities, and as were indicated recently, providing health benefits against cancer and heart diseases [1,2]. Moreover, 3-hydroxyflavones have a particular interest as sensors since they are sensitive compounds to their environment resulting from their excited state intramolecular proton transfer reaction [3, 4]. As flavonoids are among the most interesting class of antioxidants, investigating their antioxidant effects is an important task, and although they have been studied widely applying various methods, to our best knowledge, there is no report on determination of antioxidant activity of 3-Hydroxyflavone using electrochemistry as a tool.

In this work, considering that the electrochemical behaviors of rings A and B of flavones, including 3-hydroxy flavones, were well studied leaving the ring C out. Electrochemical behavior of ring C, which incorporates an hydroxide and a carbonyl functional groups, was also investigated. As a simple model molecule 3-Hydroxyflavone was prepared, as it does not have any other functional group at the rings A and B, and electrochemical oxidation mechanism was investigated at different pHs, using cyclic and differential pulse voltammetry techniques. Additionally, electrochemistry was applied to the determination of the antioxidant activity in terms of trolox equivalent antioxidant capacity.

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## P092-B1

### Voltammetric Determination of Cu<sup>2+</sup> Ion Using Modified GCE

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Great progress has been made over the last few decades in the electroanalysis for the determination of ultra trace level analyte through the chemically modified electrodes (CMEs) in the successful development of voltammetric methods. CMEs are a modern approach to the electrode system that provides a utility in a wide spectrum like that basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity to electrode surface chemistry, electrostatic phenomena at electrode surfaces, and electron and ionic transport phenomena in polymers. CMEs are also useful in the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection and electro-organic synthesis [1].

Copper is an essential micronutrients for humans and plants but its presence in nutrition assumes a special relevance. Its accumulation in the body causes some toxic effects like that neurological symptoms, kidney damage, skin problems, hair loss and hypertension [2].

In this study, a selective and sensitive voltammetric method was developed for the determination of trace amounts of Cu<sup>2+</sup> by using differential pulse voltammetry with 3-hydroxyflavone modified glassy carbon electrode. The optimal conditions were determined including pH, deposition time, deposition potential. The interference of some metal ions was studied. The method developed was applied for the trace determination of copper in various samples.

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## P093-B1

### Characterization of N,N',N'',N'''-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane and its Application as an Ionophore for Potentiometric Sensor

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The aim of this work was to exam electrochemical and sensor behavior of N,N',N'',N'''-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane, one of the macrocycles which according to the many intriguing features, attracts a huge amount of research interest. The *tpmc* was studied in aqueous NaClO<sub>4</sub> solution by cyclic voltammetry at a glassy carbon electrode. The possibility of application of *tpmc* as an ionophore for potentiometric sensor was investigated, too. The potentiometric response of the PVC based coated membrane with *tpmc*, incorporating dibutyl phthalate (DBP) in composition 5:57:38 (*tpmc*:PVC:DBP) was studied in standard solutions of large number of different ions from  $1.0 \cdot 10^{-1}$  -  $1.0 \cdot 10^{-6}$  mol/dm<sup>3</sup>. The *tpmc* was proved as a very selective to the Ag<sup>+</sup>, Cu<sup>2+</sup> and Fe<sup>3+</sup> ions. The proposed electrode exhibits Nernstian slopes and can be used from pH of 1.30 to pH which is limited by hydrolysis of mentioned ions. That gives a possibility to develop selective and sensitive sensors, for determination those ions in some real samples where commercial sensors show their limitations.

## **P094-B1**

### **Carbon Surfaces for the Oxidative Quantification of Pravastatin: Glassy-Carbon vs. SPCE**

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The electrooxidative behavior of pravastatin (PRV) in aqueous media was studied by square-wave voltammetry (SWV) at a glassy-carbon electrode and at a screen-printed carbon electrode (SPCE). Maximum peak current intensities, in a pH 5.0 buffer, were obtained at + 1.3 V vs. Ag/AgCl and + 1.0 V vs. Ag for the glassy-carbon and the SPCE, respectively. The validation of the developed methodologies revealed good performance characteristics and confirmed their applicability for the quantification of PRV in the antidiabetic drug Pravastatin Alter, without significant sample pretreatment.

A global comparative analysis between the two electrode types showed no significant differences between linear range, detection and quantification limits, and precision. However, a higher sensitivity was obtained with the SPCEs, which combined with the faster analysis and small sample volumes makes this electrode surface more adequate for the proposed analysis.

## **P095-B1**

### **On-Line Stripping Voltammetry Determination of Trace Metals in Sea Water at a Flow-Through Bismuth-Film Screen Printed Electrode by Multi-Syringe Flow Injection Analysis**

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Analysis of heavy metals in sea water during oceanic campaign need rapid and affordable methods that minimize the problems with transport and conservation the samples, and reduce the cost of analysis commonly used, ICP or AAS. The application of the electrochemical sensors, as Screen Printed Sensor, coupled with a Multi-Syringe Flow Injection Analysis system (MS-FIA) make possible the determination on board of heavy metals very low levels in sea water.

This work presents a proposal about the application of a Graphite Carbon Screen Printed Electrodes associated with electro analytical instrumentation and a flow system (MS-FIA) to determined Cd and Pb in sea water. The SPE were modifying with nafion, and the metals were determined by Square Wave Anodic Stripping Voltammetry, over a Bismuth film in-situ plated in each analysis. The ICP spectroscopy was used as a reference method.

## P096-B1

### Properties of Thiolate Monolayers Prepared by Controlled Potential at Different Amalgam Electrodes

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Many sulfur containing compounds adsorb at surfaces of different metals and spontaneously form very stable monolayer films (SAM – self-assembled monolayer). Au, Ag, Pt and Hg are the common materials for SAMs formation. Liquid mercury and mercury film covered metals have ideally smooth surface which is a necessary requirement for formation of monolayers with minimal defects. Solid amalgams of different metals are wetted by mercury well and hence it is possible to prepare electrodes covered by mercury meniscus (m-MeSAE) or by mercury film (MF-MeSAE; where Me is Ag, Au, Cu, Bi, etc.). These electrodes have advantages both of mercury and of solid electrodes. Paste amalgam electrodes after their smoothing on glass are very similar to mercury film electrodes and their advantage is in an easy mechanical surface renovation. Amalgam electrodes with liquid surface (m-AgSAE, MF-AgSAE, m-CuSAE, m-BiAgSAE, etc.) were used as supports for creating monolayer films of different thiolates and optimal conditions for this purpose were found. Charge density, surface concentration, adsorption isotherms and other characteristics of monolayers were determined from the desorption peak on cyclic voltammograms. These monolayers will be used for preparation of biosensors and as first layers for model phospholipid bilayer membranes.

The range of potentials in which thiocompound monolayer on electrode can be studied is limited by its destruction (reduction of the metal – sulfur bond) in the negative potential area and by electrode material oxidation in the positive area. Dependent on electrochemical activity of the amalgam forming metal and on the strength of metal – sulfur bond, the metal reduction peak can occur in a wide potential range. Values of peak potentials on MeSAEs, where Me is metal more noble than Hg (Ag, Au, Ir, etc.), do not differ much. In case of more electrochemically active amalgam forming metals (Cu, Bi, Cd, etc.), the peak potential can move with range of several hundred millivolts. This fact is very important for creating a monolayer which will be stable in the given potential area and which would be used for study of permeability of this monolayer or of phospholipid bilayer for different ions.

#### **Acknowledgements**

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## Coulometric-Potentiometric Determination of Autoprotolysis Constant of Water Using Hydrogen-palladium Electrode

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A variety of procedures have been used for the evaluation of autoprotolysis constants, mainly based on potentiometric titrations. Classical potentiometric procedure for the determination of the autoprotolysis constant requires preparation of standard solutions of strong acid and strong base in the solvent investigated. By generating acid or base coulometrically the procedure for the potentiometric determination of the autoprotolysis constants of the solvents may be simplified<sup>1,2</sup>. In this paper, by using hydrogen-palladium electrode as generator one for potentiometric determination of autoprotolysis constant of water, we have avoided preparation of standard solutions acid. The hydrogen dissolved in palladium is ideal for the coulometric generation of hydrogen ions in water; this oxidation potential is low and it can be quantitatively oxidized<sup>3</sup>. In this procedure, a strong base generated coulometrically at the Pt-cathode *in situ* in the cell in presence of sodium perchlorate (0.1 M) as the supporting electrolyte, is titrated with hydrogen ions obtained by the anodic oxidation of hydrogen at a hydrogen-palladium electrode. The titrations were carried with a glass and calomel (SCE) electrodes at 25 °C. The concentration autoprotolysis constant of water was calculated from the expression:  $pK_w^c = (E_b^0 - E_a^0)/59.16$ , where  $E_b^0$  and  $E_a^0$  represent the specific cell constants for the basic and acid regions, respectively. The concentration constant was recalculated to obtained the thermodynamic value by using the known ionic strength. The value obtained  $pK_w = 13.90 \pm 0.06$  was in good agreement with values in the literature.

Using hydrogen-palladium electrode for the generation of strong perchloric acid the relative acidity scale (RAS) of water was determined, too. The half-neutralization potentials of strong acid and strong base in a sodium perchlorate medium were measured using both a glass-SCE and a  $(H_2/Pd)_{ind}$ -SCE electrode pairs. The values of RAS of water determined with a glass-SCE and  $(H_2/Pd)_{ind}$ -SCE electrode pairs were  $490 \pm 6$  mV and  $210 \pm 9$  mV, respectively. In accordance with obtained the range acidity scale of water, the highest potential jumps at the end-point in acid-base coulometric-potentiometric titrations with a glass indicator electrode in this solvent, were obtained.

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## P098-B1

### Multi-Analyte Imaging in One-Shot Format Sensors for Natural Waters

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The main purpose of this work is to develop and perfect an analytical procedure using an inexpensive black and white non-cooled CCD camera for image acquisition of a one-shot multisensor. In this way, a one-shot multisensor based on ionophore-chromoionophore chemistry for optical monitoring of potassium, magnesium and hardness in water is presented.

The selectivity for each species comes from the different ionophore included in every membrane, but the optical transduction uses the same chromoionophore, lipophilized Nile Blue. This is the rationale for choosing potassium, magnesium, and hardness as the targeted analytes, and a black and white non-cooled CCD camera in this proof-of-concept study.

The analytical procedure uses the CCD camera for image acquisition of the one-shot multisensor after the reaction, followed by data treatment for quantification using the grey value pixel average from a defined region of interest from each sensing area to build the analytical parameter  $1-\alpha$ . In optimised experimental conditions, the procedure shows a large linear range, up to 6 orders using the linearised model and good detection limits:  $9.92 \cdot 10^{-5}$  mM,  $1.86 \cdot 10^{-3}$  mM and  $1.30 \cdot 10^{-2}$  mg·L<sup>-1</sup> of CaCO<sub>3</sub> for potassium, magnesium and hardness, respectively. This analysis system exhibits good precision in terms of relative standard deviation (RSD %) from 2.3 to 3.8 for potassium, from 5.0 to 6.8 for magnesium and from 5.4 to 5.9 for hardness. The trueness of this multisensor procedure was demonstrated comparing it with results obtained by a DAD spectrophotometer used as a reference. Finally, it was satisfactorily applied to the analysis of these analytes in miscellaneous samples, such as water and beverage samples from different origins, validating the results against atomic absorption spectrometry (AAS) as reference procedure.

In conclusion, the use of a one-shot multisensor measured by an inexpensive black and white CCD camera seems to be an interesting option in terms of good handling, low time-consumption and analytical capabilities.

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## P099-B1

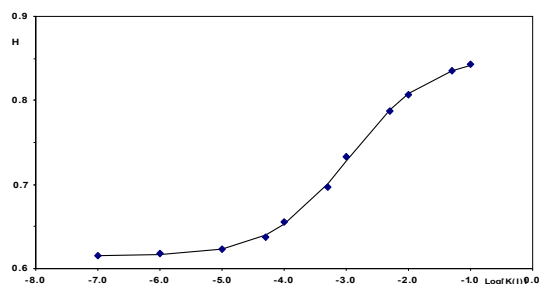
### Use of Hue Coordinate of the HSV Color Space as a Quantitative Parameter for Bitonal Optical Sensors

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The hue or H component of the HSV color space has been studied as a quantitative analytical parameter for bitonal optical sensors. The main feature of this color space is the representation of the cognitive color information in a single parameter, the H coordinate. The robust nature of this parameter affords it superior precision for the measurement of sensor materials that change colors with the speciation of some indicator molecule. This parameter has been compared to RGB intensity and RGB absorbance along with differences and ratios of both intensity and absorbance, and has been demonstrated to be 2 to 3 times superior. The H value maintains its superior precision with variations in indicator concentration, membrane thickness, detector spectral responsivity, and illumination. Because this parameter is stable, simple to calculate, easily obtained from commercial devices such as digital cameras and scanners, continuous over the entire color gamut, and bound between values of 0 and 1 it shows great promise for use in a variety of sensing applications including imaging, automated analysis, pharmaceutical sensing, lab-on-a-chip devices, and quality control applications. We have studied a one-shot sensor for K(I) based in ionophore-chromoionophore chemistry using a scanner as imaging technique. In order to check the independence of this parameter to changes in path length and chomoionophore concentration we prepare membranes of different thickness and with different concentration. The coefficient of variation obtained was about 0.5 % in both cases. We also performed a precision study, intra and intermembrane, and the results coefficient of variation obtained were above 0.3 %. Finally we calibrated the sensor (see figure) with 11 potassium concentrations obtaining five values from each solution. The calibration is similar in shape as obtained with other optical technique but the precision obtained is much more higher.



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## **P101-B1**

### **Overoxidized Polypyrrole for Sensor Application**

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Polypyrrole dopes an anionic compound, which is dedoped on overoxidation of the polypyrrole. The overoxidation can therefore be used to create a shape complementary cavity for an anionic analyte. We have been developing amperometric sensors using this simple molecular imprinting technique, and overoxidized polypyrrole films with several different complementary cavities for anionic analytes have successfully been prepared on carbon and metallic electrodes.

Here we show some of those results for amperometric adenosine triphosphate (ATP) sensors, aiming at food safety applications. The sensor has a small carbon electrode covered with an overoxidized polypyrrole film imprinted with an ATP molecule. The sensor operated in the triple pulse amperometric mode and was characterized in both the batchwise and flow-injection measurements. We have detected trace levels of ATP without employing any separate preconcentration techniques. We also confirmed excellent selectivity of the oPPy film in the flow injection studies. The ratio of ATP to AMP in the peak height was as high as 16 at the overoxidized polypyrrole electrode, while it was only 1.1 at a bare electrode (AMP, adenosine monophosphate) .



## P102-B1

### New Sulfadiazine-Selective Sensors of Molecularly-Imprinted Sol-Gel Material

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Sulfadiazine (SDZ) is one of the few sulpha drugs used today. Its analytical control is required in several kinds of samples, such as commercial drugs, and biological and food samples. Ideally, this could be achieved by non-destructive and highly selective/sensitive measurements, such as those employing ion-selective electrodes (ISEs). In the best of the authors knowledge, only one paper have reported SDZ quantification employing ISEs [1]. In that work determinations were made by using *bis*(triphenylphosphoranilidene) ammonium-SDZ, tetraoctylammonium bromide, or iron (II) phthalocyanine electroactive materials. These kinds of materials have been the vital components of potentiometric sensors over the past decades. Designing new materials that are complementary to the size and charge of SDZ could lead to more selective interactions, thus enhancing the selectivity of the sensing unit. The use of molecular imprinted polymers can fulfill these requirements as the fabricated polymers are usually region and electrostatically specific to the target molecule. This work proposes the construction of SDZ selective electrodes of imprinted sol-gel. The imprinted sol-gel was also grinded and used as electroactive material on PVC SDZ selective membranes. PVC sensors showed the best analytical behavior in terms of limits of detection and linear ranges, with slopes and detection limits ranging 33.3 – 52.7 mV decade<sup>-1</sup> and 4.78 µg mL<sup>-1</sup>, respectively. The resulting sensor is applied to the potentiometric determination of SDZ in real samples.

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## P103-B1

### Characterization of Natural Products Used in the Ancient Maya Mural Paintings as Organic Binders by Pyrolysis-Gas Chromatography-Mass Spectrometry

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The chemical analysis of Maya mural painting began in 1931 at the laboratories of the Carnegie Institution of Washington with the study of the *Chichen Itzá* site (*Yucatan, Mexico*). However, the first specialized research on the materials and techniques of these Pre-Columbian works of art arose during the last years of twenty century with the scientific support of several Research Institutions of Central America, Europe and EE.UU. Based on the historical writings dated in colonial times (16<sup>th</sup>, 17<sup>th</sup> and 18<sup>th</sup> centuries), the study presented here is focused on the chemical characterization of natural products presumably used as binders in Maya mural paintings. For this purpose, Py-GC-MS analysis of *chaka'* terpenoid resin (*Bursera simaruba*) and the gums extracted from the *holol* (*Heliocarpus spp.*), *ha'bin* (*Piscidia piscipula*), *pixoy* (*Guazuma ulmifolia*) and *piñon* (*Jatropha curcas L.*) has been performed in order to establish marker compounds able to identify these organic materials in paint samples when they are present at low concentration. On line derivatization using hexamethyldisilazane has been applied in order to improve the analytical results. *Acknowledgements:* Financial support is gratefully acknowledged from the Spanish "I+D+I MEC" project CTQ2008-06727-C03-01 and 02/BQU supported by ERDEF funds.

## P104-B1

### Optimization of Reference Materials for the Identification and Correlation of Degradation Rates of Historic Silk Textiles

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The most evident signature of the deterioration of ancient historic textiles is their lack of strength and flexibility, and thus, the impossibility of handling them in a safe way. In this study, such a qualitative visual evidence was approached from a quantitative perspective. Colorimetry, Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy, Gas Chromatography-Mass Spectrometry, Scanning Electron Microscopy, and X-Ray Diffraction analyses were performed for a set of UV aged silk samples. Basic tensile properties such as elongation at break and strength to failure were also studied. The objective of such a multi-analytical approach was to identify the degree of chemical and mechanical degradation of ancient silk samples by correlating the results obtained from these to those from new silk samples subjected to UV artificial ageing. In this study the rate of silk yellowing (as a function of specific UV ageing cycles) has been correlated to the chemical structural changes obtained by ATR-FTIR, the variations of the ratio of amino acids observed in the chromatograms, the changes of the mechanical properties initially observed as well as the microstructural changes within the crystalline and amorphous content of the protein fibers have also been investigated in detail.

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## P105-B1

### Study on the Behaviour of “Silk Pongee” for the Consolidation of Historical Textiles by SEM-EDX, Spectroscopic Techniques and Mechanical Properties

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Consolidation is one of the most delicate and decisive process to preserve any fabric. The addition of new sewed fabrics such as ‘silk pongee’ to the original textiles is a common practice nowadays to consolidate, reinforce and compensate losses in historic textiles. Pongee is a plane weave silk used as reinforcement fabric due to its low density of the yarns in both the weft and warp directions that provides a considerable transparency as well. Results obtained from this research focus on the physic-chemical characterization of pongee. Data from specimens subjected to artificial accelerated ageing (dry and wet heat and UV radiation) are also shown. A multi-method approach is proposed combining microscopy (LM and SEM/EDX) and spectroscopy (reflectance, FTIR) techniques and traction tensile tests in order to characterize the overall behavior of the ‘silk pongee’ before/after ageing processes. Tensile tests were run in weft and fill directions. Stress strain curves helped to determine the stiffness and flexibility of ‘silk pongee’ as well as their elongation and strength to failure within specific environmental conditions. Comparison of Vis and IR spectra obtained in ATR mode from different areas of the pongee textile has evidenced that the yellowness and oxidation grades increase as follows: firstly UV radiation, secondly wet heat and finally dry heat. LM and SEM examination enabled the identification of empty fiber and microfissures in different areas on the surface. The measured mechanical properties are mainly focused on the maximum deformation and strength of samples prior failure.

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## P106-B1

### **A New Way of Analysing Gum Arabic in Mediaeval Documents from al-Andalus Using Capillary Electrophoresis**

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Gum arabic is a polysaccharide used in the manufacture of inks during the al-Andalus period in Spain. It was employed as a binding material in the preparation of inks and also of paper. It was added to the inks in conjunction with other oils, sulphuric acid and water because of the viscous properties of its solutions. It made the other components of the inks easier to apply. References to the use of gum arabic are to be found in various written records concerning manuscripts from Arab times.

For an analytical bibliographer or any other scholar interested in mediaeval Arab texts it is essential to be able to analyse the substances contained in the components of these documents. The detection of gum arabic in the manuscripts of the Historical Archives of the Province of Granada (Archivo Histórico Provincial de Granada) is important both in their preservation and their classification as belonging to the al-Andalus period or not. It is of equal importance in knowing how to go about their restoration when necessary.

Samples available for analysis of these documents are limited and so any such study needs to be highly selective and sensitive. We have found that capillary electrophoresis is a very suitable technique in these circumstances. The monosaccharides that constitute gum arabic are D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid, but we have identified five others in the documents, due presumably to the use of other types of gums in their manufacture: L-fucose, D-glucose, D-mannose, D-xylose and D-galacturonic acid. We have applied the results of these analyses to classify twenty-one manuscripts as being not only of Arab origin but also specifically al-Andalus.

This work has served to improve our capacity to identify gum arabic and other gums by capillary electrophoresis by increasing the resolution of the electropherograms found in other studies.

## P107-B1

### Characterization of the Black Crusts Composition of an Historic Monument Exposed to Urban Atmospheric Pollution

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Throughout Europe, general evidence exists that historical buildings are dramatically damaged due to the attack of air pollutants present in today's atmosphere. Typically, damage layers result from wet and dry deposition processes combined with gypsum crystal growth and are composed of transformation products, deposited aerosols, and fragments of the original substrate [1]. These black crusts contain airborne organic pollutants (nitrogen dioxide, sulphur dioxide, carbon monoxide, polycyclic aromatic hydrocarbons, etc) and a wide range of particulate matter which are entrapped in the mineral matrix.

This work aims to focus on the role of atmospheric pollution, and particularly in the deposition of pollutants, in the degradation of ancient buildings.

Scanning Electron Microscopy and Fourier transformed infrared spectroscopy were used to characterize the composition of black crusts collected from the facades of one historical building, sited in Oporto metropolitan area (Portugal). Organic compounds, namely, eighteen polycyclic aromatic hydrocarbons (16 from the U.S. EPA list, dibenzo(a,l)pyrene and benzo(j)fluoranthene) were determined by liquid chromatography with fluorescence detection after microwave-assisted extraction [2]. Mean contents as high as 1348 ng/g of total polycyclic aromatic hydrocarbons ( $\Sigma$ PAHs) were measured in the black-crusts of the rain-protected façades. Approximately 81% of the detected compounds are four to six rings PAHs which have high molecular weights and being some of them classified by the International Agency for Research on Cancer as possible carcinogenic to humans. The relations between some of the individual PAHs indicated combustion of diesel as the main source of atmospheric deterioration for the studied historical building.

#### Acknowledgements

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## P108-B1

### Separation of Natural Yellow and Red Dyes Using Capillary Electrophoresis and its Application to Ancient Graphic Documents

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Since old times Man has resorted to natural substances in order to obtain dyes and pigments especially for his artistic expression. Among the broad range of natural dyes, there is a big variety of yellow and red dyes. The main objective of this study is the identification of natural yellow and red dyes commonly used in graphic documents from the 17th century onward, like gamboge, most often extracted by tapping from the *Garcinia hanbūrū* tree, saffron from saffron crocus (*Crocus sativus*), weld (*Reseda luteola* L.), and Stil de grain lake, which is a water extract from rhamnus berries (*Rhamnus catharticus*), brazilin from brazilwood (*Caesalpinia echinata*), cochineal (*Coccoidea*) and madder (*Rubia Tinctorum*).

The selected technique for this purpose is capillary electrophoresis, since it is highly efficient and low solvent-consuming.

Identification of the compounds was carried out by comparing the UV-VIS spectrum from each peak in the electropherogram with the specific UV-VIS spectrum from each dye or organic pigment standard. The method was optimised modifying the following parameters: pH, type of buffer, concentration of the electrolyte, separation voltage, injection time and influence of organic modifiers.

The analytical results were classified according to the composition of known dyes or to specific compositional patterns of unknown dyes.

A specific sampling technique was applied to avoid damage of the ancient maps.

The developed capillary electrophoretic method for analysing yellow and red dyes can be applied to the study of ancient graphic documents, in this case ancient maps of the Royal Chancellery Archives of Granada (Spain). Nineteen of them contained gambogic acid sometimes mixed with crocetin, whereas nine maps contained carminic acid. Luteolin, quercitrin, quercetin, brazilin and madder could not be identified.

The identification of dyes in these graphic documents will contribute to the restoration process and help to determine optimum conservation conditions.

## **P109-B1**

### **Scanning Electron Microscopy/Energy Dispersive Using X-Ray Analysis of Papers that Have Been Treated to Look Old**

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Often, in counterfeit documents, paper must have an aged appearance. To achieve this looking, there are some homemade methods such as a bath in tea or coffee, in Modena vinegar, etc.

In this paper a deep study about the transformation suffered by the fibers of paper when submitted to those methods have been done by scanning electron microscopy/energy dispersive using X-Ray analysis (SEM-EDX). This technique allows the document examiner to make evident the manipulation.



## **P110-B1**

### **Assessment of the Air Quality inside the Plantin-Moretus Museum – Case Study**

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The Plantin-Moretus Museum in Antwerp, Belgium, houses precious collections of old printed books and historic typographical material, among others two of the oldest printing presses in the world, and was put, in 2005, on the list of UNESCO World Heritage Sites. In order to assess the air quality inside the museum, three consecutive sampling campaigns were performed in different seasons of the year: autumn, winter and spring. For collecting of bulk particulate matter, three sampling sites were chosen: the courtyard (outside the building), the old printers' workshop on the ground floor and one of the exhibition rooms on the 1<sup>st</sup> floor. Two fractions of particulate matter, PM 2.5 and PM 10, were sampled actively for 24 hours on Teflon filters. After sampling, the weight and the bulk elemental concentration were obtained for each sample, with use of energy dispersive X-ray fluorescence (Epsilon 5 instrument, PANalytical, The Netherlands). The gas monitoring of NO<sub>2</sub>, SO<sub>2</sub> and O<sub>3</sub> was carried out by means of the diffusive samplers Radiello<sup>®</sup> (Fondazione Salvatore Maugeri IRCCS, Italy). The concentrations of gases were determined by ion chromatography and UV/VIS-spectrophotometry. The mass concentrations of PM 2.5 and PM 10, during all three seasons, were found lower inside the museum than outside (the highest I/O ratio reached the value of 0.71). Additionally, for PM 2.5, I/O ratios were higher on the 1<sup>st</sup> floor than on the ground floor, meanwhile for PM 10 these values were comparable. Nevertheless, all the ratios have been decreasing with the passing seasons. The concentrations of gaseous pollutants were generally higher outside than inside, indicating, as in case of particulate matter, small air exchange indoors/outdoors, although the differences in average concentrations of SO<sub>2</sub> and O<sub>3</sub> in galleries and in showcases of the museum, regarding changing seasons, are not yet clear.

## P111-B1

### Comparative Performances of Different Hydrophilic Interaction Liquid Chromatographic Supports in a Catecholamine Analysis Perspective

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Introduced by A. Alpert<sup>1</sup> in 1990, hydrophilic interaction liquid chromatography (HILIC) was claimed to be a valuable alternative chromatographic strategy for very hydrophilic solutes. HILIC mode requires a polar stationary phase in association with a hydro-organic mobile phase to elute polar solutes in an increasing polarity order.

The up going success of HILIC led to an important increase of the number of commercially available supports. Due to the fact that the retention mechanism was not clearly explained, one might be lose when having to choose the appropriate column.

The different commercially available HILIC supports can be classified in relation to their functional group as: neutral (diol, amide, and cyano), positively charged (amino, triazole), negatively charged (bare silica as wholly porous particles or fused core particles) and zwitterionic (sulfobetaine).

Catecholamines represent a good compounds family for column testing as they contain both basic and acidic compounds at a given pH value in a large pH range. The following catecholamines (adrenalin, noradrenalin, dopamin), indolamines (serotonin and 5 hydroxy tryptophan) and their precursors and metabolites (3,4-dihydroxy-phenylalanin, 3-methoxytyramin, tryptophan, homovanillic acid, tyrosin and 5-hydroxyindole-3-acetic acid) were selected for our column tests.

For the column comparison several parameters important for the HILIC optimization have to be taken into consideration. The salt nature and concentration, the organic modifier nature and percentage, the temperature and pH influence have been tested on different representative polar supports in order to obtain the best separation and to better understand the retention mechanism.

## P112-B1

### Capillary Chromatography System Using an Open Fused-Silica, Polyethylene, or Poly(Tetrafluoroethylene) Tube that Works under Laminar Flow Conditions

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Capillary chromatography including capillary electrochromatography, micellar electrokinetic capillary chromatography, and capillary high-performance liquid chromatography using packed and monolithic capillary column have been attracted a great attention in the research area of analytical chemistry and separation science since the last century. Most of capillary chromatography systems feature rapid measurement, easy procedure, inexpensive and small-size apparatus, small sample volume, and low cost. However, as far as we know, a new concept concerning capillary chromatography has been little proposed for the last decade.

In this study we developed the capillary chromatography system using open capillary tubes, fused-silica, polyethylene, and poly(tetrafluoroethylene) tubes, and an aqueous-organic solvent mixture (water-acetonitrile-ethyl acetate mixture) as a carrier solution; the system worked under laminar flow conditions. Model analyte mixture solutions, such as 2,6-naphthalenedisulfonic acid and 1-naphthol, eosin Y and perylene, bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein and 1,1-bi-2-naphthol, and 2,7-naphthalenedisulfonic acid and p-nitroaniline, were injected into the capillary tube by a gravity method. The analyte solutions were subsequently delivered through the capillary tube with the carrier solution by a micro-syringe pump. The analytes were separated through the capillary tube and detected on-capillary by an absorption detector. For example, when using poly(tetrafluoroethylene) tube, 2,6-naphthalenedisulfonic acid and 1-naphthol were detected in this order with a carrier solution of water-acetonitrile-ethyl acetate (volume ratio 15:3:2), while they were detected in the reverse order with a carrier solution of water-acetonitrile-ethyl acetate (volume ratio 2:9:4). The other analyte solutions were similarly separated by the system; the elution times of the analytes could be easily reversed by changing the component ratio of the solvents in the carrier solution. The separation performance of the system is discussed based on the results obtained using a fused-silica, polyethylene, and PTFE capillary tube.

## P113-B1

### Functionalized MWCNTs as Stationary Phase for Gas Chromatography

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Carbon nanotubes (CNTs) are known to have higher thermal and mechanical stability. For these characteristics and for their capability to give  $\pi$ - $\pi$  stacking interactions with aromatics and unsaturated compounds, they have been employed as gas-chromatographic packing material [1]. In this study commercially available pristine multi-walled carbon nanotubes (MWCNTs), o.d. 30-50 nm, length 10-20  $\mu$ m, and MWCNTs functionalized with amino or carboxy terminating chains have been used as stationary phase for GC-FID separation of different classes of analytes and characterized for their thermodynamical and adsorption properties; the interaction of the derivatized MWCNTs with different analytes is strictly dependent on their nature, being acidity and polarity the main discriminant for the interaction. Aromatic hydrocarbons interacts well with both pristine and derivatized MWCNT, as  $\pi$ - $\pi$  stacking interactions play a main role.

Glass column (3 mm i.d., 50 cm length or 1 mm i.d., 90 cm length) has been filled with 2 g of MWCNTs. A carrier flow rate not lower than 50  $\text{cm}^3/\text{min}$  is necessary to achieve good separations in reasonable times. Actually, CNTs give high resistance to the flow of the carrier gas, due to their aggregation as a consequence of Van der Waals interactions.

Satisfactory results have been obtained in the separation of C5-C8 alkanes (temperature from 240 to 310°C, 4°C/min) and a quantitative determination of components of lighting gas was possible in these conditions. Identification of GPL components (propane, butane, isobutane, pentane and isopentane) was achieved (temperature from 70 to 85°C, 3°C/min). Strong adsorption was observed in the separation of aromatic hydrocarbons such as benzene, toluene and p-xylene (290-320°C, 3°C/min). Efficient separation of alcohols (C1-C4) was also gained with a temperature program from 165 to 240°C, 5°C/min.

Halogenated hydrocarbons (i.e. dichloromethane, chloroform, dichloroethane) were well separated with elution within 10 minutes working in isothermal conditions (170°C).

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## P114-B1

### Characterization and Evaluation of HPLC Stationary Phases Based on Novel Core-Shell Silica Particles for Fast Separation Analysis of Environmental and Biological Important Compounds

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This research examines the characterization of HPLC stationary phases, based on novel core-shell silica particles, prepared by a novel synthetic route that is referred to as Seeded Growth Mesoporous Shell (SGMS). The SGMS core-shell silica particles has BET surface area of 205 m<sup>2</sup>/g, average pore diameter of 90 Å and pore volume of 0.33 cc/g. We presented results of the derivatisation of the SGMS core-shell silica surface with several stationary phases (including: phenyl and fluorinated phenyl phases, polar embedded and hydrophilic interaction (HILLIC) phases). The resultant bonded material having a total surface coverage of 3.4 – 3.7 μmole/m<sup>2</sup>, were fully characterized by elemental and thermogravimetric analysis (TGA). In addition, <sup>29</sup>Si and <sup>13</sup>C solid state NMR characterization was employed to investigate the surface bound material on the SGMS core-shell silica particles. To maintain a significant level of full comparison, the resultant bonded phases were packed in a 2.1 x 50 mm stainless steel column. The separation of 16 priority polyaromatic hydrocarbon (PAH) was evaluated on a polymeric and monomeric C18 phases that were prepared on the SGMS core-shell silica particles. In a similar manner, the phenyl and fluorinated phenyl phases prepared on the novel SGMS core-shell silica columns was used for further studies of priority PAH's. Further evaluation of the rapid separation analysis of 4 tricyclic antidepressant drug compounds, 5 nucleotide bases, an anti-mitotic anticancer drug and 2 biological important (antioxidant) dipeptide were conducted using the polar-embedded and HILLIC phases prepared on the SGMS core shell silica particles. From the chromatography evaluation data obtained, the SGMS core-shell silica have shown to be a valuable new material for the advances in column technologies, facing the challenging applications in environmental and biological analyses.

## P115-B1

### New TLC Mode with Gas-Phase Control

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When optimizing the separation process in TLC most attention is usually paid to selection of the optimum combination of mobile phase (MP) and stationary phase. However, separation quality in TLC is influenced also with the gas phase inside the chromatography chamber, consisting of MP solvent vapors and the ambient air. Therefore, the effect of humidity or some other solvents vapors modifying adsorbent activity is taking usually in consideration.

We have shown that the gas phase can play an active role in TLC, in particular, the acidic or basic gas phase can interact with stationary and mobile phases and also with the moving along the TLC plate dissolved sample components [1,2]. Control of pH in the approach proposed here differs from standard practice because it is based on the forced dynamic or static creation of an acidic or basic gaseous atmosphere in a special TLC chamber that replaces the initial gas phase during the chromatographic process. In this case the acidity of MP changes gradually directly on the surface of the TLC plate (what is the same as dynamic pH gradient) and the acid-base speciation for each mixture component changes at different time which depends on the  $pK_a$  values of substances. This approach enables selective alteration of interaction of sample components with the eluent and adsorbent and, therefore, changes the  $R_F$  values, selectivity factor and resolution in TLC.

This communication describes the use of gaseous carbon dioxide, acetic acid and ammonia vapors to improve a separation of mixtures of benzoic acids or aromatic amines on normal-phase, reversed-phase and polyamide TLC plates. Different types of TLC chambers for new TLC method are considered and discussed. A detailed analysis of the time, static and dynamic mode of gas exposition as well as nature of MP and TLC plates is performed. The chromatographic parameters describing the efficiency and resolution TLC separation of the substances mentioned using new mode in comparison with traditional technique are presented. The proposed method is a novel means of control of the chromatographic process and in this way extends the analytical capabilities of TLC.

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## P116-B1

### Multisyringe Flow Injection Technique Allows Fast and Sensitive Ion Chromatographic Separations Based on the Use of Surfactant Coated Short Monolithic Columns and Post-Column Chemiluminescence Detection

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An emerging issue in separation techniques is the use of mesoporous silica monoliths as stationary phases for liquid chromatography. Columns made from this material, have a porous silica skeleton with covalently bonded octadecyl (C18) groups. So, these reverse phase columns can be coated with cationic surfactants, allowing the possibility to achieve anion separations. The use of short monoliths (e.g. 10mm) allows separations in aqueous medium at high flow rates (4mL min<sup>-1</sup> flow rate is achieved using a low-pressure syringe pump, max. pressure 6 bar), but with a concomitant decrease in terms of selectivity in comparison with classic ion chromatographs. This drawback could be balanced by using more selective detection systems, such as chemiluminescence detection.

In this work, is proposed a new method that combines the last two features for the determination of trace oxalate in diverse types of fluidic samples. For the implementation of these two sample treatments in an in-line and completely automated fashion, it has been implemented in the multisyringe flow injection technique. This technique allows the simultaneous use of up to 4 syringes. In this work, one syringe is used for the chromatographic separation, and two additional syringes are used for the in-line post-column injection of the chemiluminescent reagents.

For the separation of oxalate from sample matrix components, a silica-C18 short monolith (10 x 4.6mm) is coated with 5mmol L<sup>-1</sup> CTAB solution (5% ACN/ 95% H<sub>2</sub>O). As eluent a 2.5mmol L<sup>-1</sup> KHF solution (pH = 7) was selected. Selected reagents for chemiluminescent detection were 5mmol L<sup>-1</sup> Tris(2,2'-bipyridyl)dichlororuthenium(II) and 5mmol L<sup>-1</sup> Cerium(IV) in 0.1mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. The proposed method presents a LOD and LOQ of 0.025 and 0.034mg L<sup>-1</sup> respectively, and it has a linear response of up to 12mg L<sup>-1</sup> of oxalate. Furthermore, it has an injection throughput of 33h<sup>-1</sup>.

## P117-B1

### Options of Electrolyte Systems for Analysis of Weak Anions by Coupling of Capillary Isotachopheresis with Mass Spectrometry

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Although coupling of capillary zone electrophoresis (CZE) with mass spectrometry (MS) belongs nowadays to frequently used powerful technique, coupling of capillary isotachopheresis (CITP) with MS was applied sporadically and almost exclusively for analyses of cations. For CITP-MS, choice of suitable electrolyte systems is limited to several volatile electrolytes where correct migration and separability has to be ensured. The analysis of anions in these systems is complicated; not only in the case when directly  $\text{OH}^-$  is used as terminator, hydrocarbonate can be present in the system and affect the separation process by formation of mixed zones. Each of two components of the mixed zone can form its own zone when its concentration ratio exceeds the value corresponding to the composition of the mixed zone and thus for two analytes two zones can be detected but only one of them corresponds to the analyte itself. The set of analytes that can form mixed zones (not only) with hydrocarbonate can be estimated by contours in zone existence diagrams (ZED) constructed for a given electrolyte system. Here, ZEDs constructed for several anionic electrolyte systems using chloride, acetate, or hydrocarbonate as leading ions, and ammonium and some weak bases as counter-ions with  $\text{OH}^-$  as potential terminator will be presented. CITP analyses of model and serum samples performed in some of these systems will be shown and applicability of the systems will be discussed.

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## P118-B1

### Ion Chromatography in Analysis of Imidazolium and Pyridinium Ionic Liquid Cations

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Ionic liquids (ILs) are salts with melting point often below 100°C, comprised of molecular ions, organic cations and organic or inorganic anion. Despite of their ion character, generally, the separation and analysis of ionic liquid cations, reported so far, are based on high performance liquid chromatography methods employing a variety of column packings and mobile phases.

This work is focused on the study of effect of concentration and pH of methanesulfonic acid as well as volume percent of acetonitrile in the mobile phase mixtures, on retention of imidazolium and pyridinium IL cations by ion chromatography. Analyses were performed with the use of cation-exchange column employing crown ether and carboxylate and phosphonate cation-exchange sites under the isocratic elution mode. The IL cations eluted by 2.5, 5, 7.5 and 9mM MSA followed the elution order typical for reversed-phase HPLC. Changes of elution order were observed if IL cations were eluted by 1mM MSA. Especially using a higher percentage of acetonitrile in the mobile phase (in excess of 65% (v/v)) the analytes showed normal phase HPLC retention behaviour for IL cations. With increasing MSA concentration, i.e. decreasing pH, the retention of IL cations decreased. Two concentration of MSA, 2.5 and 5mM were chosen to separate IL cations. Unfortunately, cations, such as EEIM, PMIM and BEIM, MBPy and EBzMIM, HMPy, HMIM were not separated properly. The low percentage of acetonitrile can help to separate EEIM and PMIM, but simultaneously longer analysis times preclude the detection of HMPy, HMIM and OMIM because of peaks bordering.

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## **P119-B1**

### **Special Features of Portable GC-MS for Verification of Chemicals Related to The Chemical Weapons Convention**

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The Organisation for the Prohibition of Chemical Weapons (OPCW) is the implementing body of the Chemical Weapons Convention (CWC) which prohibits the development, production, acquisition, stockpiling, retention, transfer or use of chemical weapons by States Parties. One of the verification activities that are mandated by the CWC involves the analyses of samples conducted by OPCW inspection teams. On-site analysis of samples is often necessary to fulfil the inspection mandates. Hyphenated gas chromatograph - mass spectrometer (GC-MS) has proved to be the most dependable analytical equipment for precise identification of the CWC-related chemicals.

With the experience gained over the years during on-site analysis the inspection GC-MS has undergone several modifications and incorporated some unique add-on features. Besides the general operational requirements such as ease of use, including operation under full chemical protection and security against possible tampering, inspection GC-MS supports liquid injection as well as classical mass spectra. It is also equipped with specially designed analytical database for CWC-related compounds. In order to preserve confidentiality in inspected sites, the instrument is often operated in a blinded-mode for non-targeted identification. A unique automatic software search for the presence of one or more target compounds in the analysed sample is done in such a way that even in the case of co-eluting compounds, without the intervention of the inspectors, and with the identity of the compounds in the sample remaining concealed, a target compound is identified. The chromatographic as well as mass spectrometric critical parameters for the compound are also monitored

The present poster traces the development of portable GC-MS as an inspection tool and elaborates on its special add-on features and modifications. A summary of performance evaluation of various models is also briefly presented that guided the selection of current GC-MS.

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## P120-B1

### **Bilayer Lipid Discs as Pseudostationary Phase in Capillaries Coated with Noncovalent Polyvinylpyrrolidone-Based Copolymer: Tool for Partition Studies by Capillary Electrophoresis**

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Artificial membranes are used as models of the biological membranes for studying the properties and processes of membranes. Polyethylene glycol (PEG)-lipid stabilized bilayer discs, that are planar and circular in shape and exhibit excellent long-term stability, have also been employed as model membranes in partition and interaction studies. These discs are found in lipid mixtures containing polyethylene glycol lipids where the combination of a high bending rigidity and low PEG-lipid/lipid miscibility favors disc formation.

The potential of bilayer discs in the partitioning of pharmaceuticals has been studied for the first time by capillary electrophoresis by Boija et al. [1]. In our study DSPC/cholesterol/DSPE-PEG5000 lipid discs were employed as a pseudostationary phase in the partial filling mode of capillary electrophoresis. Capillaries were coated non-covalently with poly(1-vinylpyrrolidone)-based copolymer [2]. Copolymer coating masked negative charges of the capillary wall and minimized interactions between pharmaceuticals and capillary wall. It also reduced the migration of the pseudostationary phase. Time needed for the pharmaceuticals to pass through the capillary was found to be proportional to the lipid amount used in the pseudostationary phase. Partitioning constant, logK, could be successfully determined for positively charged pharmaceuticals selected for the study. Opposite to drug partition chromatography the discs were not immobilized on a matrix or on the surface. The stability of the discs and the possibility to easily vary the disc composition strengthens the use of bilayer discs as pseudostationary phase in partial filling mode of CE for partition studies.

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## P121-B1

### Relationship between Structure and Thin-Layer Chromatographic Lipophilicity Parameter of Some Arylpiperazines

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Arylpiperazines have affinity for dopaminergic and serotonergic receptors. They have been known for some time to have activity profiles similar to those of „atypical“ antipsychotics (i.e. they have mixed  $D_2/5HT_{1A}$  activity). In our previous paper [1-3] chromatographic behaviour of some arylpiperazines, which exhibit affinity towards dopaminergic and serotonergic receptors, was investigated. Various normal- and reversed-phase chromatographic systems were chosen to study the effect of nature of substituents in aryl part of molecule on their retention.

New compounds with halogen atom introduced into benzimidazole and benzimidazole-2-thion dopaminergic pharmacophore of the 6-[2-(4-arylpiperazin-1-yl)ethyl]-1*H*-benzimidazoles and 4-{2-[5-(1*H*-benzimidazole-2-thione)]-1-arylpiperazines} in which arylpiperazine part of molecule were selected according to known structure-affinity requirements, have been synthesized. Based on our interest in the biological activity of the aforementioned newly synthesized compounds, in this work the lipophilic character of arylpiperazines was studied by means of thin-layer chromatography, using different aqueous solvent systems and two stationary phases, RP-18 silica and alumina.

From the results obtained it can be seen that an increase in water content of the mobile phases results in increased both selectivity and retention. In all cases a linear dependence between volume percent of organic modifier and corresponding  $R_M$  values was obtained. On this way evaluated lipophilicity were correlated with other solute-related parameters, which reflect their structural features, by multiple linear regression. The obtained model provides valuable data regarding the evaluation of lipophilicity of solutes, the prediction of relative biological activity within a set of compounds, and understanding the separation mechanism in a given chromatographic system.

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## **P122-B1**

### **HPLC-Chip/MS Approaches for High Sensitive Analysis of Metabolites and Pharmaceuticals in Biological Matrix**

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Analysis and quantitation of drugs as well as their metabolites in the early drug discovery phase is usually challenged by small sample volumes and low concentration levels in complex biological matrices. DMPK (drug metabolism and pharmacokinetics) studies are preferentially performed using small animals (mice), what significantly helps to decrease breeding and sustainment costs. This in turn makes great demand on the sensitivity of the employed analytical technique.

HPLC-chip/MS is an easy to use nano-LC platform that can handle smallest amounts of sample and achieves superior sensitivity due to an integrated nano-ESI emitter. Its application to the analysis of pharmaceuticals in biological matrices is presented and discussed. The chip-based technology overcomes problems and limitations of classical nano-LC approaches (cumbersome operation, inadequate extra-column volume and thus band broadening, lacking reproducibility, leaks), as all HPLC parts, like enrichment and separation column, valve switching, connectors, unions, tubings, frits and ESI tip, are integrated on one laminated polymeric chip.

Novel chip designs, tailored for the analysis of small pharmaceutical compounds with strongly different polar properties (logP range 0-5), are introduced. As reversed phase (RP) chips are restricted to the enrichment and separation of compounds in highly aqueous sample solutions, different combinations of trap and analytical column stationary phases (HILIC and IEX materials) have been studied in order to simplify the typical DMPK workflow, which mostly ends up with highly organic samples (60-80 % organic) after sample preparation (protein preparation). The capability of the different chip-based approaches will be compared and discussed.

## P123-B1

### Determination of Indole Alkaloids Based Pharmaceuticals with DNA-Amperometric Sensor and Enzyme Immunoassay Test-System

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The development of the new methods of pharmaceutical analysis of multicomponent mixtures aiming at choosing correct dosage and administration, studying their pharmacokinetics and reducing possible adverse effects is a relevant task of analytical chemistry. The use of electrochemical methods in combination with bioaffine interactions provides the opportunity to study and simulate anticancer drug interaction with DNA. In addition, electrochemical DNA-sensors allow rather fast, omitting long sample preparation, but highly sensitive and selective determination of anticancer medicines.

The study is devoted to the development of the new methods of analysis of indole alkaloids based medicines such as vincristine (oncovine) and ajmaline acting as antumor agents.

Bioaffine based methods of determination of ajmaline being known as a cytostatic agent and a heart medicine were developed. The methods are based on the use of the amperometric DNA-sensor and enzyme immunoassay test-system with spectrophotometric registration of analytical signal. It is possible to concentrate the alkaloid efficiently at the biosensor from the examined solutions due to its complexing with native immobilized DNA. Both optimal conditions of the concentration and optimal conditions of biosensor's renewal letting it to be used repeatedly were found. The analysis takes 25 – 30 min,  $c_{\min}$  is  $3.0 \times 10^{-10}$  mol/l ( $s_r$  0,33).

Immune interaction of ajmaline with corresponding antibodies labeled with horse-radish peroxidase was employed in the test-system providing  $c_{\min}$  of  $4.0 \times 10^{-9}$  mol/l ( $s_r$  0,33). Ajmaline determination in model solutions of blood serum and dosage forms (pellets and injections) was carried out by both methods.

Anti-cancer vincristine (oncovine) interaction with DNA was studied and determination was carried out using the amperometric DNA-sensor. Renatured DNA (r-DNA), being covalently immobilized in cellulose nitrate matrix, was used for the preparation of sensor's biosensitive part taking into consideration the DNA-vincristine interaction mechanism. Bioaffine membrane concentration and reactivation of the sensor was performed followed by measuring of anti-cancer drug's reduction peak current at  $-0.9$  V which was used as an analytical signal. Vincristine complexing with immobilized molecules of r-DNA (r-IDNA) was studied in order to estimate effector's affinity to the specific form of DNA and to optimize the developed method of analysis. The affinity binding constant  $K_{\text{bind}}$  for vincristine and r-IDNA couple was calculated by Scatchard's method based on voltammetric data to quantify a specificity of the complexing. The obtained  $K_{\text{bind}}$  value is high enough  $((5.0 \pm 0.4) \times 10^5$  l/mol) thus confirming high specificity of the complexing with r-IDNA. The duration of the assay is 40 min. The developed bioaffine method of vincristine determination is characterized with  $c_{\min}$  of  $2.0 \times 10^{-9}$  mol/l ( $s_r$  0,33), with the lack of the necessity for long sample preparing and was examined for blood serum assay and dosage form analysis for the active substance.

## P124-B1

### Manganese Mapping by SR-XRF in Protein Spots of Plasma and of Liver and Muscle Tissues of Nile Tilapia (*Oreochromis Niloticus*)

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Metalloproteins and metal ions bound to proteins represent a large portion of the total number of proteins. It has been estimated that approximately 40% of all proteins and enzymes require a metal ion to become biologically active [1]. Manganese is an essential micronutrient in fish nutrition. Mapping manganese in various tissues, particularly in proteins of these tissues separated by electrophoresis, can provide important information for the area of fish physiology and nutrition [2]. In view of the foregoing, the present study aimed to map manganese in protein spots of samples of plasma, muscle and liver of Nile tilapia (*Oreochromis niloticus*). The proteins of the plasma, muscle and liver samples were separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The spots were cut out from the gel, dried under an infrared lamp, and analyzed by synchrotron radiation X-ray fluorescence (SR-XRF) with a 500  $\mu\text{m}$  beam, with readings taken at two points of the spot, at a time of 200 s. The spectra obtained from the analyses of each spot of protein were processed using the AXIL program, which allowed the variation of the intensity of the synchrotron radiation beam to be corrected in order to normalize the peak area of the chemical species detected by counting the argon peak. The analysis of the gels revealed manganese in 1 protein spot of plasma, 2 protein spots of muscle and 6 of liver. Of the 9 proteins found in three types of samples, most of the manganese was distributed in proteins with a molecular weight of less than 45 kDa and with pI in the range of 4.5 to 8.6. The only exception was in the muscle sample, which showed a protein with molecular weight and pI of approximately 97.0 kDa and 5.4, respectively.

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## P125-B1

### Analysis of Multicomponent Medicines without THEIR Separation: Concentration Calculations for Nonadditive Analytical Signals

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Spectrophotometry has been used for a long time as a method of analysis of some unseparated multicomponent pharmaceutical preparations (medicines). This easy, fast and sensitive method has the only drawback – low selectivity, connected with a spectral overlap and/or an interaction of components. The second factor leads to nonadditivity of the absorbances and therefore to systematic errors ( $\Delta C$ ) when component concentrations are calculated. The aim of our investigation is to estimate the effect of nonadditivity on the results of unseparated mixture analysis by three chemometric algorithms. Namely, we used Firordt method (FM), method of multiple linear regression (MLR) and projection on latent structures method (PLS). Individual organic substances were determined in some model mixtures and in real medicines, which contained  $n$  (2 – 7) analytes; concentration ratio of the components was from 1:1 to 1:20. Some analyzed mixtures had the deviations from additivity ( $\Delta A$ ) which were statistically significant. The spectra of aqueous solutions of model substances ( $10^{-6}$ - $10^{-4}$  M) were measured in the UV-region without addition any reagent, spectral data were processed by using the adequate programs.

The compared algorithms differ from each other in their analytical potentialities even in the trivial case when  $\Delta A = 0$ . It was established that the demanded accuracy of analysis ( $\Delta C < 5\%$  rel.) may be obtained with FM only for simplest mixtures ( $n = 2-3$ ); MLR is also suitable for more complicated systems ( $n \leq 5$ ). PLS-method leads to desirable results even for  $n = 6-7$ .

Small deviations from additivity do not prevent to use FM and MLR, but in such cases analytical wavelengths (AWLs) had to be selected in special way. New criterion to select AWLs for FM was proposed. Calculation formulas were derived relating the deviations from additivity of the absorbance of a random binary mixture and systematic errors of determining components by the Firordt method. An algorithm was developed and tested on model mixtures, used to predict the possibility of the simultaneous determination of both components of the mixture with errors below a specified limit. Within the limits MLR it is expedient to calculate absorption coefficients for all components with spectral data concerning mixtures with well-known concentrations of analytes. This mode diminishes the effect of nonadditivity, and  $\Delta C$ -meanings are decreased 3-5 times. When  $\Delta A > 0$ , PLS-method leads to more correct results than FM and MLR. All components of multicomponent medicines were determined with PLS, and in all cases  $\Delta C < 2\%$  rel. Construction of corresponding mathematical models demands no more than  $(2n+1)$  model mixtures.

Obtained theoretical rules were used to work some express methods to analyze multicomponent medicines (papaverine + dibazol, etc) as well as polyvitamines. The results approximately coincide with the other method results (HPLC) and with nominal composition of examined substances.

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## **P126-B1**

### **Determination of Capsaicin in Pharmaceuticals by Oil-in-Water Microemulsion Liquid Chromatography**

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Capsaicin is the main capsaicinoid, isolated from chili peppers. Capsaicin is currently used in topical ointments to relieve the pain of peripheral neuropathy such as post-herpetic neuralgia. It may be used as a cream for the temporary relief of minor aches and pains of muscles and joints associated with arthritis, simple backache, strains and sprains. The main problem dealing with the analysis of many cream pharmaceuticals is their oil-based matrix, what requires more complicated procedure of sample pretreatment.

The technique of microemulsion liquid chromatography (MELC) was first reported in 1992 and has been successfully used for the analysis of different pharmaceuticals since then. Due to high content of aqueous phase oil-in-water microemulsions are compatible with usual reversed-phase columns. Using microemulsion as a mobile phase enables the isocratic separation of both hydrophilic and hydrophobic compounds. Moreover, oil constituent of the microemulsion gives the ability to solve non-polar matrices.

In present work a simple and rapid technique for the determination of capsaicin in cream pharmaceuticals was suggested. The influence of the microemulsion compound on the separation of the components was investigated. Microemulsion of 3.3% SDS, 1% heptane, 8% n-butanol and 0.05% TFA was shown to be the most suitable for the analysis. Different ways of the capsaicin extraction were compared and extraction with the microemulsion was showed to be the best one. It should be noted that the main disadvantage of microemulsions is their high viscosity and large pressure in the system. Using a monolithic column as a stationary phase allowed to reduce the pressure and increase the flow rate. The time of the analysis in this case was 2.5 minutes. The described method was successfully applied for comparative analysis of a number of capsaicin-containing pharmaceuticals.

## P127-B1

### Determination of Phenolic Substrates of Plant Peroxidases in Water Miscible Ionic Liquids and Polar Organic Solvents

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The sensitive, selective and rapid determination of toxic phenolic compounds in water-insoluble samples and organic extracts is an actual problem of the chemical analysis. The application of enzymes, plant peroxidases especially, for the determination of their phenolic substrates in polar organic solvents is limited by the denaturative action of media on proteins. Nowadays, a novel class of nonaqueous but polar solvents, ionic liquids (ILs), has attracted increasing attention as an alternative reaction medium for homogeneous biocatalysis.

In the present report the analytical possibilities of two commercial hydrophilic ILs, such as tetrafluorophosphates of 1-butyl-3-methylimidazolium and N-alkylpyridinium, in the reactions of guaiacol and *o*-chlorophenol oxidation by *tert*-butyl hydroperoxide catalyzed by horseradish and soybean peroxidases were studied. Analogous experiments were carried out in the aqueous mixtures of acetonitrile and DMSO, which have a polarity comparable with that of the investigated ILs. The indicator reaction rate was monitored spectrophotometrically ( $\lambda_{\text{max}}$  470 and 415 nm for guaiacol and *o*-chlorophenol, respectively). It has been shown that ILs/buffer solution combinations are more suitable media for phenols biotransformation than their analogues with molecular organic solvents. The important advantage of using ILs for phenols determination is the possibility of carrying out the indicator reactions in 70 - 80% (v/v) of IL whereas peroxidase catalysis in aqueous mixtures of acetonitrile and DMSO is observed if the content of organic solvent doesn't exceed 25 - 40% (v/v). It has been found that the catalytic activity of plant peroxidases, and the sensitivity of phenols determination as a result, depend significantly on the nature of the enzyme, IL cation, and buffer solution. The water content in ILs preparations is also important. The procedures for guaiacol and *o*-chlorophenol determination in 70-80 % (v/v) of ILs have been developed and approved successfully in analysis of the water-insoluble pharmaceuticals.  
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## P128-B1

### Electrocatalytic Oxidation of Atenolol and Acetaminophen on a Gold Nanoparticles-Modified Carbon Paste Electrode

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A simple and selective electrochemical method was developed for Simultaneous determination of atenolol and acetaminophen in Britton-Robinson buffer solution on a gold nanoparticles carbon paste electrode (GN-CPE) using differential pulse voltammetry (DPV). Atenolol ( $\beta$ -adrenoceptor blocking drug) is of therapeutic value in the treatment of various cardiovascular disorders, such as pectoris, cardiac arrhythmia and hypertension [1]. Acetaminophen also is a drug with antipyretic and analgesic action, frequently used in therapy and which has low toxicity when used at the recommended doses [2]. Electrochemical techniques are suitable in samples containing complex matrix such as syrups, tablets, creams or biological fluids [3]. The purpose of this work is to investigate the DPV behavior of atenolol and acetaminophen using nanogold modified CPE. The experimental results revealed that gold nanoparticles promote the rate of atenolol and acetaminophen oxidation by increasing the peak current ( $i_p$ ) and oxidizing at lower peak potentials as compared to the respective bare electrodes due to the electrocatalytic effect. The linear response ranging from  $1.0 \times 10^{-6}$  mol L<sup>-1</sup> to  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> for both compounds. The detection limits for the simultaneous determination of atenolol and acetaminophen were  $5.0 \times 10^{-8}$  mol L<sup>-1</sup> and  $1.0 \times 10^{-7}$  mol L<sup>-1</sup> respectively. The proposed method was successfully applied in simultaneous determination of atenolol and acetaminophen in several pharmaceutical formulations.

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## P129-B1

### Simultaneous Determination of Telmisartan and Hydrochlorothiazide by HPLC-DAD Technique in Pharmaceutical Dosage Forms

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Telmisartan (TMS) and /hydrochlorothiazide (HCT), a combination medicine, is often prescribed for the treatment of high blood pressure. An HPLC-UV method is presented for the simultaneous determination of TMS and HCT. In this method; a reversed-phase column (Inertsil ODS 3V ; 5  $\mu$ m, 4.6x150 mm I.D.) with a mobile phase of acetonitrile: NaH<sub>2</sub>PO<sub>4</sub> buffer (pH 3.0 ; 1.7 mM) (46 : 54 ; v/v ) at 1.5 ml/min flow rate was used to separate both compounds with a detection of 270 nm. The chromatographic separation was performed at 30 °C. Irbesartan was chosen as the internal standard (IS) because it showed a shorter retention time with better peak shapes and better resolution, compared to other potential internal standards. The proposed HPLC method gives a good resolution between TMS, HCT and internal standard within a short analysis time. Using these conditions, the retention times were obtained as 3.02 min for TMS, 2.35 min for HCT and 5.10 min for IS. The proposed methods have been extensively validated. System suitability tests were also carried out. Linearity was obtained in the concentration range of 0.0227-745  $\mu$ g mL<sup>-1</sup> for TMS and 0.00476-156  $\mu$ g mL<sup>-1</sup> for HCT. In order to demonstrate the validity and applicability of the proposed HPLC method, recovery tests were carried. High percentage recovery shows that the method is free from the interferences of the commonly used excipients and additives in the formulations of drugs. The present HPLC study purposes a rapid, simple, sufficiently precise and accurate method for the simultaneous determination of TMS and HCT, in raw material and pharmaceutical formulations. The proposed method is suitable for quality control laboratories, where economy and time are essential.

## P130-B1

### Determination of the Fluorescence Properties of the Local Anaesthetics in Different Solvent and Organic Solvent-Water Mixture

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Drugs which block impulse conduction when applied to the nerve tissue in appropriate concentration are called "local anesthetics". The important advantage of local anesthetics is that the effect is reversible. They are usually composed of a benzene ring which is a lipophilic group and a hydrophilic group which is a tertiary amine. These two groups are connected with an intermediary structure composed of an ester or amid. Lipophilic group (free base) provides the penetration of the solution through the nerve membrane. Hydrophilic group (cationic form) is the pharmacologically active part. Within the nerve cell, the non-ionized (lipophilic, free base) and the ionized (hydrophilic, cationic form) parts come to equilibrium, the ionized part which is pharmacologically active, affects the receptor. These drugs are effective on all types of nerve fibers and in anywhere in the nervous system [1].

Solvation dynamics of fluorescent molecule with its environment has an important effect on kinetics of many photochemical parameters. The Stokes' shift observed in fluorescence emission has generally invoked the interaction between the solvent and fluorescent molecules.

In this study, the effect of solvent on the fluorescence properties (the positions of excitation [ $\lambda_{ex}$ ] and emission wavelengths [ $\lambda_{em}$ ] and on the fluorescence intensity) of local anesthetics such as tetracaine, dibucaine and procaine have been studied in a series of solvents with different polarities and proton-donating abilities and in certain percentages of acetonitrile-water, DMF-water and methanol-water binary mixtures at room temperature. The solvent effects on ACE inhibitors were determined. For this purpose, emission spectra of ACE inhibitors in methanol, ethanol, DMSO, DMF, acetonitrile, water (Fig.) and in 10-90% of organic solvent-water binary mixture were measured by changing excitation wavelengths and then fluorescence intensities at excitation ( $\lambda_{ex}$ ) and emission wavelengths ( $\lambda_{em}$ ) were determined in these solvents and compared with each other. Furthermore, solvatochromic correlations were used to estimate the ground-state ( $\mu_g$ ) and excited-state ( $\mu_e$ ) dipole moments. The excited-state dipole moments for all the three molecules are found to be larger than their corresponding groundstate dipole moments. Further, the changes in dipole moment ( $\Delta\mu$ ) were calculated both from solvatochromic shift method [2] and on the basis of microscopic empirical solvent polarity parameter ( $E^N_T$ ) and the values were compared.

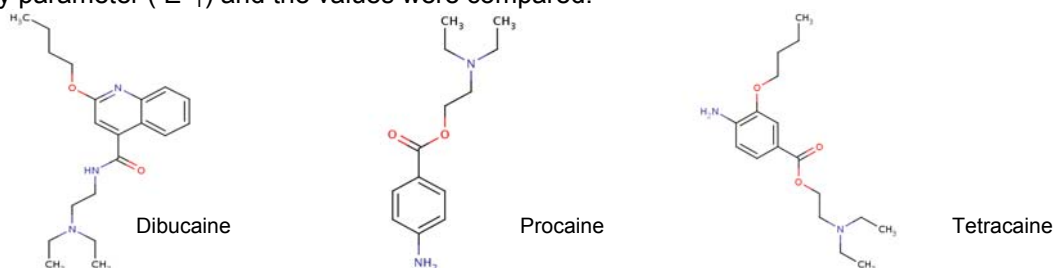


Fig. Molecular structure of the local anesthetic compounds

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## P131-B1

### **Development of a Classification and Ranking Method for the Identification of Possible Biomarkers in Proteomics Based on Principal Component Analysis and Variable Selection Procedures**

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2D gel-electrophoresis, one of the most exploited techniques for protein separation, provides 2D-maps where proteins appear as spots separated according to their isoelectric point and molecular mass. Sets of maps from different classes (e.g. control/diseased) can be used for the identification of possible biomarkers in several fields (clinical proteomics, drug design, botany) through the analysis of “spot volume datasets”, where each map is described in terms of the volumes (related to the amount of protein) of the spots identified. This analysis can be quite difficult since the small number of samples usually obtained (<10 for each class) is in general described by a large number of spots (hundreds or thousands). Usually, Student t-tests are used to provide the relevant up- or down-regulated proteins; a better approach is certainly the use of multivariate methods taking into consideration the relationships between the variables.

The final aim in proteomics is the identification of all possible biomarkers: this scope is generally in contrast with standard variable selection procedures adopted in chemometrics where the smallest possible subset of variables providing a good classification is selected. To overcome this problem, a novel approach is proposed here, based on a particular use of Principal Component Analysis (PCA). PCA is applied as ranking method combined to a variable selection procedure in forward search: the variable allowing the best separation of the two classes (largest distance between centroids) is first selected, then a variable at a time is added to the model. At each cycle, the variable selected is the one providing the largest increase of distance between the centroids of the classes represented in the space of the relevant PCs. Selection is performed in cross-validation. The use of the relevant PCs allows to take into consideration the relationships between the variables and assures a proper variable reduction that can be successfully applied to under-determined datasets. The most discriminant variables are then ranked according to their discrimination ability. The procedure is successfully applied to different datasets and indexes are calculated to evaluate the performance of classification.

## P132-B1

### Extraction and Purification of Proteins from Lupine and Hazelnut

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In the past decade, research on cumulative risks has raised awareness for the control of health risks. Food consumers demand more rigorous food safety testing as far as finished food products may contain a wide range of contaminants from both natural and anthropogenic sources. Allergenic proteins are an example for naturally occurring contaminants which have to be avoided by the allergic consumer. In the field of allergenic food contaminant analysis, with the absence of clear threshold levels and the risk associated with cross-contamination, a major focus is the detection and characterization of food allergens listed in Annex IIIa of the EC-Directive 2007/86/EC in different foodstuffs. In this context the extraction of allergenic proteins from foodstuffs is a crucial task of the work. For this purpose, innovative sample extraction techniques were introduced and existing strategies and methods such as using cooled acetone to defat the nuts for efficient extraction of target allergenic proteins from foodstuffs were addressed.

In this poster the extraction methods, which have been established for extraction of protein from hazelnut and lupine, will be presented and the profiles of extracted protein will be compared.

For extraction of hazelnut, the nuts were ground and defatted. After vacuum-filtration, the remaining sediment was dried overnight in a fume-hood. Extraction of the proteins was done with three different buffer systems. Due to the mild isolation conditions, denaturation of proteins during extraction was minimised. Furthermore the extracts were dialysed to remove excessive salts for further electrophoresis.

For lupine also different buffers were used for extraction. Comparing them there were no real differences in the obtained protein fractions observable. Therefore another separation procedure: the classic "Osborne fractionation" was used.

All of the fractions were characterised with electrophoresis to get a picture about the differences between the extracts.

This work is part of the Christian Doppler Pilotlaboratory for Rapid Test Systems for Allergenic Food Contaminants.

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## P133-B1

### Application of Capillary Electrophoresis with Contactless Conductivity Detection in Pharmaceutical Analysis

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After ten years of its existence the axial capacitively coupled contactless conductivity detection ( $C^4D$ ) became an economical, simple, sensitive and versatile detection tool in capillary electrophoresis (CE). Compared to the UV detection that is most frequently applied in CE, the main merit of  $C^4D$  consists in making possible detection of non-UV-absorbing species without any need of using derivatization or indirect detection.

A brief overview presented maps applications of CE/ $C^4D$  in the field of pharmaceutical analysis. The applications discussed cover the determination of main active components in pharmaceutical preparations (e.g., suxamethonium [1] and glucosamine [2]) as well as monitoring of active principals in body fluids. The topic of chiral separations with CE/ $C^4D$  is discussed as well.

Besides this overview first original results concerning the development of CE/ $C^4D$  assay for the determination of quaternary ammonium salts of pharmaceutical interest (cationic surfactants such as, e.g., septonex) are demonstrated. Septonex (*carbethopendecinium bromide*) acts as antimicrobial, antiseptic and germicide agent. This substance is formulated as main active principle in dermatological sprays, eye drops and ointments.

Strategy of the CE/ $C^4D$  method development involves the selection of suitable buffering system of the background electrolyte (BGE), the choice of appropriate cyclodextrin to enhance the selectivity of the method and the choice of optimal organic modifier to suppress possible interaction of the analyte with the inner fused-silica capillary walls. The results of quantitative CE/ $C^4D$  analyses of real samples will be also presented.

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## P134-B1

### Quantification of Chlorpheniramine Maleate Enantiomers by Spectroscopy and Chemometrics Methods

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Chlorpheniramine maleate (CLOR) is an alkylamine derivative and it is an antihistamine used to treat allergic rhinitis and other allergies. Significant stereoselectivity has been reported in pharmacodynamics and pharmacokinetics of this drug. In this work, chlorpheniramine maleate enantiomers were quantified by near infrared and ultraviolet spectroscopy with partial least squares regression. The CLOR samples ( $1.0 \times 10^{-3}$  mol/L) were prepared by an inclusion complex with  $\beta$ -cyclodextrin ( $2.0 \times 10^{-3}$  mol/L) and 1-butanol (0.05 mol/L) and mole fractions in the range of 50 to 100% (S)-CLOR. For the multivariate calibration, the outliers were detected and excluded and a variable selection was performed by interval partial least squares and genetic algorithm. The best model was obtained by ultraviolet spectroscopy using the whole spectra. Figures of merit showed results for accuracy of the 3.63 and 2.83 % (S)-CLOR for root mean squares error of calibration and prediction, respectively. Precision, limit of detection, limit of quantification and analytical sensitivity were 0.57,  $1.10 \times 10^{-3}$ ,  $3.50 \times 10^{-3}$  and 0.50 % (S)-CLOR, respectively. The sensitivity, selectivity, adjust of the net analyte signal and signal to noise ratio were also determined. The method was validated by paired test-t with the results obtained by the high performance liquid chromatographic (HPLC) propose by European Pharmacopoeia and circular dichroism (CD). The results presented no significant difference between the methods at 95% confidence. The proposed methodology based on spectroscopy and chemometrics is an alternative to the HPLC and CD methodologies. Acknowledgements: FAPESP 05/56188-1.

## P135-B1

### Investigation of the Applicability of Zernike Moments for the Automatic Classification of Proteomics Maps

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Nowadays, researchers of several scientific areas are requested to solve problems characterised by many variables. Proteomic is an example of a discipline where researchers need statistical and mathematical background in addition to chemical and biological knowledge for manipulation of multivariate datasets.

In proteomic research one of the most applied techniques for protein separation is SDS 2D PAGE (Sodium Dodecil Sulphate 2 Dimensional Gel Electrophoresis). Almost every mixture of protein coming from biological tissues or from biological fluid can be separated using this technique. The result of the separation is a two dimensional gel, where usually thousand of spots are present. Every spot contains one or more protein families. This information is useful in understanding diseases mechanism, as a diagnostic tool or for new biomarker detection. Recently SDS 2D PAGE have been analyzed and classified using multivariate tools or algorithms for automatic spots detection. Usually classification is performed using standard techniques like PLS-DA (Partial Least Squares Discrimination Analysis) or LDA (Linear Discrimination Analysis). The aim of these work is to correctly classify new gels coming from different tissues using global image descriptors such as Zernike moments or local descriptors such as Wavelet decomposition or pixel analysis. Unfortunately 2D gel-electrophoretic maps are often distorted and not completely overlapping. The deformation of the images can influences the quality of the classification models. The aim of the present work is to study this phenomena using simulated datasets of gel-electrophoretic maps of increasing complexity, that mimic the original maps. Using this strategy it is possible to investigate the robustness of the classification and is also possible to define acceptance criteria such as the level of deformation archived from the set of maps and the minimal number of gel-electrophoresis maps that is needed for obtaining realistic results from standard classification techniques.

## **P136-B1**

### **Evaluation of an Analytical Method for Determination of Inorganic Species in Enteral Nutrition Using ICP OES**

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An analytical method for the determination of inorganic species (Na, Ca, K, Mg, Zn, Fe, Cu, Mn, Co, Ni, Mo, Cd, Cr, Ba, V, Pb, Se, Al, Sb and Hg) in liquid enteral nutrition is presented. Enteral nutrition is the nutrition provided through the gastrointestinal tract via a tube, catheter, or stoma that delivers nutrients distal to the oral cavity to people who are unable to meet their needs with food and beverages alone. The method proposed is based on Inductively Coupled Plasma Optical Emission Spectrometry (Perkin-Elmer, Optima 3000DV, cross flow nebulizer) and allowed the analysis of samples after a mineralization step in a closed microwave furnace. The experimental parameters (plasma power, nebulizer flow, torch configuration, yttrium as internal standard and emission wavelengths) were evaluated and the signal to background ratio (SBR) and the plasma robustness were considered to find parameters optimized. Since there is not a certified reference material for these matrices, the accuracy of the method was evaluated employing analyte addition and recovery experiments. The recovery values were between 90 and 110% for the majority of the analytes and the RSDs obtained were, in general, lower than 5%. The method allows the determination of Na, Ca, K, Mg, Zn, Fe, Cu and Mn presenting in the commercial formulas.

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## P137-B1

### Application of CE-ICP-MS to Proteomics Studies Related to Metallodrug Discovery and Development

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Capillary electrophoresis (CE) hyphenated with inductively coupled mass spectrometry (ICP-MS) has become a powerful analytical platform for metalloproteomic studies, including characterization of metabolic transformations for metal-based drugs. The present work is focused on CE-ICP-MS examination of in vitro interactions between tris(8-quinolinolato)gallium(III), a potential anticancer drug, and serum transport proteins, albumin and transferrin, as well as human serum. The instrumental setup, comprising commercial CE and quadrupole ICP-MS units interfaced using a microconcentric nebulizer CEI-100, was adopted from our earlier metallodrug-protein binding studies. However, separation conditions previously used for mapping the protein adducts of platinum(II) and ruthenium(III) drugs were found unacceptable to unambiguously establish the adduct formation for the gallium drug on interest (the recorded peaks were too broad and migration times not reproducible). On the contrary, satisfactory results have been obtained with an electrolyte system based on biologically compatible HEPES buffer (at the physiological pH of 7.4). Separations performed under such conditions take a shorter time and provide fairly reproducible migration times (RSD<4.5%). Furthermore, the detection sensitivity was much better due to more efficient peak shapes.

The modified CE-ICP-MS system allowed us to monitor metal-specifically the formation of drug-protein adducts. In the case of transferrin, three gallium-bound forms were quite unexpectedly observed, their relative abundance being time-dependent. The gallium speciation profiling resulted from drug's binding to serum in general resembled that of transferrin. A comparatively low abundance of albumin adduct may be inferred that mainly transferrin takes the role of gallium transporter in the body.

## P138-B1

### RP-HPLC-DAD and RP-HPLC-MS Methods in Stability Profiling of Rizatriptan Benzoate through Forced Degradation Studies

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The aim of the present study was to report the stability profile of novel antimigrain drug Rizatriptan benzoate based on the information obtained from forced degradation studies. These studies are often used as a part of the drug development strategy in order to establish the degradation pathways, identify the likely degradation products and the intrinsic stability of the drug molecule. In that manner, it is possible to upgrade the drug quality as well as the safety and effectiveness of pharmacotherapy. According to ICH recommendations, the drug was subjected to acid (0.1–1 M HCl), neutral and base (0.1–1 M NaOH) hydrolysis and oxidative decomposition (3–30% H<sub>2</sub>O<sub>2</sub>) at room temperature. Photolysis and thermo degradation at 75°C were carried out in water solution and in solid state with both bulk drug and tablet formulation in order to additionally investigate the possible drug interactions with the tablet excipient. The formed degradation products were afterwards analyzed by RP-HPLC-DAD and RP-HPLC-MS methods. Based on the results, it was concluded that the drug was stable towards every stress condition but oxidation. The stability was not jeopardized even after exaggerating the stress conditions by increasing the temperature, strength of acid/alkali solutions and prolonging the testing period. Consequently, new stability indicating method was developed with the assistance of experimental design methodology and validated with respect to ICH guideline. Separations were achieved on a C<sub>18</sub> column (*Waters XTerra*<sup>TM</sup>, 150 mm × 3.9 mm, 5 μm) methanol–water solution of TEA (pH 6, 1%, v/v) (6:94, v/v) as the mobile phase pumped at 1 mL/min flow rate and with 25°C column temperature. The detection was initially performed at 225 nm. But, the simultaneous detection on several wavelengths using diode-array detection was included in case that formed degradation products have different absorption characteristics than the parent drug. The peak purity was also investigated to insure the absence of co-elution and therefore appropriate selectivity of the method. Mass spectrometry was employed in order to detect the possible degradation product without UV/visible chromophores. Since it also gives valuable data about the molecular weight, it was possible to furthermore characterize the chemical structure of the degradation products and outline the possible degradation pathway.

## P139-B1

### Thermoanalytical Study of Cefadroxil and its Mixtures with Different Excipients

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In the present study, the thermal decomposition of cefadroxil and some mixture with excipients containing the same active compound was studied using thermogravimetry / derivative thermogravimetry (TG / DTG) and differential scanning calorimetry (DSC).

The thermal methods of analysis are widely employed in the study of stability and thermal decomposition of substances used in medicine, especially drugs.

Differential scanning calorimetry (DSC) study was performed on DSC 204 equipment (Netzsch) under nitrogen atmosphere and under dynamic conditions (with 5, 7, 10, 12 and 15 °C·min<sup>-1</sup>)

Thermogravimetical analysis (TGA) was performed on TG 209 equipment under nitrogen atmosphere and under dynamic conditions (with 5, 7, 10, 12 and 15 °C·min<sup>-1</sup>) between 20 and 550 °C in order to obtain the activation energy, pre-exponential factor A and reaction order n for the degradation processes of cephadroxil and the mixtures with excipients.

The kinetic parameters were obtained from TG curves using the following methods: Friedman, Flynn–Wall–Ozawa and Chang, respectively a nitrogen dynamic atmosphere and different heating rates: 5, 7, 10 and 15 °C·min<sup>-1</sup>.

The kinetic data showed that the cefadroxil– active substance is thermally more stable than the mixtures with any excipient. The decrease in stability was attributed to the presence of excipients in formulation and to the possible interactions of them with the active substance. The difference in stability is bigger between the pure substance and the mixture with the magnesium stearate.

The decomposition process has been relatively complex for a simple and easy correlation of the kinetic data with the molecular structure's characteristics.

**KEYWORDS:** cefadroxil; degradation behaviour; excipient; TG; DSC; activation energy; IR spectra.

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## P140-B1

### Immobilization of *Candida Rugosa* Lipase on Sporopollenin for Kinetic Resolution of Chiral Drugs

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Sporopollenin is a natural polymer obtained from *Lycopodium clavatum* which is highly resistant to chemical attack, has a high capacity, is stable, has a constant chemical structure and occurs naturally as a component of spore walls, and exhibits very good stability even after prolonged exposure to mineral acids and alkalies [1,2]

*Candida rugosa* lipase (CRL) is an important industrial lipase due to its wide substrate specificity, which provides its successful utilization in a variety of hydrolysis and esterification reactions; and due to its high stereoselectivity and regioselectivity, which make possible the synthesis of several pharmaceuticals [3]. S-Naproxen [(S)-(+)-2-(6-methoxy-2-naphthyl) propionic acid], which belongs to non-steroidal, anti-inflammatory drugs, is used as a single enantiomer since the physiological activity of the S-Naproxen is 28-fold higher than that of the R-form [4].

To our best knowledge, there exists no report on the use of sporopollenin from *Lycopodium clavatum* as support for immobilization of lipase. We think that it could be interesting for lipase immobilization by covalent binding. Therefore, we now report the use of sporopollenin on lipase immobilization and explore the effect of these material in the enantioselective hydrolysis reaction of (RS)-Naproxen methyl ester. The effect of temperature, pH, thermal/storage stability were also investigated.

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## P141-B1

### Optimization of LC Methods for the Determination of Angiotensin II Receptor Antagonists in their Pharmaceutical Dosage Forms

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Angiotensin antagonists are the first major innovation in hypertension management in over a decade. Several HPLC methods have been previously reported for the determination of ARA II in pharmaceuticals. The optimization of chromatographic separation for the determination of ARA II compounds in their pharmaceutical dosage forms is very scarce.

Because most compounds of interest in pharmaceutical and biomedical applications are ionizable, their retention mechanisms in RPLC have become of great interest. The optimization of the chromatographic conditions is essential to achieve their proper separations and accurate quantitation. Retention in LC is ruled by a complex mechanism depending on a various types of interaction which a solute can undergo in the mobile and stationary phase. Due to specific acid base characteristics of ionogenic solutes, two most important adequization parameters are the organic modifier content and pH of the mobile phase.

In order to study the influence of the pH of the mobile phase on the chromatographic behavior of the ARA II compounds, the capacity factors over a range of mobile phase were determined. The affect of the efficiency, selectivity and retention terms in the fundamental resolution equation were evaluated. Throughout this study, the mobile phase assayed were ACN - water containing 50 mM phosphoric acid. The results indicate that good chromatographic separation can be obtained for the ARA II compounds with 50 % (v/v) of acetonitrile when the pH of the mobile phase is 4.0. Due to the excellent separation efficiency the proposed method is suitable for complex mixture of ARA II determination. The method developed was successfully applied to the simultaneous determination of ARA II compounds in their commercial dosage forms. Four pharmaceutical dosage forms were analyzed using this optimized method. The calibration curves and equations for irbesartan, telmisartan, losartan potassium and valsartan were calculated by plotting the peak area ratios of ARA II to I.S. (naproxen) versus concentration of the compounds in the range of 50–400  $\mu\text{g}\cdot\text{mL}^{-1}$  for irbesartan, 20–200  $\mu\text{g}\cdot\text{mL}^{-1}$  for valsartan, 20–150  $\mu\text{g}\cdot\text{mL}^{-1}$  for losartan potassium and 30–80  $\mu\text{g}\cdot\text{mL}^{-1}$  for telmisartan. When working on standard solutions and according to the obtained validation parameters, results encourage the use of the proposed method described for the assay of irbesartan, valsartan, losartan potassium and telmisartan in their pharmaceutical dosage forms. The quantities found were in conformity with the values claimed by the manufacturers.



## P142-B1

### Studies on Alternative Techniques for Rapid and Robust Routine Cleaning Validation in Pharmaceutical Industries

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Cleaning validation is an important topic in pharmaceutical industries. Methods allowing the analysis of the solvent employed for cleaning are responsible for guaranteeing the success of the cleaning procedure and keeping idle time of production plants as short as possible. The most common analytical techniques for determining organic residues in cleaning processes are high-performance liquid chromatography (HPLC) and gas-chromatography (GC). Although these methods are able to provide very reliable results with narrow confidence intervals, analysis times are often regarded as too long, resulting in abdicable delays with respect to the start of the next production campaign. On this account the search for novel, alternative and primarily fast analytical techniques for cleaning validation is of substantial importance.

Basically the solvent used for the so called "last rinse" includes not more than a few analytes, possible decomposition products included, and therefore demands made on the separation system are rather low. This fact implies potential for further development, as a series of analytical methods allows the simultaneous determination of such contaminants in much shorter time than chromatographic techniques. Nonetheless, suppression effects possibly leading to biased results have to be considered.

The aim of the project is to evaluate the capabilities, advantages and limits of applicable analytical techniques for cleaning validation. These include ion-mobility-spectrometry (IMS), direct analysis in real time (DART), as well as flow injection analysis (FIA) with electrospray ionization (ESI), atmospheric pressure photo-ionization (APPI) and atmospheric pressure chemical-ionization (APCI) mass spectrometry (MS). Any of these techniques are auspicious options for chromatographic systems, predominantly allowing keeping analysis time very short. A set of six derivatives of a pharmaceutical product with different functional groups has been employed for a critical evaluation of these different techniques and their comparison with well established chromatographic methods for cleaning validation.

## P143-B1

### Investigation on the Possibilities to Use Capillary Zone Electrophoresis for the Assay and Analytical Characterization of Ondansetron

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Our study was focused on testing the possibilities to use capillary zone electrophoresis (CZE) both in determining apparent pKa value and in separating and assay of ondansetron in a complex mixture containing some of its synthesis impurities.

The apparent pKa of ondansetron, estimated from effective electrophoretic mobility was consistent with the value in literature (7.4).

A new CZE method with diode-array detection for the assay of ondansetron and (3RS)-3-[(dimethylamino)methyl]-9-methyl-1,2,3,9-tetrahydro-4H-carbazol-4-one (impurity A) was developed. The cassette temperature (15°C), the separating voltage (25 kV), the type and the pH of the running buffer (35 mM phosphate buffer, pH 6) were optimized. The UV detection wavelength used was 216 nm.

The newly established method was validated with respect to specificity, linearity, precision, limits of detection and quantification. The method proved to be linear in the concentration range between 20 and 200 µg/mL for ondansetron ( $r = 0.9995$ ) and between 5 and 25 µg/mL for impurity A ( $r = 0.9998$ ). The detection limits were 6 µg/mL and 4 µg/mL, respectively.

The proposed method is simple, fast and reliable, with very good results in the assay of ondansetron and its impurity in bulk, but mostly in pharmaceutical dosage forms.

We, also, checked the discrimination capacity of  $\beta$ -cyclodextrin and some of its substituted derivatives for the CE chiral separation of ondansetron and impurity A, respectively. The significant polarity of these molecules resulted in a better enantioselectivity obtained using hydroxy-propyl- $\beta$ -cyclodextrin at pH 5-6.

## P144-B1

### In-Capillary Approach to Drug Metabolites Generation with Subsequent Electrophoretic Separation

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Studies of drug metabolism are essential for pharmacology related research. Human liver microsomes represent the generally accepted *in vitro* system for these studies. They credibly mimic liver functions. Cytochrome P450 enzymes, the most abundant proteins of microsomes, are responsible for biotransformation of xenobiotics. Dextromethorphan (DEX) chosen as a drug probe is transformed into three metabolites. The most involved cytochrome P450 (CYP) isoforms are CYP 3A4 and CYP 2D6 at formation of 3-methoxymorphinan, 3-hydroxymorphinan and dextrorphan.

Methods base on capillary electrophoresis (CE) have potential to be a useful tool in high throughput screening of drug metabolites. Electrophoretically mediated microanalysis (EMMA) is a technique derived from CE. In EMMA method the separation capillary serves as a reaction vessel and thus it represents a suitable format for on-line enzymatic microreactions. Different mobility of an enzyme and its substrate are utilized to mix the zones together and to accomplish the biotransformation and subsequent separation of the substrate and its metabolites. Such configuration is suitable for automation and control of the time of contact between the enzyme and the substrate.

In this work we focused on development of a method for simultaneous determination DEX and its metabolites generated by at-capillary (i.e., capillary inlet) incubation at direct injection of microsomes or recombinant CYP 2D6 isoenzyme. The optimal separation was reached in tetraborate buffer (80 mM, pH 9.8) with addition of 2-propanol (8 %, v/v) at 37°C and allowed to perform the separation of both off-line and on-line incubated samples. The partial filling method enabled to combine separation and incubation buffers within one capillary. Main parts of optimization, operation settings and injection parameters, will be discussed. The results of incubations with microsomes and recombinant CYP2D6 isoenzyme will be compared.

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## **P145-B1**

### **Gaining a Representative Glycan Sample for HPLC-FL Analysis**

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One of the most important issues for the characterization of glycoproteins used as biopharmaceuticals is the analysis of the glycan pattern. For this purpose, N-glycans are usually cleaved by an enzyme and labeled with a fluorescence dye to enable for sensitive detection. Avoiding selective losses in any of these steps is a prerequisite to gain meaningful results.

Hence, a sample preparation procedure for glycan analysis by HPLC was optimized to avoid selective depletion of analytes. In the first step glycans are completely released by PNGase-F, as verified by CE analysis, and separated from the protein by ultrafiltration. The glycans are evaporated to dryness in a centrifugal evaporator and labeled with 2-aminobenzoic acid (2AB), a commonly used fluorescence label for oligosaccharides. To avoid hydrolysis of sialic acids, a well-known side reaction at elevated temperatures, which influences the glycan pattern of the sample, the reaction temperature was reduced compared to protocols published in literature. On the other hand, the reaction time had to be increased to achieve acceptable yields. The excess label is depleted by gel filtration utilizing Sephadex G-10 columns.

The obtained sample was analyzed by HPLC in several modes employing columns with high efficiencies and alternative selectivities (RP, HILIC, PGC).

## P146-B1

### Development, Validation and Application of a Capillary Electrophoresis Method for Quantification of Clopidogrel and its Carboxylic Acid Derivative

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Clopidogrel is a potent antiplatelet and antithrombotic agent which acts as a prodrug, requiring oxidation by the hepatic cytochrome P450 and subsequent hydrolysis to generate the active metabolite, a thiol compound. However, after oral administration, the main metabolite circulating in plasma (85 %) is the inactive carboxylic acid derivative, formed by hydrolysis of the ester function by carboxylesterases [1, 2].

A few analytical methods have been reported in the literature for the determination of clopidogrel and/or its carboxylic acid metabolite. These are based on high-performance liquid chromatography (HPLC) with UV absorbance or mass spectrometry (MS) detection and gas chromatography with mass spectrometry detection [3]. Recently a CE study was published for the determination of clopidogrel and its impurities in pharmaceutical formulations [4].

The aim of the present study was to develop and validate a fast electrophoretic method for the quantification of clopidogrel and its carboxylic acid metabolite in biological samples. Prior to development of the method the ionization profiles of both compounds were also determined by capillary electrophoresis.

The method demonstrated to be selective and linear in the concentration range 2–150  $\mu$ M for both compounds. For the standards the limits of detection and quantitation were, respectively, 1.2  $\mu$ M and 3.7  $\mu$ M for clopidogrel and 1.1  $\mu$ M and 3.2  $\mu$ M for the carboxylic acid metabolite. For plasma samples spiked with both compounds, the limits of detection and quantitation were, respectively, 1.6  $\mu$ M and 4.9  $\mu$ M for clopidogrel and 1.3  $\mu$ M and 4.1  $\mu$ M for the carboxylic acid metabolite. Moreover, method validation demonstrated acceptable results for the precision (RSD<5%) and the accuracy (Er>75%). The proposed method was successfully applied in a kinetic study corresponding to the inhibition of enzymatic hydrolysis of clopidogrel with the flavonoid aglycones diosmetin and hesperetin.

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## P147-B1

### A Rapid and Simple High – Performance Liquid Chromatographic Method for the Analysis of Ceftriaxone in Pharmaceutical Formulations and Perilymph Fluid

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Ceftriaxone, a semisynthetic third-generation cephalosporin, is effective against a wide variety of Gram-positive and Gram-negative bacteria. It is indicated in several infectious diseases such as lower respiratory and urinary tract infections, acute bacterial otitis media, gonorrhea, pelvic inflammatory disease. The administration of ceftriaxone may reduce the incidence of postoperative infections in patients undergoing surgical procedures. A simple, sensitive and selective high - performance liquid chromatographic method for analysis of ceftriaxone in pharmaceutical formulation and perilymph fluid by direct injection without any sample pretreatment is described. Several parameters affecting the analysis of ceftriaxone were studied, including pH and concentration of the citrate buffer, methanol content of the mobile phase and flow rate. Analysis was carried out on Nucleosil C<sub>18</sub> 100-3 (125 mm x 4.6 mm, i.d., 5µm) column with a mixture of methanol:citrate buffer (30 mM, pH 3.0) (25:75, v/v) as the mobile phase, at a flow rate of 1.0 mL min<sup>-1</sup> and at 270 nm using ultraviolet detector. Sulfisoxazole was used as an internal standard. The optimized method was validated in terms of linearity, sensitivity, specificity, accuracy, precision, recovery, ruggedness and robustness. The linearity range was found to be 0.05 - 1.00 µg mL<sup>-1</sup>. The developed method was also successfully applied for quantitative determination of ceftriaxone in its pharmaceutical formulations and to both spiked perilymph fluid and perilymph fluid of patients' intravenous administered ceftriaxone. The recovery from spiked perilymph fluid found to be 98.35 ± 1.09 % with RSD of 1.92 at a concentration level of 0.25 µg mL<sup>-1</sup> of ceftriaxone.

## P148-B1

### RP-HPLC Determination of Lipophilicity of Beta-lactams Used in Treatment of CNS Infections

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80 years have passed since Fleming published discovery of penicillin. Beta-lactams have been for years the safest and most efficient antibiotics owing it to the specific action on bacterial cell wall. Nowadays, beta-lactams are in their renaissance as multipotent agents against neurological diseases (e.g. amyotrophic lateral sclerosis, Alzheimer, Parkinson and prion diseases).<sup>1</sup> Thus their lipophilicity, as major characteristic determining passage through blood-brain barrier, is of great importance for their targeted delivery.

Eight most commonly used beta-lactams for treatment of CNS infections (amoxicillin, ampicillin, benzylpenicillin, cloxacillin, cefuroxime, ceftazidime, ceftriaxone, cefotaxime) were analyzed on C<sub>18</sub> XBridge™ column (Waters, USA) using binary mobile phase of water and acetonitrile on Agilent 1100 HPLC. Using linear solvent strength model  $\log k_{w0}$  and  $\phi_0$  were determined.<sup>2</sup> Lipophilicity  $\log P$  parameters were calculated using different programmes available on the Internet (ALOGPs, CLOGP, IA\_logP, KowWin, MiLogP, MLOGP, XLOGP).

Cloxacillin was the most lipophilic beta-lactam analyzed ( $\log k_{w0} = 1.53$ ). Generally penicillins had greater lipophilicity compared to cephalosporines. HPLC parameters  $\log k_{w0}$  and  $\phi_0$  were correlated to calculated  $\log P$  values and significant correlation of  $\phi_0$  and XlogP was observed.

This RP-HPLC method is very quick and efficient for determining lipophilicity of different classes of beta-lactams.

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## P149-B1

### Mf-MELDI-MS: Synthesis and Application of a New MELDI Material for the Determination of Carbohydrates in Medicinal Herbs

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Matrix assisted laser desorption / ionization mass spectrometry (MALDI-MS) has been used for the identification of low molecular weight compounds using normal matrices [1, 2] The use of normal matrices for these analyses faces some difficulties like back ground signals for the matrix especially in low mass range what makes the analysis of low molecular weight compounds very difficult [3]. In order to overcome this problem matrix-free material enhanced laser desorption / ionization mass spectrometry has been applied successfully for such analysis [3, 4].

The study relates to the synthesis of a new material for mf-MELDI-MS prepared by immobilizing bradykinin – a peptide on silica gel coupled to 4-(3-triethoxysilylpropylureido)-azobenzene. This material was applied for the identification of carbohydrates in plant extracts.

This modification of silica particles enables the absorption of laser energy sufficient for desorption and ionization of low molecular weight molecules like carbohydrates. In fact, mono-, di-, tri- and tetrasaccharides can be easily detected within a complex plant extract. Further also higher oligomerization degrees of glucose are detected at high signal to noise ratios.

Finally the newly synthesized material proved its high performance delivering excellent results in respect to signal to noise ratio and sensitivity.

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## P150-B1

### Characterization of Volatile Biomarkers in Individuals with Oncologic Pathologies by HS-SPME-GC-qMSD

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Nowadays, there is an increase interest on the determination of volatile biomarkers for clinical diagnosis and therapeutic monitorization. Since the early 90's, several groups of investigators described some volatile compounds as markers of metabolic processes and clinical diagnosis from various pathologies, including cancer. The existence of this pathologic state leads to the production of determined volatile organic compounds (VOC's) namely, aldehydes (pentanal, hexanal, octanal, nonanal), alkanes (decane, *n*-undecane) and aromatic hydrocarbons (benzene, xylene, toluene), in biological fluids like blood and urine [1].

Several methods have been employed for the discovery of biomarker patterns of major human diseases, especially for various types of cancer. Current sample preparation is one of the most time consuming aspect in analytical chemistry. The development of solid-phase microextraction (SPME) has experienced significant growth since its introduction as a new approach to sample preparation in the 90's [2]. The biological samples used in this work were obtained from 27 patients with breast cancer and 21 controls (healthy volunteers) in Serviço de Hemato-oncologia and Banco de Sangue of Centro Hospitalar in Funchal.

The purpose of this work was to identify the volatile organic compounds in biological fluids by means of HS-SPME-GC-qMSD (headspace solid phase-microextraction) described in the literature as possible biomarkers of cancer from individuals with oncologic pathologies (oncologic group) and without pathology (control group). More than 80 volatile compounds were identified in both groups, belonging to several chemical families, namely aldehydes, ketones, benzene derivates, among others. The major chemical families identified in control group were ketones, sulfur compounds, and for oncologic group were ketones, sulfur compounds and volatile phenols. The most representative compounds of these families were 4-heptanone, methanethiol and 4-methyl-phenol. Acetone, 4-heptanone and 2-pentanone were common in both groups, with higher values for the oncologic group.

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## P151-B1

### Aspects of Phenol Derivatives Separation through Bulk Liquid Membrane

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The separation of phenol derivatives in a membrane system is widely used by scientists. Most of the published papers on this topic followed up the separation of the phenol derivatives using emulsion liquid membranes or supported liquid membranes.

The aim of this study was to separate some phenol compounds (nitrophenols) using the technique of bulk liquid membranes. In a diffusional transport the moving force that ensures the translocation of the organic substrate from the feed phase in the receiving phase is the pH gradient. In order to obtain a good separating efficiency the repartition of bi- and three-phase equilibrium were studied. The optimum parameters as: the nature of the organic membrane solvent, the pH of the receiving phase, the pH of the feed phase and the transport time were established.

Using chloroform as a membrane solvent, an acidic pH for the feed phase (pH = 2) and an alkaline pH of the receiving phase (pH = 12) the separation efficiency of the transport exceeded 90 % (for example: the transport efficiency for p-nitrophenol in the mentioned conditions was 97.76 %).

In mean time, the obtained experimental results allowed us to calculate the repartition constants and the  $pK_a$  values for the studied phenolic compounds within the water – solvent membrane system.

## P152-B1

### Immobilized Ionic Liquids for Solid-Phase Extraction of Trace Elements

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Ionic liquids are successfully applied in different areas of analytical chemistry, particularly as alternative solvents for liquid-liquid extraction, stationary phases in gas chromatography, running electrolytes in capillary electrophoresis, etc. The ability of ionic liquids to be kept on solid surfaces provides the preparation of new materials, for example sensors and membranes, as well as solid-phase extractants. The last application is the area of growing importance. Development of novel sorbent materials by immobilization of ionic liquids on solid matrices seems to be promising for trace element preconcentration due to the possibility of ion-exchange and complexing interactions. In the present work ionic liquids have been applied to synthesize novel solid-phase extractants for selective recovery of noble metals and radionuclides from acid solutions. For these purposes the imidazolium and phosphonium ionic liquids were non-covalently immobilized on different solid supports. The effects of cation and anion parts of ionic liquids were investigated to ensure their good retention in acid solutions and sufficient stability of the materials. The matrices with large surface and high sorption ability were chosen as supports: multi-walled carbon nanotubes and highly cross-linked polystyrene. The study of sorbent properties has shown the efficiency of prepared solid-phase extractants for trace element preconcentration from hydrochloric and nitric acid solutions. The solid-phase extractants based on 1-hexadecyl-3-methylimidazolium bromide have been used for Pt(IV), Pd(II) and Au(III) preconcentration from 1M HCl in both batch and dynamic regimes. High selectivity, good kinetics and the possibility of fast quantitative desorption of noble metals give an opportunity to the application of the novel solid-phase extractants for instrumental element determination, including on-line mode. As an example the proposed approach was successfully applied to the ETAAS-determination of platinum, palladium and gold at 0.001-1 ppm levels in the certified reference materials of different ores.

#### *Acknowledgements*

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## **P153-B1**

### **Separation of $^{90}\text{Y}$ from $^{90}\text{Sr}$ Using a TODGA Based Solvent Extraction Method**

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$^{90}\text{Y}$  based radiopharmaceuticals are widely used for cancer treatment as well as in radiation synoviorthesis.  $^{90}\text{Y}$  of high specific activity and very high chemical purity is essential for targeted therapy. There are several methods for the separation of pure  $^{90}\text{Y}$  from  $^{90}\text{Sr}$  viz. precipitation, ion exchange, electrochemical methods, etc. However, each method has its own disadvantage. It is, therefore, required to develop efficient separation technology for the production of high pure  $^{90}\text{Y}$  tracer with minimum  $^{90}\text{Sr}$  contamination.

N,N,N',N'-tetraoctyldiglycolamide (TODGA) displays unique extraction behaviour as the trivalent metal ions such as the actinides and lanthanides are extracted to much higher extent as compared to the tetra- and hexa-valent actinide ions. It is also reported to extract Sr(II) from nitric acid medium. We have reported that tuning the extraction conditions can lead to decrease in Sr(II) extraction. The relative extraction efficiency of TODGA towards  $\text{Y}^{3+}$  and  $\text{Sr}^{2+}$  was investigated from  $\text{HNO}_3$  as well as HCl media. Based on the high decontamination factor values obtained in the HCl media, a separation method for  $^{90}\text{Y}$  from  $^{90}\text{Sr}$  +  $^{90}\text{Y}$  mixture has been developed in the present work. The extraction kinetics, thermodynamics are also investigated. An attempt has been made to determine the  $^{90}\text{Sr}$  contamination in the  $^{90}\text{Y}$  tracer obtained by the multiple extraction method.

## P154-B1

### GC Determination of Aniline, Cyclohexylamine and Dicyclohexylamine

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Aniline, cyclohexylamine and dicyclohexylamine are chemical compounds having their origin in many pharmacologically active substances containing nitrogen. They are formed as a degradation or biotransformation products and could be potentially harmful for human health. We developed GC method for determination of these compounds using Rtx-35amine column [35% diphenyl-65%dimethylpolysiloxane, Restek] and FID detection. Sample preparation was made with SPE [Oasis HLB tubes, Waters] using buffer pH=10 promoting retention of the appropriate compounds to the sorbent and methanol as elution solvent.

GC method was validated by determination of the following parameters: selectivity [aniline RT 9.160 min, cyclohexylamine RT 6.918 min, dicyclohexylamine RT 13.472 min], precision [aniline 6.49%, cyclohexylamine 2.38%, dicyclohexylamine 3.19%], linearity [aniline  $r = 0.9996$ , cyclohexylamine  $r = 0.9993$ , dicyclohexylamine  $r = 0.9997$ ], detection limit [aniline 1.8 ppm, cyclohexylamine 5.5 ppm, dicyclohexylamine 3.2 ppm] and quantitative limit [aniline 6.2 ppm, cyclohexylamine 18.0 ppm, dicyclohexylamine 10.7 ppm]. Presented data show the suitability of chromatographic conditions for the determination of these compounds.

## P155-B1

### Sample Preparation for Characterization of Guitar Strings

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Skin allergy occurs frequently in musician population: 22% of musicians have skin problems and 19% of them have skin dermatitis. The most problematic metal is nickel since 20% of female and 6% of male population is allergic to it. Therefore the EU Nickel Directive *The Dangerous Substances and Preparations (Nickel Safety) Regulations* limits the extractable nickel in everyday metal items that are coming into direct and prolonged contact with the skin to the amount of 0.5 µg Ni/cm<sup>2</sup>/week. Unfortunately, the existing testing procedures are not appropriate for the musical string analysis due to the relative mild testing conditions. Therefore, in this work we emphasize the importance of careful sample preparation for proper monitoring of nickel and other allergenic metals on surface of nickel plated steel strings, since those can be extracted by playing the guitar. Since the nickel in guitar strings may be present in the steel as alloying element, or as the coating on strings, sample preparation included three separate processes: for monitoring of extractable metal ions in artificial sweat solutions, for chemical analysis of bulk string samples and for analysis of metal distribution through sample cross section. Firstly, the samples of strings were immersed in artificial sweat solution at 37 °C according to EN 1811:1995 for monitoring of extractable metal ions. Secondly, the dissolution in nitric acid was performed for chemical analysis of string samples. All the analyses were performed by inductively coupled plasma - optical emission spectrometry and flame absorption spectrometry. In order to understand the metal elution process in artificial sweat solution, it is important to know the distribution of metals through sample. Therefore, scanning electron microscope equipped with EDS detector was applied. Prior to SEM-EDS analysis, samples were cut, embedded in resin DuroFix-2 Kit, grounded and then polished up to 1 µm with a diamond paste until a mirror-like surface was achieved.

Keywords: sample preparation, metal; guitar strings; allergy; SEM-EDS; AAS; ICP-OES;

## P156-B1

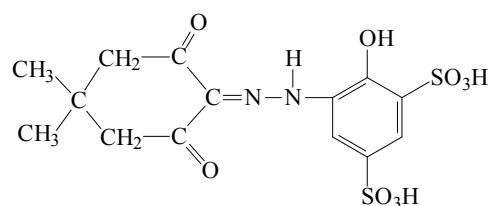
### Determination of Copper(II) in Seawater, Igneous Rock and Nickel-Based Alloys Using Ion Pairs of 5,5-dimethyl-2-(2-hydroxy-3,5-disulfophenyl-hydrazo)cyclohexane-1,3-dione with Cationic Surface-Active Substances

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5,5-Dimethyl-2-(2-hydroxy-3,5-disulfophenylhydrazo)cyclohexane-1,3-dione (**H<sub>2</sub>L**, see Scheme) was synthesized using the Japp-Klingemann conditions [1].



The dissociation constants of **H<sub>2</sub>L** were determined by potentiometric titration and found to be  $pK_1 = 5.90 \pm 0.03$  and  $pK_2 = 9.67 \pm 0.04$ . The interaction in the system of **H<sub>2</sub>L** and cationic surface-active substances (**CSAS**) [cetylpyridinium chloride (**CPCI**), cetylpyridinium bromide (**CPBr**), and cetyltrimethylammonium bromide (**CTABr**)] was studied in the absence and presence of copper(II). It was recognized that **CSAS** electrostatically interacts with the sulfonate groups of **H<sub>2</sub>L** and form ion pairs. The stoichiometry and stability constants of these ion pairs and their complexes with copper(II) were determined and it was found that the detection limit of copper(II) in the presence of **H<sub>2</sub>L(CSAS)<sub>2</sub>** ion pairs decreases. The stability constants of their complexes grow in the order  $\text{Cu}[(\text{HL})(\text{CPCI})_2]_2 > \text{Cu}[(\text{HL})(\text{CPBr})_2]_2 > \text{Cu}[(\text{HL})(\text{CTABr})_2]_2$  with the increase of the stability of ion associates ( $(\text{H}_2\text{L}-\text{CPCI})_2 > \text{H}_2\text{L}(\text{CPBr})_2 > \text{H}_2\text{L}(\text{CTABr})_2$ ). Also it was found that the mechanism of complexation between copper(II) and **H<sub>2</sub>L** is the same as that for **H<sub>2</sub>L(CSAS)<sub>2</sub>** ion pairs and that these complexes occur as monomers rather than polymers.

The effect of foreign ions and masking substances on the complexation was studied. A comparison of the selectivity of the reagents for the determination of copper(II) listed in the assortment with that of **H<sub>2</sub>L(CSAS)<sub>2</sub>** shows that **H<sub>2</sub>L** is more selective in both the presence and absence of **CSAS**. A procedure for the spectrophotometric determination of copper(II) in seawater, igneous rock and nickel-based alloys was developed based on the above mentioned observations and will also be reported.

**Acknowledgement.** This work has been partially supported by the Foundation for Science and Technology (FCT), Portugal ([SFRH/BPD/43629/2008](https://doi.org/10.54720/SFRH/BPD/43629/2008)) and by Baku State University.

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## **P157-B1**

### **The Impact of Microwaves on Analytical Chemistry**

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Sample preparation is usually the most time-consuming step in the analytical chain, besides it also has an enormous impact on the accuracy of the subsequent analysis.

Moreover, modern analytical labs have to cope with a variety of different analytical tasks, thus requiring sample preparation techniques just as versatile.

Scientifically accepted already for years, microwave-assisted sample preparation has been developed over time into an indispensable tool to bridge the gap between sample and analysis.

As a consequence, a modern microwave reaction system has to provide capabilities to handle different sample preparation tasks like wet-chemical digestion and solvent extraction, drying and evaporation, UV digestion and oxygen combustion, preferably within one instrument.

Focusing on environmental applications, selected examples will be presented while illustrating the advantages and benefits over conventional methods.



## Synergistic Solvent Extraction and Separation of Cobalt(II) Ions with Mixtures of Oxime Extractants and Neutral Donors

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Extractability and selectivity improvements among many metal ions have been one of the important subjects concerning solvent extraction. The synergic extraction of metal chelates has been mostly explained by an increase in the hydrophobicity of extracted chelates by a replacement reaction of water molecules bound to the central metal ion by basic neutral organic molecules [1] or by hydrogen-bonding between metal chelates and phenol derivatives in the organic phase [2].

Cobalt is an important trace element necessary for animal nutrition. Ruminant grazing upon deficient pasture exhibit retarded growth, loss of appetite and anemia. Rapid recovery from these symptoms occurs upon feeding the animal with cobalt supplemental diet. Although small amounts of cobalt are invaluable in the treatment of pernicious anemia in sufficiently large doses, the metal becomes toxic. It causes irritation in the gastrointestinal tract, nausea, vomiting and diarrhea [3].

The importance of cobalt provokes the extraction of this element to be considered by many research groups. A part of these investigations is concerned to the application of a mixed extractants (synergistic extraction) for ameliorating the extraction efficiency [4].

In the present communication, we report the synthesis oxime extractants named 1-hydroxy-2-naphthaldoxime ( $L^1$ ) and 2-hydroxy-1-naphthaldoxime ( $L^2$ ). These molecules were characterized using UV-Vis, FT-IR,  $^1H$ NMR and  $^{13}C$ NMR spectroscopic techniques, and were used as extractant in the synergistic extraction of cobalt ions in the presence of neutral ligands triethylamine (TEA), tributylamine (TBA), trioctylamine (TOA), tri-n-octylphosphine oxide (TOPO), tributyl phosphate (TBP), dimethylsulfoxide (DMSO) and tris(2-ethyl) phosphate (T2EHP).

The analysis of the extraction data allows evaluating the synergistic coefficients and apparent stability constants of adduct formation. The order of synergistic coefficient for both oximes in the presence of the neutral donors was found to be as: TBA>TOA>DMSO>T2EHP>TOPO>TBP.

It is shown that the stoichiometry of adducts formed in the organic phase by both  $L^1$  and  $L^2$  in the presence of TOPO depends on the concentration of the oximes. In fact, the stoichiometry deduced for the adduct at the low concentration of  $L^1$  or  $L^2$  was  $[Co(L^{1(2)})(TOPO)Cl]$ , whereas this stoichiometry is found to be as  $[Co(L^{1(2)})_2(TOPO)]$  at high concentration of oximes. The effect of organic diluent on the synergism was investigated. This investigation shows the importance of this parameter on the extraction efficiency of the synergistic process. The application of method for the extraction-recovery of cobalt ions from the leach liquor of spent Ni-Cd batteries was assessed.

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## **P159-B1**

### **Determination of Phosphorus in Different Detergent Samples by UV-VIS Spectroscopy and GFAAS**

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Phosphates had been widely used as assistant detergents. Phosphates are incorporated in synthetic detergent formulations to sequester metal ions, re-dissolve salts of metal ions, soften the water and enhance the performance of surfactants. Though it is considered to be related to the eutrophication of waters and was forbidden or limited to use in detergents in many places, phosphates are still used in detergents especially in developing countries. Eutrophication, although reversible and based on natural effects, is a real and major environmental problem.

In Turkey, according to the examination of sewage systems in the receiving environment of phosphate, 70% comes from detergents. These detergents, despite all their required limit of phosphate involve  $P_2O_5$  more than 30%. A ban on the use of phosphate based detergents can achieve a phosphorus load reduction of up to 40% entering surface water bodies, which is not sufficient in isolation to result in any substantial improvement.

The purpose of this study is to improve the applicability of the developed method for determination of phosphorus in different detergents. UV-Visible Spectroscopy and GFAAS was used to determine the phosphorus contents of detergents and compare the results. Phosphorus determination by UV-Visible Spectroscopy which provides a detection limit of 2.97 ppb, is based on the formation of phosphomolybdate in the presence of ascorbic acid. Two different sample solubilization techniques for GFAAS were developed as ash drying and microwave solubilization. The maximum and minimum phosphorus contents in detergent samples found by GFAAS were 17.50% and 11.17%, respectively. After comparison of two different spectroscopic methods applied in different detergent samples, it was shown that the results were in agreement.

## P160-B1

### Kinetic Quantification of Sodium Salicylate in Human Serum and Wine

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A rapid, relatively sensitive and simple spectrophotometric method for determining water-soluble sodium salicylate content in human serum and wine has been developed and validated. This method was based on the Fenton reaction and involved mixing of ferrous ions, hydrogen peroxide and sodium salicylate in acetic buffer medium. Ferrous ions, oxidized by hydrogen peroxide, formed with sodium salicylate purple complex whose degradation started immediately, influenced by oxidation of sodium salicylate. This effect was measured by the decrease of absorbance at 525 nm. The absorbance increased linearly with the increment of sodium salicylate concentration. The system obeyed Beer's law in a range of 0.93-9.3  $\mu\text{g cm}^{-3}$  of sodium salicylate concentration. The least squares' equation for the calibration graph and correlation coefficient ( $r$ ) for the determination of sodium salicylate were calculated under the optimal reaction conditions ( $c_{\text{Fe}^{2+}} = 4.67 \times 10^{-5} \text{ M}$ ,  $c_{\text{H}_2\text{O}_2} = 6.67 \text{ mM}$ ,  $c_{\text{buffer}} = 8.60 \text{ mM}$ ,  $\text{pH} = 3.86$ ,  $t = 26 \pm 0.10^\circ\text{C}$ ):

$$\text{tg } \alpha \times 10^2 = 0.2530 + 0.3553 \times c_{(\text{Na-salicylate})}; r = 0.9983.$$

The calculated values for the detection limit, according to two formulas, available in the literature, were found to be 0.67  $\mu\text{g cm}^{-3}$  and 0.48  $\mu\text{g cm}^{-3}$ . The variables affecting the rate of the proposed reaction were investigated. The relative standard deviations for five-replicate determinations of 0.93, 3.31 and 9.3  $\mu\text{g cm}^{-3}$  of sodium salicylate were calculated to be 6.8, 2.95 and 1.71%, respectively. The proposed kinetic method has been successfully applied to determining sodium salicylate in human serum and wine and validated by HPLC (High-Pressure Liquid Chromatography) referent method.

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## P161-B1

### Cadmium Ion-Selective Sorbent Preconcentration with Posterior Determination by FAAS Using a Column Packed with Ion Imprinted Poly(vinylimidazole) (PVIIm)

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The present study focuses on the preparation of an ion-imprinted polymer (IIP) for selective extraction/preconcentration of  $\text{Cd}^{2+}$  ions from aqueous solution with further determination by FAAS using a flow system. The polymer is able to specifically recognize the cadmium ion. It was synthesized by bulk method, where cadmium nitrate salt  $[(\text{Cd}(\text{NO}_3)_2)]$  and 1-vinylimidazole were used as template and monomer, respectively dissolved in acetonitrile. Ehtylene glycol dimethacrylate (EGDMA) and 2,2'-azobis-isobutyronitrile (AIBN), used as crosslinking agent and initiator, respectively, were added to the reaction mixture, which was maintained under nitrogen flow. After polymerization, the  $\text{Cd}^{2+}$  ions were removed from selective cavities of the polymer by using successive washing with  $1.0 \text{ mol L}^{-1}$   $\text{HNO}_3$ . Sorbent extraction/preconcentration system was optimized by using factorial design and Doehlert matrix, where the following variables were studied: sample pH, buffer concentration, preconcentration flow rate and type of eluent ( $\text{HNO}_3$  or  $\text{HCl}$ ) using sensitivity efficiency as analytical response. Under optimized conditions by preconcentration of  $15.0 \text{ mL Cd}^{2+}$  solution buffered with  $0.02 \text{ mol L}^{-1}$  phosphate buffer at pH 6.77 through  $120 \text{ mg}$  of ion imprinted polymer packed into a minicolumn, followed by the elution step using  $0.4 \text{ mol L}^{-1}$   $\text{HNO}_3$  solution at flow rate of  $5.6 \text{ mL min}^{-1}$ , limits of detection and quantification of  $0.11$  and  $0.38 \text{ } \mu\text{g L}^{-1}$ , respectively, were obtained. The linear analytical curve was from  $1.0$  up to  $50 \text{ } \mu\text{g L}^{-1}$  ( $r = 0.993$ ). The preconcentration factor (PF), consumptive index (CI), concentration efficiency (CE) obtained were  $38.4$ ,  $0.65 \text{ mL}$  and  $14.3 \text{ min}^{-1}$ , respectively. When the selectivity coefficient of IIP was compared with the selectivity coefficient of NIP (*non imprinted polymer*) from  $\text{Cd}^{2+}/\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}/\text{Cu}^{2+}$  and  $\text{Cd}^{2+}/\text{Ni}^{2+}$  binary mixtures, respective values of relative selectivity coefficient ( $k'$ ) were  $158.5$ ,  $4.4$  and  $1.8$ , demonstrating higher recognition sites of IIP for  $\text{Cd}^{2+}$  ions.

## P162-B1

### Optimization of Native Agar from the Red Seaweed *Gracilaria Vermiculophylla* by Microwave-Assisted Extraction Using Response Surface Methodology

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Due to its excellent gelling and thickening properties, agar is a commercially valuable biopolymer extracted from red seaweeds composed mainly by two fractions: a neutral polymer with high gel strength, agarose, and a sulphated polysaccharide with low gel strength, agaropectin. In spite of exhibiting lower gel strengths when compared with other red algae genera, *Gracilaria* genus is the largest world-wide agar source existing in large quantities in temperate and tropical regions. Agars with gel strengths greater than 700 g cm<sup>-2</sup> in a 1.5% (w/w) solution, are considered high quality agars and have a large range of applications specially in the manufacture of food and pharmaceutical/cosmetic products. Nevertheless, high gel strength is not always the most desirable quality for hydrocolloids since agar with lesser gel strength (30-100 g cm<sup>-2</sup>) may have potential uses in new industrial applications, including liquid and spread foods, soft-texture confectionery and fat replacers [1]. In this emerging path to be exploited, agar from *Gracilaria* reveals excellent characteristics [2].

The objective of this work included the study and optimization of a new eco-friendly native agar extraction process based on microwave-assisted extraction. This technique presents significantly lower extraction times, requires less energy and solvent volume when compared with the traditional agar extraction process. In the present study, agar was extracted from *Gracilaria vermiculophylla*, an invasive species, newly established at Ria de Aveiro (north-western, Portugal). Response surface methodology was used to determine optimum extraction conditions in terms of yield, gel strength, gelling and melting temperatures, as well as, sulphate and 3,6-anhydro-L-galactose contents. Characterization of the extraction parameters effects and their interactions was conducted using a 2<sup>4</sup> experimental design.

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## P163-B1

### Analysis of Chlorophyll Biodegradation Products in Autumnal Leaves of the Hamamelidaceae Family

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The presence of the chlorophyll biodegradation products was investigated, in the crude leaf extract, in the following autumnal leaves of the Hamamelidaceae family: *Corylopsis pauciflora*, *Corylopsis spicata*, *Forthergilla major*, *Hamamelis japonicum*, *Hamamelis japonicum* var. *flavopurpurea*, *Hamamelis virginiana*, *Parrotiopsis jacquemontiana* and *Parrotia persica*.

The chemical composition of the chlorophyll biodegradation products present in the crude leaf extract, of the plant species investigated, was determined qualitatively by the LC/MS analysis on a reversed – phase C4 (RP C4) column under gradient conditions. Observed retention data were used to calculate the capacity factors ( $k'$ ) of the chlorophyll biodegradation products present in the crude autumnal leaf extract.

The data obtained from the LC/MS analysis can be useful for the detection of chlorophyll biodegradation products in samples analyzed and for the identification of the chlorophyll biodegradation pathway in other plant species.

## P164-B1

### Microfluidics Device for Generation of Temporal Gradients to Study Cell Dynamic Response

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Microfluidics with programmed pneumatic valves was fabricated to generate concentration gradient and concentration oscillation to study HeLa cells water absorption in dynamic control over environmental conditions. The three layer poly(dimethylsiloxane) device contained eight pneumatic valves and a 12cm mixing channel. Different solutions were injected into channel by syringe pump and controlled by programmed valves. By adjusting valve open-close frequency, the concentration oscillation can be easily manipulated and changed in time. We used fluorescent dye to demonstrate a concentration gradient formed along the channel and at the channel cross section. We used the device to monitor HeLa cells response in water and medium oscillation indicating the system will be useful in future studies of cellular physiology.

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## P001-B2

### LC-MS/MS Determination of Sulfur Mustard Metabolite in Human Plasma

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Sulfur mustard is a widely known chemical warfare agent. In Russia and former USSR republics heavy stocks of ammunition containing this agent are stored. Industrial destruction of these dangerous objects has been taking place recently. Control of environmental and human safety in regions located near factories is an important analytical task. Another reason of creating analytical technique for the determination of chemical warfare agents is a threat of application of sulfur mustard in acts of terror. After human intoxication by sulfur mustard it metabolizes producing different metabolites. Some of them are not stable, but one - 1,1'-sulfonyl-bis-[2-S-(N-acetylcysteiny)etan is stable enough to be found in plasma for several days. This compound is the most suitable for being used as a sulphur mustard intoxication marker.

In present work LC-MS/MS technique was used for the determination of 1,1'-sulfonyl-bis-[2-S-(N-acetylcysteiny)etan in plasma samples. The procedure of sample pretreatment included steps of plasma deproteinization with 10 % water solution of hydrochloric acid, and solid phase extraction (Strata SDB-L cartridges) to clean the sample. HPLC separation was carried out on a reversed-phase column (Synergi RP Hydro (150x2.1 mm) using water-acetonitrile as a mobile phase (pH 5.4)). The detection in a negative electrospray ionization mode provided good analytical signal. Deprotonised molecular ion ( $m/z = 443$ ) and its fragments ( $m/z = 163.5$  and  $127$ ) were used for the detection in MRM (multi reaction mode). Limit of detection was about 50 ppt. Validation of technique was carried out using plasma of rats, intoxicated by sulfur mustard (about  $\frac{1}{2}$  LD<sub>50</sub>). 1,1'-sulfonyl-bis-[2-S-(N-acetylcysteiny)etan was found in rat plasma up to 14 days after intoxication.



## P002-B2

### Real-Time Breath Analysis with PTR-MS

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Breath gas analysis is a field of intense research with many potential applications. So far most experimental approaches employ off-line sampling, where the breath is stored in a vessel and subsequently analyzed by GC-MS. The possibility to analyze breath samples in real-time (online) offers several advantages: immediate results, no sample storage, and fast monitoring with breath-to-breath time resolution. Proton-transfer-reaction mass-spectrometers (PTR-MS) are ideal tools for online breath analysis: fast response times (below 100ms), and no sample preparation. Using the PTR ionization technique, the majority of volatile organic compounds (VOCs) can be detected with high sensitivity (LOD in the ppqv range). The high signal linearity and the low degree of fragmentation make PTR-MS especially useful to analyze complex gas samples, such as human breath.

We present several medical applications for online breath analysis with PTR-MS. By monitoring the exhaled concentrations of an administered drug, pharmacokinetics, i.e. the uptake and distribution of drugs in the body, can be monitored in real-time. Furthermore, metabolic processes can be studied, by monitoring the metabolites in the breath. For a better separation of a metabolic process isotopically labeled compounds can be used. Another large research field is devoted to the search for disease markers, e.g. for lung cancer, in human breath. We will present a setup for the online measurement of a large number of VOCs in a relatively short amount of time, which is currently employed in a large clinical study.

Finally, we present the next generation of PTR instrumentation. A time-of-flight (TOF) mass spectrometer in combination with the soft proton-transfer reaction offers numerous advantages for on-line breath analysis. First, these PTR-TOF-MS instruments measure a complete mass spectrum within a fraction of a second. Second, their high mass resolution of  $> 5000 \text{ m}/\Delta m$  (FWHM) enables separation of isobaric molecules and identification of their chemical composition.

## P003-B2

### HS-GC-MS as a Tool for Identification of Anaerobic Propionic Acid Degradation Metabolites

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Anaerobic biowaste treatment is a possibility in the waste management system to decrease the amount of waste with concomitant production of a fertilizer and the renewable energy source-methane. Disturbances of the anaerobic food chain by i.e. substrate overload cause often problems like increasing concentrations of volatile fatty acids, especially propionic acid (Prop), a rapid decrease of the pH and failure of biogas production. To avoid deficiency of the anaerobic digestion process, a deeper insight in metabolic pathways and involved bacterial species is necessary. HSS Dani 3950 with Varian 431-GC and Varian 210-MS is used to analyze the products of anaerobic Prop metabolism.

Two main Prop degradation pathways are described in the literature, the methyl-malonyl-CoA-pathway, and the C-6-dismutation-pathway. They can clearly be distinguished if <sup>13</sup>C-Prop is supplied. The amount of produced acetic acid and the distribution of <sup>13</sup>C in the methyl and carboxyl moiety of this metabolite allow differentiating between both pathways. The bacteria cultures from a full-scale anaerobic digester are being fed with 1-<sup>13</sup>C-spiked Prop and incubated at appropriate conditions. The rate of Prop degradation and concentration of metabolism products is analyzed with HSS-GC-MS. Evaluation of metabolites' amount should allow identification of preferred Prop degradation pathway and involved Prop degraders.

The application of HSS allows injection of volatile fatty acids into GC-MS without complicated sample preparation steps from biowaste (purification) and without derivatisation. The mass spectrometry is a possible tool for metabolites identification due to simplicity in the comparison of their molar mass and the masses of fragmentation products. The opportunity of direct examination of masses intensity ratio between "light" and "heavy" analytes is also an advantage of this method.

As there are nowadays 4891 biogas producing facilities in Germany, investigations of propionate degradation and of its limits are essential. First results obtained with described method are promising.

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## P004-B2

### Development of an LC-MS/MS Procedure for the Quantification of Thiouracil in Urine without Derivatisation

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Thiouracil (TU) belongs to the xenobiotic thyreostats, forbidden in Europe since 1981 (Council Directive 81/602/EC). These illegal growth-promoting agents do not only alter the quality of the meat derived from these treated animals, but also represent a possible human health risk due to their listed carcinogenic and teratogenic properties (International Agency for Research on Cancer). Sensitive and specific analysis methods are developed to monitor the illegal use of thyreostats in livestock breeding, where the most sensitive one exploits 3-iodo benzylbromide derivatisation. This method has been used to establish a correlation between thiouracil in bovine urine and a Brassicaceae diet, known to contain precursors of natural-occurring thyreostats. Thiouracil itself was not detected in the diet, which in turn provokes the question: Does thiouracil have a semi-endogenous status or concerns false-positive results due to the derivatisation? It is important in the framework of the national control plans to eliminate false results, therefore, the goal of this study was to develop an analytical method for the quantification of TU from urine without derivatisation.

A critical step in the determination of TU from a urine matrix is the sample extraction itself. TU, as a result of its polar nature and different tautomeric forms, undergoes interferences from matrix constituents, resulting in the disability of widely accepted techniques such as liquid-liquid extraction (LLE) and solid-phase extraction to extract TU. Various techniques such as desalting, denaturation, hydrolysis, reducing agents and mechanical disruption were tested during this study to evaluate the most optimal extraction conditions. Best results were obtained by using mechanical disruption at elevated temperatures, followed by LLE with ethyl acetate. Analysis was performed using LC with tandem MS on a TSQ triple quadrupole mass analyzer (Thermo Electron, San Jose, USA), detection performed in positive ion mode in MS<sup>2</sup>.

An effective LC-MS/MS method was developed for the detection of TU in urine. Future research will include all xenobiotic thyreostats and focus on the validation of this procedure regarding the criteria as described in the European Criteria EC/ 2002/657.

## P005-B2

### **Magnetic Bead-Based Bio-Barcode Fluorescence Immunoassay for Aflatoxins in Food Using Bio-Functionalized Silica Nanoparticles as Recognition Elements**

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The emerging research field of the nanoparticle-enabled bio-barcode technology provides excitingly new possibilities for advanced development of new analytical tools and instrumentation for bioanalytical and biotechnological applications. One major merit of using a bio-barcode assay (BCA) is that it enables the detection of biomolecules like proteins and DNA in specimens with sensitivity several orders of magnitude greater than is possible with, for example, ELISA or mass spectrometry ([http://medgadget.com/archives/2005/12/biobarcode\\_tech.html](http://medgadget.com/archives/2005/12/biobarcode_tech.html)).

Aflatoxins, among the mycotoxines, have assumed significance due to their deleterious effects on human beings, poultry and livestock. Various methods and detection techniques have been applied for their determination. However, despite many advances in this field, it is still a challenge to find new approaches that could improve the simplicity, selectivity, and sensitivity of the analytical methods. Hence, the project is focused on the development of a highly sensitive BCA fluorescence immunoassay for aflatoxins in food. The assay involves two types of particles. One, magnetic nanoparticles (MP), have the target analyte(s) of interest, e.g., aflatoxins, covalently attached to its surface. MP are attractive because they have good biocompatibility and can be separated very easily from reaction mixtures by an external magnetic field. The second, Rhodamine-doped silica nanoparticles (SP), are bio-functionalized with anti-aflatoxin antibodies as recognition elements. SP have proved to be an ideal protein host due to high chemical and thermal stability, fine suspendability in aqueous solution, and good compatibility with the environment. In a competitive assay, the two types of particles are incubated with the sample, forming MP-aflatoxin-Rhodamine/SP-antibody complexes, depending on the aflatoxin concentration in the sample. A magnetic field is used to localize and collect the magnetic particles, while unattached bio-functionalized Rhodamine-SP are washed away. Finally, fluorescence intensity is measured by an appropriate reader system.

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## P006-B2

### Gas Chromatographic-Mass Spectrometric Determination of Brain Levels of Lathosterol

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Lathosterol is one of the most important precursors of the cholesterol synthesis. The interest in brain cholesterol metabolism is growing nowadays since it was described to play an important role in some neurodegenerative disorders such as Alzheimer's disease and Multiple Sclerosis. Quantification of lathosterol is complicated by the presence of numerous other lipids and the low concentration of the compound relative to cholesterol (ratio lathosterol / cholesterol is approximately 1/100). Therefore, it is of crucial importance to establish sensitive methods to quantify this compound.

In this work a gas chromatographic-mass spectrometric (GC-MS) method for the detection and quantification of lathosterol in rabbit brain is proposed. The analytical methodology proposed involves a liquid-liquid extraction procedure followed by a silylation step previous to the GC-MS analysis. The chromatographic separation was performed by using a low bleed HP5-MS fused silica capillary column. A clean up is not necessary when using single-ion monitoring mode.  $\alpha$ -Naphthol was used as an internal standard and the detection limit obtained was  $0.09 \mu\text{g mL}^{-1}$ .

The proposed method was successfully applied for the determination of brain lathosterol levels in rabbits fed with different types of diets (control and atherogenic, supplemented or not with natural polyphenolic antioxidants). The quantification of the compound in samples showed after a month, a reduction of this precursor of cholesterol synthesis in rabbits fed with antioxidant supplemented diets.

## **P007-B2**

### **Next Generation Mass Spectrometers: Advancing the Impact of Bioanalytical Data in Support of the Drug Analysis**

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In order to support discovery and development studies within the pharmaceutical industry there has been an increased use of innovative analytical technology. Development of assays for pharmaceutical compounds in biomatrices such as plasma, urine and tissue can be very challenging. The challenge focuses on the quantification of drugs and metabolites at very low concentrations, in an excess of biological matrix and in a high-throughput manner. Recent technological advances in mass spectrometric detection can be successfully applied to overcome these challenges.

With the aim of enhancing data quality we evaluated the performance of the next generation triple quadrupole mass spectrometers Waters Xevo™ TQ, API 5500™ QTrap (Applied Biosystems/MDS SCIEX), Thermo Scientific TSQ Vantage and Agilent 6460 JetStream for their analytical capabilities.

A test set of eleven drugs with a wide range of physico-chemical properties was identified and blank plasma samples were spiked. Working solutions were prepared (0.001-1000 ng/mL) and run (n=10) to evaluate LOD and linearity. Protein precipitation was performed for sample clean-up. Chromatography was carried out using a 100x3 mm monolithic C18 column using gradient elution at 1mL/min flow rate. A batch of samples was run initially using an API 4000 mass spectrometer (Applied Biosystems/MDS SCIEX) and data generated were used to benchmark the instrument. The same samples were then run on each of the different mass spectrometers evaluated. Experimental results, which included sensitivity, imprecision and inaccuracy, carryover, matrix effect, dynamic range, operating mass range, scan speed, ease of use, robustness, flexibility were recorded for all the MS platforms.

Results obtained during the evaluation demonstrated the new instrumentations to be extremely sensitive providing up to 50-fold increase in sensitivity and yield enhanced spectral quality. This data will be displayed in the poster.

No previous data obtained from the same samples run on the four top manufacturer's machines have been published.

## P008-B2

### Modified-RNA Aptamer-Tobramycin Interaction. SPR Characterization and Impedimetric Detection

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Tobramycin is a broad spectrum aminoglycoside antibiotic. Like other aminoglycosides, the use of tobramycin is limited by a narrow therapeutic range (2-10 µg/mL in serum), because of the potential side-effects of ototoxicity and nephrotoxicity that can appear. Therefore, careful monitoring of the drug levels in patients serum is required.

The major trouble found in aminoglycoside analysis is their lack of electrochemical and spectroscopic properties, so it is of great importance the development of new analysis methods where derivatization or labels are not required.

For this purpose we suggest the use of an RNA anti-tobramycin aptamer<sup>[1]</sup> as molecular recognition element coupled to Faradaic Impedance Spectroscopy (FIS) measurements for tobramycin determination. The RNA aptamer was modified at 2' ribose position with a –OMe group to increase its nuclease resistance. A displacement assay format is proposed based on a general format assay for the determination of small molecules reported previously<sup>[2]</sup>. The diminution in the electron transfer resistance ( $R_{et}$ ) is related to the aptamer displacement from its complex with a surface modified antibiotic by free tobramycin in solution. The sensing surface was evaluated in terms of its reproducibility, detection limit, useful analytical range and selectivity from other aminoglycosides with similar structure.

Interaction between the aptamer and the antibiotic is also studied using Surface Plasmon Resonance Spectroscopy (SPR). The dissociation constant was calculated from the equilibrium data obtained adapting the same format assay used for the generation of FIS data.

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#### Acknowledgements

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## P009-B2

### Evaluation of Quality Control Parameters for the Determination of Major and Trace Elements in Urine by Sample Dilution and Quadrupole ICP-MS

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Urine is an interesting biological matrix for the analytical evaluation of major and trace elements. Many toxic elements are excreted in urine, making it an attractive matrix for estimation of exposure. In addition, some major and trace urinary elements are essential for life, and thus related with diverse physiological processes that can be investigated through urine analysis. ICP-MS, due to its sensitive multielement capability, is the preferred analytical technique for elemental profile determinations in urine (1), but sample preparation adjustments and instrumental specifications are critical points for accurate measurement, especially for a range of elements that are affected by spectral and matrix interferences (2). ICP-MS technological improvements like collision/reaction cells, use of high resolution mass analyzers, or sample digestion protocols are available alternatives to manage the problem, but in some circumstances it can be necessary to apply a simple protocol based on dilution plus determination at simple quadrupole instruments, for instance in screening studies. These simple methods must be carefully checked for accuracy of individual elements results to obtain useful information. In this work, we have tested 1/5 urine dilution followed by quadrupole ICP-MS methodology for the screening of the elemental profile (33 major and trace elements), on urine samples collected in the frame of a clinical investigation about menopause and physical activity. Quality control was performed by blank values evaluation and Seronorm™ Trace Elements Urine reference material elemental recoveries. Applicability of the simple dilution protocol is limited by spectral interferences, especially for As, Cr, Cu, Se, Ti, V, or Zn, whereas a large group of some 25 elements can be accurately measured. Analytical strategies based on digestion procedures and isotopic selection to solve the interferences encountered are presented and discussed.

#### Acknowledgements:

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## P010-B2

### Electrochemical Methods for Determination of Trace Amount of Dopamine and Uric Acid Using Carbon Paste Electrode Modified With $\alpha$ -Cyclodextrine Incorporated Multi Walled Carbon Nanotube

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The electrochemical study of Dopamine and Uric acid has been investigated by using a bare and modified carbon paste electrode (MCPE) as working electrode and pt as counter electrode, SCE as reference electrode, respectively. In 0.2 M Britton-Rabinson buffer solution (pH 6.0), the carbon paste electrode modified with  $\alpha$ -cyclodextrine incorporated multi walled carbon nanotube (> 95%) exhibits a strong catalytic effect toward the electro-chemical oxidation of dopamine (DA) and uric acid (UA). The peak current increases linearly with the concentration of DA in the two range of  $8.73 \times 10^{-7}$  -  $1.47 \times 10^{-5}$  and  $1.47 \times 10^{-5}$  -  $1.62 \times 10^{-4}$   $\text{ML}^{-1}$  and for UA in the range of  $1.17 \times 10^{-6}$  -  $2.15 \times 10^{-5}$   $\text{ML}^{-1}$ . The detection limit (was  $S/N > 3$ ) for DA is  $1.7 \times 10^{-7}$   $\text{ML}^{-1}$  and for uric acid is  $7.7 \times 10^{-7}$ . In this work was used voltammetric methods such as cyclic voltammetric, chronoamperometry, chronocoulometry and square wave voltammetry. Cyclic voltammetry was used to investigate the redox properties of this modified electrode at various scan rates. The diffusion coefficient ( $D = 4.2 \times 10^{-5}$   $\text{cm}^2 \text{s}^{-1}$ ) for DA were determined using electrochemical approaches. Using square wave voltammetry (SWV) for simultaneous measurement, we were obtained two peaks for DA and UA in the same solution which the separation between the two peaks was about 170 mV. The recovery for Dopamine injection was obtained to be in average of 101.07 % and for uric acid in human urine sample in average of 99.4 %.

## P011-B2

### Separation of Several Bioactives Molecules on Mixed-Mode Stationary Phase

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Mixed-mode silica-based chromatography materials developed in our laboratories combine both reversed phase (RP) and weak anion exchange (WAX) characteristics and offer a wide range of applicability for the separation of neutral, acidic, basic, or amphoteric compounds. Chromatographic runs can be performed in many different interaction modes such as reversed phase, hydrophilic and hydrophobic interaction, ion exclusion, ion exchange, or a combination of several of those modes. Due to this high flexibility in usage such multi-modal material allows separation of compounds which are difficult or even impossible to separate on standard column.

The first step of our work consisted in optimizing separation of molecules like betablockers, fluoroquinolone or antidepressants on RP-18 material in order to determine best possible separation with usual chromatographic conditions. In a next step we performed similar method optimization on RP-WAX material. Using non-conventional gradient systems on multi-modal material allowed us to highlight new selectivities, which lead to better chromatographic separations.

After having determined the optimal separation conditions for each mixture of compounds the respective methods were submitted to run-to-run reproducibility tests in order to show their applicability in routine analysis.

## **P012-B2**

### **Assessment of Heavy Metals Levels in Vegetables By AAS And EDXRF Methods**

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Vegetables, an important part of human diet which contain, among others minerals and trace elements, grown at contaminated sites could take up and accumulate metals at concentration that are toxic for human health.

The objective of this study was to analyze the heavy metal concentrations in some leafy vegetables (cabbage, spinach, celery and lettuce) and in their growing soil from sites with different industrial activity by using atomic absorption spectrometry (AAS) and energy dispersive X-ray fluorescence (EDXRF) analysis.

The data sets were analyzed to evaluate the comparability of the used instrumental methods and to determine the levels of heavy metals (Cr, Mn, Fe, Co, Ni, Zn, Cd, and Pb) in the samples. In general, the results obtained were comparable and the strengths and weaknesses of the used instrumental analysis methods were established.

A quantitative evaluation of the relationship of element uptake by the vegetables from soil was made by calculating the coefficient accumulation. The results reveal that cabbage was the least accumulator and lettuce was the most accumulator of heavy metals. The element concentrations of analysed vegetables were within safety baseline levels for human consumption

## P013-B2

### Liquid Chromatographic Fingerprint Analysis of *Ganoderma* spp. Extracts in Hydrophilic Interaction and Reversed-phase Elution Modes Using UV and Evaporative Light-Scattering Detection

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Chromatographic fingerprint analysis is gaining popularity, for example for species differentiation and quality control of medicinal herbs and mushrooms. With respect to liquid chromatography, separations are almost exclusively carried out using reversed-phase (RP) columns. This, however, bears risk of losing fingerprint information for very polar compounds. On the other hand, a combined use of quasi-orthogonal separation modes such as RP and hydrophilic interaction liquid chromatography (HILIC) is a viable means in order to cover a broader range of analytes. In the present study extracts of *Ganoderma* spp., a mushroom widely used in Traditional Chinese Medicine, were investigated. Six polar stationary phases with different separation properties were used for HILIC runs and a C<sub>18</sub> column was employed for separations in the RP elution mode. Chromatographic fingerprints of eleven *G. lucidum* samples served as training set for the construction of column-specific simulative mean chromatograms based on UV detection. Validation with twelve samples covering *G. lucidum*, *G. sinense*, *G. atrum* and *G. tsugae* by correlation coefficient based similarity evaluation of peak patterns showed that a discrimination of *G. lucidum* from other *Ganoderma* species is possible on all columns, except of a bare silica column. Despite differences in the retention and selectivity patterns of different polar-bonded silica packings their sample discrimination capabilities by chemometric fingerprint analysis may be largely equal. Furthermore, the combined use of RP and HILIC was found to be helpful in reducing the risk for sample misclassification. To further investigate the role of experimental variables on fingerprint information additional experiments were carried out which compared fingerprints obtained by UV detection (at different wavelengths) with those delivered by less selective evaporative light scattering detection.

## P014-B2

### Enantioanalysis of (-)Butaclamol Using Vancomycin and Teicoplanin as Chiral Selectors

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(-)-Butaclamol is an antipsychotic drug used in the treatment of schizophrenia. Macrocyclic antibiotics: vancomycin and teicoplanin, are proposed as chiral selectors for the design of the enantioselective, potentiometric membrane electrodes (EPMEs) for the assay of (-)-butaclamol. The slopes of the electrodes are near-Nernstian for the assay of (-)-butaclamol with linear concentration ranges between  $10^{-10}$  and  $10^{-7}$  mol/L and between  $10^{-9}$  and  $10^{-7}$  mol/L for vancomycin and teicoplanin based EPMEs. The electrodes were reliably used for the enantioanalysis of (-)-butaclamol in serum samples.

## **P015-B2**

### **Speciation in Aluminium(III) – Fluoroquinolone Family Members Solutions**

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Aluminium can be regarded as detrimental and in particular neurotoxic element. The iatrogenic aluminum originates mainly from aluminum-containing phosphate binders and aluminum-containing antacids administered to uremic patients or those with gastric or duodenal ulcer.

Fluorinated quinolones are chemically weak substituted heterocyclic amino acids which primarily find use in the treatment of urinary and respiratory infections. Clinical investigations have shown that concomitant intake of fluoroquinolones and aluminum-containing compounds (eg. antacids) results in reduced maximal plasma concentration accompanied by the decrease in AUC.

In our previous works we have investigated complex formation equilibria of aluminum with fluoroquinolones norfloxacin, ofloxacin, fleroxacin and moxifloxacin. Composition and stability of complex species were determined.

The objective of this work was to assess the influence of fluoroquinolones, on bioavailability of aluminum through computer-aided speciation calculation.

The calculations were performed with the program Hyss2006 and the model for human plasma was taken from the literature. The main binding species for aluminium in blood are transferrin, albumin, citrate, phosphate and hydroxide. Very low concentration of fluoroquinolones in plasma do not affect the plasma levels of Al-citrate and Al-phosphate. At concentration levels of fluoroquinolones higher than 0.02 mmol/L, the levels of citrate and phosphate complexes begins to decrease and in some time the concentration of Al-fluoroquinolone complexes increases. These complexes are charged and could be excreted by kidneys. Thus in case of toxic levels of aluminum in plasma the fluoroquinolone would be able to partly bind aluminum through chelation.

## **P016-B2**

### **Trace Element and Sulphur Isotope Ratio Analysis of Single Hair by LA-(MC)-ICP-MS**

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Human hair is known to have the potential to store time resolved information about the trace element status of a person and can be used as an important biomarker for provenance and forensic studies. Hair is easy to sample, store and ship. Moreover, the information is stored for an extended period of time and thus makes hair an excellent tool to provide information about historic individuals. Longitudinal ablation of a single hair by laser ablation and subsequent analysis by ICP-MS for elemental analysis (or MC-ICP-MS for isotope ratio analysis) allows the direct assessment of this time resolved information. Analytical challenges such as e.g. the proper calibration strategy for quantification, the discrimination of endogenous and exogenous origin or the validity of the results have to be overcome.

In this study, elemental analysis was applied to recent and historical samples in order to evaluate the actual elemental status or possible intoxication of well known personalities (e.g. Mozart, Beethoven or Schiller). Moreover, sulphur isotope ratios ( $^{34}\text{S}/^{32}\text{S}$ ) were determined to prove the authenticity and provenance of single hair samples (especially if e.g. DNA analysis cannot be performed).

## P017-B2

### Strontium Treatment does not Affect the Mineralisation of Calcified Tissue in Rats and Dogs

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Molar ratios of key elements in calcified tissue of rats and dogs after treatment with high doses of strontium malonate (SM) were investigated. It was found that while the concentration of strontium increased with dose, the mineralisation remained unchanged. In the present study, 180 rats and 32 dogs were treated with three different doses of SM for 26 and 52 weeks respectively. Following removal of all soft tissue, the concentrations of Sr, Ca, Mg and P in rat incisors and dog femur were determined using inductively coupled plasma mass spectrometry (ICP-MS). The large number of data facilitated a reliable separation of the biological variation from the uncertainty of measurement. Strontium is known to have a positive effect on osteoporotic bone tissue and to reduce the incidence of bone fractures. Strontium is predominantly incorporated into the hydroxyapatite matrix by ionic substitution of calcium, and previous studies investigating the mineralisation of calcified tissue following strontium treatment have frequently reported results on a mass-to-mass basis (mg element / g bone). However, owing to strontium's high molar mass (88 g/mol) compared with that of calcium (40 g/mol), this approach may lead to an overestimation of the resorption of calcium and phosphorous when large amounts of strontium are incorporated into the bone matrix. After converting to molar ratios and correcting for mass contributions from water and organic bone tissue (collagen), it was concluded that even at doses of 1000 mg/kg/day, no significant changes in calcium and phosphate concentrations had occurred.



## **P018-B2**

### **Solid Phase Extraction for Sample Preparation to Determine Selenium Species in Human Urine by HPLC – ICP/MS**

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Selenium has been characterized as an oligoelement and more recently some properties for cancer prevention and other illness have been shown. However, its toxicity requires monitoring of the ingested amount of selenium and an improvement of knowledge in metabolic transformations. For this purpose, monitoring of Se species in human urine is usually performed.

Most of the authors working in this field generally use a tenfold sample dilution to reduce the complexity of urine matrix prior to injection into HPLC – ICP/MS. However, due to the low level of selenium in these samples, such dilution hampers the detection of some species which are below the detection limits of analytical method.

The aim of this research is to develop an alternative sample preparation to avoid or to minimize sample dilution. The analytical procedure is based on urine matrix purification solid phase extraction (SPE). The optimisation of sample purification has been performed by using different stationary phases and testing parameters such as conditioning solution for cartridges, use of ion pairing agents.... After this treatment, the sample is injected, without any further dilution, into HPLC coupled with ICP/MS. Two different stationary phases (porous graphitic carbon and C18) are then used to confirm the identity of Se species.

This method, which shows reproducible results, allows the elimination of part of the matrix without any species losses or degradations. By comparing chromatograms of urine diluted in water (1:10), and urine treated by SPE with only a dilution (1:1) in water, an increase of peaks intensity and the emergence of uncharacterized peaks has been observed. Identified species for which standards are commercially available were quantified by standard additions, and unknown compounds should be further identified by molecular mass spectrometry.

## P019-B2

### Immediate in Situ Derivatization of Urinary Prolyl-4-hydroxyproline Dipeptide with Alkyl Chloroformates for Liquid Chromatography – Tandem Mass Spectrometry

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A polar dipeptide of prolyl-4-hydroxyproline (PHP), examined recently as a potential urinary resorption osteomarker, was treated with a series of alkyl chloroformates in aqueous media; the reaction products were investigated by electrospray mass spectrometry (ESI MS). Direct derivatization of PHP in a urine substitute using a combined action of isobutanol (IBOH) and isobutyl chloroformate (IBCF) converted the metabolite into N-isobutoxycarbonyl-O-isobutyl ester (IBOC-IBE). The derivative afforded a highest ESI  $[M+H]^+$  response from all the analytes examined, including the native PHP. HPLC/MS conditions for the IBCF-treated PHP product were easily set-up on a common RP-C18 HPLC column using an elementary isocratic elution with 10 mM ammonium formate-methanol and tandem mass spectrometry (MS/MS) of the diagnostic m/z 128, 218 ions of the product. Using the heptadeuterated analog (PHP-d7) as internal standard, the novel HPLC/MS method was validated for the quantitative determination of PHP in urine. It has been demonstrated that the derivatization of polar analytes with alkyl chloroformates presents a useful sample preparation approach in HPLC/MS analysis.

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## P020-B2

### A Validated Method to Determine New CB<sub>1</sub> Antagonists in Rat Blood and Brain by Liquid Chromatography – Tandem Mass Spectrometry

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According to interesting pharmacological profile and to wide therapeutic applications of cannabinoid compounds, CB<sub>1</sub> and/or CB<sub>2</sub> derivatives have been extensively investigated in different *in vitro* and *in vivo* assay models.

A new series of cannabinoid ligands have been obtained by PharmaNess. Among this new class of compounds, we have synthesized Rimonabant<sup>®</sup> derivatives bearing thiophene substituen in position 5 of the pyrazole ring. In particular CB<sub>1</sub> antagonist compounds NESS014A and NESS006A were obtained characterized by CB<sub>1</sub> affinity of 35.0 and 14.3 nM, respectively (expressed as K<sub>i</sub>).

To evaluate the effect of the chemical structure of the compounds on the blood brain barrier permeability, bioanalytical methods based on high-performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) in both rat brain and blood were developed and validated.

A HPLC system (Waters separation module Alliance 2695) was used to inject 5µl aliquots of processed samples. HPLC separation was performed by means of Xbridge C18 2.1\*100, 3.5µm, at 35°C using a gradient mobile phase of acetonitrile and water (both charged with 0.2% Formic Acid). The analytes were detected by electrospray ionization in positive mode and multiple reaction monitoring with a Quattro Micro triple quadrupole mass spectrometer (Micromass, Waters).

Standard stock solutions (1mg/ml) of the compounds were prepared in methanol. Analytical standard samples were prepared by spiking known quantity of standard solutions into blank rat brain or blood using internal standards. The biological samples were purified using protein precipitation technique and solid-phase extraction.

After drug administration into jugular vein (1 mg/kg) both brain concentration and blood – time profiles of NESS014A, NESS006A, and Rimonabant<sup>®</sup> were compared.

Satisfactory results in both rat brain and blood analysis in terms of linearity, selectivity, precision, accuracy, lower limit of quantification and extraction recovery were obtained.

Adopting the developed methods, significant differences were highlighted between the pharmacokinetic profiles of the assayed cannabinoid ligands.

## P021-B2

### The Isolation and Determination of Paeoniflorin in *Paeonia Masculata* and Evaluation of its Antiseizure and Antinociceptive Effects in Mice

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Paeoniflorin is a major monoterpene glycosides in *Paeonia species*. Paeoniflorin was isolated from *Paeonia masculata* ssp. *bodurii* by chromatographic methods and the structure identifications were achieved by spectrophotometric methods.

A reversed phase high performance liquid chromatographic method is used for the determination of Paeoniflorin. The analysis of Paeoniflorin and the internal standard metronidazole (IS) were determined using reversed-phase column (Nucleosil 100-5 C<sub>18</sub> 5µm, 250x4.6 mm) eluted with a mobile phase containing acetonitrile: 10mM pH 3.5 phosphate buffer (20:80 v/v) at a flow rate of 1mL/min. Photodiode array detector was set to 230 nm. The retention times of IS and paeoniflorin were 5.2 and 7.8 min, respectively.

Paeoniflorin (i.p.) exerted antiseizure activity in the maximal electric shock (MES) in mice. In a MES intensity setting where 50% of the mice had convulsions, paeoniflorin at 250 and 500 mg/kg, inhibited convulsions in 7 out of 10 and 9 out of 10, respectively. The same dose of paeoniflorin also inhibited acetic acid-induced writhing by 30%, similar to that elicited by 200 mg/kg acetylsalicylic acid ( $P<0.05$ ). We demonstrated that paeoniflorin at doses higher than 250 mg/kg inhibit seizure activity and attenuate inflammatory pain.

## P022-B2

### 3-Hydroxy-2-Methyl-1-(*p*-Aminophenyl)-4-Pyridone and its Role as Complexing Agent

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4-Pyridone derivatives are excellent complexing agents and therefore can be used in the extraction and spectrophotometric determination of metal ions.[1] Some pyridone derivatives are inhibitors of bacterial enzymes [2] and are potential antibacterial agents used for bacterial infections.

Numerous spectrophotometric methods, especially the more sensitive ones, are based on the formation of the complexes between the chelating reagents and metal ions.

The analytical application of few 3-hydroxy-4-pyridones has been outlined in the preceding communications. [3]

Recently, there has been a significant interest in these compounds as sequestering agents for various metal ions.

In this work we have explored the application of the new synthesized 4-pyridone derivative 3-hydroxy-2-methyl-1-(*p*-aminophenyl)-4-pyridone (HZ) as a complexing agent for different metal ions in aqueous solution, as well as the possibility of the extraction of formed complexes in organic phase.

Due to high metal chelating affinity HZ has potential pharmacological usefulness, mostly related to removal therapies of unbalanced metal ions in the human body.

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## **P023-B2**

### **Biological and Environmental Monitoring of Occupational Exposure to Heavy Metals in Metallurgical Factories in Khartoum State, Sudan**

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The main purpose of this presentation is to assess the occupational exposure to some heavy metals in two metallurgical factories in the Khartoum State, Sudan using biological and environmental samples as indicators.

Urine and scalp hair samples were collected from workers in a mint and foundry factories as well as from residents far from emission of metallurgical processes. In addition, indoor air samples were collected from the same factories. Concentrations of Cr, Fe, Co, Ni, Cu, Zn and Pb were determined. For realization purpose, samples were analyzed by both X-ray fluorescence spectrometry and flame atomic absorption spectrophotometry.

Comparing with Fe content in urine samples of residents in the control area, higher Fe content (5-fold) in urine samples of workers in the foundry factory was recorded indicating serious occupational exposure. Elevated Cr, Ni and Cu contents in urine samples of workers the mint factory (2- to 3-fold) were also recorded. In addition, Fe and Zn contents in hair samples of workers in the mint and the foundry factories were higher (> 2-fold) than those of residents in the control area. Furthermore, Cr, Ni Cu and Pb contents in hair samples of workers in the foundry factory were higher (1.5 fold) than those in control area. On the other hand, Fe content in indoor air of the foundry factory was higher than that of the mint factory, and vice versa for Zn. Furthermore, a correlation study between metal contents in hair, urine and air was conducted. Positive correlation with different levels, was recorded. It could be concluded that both biological and environmental indicators give more reliable assessment of occupational exposure to heavy metals, rather than using a single type of sample. Additionally, comparative study between results obtained in the current control area with other worldwide control areas exhibits significant variation, which is due to variation in climate and race. This finding emphasizes that the inclusion of a control area, in the same region of the study area, is essential to assess occupational exposure to heavy metals.

#### **Acknowledgements**

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## P024-B2

### Simultaneous Determination of Quinolone Antibacterials in Bovine Milk by Liquid Chromatography–Mass Spectrometry

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Quinolones are a group of structurally related antibiotics that are widely used in human and veterinary medicine for the treatment and prevention of pulmonary, urinary and digestive infections. The occurrence of pharmaceuticals –particularly antibiotics– in the environment and in food has generated increasing attention. Long-term exposure to antimicrobial agents has been associated with an increased risk of development and spread of antibiotic resistance.

The 1998 World Health Report of the World Health Organization described the increasing occurrence of resistant bacteria and their quick spreading in the world population as one of the biggest health problems of the 21<sup>st</sup> century. In order to ensure the safety of human foodstuffs, the European Union (EU) has set tolerance levels for quinolones in products of animal origin (EU Commission Regulation No. 2377/90, 1990; EU Commission Directive No. 96/23/EC, 1996). Thus, the establishment of sensitive methods for the analysis of residual amounts of these drugs is required for the quality control of food products for consumers and to evaluate the correct application of withdrawal times.

In this work a new liquid chromatography–mass spectrometry method for the simultaneous determination of eight quinolone antibacterials for veterinary use in processed bovine milk samples is presented. The studied quinolones include marbofloxacin, ciprofloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin, oxolinic acid and flumequine. A new sample-treatment procedure has been used for extraction and preconcentration of these compounds. Norfloxacin was used as internal standard. The limits of quantification found (2–7 ng g<sup>-1</sup>) were in all cases lower than the maximum residue limits tolerated by the EU for these compounds in milk. The proposed method was successfully applied to determine these compounds in a large number of processed milk samples obtained from different supermarkets in Granada (South of Spain).

## P025-B2

### Gas Chromatographic-Mass Spectrometric Study of the Degradation of Phenolic Compounds in Wastewater Olive Oil by *Azotobacter Chroococcum*

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During the process of olive oil production, a highly contaminating residue is generated as a by-product of the mechanical extraction. This waste – commonly called *wastewater olive oil* (WOO) – is a complex mixture of water (83-96%), sugar, nitrogenous substances, organic acids, pectins, mucilages, tanins, lipids and inorganic substances. In addition, it presents a poor biodegradability and a high phytotoxicity due to the presence of a large amount of phenolic compounds (PCs), free fatty acids and inorganic salts (mainly potassium salts). In recent years, there has been growing interest in PCs because they have been described as the most powerful contaminant compounds presents in these wastewaters. Therefore, it is important to have analytical methodology to check the evolution of polyphenols content into this matrix throughout the degradation process.

It is known that WOO is degraded by different microorganisms that reduce PCs content. Thus *Azotobacter chroococcum* is capable of increasing biological activity in grounds which contains phenolic residues. This affirmation suggests that these microorganisms could contribute to the biotransformation of the residue.

In this work, the compounds present in the WOO used in metabolic pathways of *A. chroococcum* have been investigated. Microbiological and analytical techniques have been used to follow up the evolution of the experiments. The degradation curves for the compounds capable of being degraded by this microorganism have been individually studied. It has been shown that in batch culture, PCs such as protocatechic acid and *p*-hydroxybenzoic acid facilitate the growing up of *A. chroococcum*. What is more, the maximum concentration in which bacteria can grow was 0.3% (w/v) for both polyphenols. At higher concentrations, substrate inhibition was observed. Therefore *A. chroococcum* can grow up using PCs as an individual source of carbon and energy supply but it is dependent on the type of the compound and on its concentration.



## P026-B2

### A Sensitive Gas Chromatographic-Mass Spectrometric (GC–MS) Method for the Determination of Bisphenol A in Rice-Prepared Dishes

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Certain chemicals possess the potential to modulate endocrine systems, and thereby interfere with reproductive and developmental processes. Bisphenol A is suspected to be one of them. This compound is widely used as a plastic additive, lacquer, resin, or plastic and can usually be found in food samples.

Some workers have investigated the effects of the food processing conditions on the migration of BPA from food or from simulating liquids. Sterilization features are relatively extreme (high temperature and sterilization time). In view of the potentially long shelf-life of most canned foods and the current interest surrounding the exposure to BPA from food packaging, it is important to obtain information on all of these factors. Therefore, it is of crucial importance to devise an analytical methodology for detecting and quantifying these compounds in food.

This work presents an accurate and reproducible gas chromatographic-mass spectrometric (GC–MS) method to detect and measure trace amounts of Bisphenol A in rice-prepared dishes samples. A solid-liquid extraction with acetonitrile was carried out in order to isolate and preconcentrate the analyte. Later, solvent was removed and a silylation step using *N,O*-bis(trimethylsilyl)trifluoroacetamide/pyridine (BSTFA/PYR) was carried out. The silylated compound was identified and quantified by GC–MS using a DB-5 MS column. Bisphenol F was used as a surrogate. The detection limit found was 2.0 ng g<sup>-1</sup> while inter- and intra-day variability was under 6%. The method was validated using standard addition calibration and a recovery assay. Recoveries for spiked samples were over 90% and under 105%.

The proposed method has been successfully applied to spiked and non-spiked rice samples. Anyway, it has been used routinely for monitoring the presence of bisphenol A in the production of rice-prepared dishes and no contamination has been found.

## P027-B2

### Screening and Quantitative Non-Chromatographic Procedures for the Speciation of Arsenic in Rice Flour Using Electrothermal Atomic Absorption Spectrometry

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It is well known that arsenic can be found mainly in natural systems as As(III), As(V), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AsB), the toxicity of these species being quite different. An appropriate procedure to carry out the speciation of these arsenic compounds is to use high performance liquid chromatography linked to inductively coupled plasma mass spectrometry (HPLC-ICP-MS), a sensitive and reliable way provided that this highly specific detector be available. It is clear that alternatives based on the use of other relatively low-cost analytical techniques commonly present in the labs could be of practical usefulness.

In the course of a study of the behaviour of arsenic species when submitted to a heating programme by means of an electrothermal atomizer it was found these compounds can be selectively volatilized in the presence of chemical modifiers. This allowed a procedure for the rapid screening of arsenic species to be outlined. Rice flour was used as a real matrix to prove the practical usefulness of this finding, and our studies proved it was unnecessary for the purpose to completely dissolve the sample. The use of a 10% rice flour suspension prepared in a 1M tetramethylammonium hydroxide (TMAH) solution proved suitable to carry out the measurements. Selective volatilizations obtained using different chemical modifiers allowed to show the presence of the most toxic species (As(III), As (V) and MMA) above 100 ng/g. By changing the heating programme it was possible to distinguish between DMA and AsB.

On the other hand, if quantitation is required, a fractionation of the compounds can be carried out by means of selective retentions in ion-exchange columns followed by elution and preconcentration by dispersive liquid liquid microextraction before measurement by ETAAS. In this case, the detection limit was 1ng/g arsenic in the rice flour sample that, as in the screening procedure, was used as a 10% suspension prepared in TMAH.

## P028-B2

### Surface Characterization of Raw and Chemically Treated Walnut Shells

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Considerable attention has been given, in recent years, to agricultural by-products as raw materials for water pollution control. These biomaterials have been considered as an alternative to more conventional adsorbents, such as activated carbon, due to their low cost and availability [1]. The interest is also to convert these raw materials into useful products since they represent, in most cases, unused resources and present disposal problems.

Frequently, agro-wastes biosorbents are chemically modified to increase their sorption capacities or to remove non-structural constituents such as tannins, terpenes, or phenolic compounds [2]. Biomass chemical modifications may include delignification, esterification of carboxyl groups, methylation of amino groups, and hydrolysis of carboxylate groups.

In this study walnut shells were pretreated with NaOH (alkali treatment) and with HCl (acid treatment).

Alkali treatment results in swelling of fibres and higher cell wall permeability, facilitating extraction of soluble polymers and removal of non-structural constituents and degradation products from the lignocellulosic matrix [3]. Some alkali-labile linkages between lignin monomers, or between lignin and polysaccharides, might be broken by the treatment. Acidic moieties such as carboxylic or phenolic groups, ionized in alkaline solution, might also promote the solubilization of the lignin, either by increasing the solubility of individual fragments or by inducing the swelling of the cell wall.

Acid treatment of lignocellulosic materials has received considerable research attention over the years. Numerous methods have been proposed using mineral acids to solubilize and hydrolyze hemicellulose and provide hemicellulosic sugars for bioconversion to fuel ethanol [2]. The major effect of this treatment is the removal of hemicellulose fraction from the biomass.

Scanning Electron Microscopy (SEM), [Fourier Transform](#) Infrared Spectroscopy (FTIR), and X-Ray Photoelectron Spectroscopy (XPS) were applied, in this study, to investigate the effect of the chemical treatments on the shell surface morphology and chemistry.

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## P029-B2

### Advantages for the Determination of Antioxidants Phenolic Acids in Virgin Olive Oil by CE Coupling Adapted Orthogonal Position from Commercial Co-Axial ESI-MS Interface

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A sheath liquid flow capillary electrophoresis-mass spectrometry system just designed to be coupling means co-axial interface using an electrospray ion source, has been coupled using an adapted orthogonal interface no available commercially designed for liquid chromatography coupling (*LCQ DECAX Plus from Thermo Finnigan*), after affordable structural modifications from the original design, in order to improve the analytical parameters.

A first capillary electrophoresis separation currently published (*Talanta, 2009 in press*) using commercial coaxial source ESI-MS interface for determination of antioxidants compounds phenolic acids in virgin olive oil samples, was compared with the results obtained using laboratory-made orthogonal position. The separations were done on 80 cm fused-silica capillaries (75  $\mu\text{m}$  i.d., 375  $\mu\text{m}$  o.d.), using a 10 mM ammonium acetate/ammonium hydroxide buffer solution at pH 10.0 and applying a constant voltage of 30 kV. The sheath liquid consisting in 75 % isopropanol and 5 mM running buffer at a flow rate of 2  $\mu\text{L min}^{-1}$ , 200 °C as heated capillary temperature and 2.25 kV electrospray needle voltage using negative ionisation mode.

The main advantages that home-made design showed were related to the sensibility, obtaining around 10 fold higher peak absolute area values, stability of the signal since in all cases lower relative standard deviation values (less 5 %) in term of repeatability were got, and on selectivity regarding to signal/noise ratio. Besides lower values of interface parameters as electrospray voltage, sheath liquid and sheath gas flows were required due to using the orthogonal position the ion sampling excludes larger droplets that have higher momentum along the axis, which are drained away through the sink at the bottom of the ion source, and so the sample is ionized more easily, the electrospray stability is bigger and essentially this is translated to improve the signal intensity.

## P030-B2

### An LC-MS/MS Based Reference Method for the Detection of Hidden Milk Allergens in Food

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Allergenic proteins represent one large group of contaminants in food, whose harmful effects towards allergic consumers should not be underestimated, and thus have to be avoided, by this susceptible population group. Analytical methods for identification of food contaminants are necessary to ensure safe food. Besides rapid immunoanalytical methods such as ELISA and lateral flow devices, advanced analytical methods based on liquid chromatography – tandem mass spectrometry (LC-MS/MS) are highly desirable for structural investigation and quantitation of (hidden) allergenic proteins, even in complex food matrices.

Here we describe the development of an LC-MS/MS based reference method for the determination of four allergenic proteins occurring at high abundance in milk: alpha-casein, beta-casein, alpha-lactalbumin and beta-lactoglobulin. Sample preparation consists in extraction of protein from food samples, followed by enzymatic digestion of protein using Trypsin and desalting of the tryptic peptides using solid-phase extraction. Then, milk samples were screened by LC-MS full scans for tryptic peptides of the corresponding allergenic proteins, retrieved by theoretical digestion using "PeptideMass" (ExpASY website). Afterwards, their amino acid sequences were determined by MS/MS product ion scans and checked with the Peptide Fragment Ion Analyzer software. For each of the four milk proteins, two tryptic peptides (with highest signal intensities observed in the LC-MS/MS experiments) were purchased as analytical standards and applied for creating a selective and sensitive LC-MS/MS method based on selected reaction monitoring (SRM). This LC-MS/MS profiling method allowed the simultaneous determination of eight tryptic peptides derived from four milk proteins in a single run. Various food samples such as milk, yogurt, cheese and whey drink have been analysed successfully with this method, yielding characteristic peak patterns. In the near future, this reference method will be further optimised, validated and used for the quantitation of tryptic peptides of (hidden) allergenic milk proteins in (processed) food samples.

## **P031-B2**

### **Screening of Cephalosporin Antibiotic in Milk Samples**

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The cephalosporins are a class of  $\beta$ -lactam antibiotics, they has used in veterinary medicine for the treatment and prophylaxisof livestock animals deseases. Unfortunately nowadays this kind of  $\beta$ -lactam antibiotics are used for weight increase promotion of animals. Also they are illegal food additives. Therefore the problem of livestock products screening for cephalosporins determination is very actual .

For determination of these antibiotics fluorecence polarization immunoassay (FPIA) was used. It is a competitive homogeneous method which is characterized by good sensitivity, high specificity, considerable rapidity of several samples analysis. Now small-size equipment allows to work in any laboratory, even unequipped for this investigations and permits to make a procedure of screening less distant.

The goal of checking of milk products is bound up with constant presence of it in our routine food. For this investigation eight tracers (cefalexin and cefalotin were labeled by different kinds of fluorescent marks) and four conjugates for antibody producing are synthesized, after this a binding between of immunoreagents is checked. Two pairs of tracer – antiserum, which are characterized by good binding are chosen, calibration curves are built. The limit of detection was 5 ppb. It made it possible to select conditions for cefalexin and cefalotin detection in milk samples.

The problem of milk samples screening is very complicated because these objects are characterized a very high matrix effect. Therefore, development of clean-up and pretreatment procedure was very important step of this investigation. It was found out that liquid extraction by methanol with following evaporation was optimal for subsequent FPIA analysis.

The limit of detection for cefalexin in milk sample was 8 ppb, the sensitivity of assay (IC 50 value) was 98 ppb. It allowed us to screening different samples.

## P032-B2

### Determination of Micro Contamination in Danube River by Gas Chromatography-Tandem Mass Spectrometry, as Trimethylsilyl (oxime) Derivatives

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Pharmaceuticals have become one of the major targets in environmental chemistry due to their presence in waste-, surface-, ground- and even in drinking water. Our aim was to further develop our original gas chromatography mass spectrometry single ion monitoring (GC-MS SIM) method in order to get lower limits of quantitation (LOQ) and to get higher number of target compounds (pharmaceuticals, food additives, xenobiotics, bile acids and hormones). The applied analytical method is based on a solid phase extraction (SPE) with Oasis HLB cartridges, followed by gas chromatography tandem mass spectrometry (GC-MS/MS), as their trimethylsilyl (oxime)-ether/ester derivatives.

This hyphenated detection technique allows the isolation of only one ion from the matrix. Product ions are formed from the parent ion by collision induced dissociation (CID) with helium gas in the ion trap. Conditions for the dissociation of the selected parent ions were optimized using the automated method development (AMD) in the resonant mode, by the Varian Saturn GC-MS/MS software. The signal-to-noise ratio has increased 2-12 times compared to the GC-MS SIM method. As a result of this ion preparation technique, not only the sensitivity was increased, but the confidence of the spectrum identification was improved also. Detailed spectrum study was performed and fragmentation pathways were given. The LOQ depending on the parent ion chemical structure were 0.20-1 ng/L. During the SPE method development an alkylation step was introduced (500µL 7M NH<sub>3</sub> in methanol was added) before the evaporation in order to get better recoveries for the most volatile benzoic acid-derivatives. Recoveries were varied between: 65-110 %. The method was successfully applied for the analysis of 42 different micropollutants in Danube River waters.

## P033-B2

### Internal Standard Calibration for the Analysis of Volatile Organic Compounds in Ambient Air by Thermal Desorption – Gas Chromatography – Mass Spectrometry

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Mass spectrometry hyphenated to gas chromatography with thermal desorption injection (TD-GC-MS) belongs to the most common techniques for the analysis of volatile organics (VOCs) at low concentrations ( $\text{pg L}^{-1}$  -  $\text{ng L}^{-1}$ ). Despite its advantages, there remain at least three widely encountered but poorly addressed bottlenecks, limiting precise and accurate quantification. This presentation brings forward solutions to improve calibration.

The first issue deals with the instability of a MS detector for quantification purposes. This results in a limited precision, exemplified by high relative standard deviations (up to 40%,  $n = 5$ ) on response factors of a set of 69 selected VOCs. The addition of [ $^2\text{H}_8$ ]toluene as an internal standard improves this imprecision by a factor of 5.

The second point deals with the matrix in which the standard is dissolved. It is shown that the common practice of using liquid calibration mixtures for gaseous samples may be questionable when using external calibration. Quantification of gaseous VOCs loaded on a sorbent tube using response factors obtained with liquid standards results in systematic deviations of 40-80%. Relative response factors (RRF) determined by analysis of sorbent tubes loaded with both the analytes and [ $^2\text{H}_8$ ]toluene from a liquid phase offer a reliable alternative for quantification of airborne VOCs, without need for expensive and often hardly available gaseous standards.

A third bottleneck deals with complex multi-residue analysis as typically encountered in environmental matrices. Apart from the challenge to identify all VOCs correctly, the analyst is confronted with the difficulty having a proper calibration mixture. Here, a strategy is proposed involving the determination of a RRF being representative for a group of analytes with similar functionalities and electron impact fragmentation patterns. This group method approach indicates to be useful ( $\text{RSD} \approx 10\%$ ) for quantifying analytes belonging to that class but having no standards available.



## P034-B2

### Determination of Aliphatic Alcohol in Water by Gas Chromatography Using Headspace Solvent Microextraction

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The possibility of Applying headspace microextraction into a single drop for the determination of alcohols in aqueous solutions is demonstrated. A drop of Benzyl Alcohol containing 2-Butanones as an internal standard was suspended from the tip of a syringe needle over the headspace of stirred sample solutions for extraction. The drop was then injected directly into a GC. The total chromatographic determination was less than 10 minutes. Optimization of experimental conditions (sampling time, sampling temperature, stirring rate, ionic strength of solution, concentration of reagents, time of extraction and organic drop volume) were investigated and the linear range and the precision were also examined. Calibration curves yielded good linearity and concentrations down to 2.5 ng/ml were detectable with RSD values ranging from 6.0 to 12.0% and the method was applied to the determination of alcohols in tap and river waters.

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## P035-B2

### Determination of Trace Amounts of Nickel by Modified Graphite Furnace Atomic Absorption Spectrometry after Liquid Phase Microextraction

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The liquid phase microextraction (LPME) was combined with the modified Graphite furnace atomic absorption spectrometry (GF-AAS) for determination of nickel in the water and solid samples. In a preconcentration step, nickel was extracted from a 2 ml of its aqueous sample in the pH =5 as lead cationic complex into a 4  $\mu$ l drop of dichloroethane and ammonium tetraphenylborate as counter ion immersed in the solution. In the drop, the nickel-Pyrimidine-2-thiol ammonium tetraphenylborate ion associated complex was formed. After extraction, the micro drop was retracted and directly transferred into a graphite tube modified by [W.Pd](c). Some effective parameters on extraction and complex formation, such as type and volume of organic solvent, pH, concentration of chelating agent and counter ion, extraction time, stirring rate and effect of salt were optimized. Under the optimum conditions, the enrichment factor and recovery were 525 and 94%, respectively. The calibration graph was linear in the range of 0.03-15  $\text{ng ml}^{-1}$  with correlation coefficient of 0.9985 under the optimum conditions of the recommended procedure. The detection limit based on the  $3S_b$  criterion was 0.0062  $\text{ng ml}^{-1}$  and relative standard deviation for (RSD) for eight replicate measurement of 0.1  $\text{ng ml}^{-1}$  and 0.5  $\text{ng ml}^{-1}$  nickel was 3.5 and 3.1% respectively. The characteristic concentration was 0.0055  $\text{ng ml}^{-1}$  equivalent to a characteristic mass of 22 fg. The results for determination of nickel in reference materials, spiked tap water and seawater demonstrated the accuracy, recovery and applicability of the presented method.

**Keywords:** Liquid phase microextraction, Preconcentration, Graphite furnace atomic absorption spectrometry, Nickel.

## P036-B2

### Single Drop Microextraction of Gold and Determination by Electrothermal Atomic Absorption Spectrometry

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The determination of gold has attracted interest not only because of the widespread use of gold compounds to disinfect drinking water but also because of its hazardous effect on human health.

In this work 2-pyridyl mercaptan has been immobilized on microcrystalline naphthalene. The resulting sorbent has been air dried and was used for preparation of micro-column. Sample solution passed through the micro-column and gold retained as a complex in the column. The column was washed by 2 mL of a solution of ethanol and hydrochloric acid to elute the adsorbed gold. Absorbance of eluents was determined by flame atomic absorption spectrometry. Several parameters such as pH of the sample solution, ligand concentration, volume of sample, flow rate and concentration of acid were investigated. At the optimized conditions, a large enrichment factor of 150 fold can be achieved. The detection limit was calculated to be  $0.8 \text{ ng mL}^{-1}$  based on  $3S_b$ . The relative standard deviation RSD ( $n=5$ ) was 1.5%, while the calibration curve was linear in the range of 0.8 to  $65 \text{ ng mL}^{-1}$ . In order to evaluate the accuracy and recovery of the presented method the procedure was applied to the analysis of drinking water and reference materials.

**Keywords:** Gold; 2-pyridyl mercaptan; Solid-Phase Extraction; microcrystalline naphthalene; Flame atomic Absorption Spectrometry

## P037-B2

### Liquid Chromatography-Tandem Mass Spectrometry Determination of UV Filters: Atmospheric Pressure Photoionization versus Electrospray Ionization

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Over the last years, the combination of liquid chromatography-mass spectrometry (LC-MS) electrospray ionization (ESI) mode has become the method of choice for the analysis of polar pollutants. On the other hand, atmospheric pressure chemical ionization (APCI) has proved to be suitable for the determination of semi-polar analytes. Since 2000, with the introduction of an atmospheric pressure photoionization (APPI) interface, sensitive LC-MS analysis of at least UV-absorbing, non polar substances became possible.

The study presented reports the evaluation and comparison of electrospray ionization and atmospheric pressure photoionization for monitoring 11 UV filters, 4 in negative ion mode and 7 in positive ion mode, in water samples.

For 9 of the compounds APPI generated similar response to that of ESI, but APPI signal-to-noise (S/N) ratios are 1.3 – 60 fold higher. The two most polar analyzed UV filter compounds (PBSA and BP-4) were more efficiently ionized by ESI offering higher signal intensities and lower detection limits. Furthermore, APPI was less susceptible to ion suppression than ESI when real samples were injected. In order to optimize the APPI conditions different solvents used as dopants were examined to enhance the efficiency of the photoionization process. Among the evaluated dopants, toluene was selected as a compromise. At a toluene flow rate of 10% of the solvent flow rate the ionization response increased by a factor of 40-50 over no-dopant conditions for the compounds in positive ion mode and more than 300 for the compounds in negative ion mode.

In summary, APPI exhibited superior performance for the analysis of the semi polar and lipophilic UV filter compounds. The most polar analytes, PBSA and BP-4, should be analyzed preferably with ESI in negative ion mode.

## P038-B2

### **Clean Direct Potentiometric Method for the Determination of the Ionic Liquid Butylmethylimidazolium Chloride in Contaminated Water and its Application in Water Remediation**

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Ionic liquids (ILs) are acknowledged to be 'green' alternatives to organic solvents in many processes owing to their unique chemical and physical properties. However, it has recently been demonstrated that many commonly used ILs have a certain level of toxicity. Because, environmental pollution through accidental spillage or by effluents is always possible, so the development of new methods for the determination of ILs is of particular interest for environmental analysis.

The construction and electrochemical characteristics of an ion-selective membrane electrode for the determination of butylmethylimidazolium chloride (BMIM-Cl) is presented. The sensing membrane of the electrode comprised BMIM in a plasticized PVC matrix and the influence of the membrane composition on the response was studied. The electrode showed a fast, stable and Nernstian response over a wide BMIM concentration range with a low detection limit and a wide working pH range. The electrode showed good reproducibility and selectivity with respect to some common inorganic ions. The response of the sensor was also tested towards others imidazolium chlorides ILs.

Since dissolution in water during industrial use is the most likely route for ILs to enter the environment, the new sensor was applied to the determination of BMIM-Cl in tap, ground and waste water. Good analytical results were obtained.

Other applications for the ion-selective electrode were the calculation of the ion-partition coefficients of imidazolium cations, which are related with their toxicity, and the monitoring of the water remediation process involving biosorption of BMIM-Cl by dry biomass from the aquatic plant *Posidonia Oceanic* and orange rind.

In order to develop a 'clean method', all the waste generated during the research were treated with *Posidonia Oceanic* before they are discharged into the environment.

## P039-B2

### Accelerated Solvent Extraction (ASE) and Ultra Performance Liquid Chromatography Coupled to Triple Quadrupole Mass Spectrometry (UPLC-QqQ-MS/MS): Innovative Techniques for Fast Analysis of Steroidic Compounds in Woodchips

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Laboratories are constantly working towards increased sample throughput and enhanced laboratory efficiency by e.g. reducing the sample preparation time and accelerating analysis time. In this study a combination of innovative techniques was used to develop a method for the rapid analysis of steroidic compounds in woodchips.

In wood, plantsterols, characterised by the steroid skeleton structure, are naturally occurring substances. However, knowledge on the occurrence of steroidic hormones in wood matrices is scarce despite the fact that the presence of androstadienedione in paper mill effluent has been extensively reported. This lack of information might be due to the complexity of a solid matrix such as wood.

This study describes an analytical method for the determination of specific steroidic analytes in woodchips. Based on the pathway leading from cholesterol to several sex steroids, progesterone, 17-hydroxy-progesterone, androstadienedione, androstenedione,  $\beta$ -boldenone,  $\alpha$ -testosterone and  $\beta$ -testosterone were selected as the analytes of interest. Accelerated solvent extraction was performed using an ASE350 system (Dionex Corporation, Sunnyvale, USA) allowing fast and efficient extraction of all analytes using a combination of increased temperature and pressure. Chromatographic separation was achieved in a runtime of only 5 minutes using an Accela ultra high performance liquid chromatograph (U-HPLC) (Thermo Electron, San Jose, USA). Mass spectrometric detection was carried out in the selected reaction monitoring mode (SRM) using a TSQ Vantage triple quadrupole mass analyser (QqQ-MS/MS) equipped with an electrospray ionisation interface (Thermo Electron, San Jose, USA).

Innovative techniques such as ASE and U-HPLC-QqQ-MS/MS allowed fast and efficient analysis of steroidic analytes in woodchips. Future experiments will focus on the validation this method.

## P040-B2

### Investigation of Effect of Acidity and Redox Potential on the Sorption of Chromium in the Soil

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Soil contamination with anthropogenic chromium, which mainly comes from industrial activity and traffic pollution, has received much attention in recent years. The anthropogenic chromium is easily accumulated in the topsoil, as in natural sink for contaminants, resulting potential problem like toxicity of plant and animals. Having in mind that knowledge it appeared as important to investigate the capacity of agricultural soil for chromium binding and other conditions like acidity and redox potential that could increase its solubility and mobility.

In order to investigate the influence of acidity and redox potential on the bioavailability of chromium, its solubility and mobility as well, an incubation experiment was conducted. Soil samples were spiked with standard solutions of Cr(III) and Cr(VI) at four concentration levels (40, 80, 120 and 200 ppm). Within each concentration levels, four samples, with different pH values (5.98 as natural, 5.00 and 7.0) were analyzed. Additionally, redox condition were set to be aerobic, with about 80% water holding capacity and anaerobic, too. After the incubation period of 60 days was terminated the experiment was continued.

The mobility and bioavailability of chromium depends on physical and chemical forms of this metal, so the measurements of its total content doesn't offer enough information. To get more detailed information about association of chromium with geochemical structures of the soil and to predict its behavior and potential bioavailability three stages sequential extraction with different chemical treatment (1M solution of ammonium–acetate, 0.1 M solution of hydroxylamine–chlorhydrate and a 0.2/0.2 M oxalic acid/ammonium-oxalate mixture) was performed.

For the measurements of chromium concentrations in analyzed samples atomic absorption spectrophotometry with graphite furnace (GFAAS) was applied.

Obtained results will be discussed in the way to explain potential sources of this element in the soil structures.

## P041-B2

### **A Sensitive Method for Determining Total Vanadium in Water Samples Using Colorimetric-Solid-Phase Extraction-Fiber Optic Reflectance Spectroscopy**

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In recent years sorption-spectrophotometric method has been most widely used for the separation and sensitive determination of metal ions, mainly in water samples. This methodology, termed Colorimetric Solid Phase Extraction (C-SPE), is based on the extraction of analytes onto a proper support loaded with a colorimetric reagent and then quantified directly on the adsorbent surface using reflectance spectroscopy. Reflection spectrometry is a valuable alternative for the analysis of solid samples on a support. C-SPE provides another alternative for greener sample pretreatments. By utilizing this methodology the elution step can be completely eliminated from the C-SPE process. In this work, a selective colorimetric solid-phase extraction (C-SPE) method for the determination of total vanadium in water samples was developed. This method introduced a new variation of C-SPE. The color reaction is based on the reaction of vanadium(V) ternary complex formed with 1-(2-pyridilazo)-2-naphtol (PAN) in the presence hydrogen peroxide ( $H_2O_2$ ). In this technique, the target analytes in samples are extracted onto solid matrix loaded with a colorimetric reagent and then quantified directly on the adsorbent surface by using a miniature fiber optic reflectance spectrometer. The measurements were carried out at a wavelength of 589.4 nm since it yielded the largest divergence different in reflectance spectra before and after reaction with the vanadium. The overall time required for the C-SPE procedure was ~ 20 min. The amount of concentrated vanadium is then determined in a few seconds by using miniature reflectance spectrometer. At the optimal conditions, a calibration curve was constructed, revealing a linear range of 0.05-0.52  $\mu\text{g mL}^{-1}$  and a detection limit as low as 0.01  $\mu\text{g mL}^{-1}$  while the RSD lower than 2.8%. The proposed method was applied to the determination of vanadium in tap water, seawater and certified reference material samples.



## P042-B2

### Microwave Assisted Cloud Point Extraction and Determination of PGEs from Dust Samples by ICP-MS

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Automotive catalytic converters typically contain PGEs, mainly Pt, Pd and Rh. The use of catalytic converters has led to an increase of PGEs in and around areas with heavy traffic and it is possible that, in the long run, PGEs may accumulate in the environment. Nowadays, inductively coupled plasma mass spectrometry (ICP-MS) is often used for the determination of PGEs. However, the determination of  $^{105}\text{Pd}$ ,  $^{196}\text{Pt}$  and  $^{103}\text{Rh}$  by low resolution, e.g. quadrupole ICP-MS, is hampered by spectral interferences from monoatomic and polyatomic ions produced from the matrix constituents and gases used (e.g.  $^{87}\text{Sr}^{16}\text{O}^+$ ,  $^{206}\text{Pb}^{2+}$ ,  $^{89}\text{Y}^{16}\text{O}^+$ ,  $^{179}\text{Hf}^{16}\text{O}^+$ ). Recently microwave assisted cloud point extraction (MW-CPE) procedures have been applied for the separation of e.g. PGEs from matrix before ICP-MS determination. For example, cetyltrimethylammonium bromide/Triton X-114 and 2-mercaptobenzothiazole/Triton X-100 have been used in the MW-CPE procedures.<sup>1-3</sup> In this study, the interference elimination in the Pd, Pt and Rh determinations was done using a temperature controlled MW-CPE with 2-mercaptobenzothiazole as a ligand and Triton X-100 as a surfactant. The extraction procedure used was modified from the MW-CPE method described earlier by Simitchiev et al<sup>1</sup>. It was found out, that almost all main interfering elements (e.g. Zn, Sr, Y, Hf, Pb) were eliminated using the MW-CPE. The results obtained for certified reference material (BCR-723) showed that trace concentrations of Pt and Rh can be determined in dust samples by ICP-MS after microwave digestion microwave and temperature controlled MW-CPE. Furthermore, the preliminary results from the spiking experiments indicated, that also Au, Pd and Ru are quantitatively recovered by the method used.

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## P043-B2

### Determination of Ecological Status of Ludas and Zobnatica Lakes (Vojvodina, Serbia) Using Fluorogenic Model Substrates

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The ecological status of Ludas and Zobnatica lakes (Serbia) was estimated by using both microbiological and biochemical methods. Apart from standard microbiological analyses, measurement of extracellular enzyme activity is widely used method for assessment of water ecosystem pollution. Water samples were collected seasonally in June and October 2008. Determination of heterotrophic plate count (HPC) was carried out after plating appropriate dilution on Nutrient agar (26°C, 72 h). On the basis of HPC, the water quality was categorized according to Kohl. As biochemical method, fluorogenic model substrates that release 4-methylumbelliferone (4-MU) after degradation were used to determine the phosphatase and  $\beta$ -glucosidase activity enabling hydrolytic rate measurements as low as  $1\text{ nmol (L x h)}^{-1}$ . The concentration of the reaction product was measured fluorimetrically (364 nm excitation, 445 nm emission). During the summer months, water from Zobnatica belonged to class II while from Ludas to class II-III, whereas in October water of Zobnatica Lake belonged to class I-II and water of Ludas Lake to class II. The phosphatase activity was higher in June in Ludas ( $2295.95\text{ nmol (L x h)}^{-1}$ ) than in Zobnatica Lake ( $1445.18\text{ nmol (L x h)}^{-1}$ ) as well as in October ( $1315.38\text{ nmol (L x h)}^{-1}$  and  $252.35\text{ nmol (L x h)}^{-1}$ ). The  $\beta$ -glucosidase activity was higher too in June in Ludas ( $96.39\text{ nmol (L x h)}^{-1}$ ) than in Zobnatica ( $35.01\text{ nmol (L x h)}^{-1}$ ) as well as in October ( $72.64\text{ nmol (L x h)}^{-1}$  and  $52.157\text{ nmol (L x h)}^{-1}$ ). The results indicate that the phosphatase and  $\beta$ -glucosidase activity was significantly higher in Ludas than in Zobnatica Lake in June as well as in October.

Key words: fluorogenic method, phosphatase,  $\beta$ -glucosidase, Ludas Lake, Zobnatica Lake, ecological status;

## P045-B2

### Assessment of Multiwalled Carbon Nanotube Paste Electrode for Square-Wave Adsorptive Cathodic Stripping Voltammetric (SWAdCSV) Determination of Methyl Parathion in Water Samples

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In this work the assessment of an electrode composed by multiwalled carbon nanotube (MWCNT) dispersed in mineral oil as well as its application on the electrochemical determination of methyl parathion (MP) in environmental water samples by square-wave adsorptive cathodic stripping voltammetry (SWAdCSV) is described. The suitability of the electrode for this purpose was confirmed by comparing it to a glassy carbon electrode (GCE) and to carbon paste electrode (CPE). An increase of 7.1 and 3.4 times on the signal, respectively, was achieved. In order to obtain the best performance of the method, significant factors were established by a factorial design and the optimization was made by employing the Doehlert matrix. Based on these chemometric tools the following experimental conditions were selected: 7.95, 70 mV, 205 Hz and 0.3 mol L<sup>-1</sup>, respectively, for sample pH, pulse amplitude, frequency and buffer concentration. A study of interferences was conducted through the addition of inorganic ions NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup> and Mn<sup>2+</sup> during methyl parathion analysis and no interference was noted. The method presented a linear range between 0.56 and 18.00 μmol L<sup>-1</sup> (r = 0.995), limit of detection (LD) of 0.15 μmol.L<sup>-1</sup> and limit of quantification (LQ) of 0.49 μmol.L<sup>-1</sup>. The determination of MP on environmental spiked samples showed good recovery values and repeatability.

## P046-B2

### Optimization of Thermal-Desorption Experimental Conditions in GC-MS Analysis of Environmental Samples

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Thermal desorption coupled to gas chromatography-mass spectrometry (TD-GC-MS) is a common technique used to quantify volatile organic compounds in environmental samples. With thermal desorption methods, however, a collection of experimental parameters have to be optimized, in order to satisfy desirable properties of the chromatograms, namely acceptable sensitivity and peak shapes for each compound. The optimization of these experimental factors is not an easy task. First, because the relationship between experimental factors (e.g., cold-trap temperature) and objective (e.g., peak asymmetry of compound A) is obscure. Second, because these objectives are not always compatible, yielding a situation of trade-off between signal quality and sensitivity.

To this aim, a central composite design (CCD) was run to optimize the sensitivity (peak area) and signal quality (peak asymmetry) of the chromatogram. The experimental factors to optimize were desorption time, desorption temperature, outlet split and the cold-trap maximum temperature. Peak asymmetries and peak areas were measured for several (representative) compounds of the sample. A model was build to relate objective functions with experimental conditions, Derringer functions were used to reduce the complexity of the multi-objective optimization problem to a single-objective optimization case.

Statistical analysis has shown outlet split as the only factor that significantly affects peak asymmetry, whereas cold-trap temperature and outlet split are found to be the most significant factors affecting peak area. Although the optimum conditions of the two responses are not compatible, an optimum condition finding a compromise between them has been found.

## **P047-B2**

### **Accuracy and Precision in Analysis of Certain Chlorophenols in Water at ng/L Level by SPME/GC-ECD**

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In cork industry, boiler water must fulfil several quality parameters: low organic pollution and maximum admissible values of organic and inorganic pollutants including 2,4,6 trichlorophenol (TCP), 2,3,4,5 tetrachlorophenol (TeCP) and pentachlorophenol (PCP). These phenols must be detected and quantified at ng/L level and the usual methodology is derivatization with acetic anhydride and analysis by solid phase microextraction (SPME) coupled with gas chromatography. This technique permits low detection limits but has the disadvantage to find a suitable internal standard so, sometimes, there are problems with reproducibility. A fast and simple analytical methodology, without internal standard, is presented in order to detect and quantify TCP, TeCP and PCP in low organic pollution water such as ground, tap and well water, with optimum levels of accuracy and precision, achieving repeatability within the rules laid down by Chemical Metrology as the Horwitz equation.

With this methodology, liquid samples are derivatized with acetic anhydride, heated at 55°C and target compounds are absorbed with a 100 µL polydimethylsiloxane SPME fiber in head space mode.

Afterwards, chlorophenols are desorbed in a gas chromatography injector at 260°C and a electron capture detector detects, identifies and quantifies the analytes at ng/L level. The results are good as fulfils the metrology criteria at these concentrations. The correlation coefficient is upper 0.9, the repeatability is under 15%. With reference to detection limits, the obtained results were 35 ng/L for TCP and TeCP, and 20 ng/L for PCP.

## **P048-B2**

### **Identification and Quantification of Antibiotic Residues in Natural Waters by HPLC–ESI-MS/MS**

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In this paper sensitive analytical method for analysis of selected antibiotic residues in natural waters was presented. The procedure was based on the solid-phase extraction (SPE) and the analysis of the extracts by high performance liquid chromatography–tandem mass spectrometry, with electrospray ionization (HPLC–ESI-MS/MS). Seven environmentally relevant antibiotics were chosen for the study, according to human and veterinary consumption in Serbia. Several commercial and laboratory made SPE cartridges were tested for the extraction of the analytes from the water samples. Significant matrix effect was observed and eliminated using matrix-matched standards. The developed analytical method exhibited good linearity, and low limits of detection ( $0,033\text{--}0,551\text{ ng cm}^{-3}$ ) and quantification ( $0,109\text{--}1,838\text{ ng cm}^{-3}$ ). The method was applied to real water samples for monitoring of the selected antibiotics. Results revealed the presence of azithromycin in 22% of water samples in the concentration range  $25\text{--}140\text{ ng dm}^{-3}$ .

## P049-B2

### Determination of Organic UV Filters in Water by Using Sorptive Extraction Techniques and DART-MS

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The rising awareness of the risk of direct exposure to sunlight has led to increased use of personal care products such as sunscreens containing UV filters. Sunscreens provide protection from UV radiation by producing a thin layer on the skin in which UV light is either absorbed by organic compounds or scattered, absorbed and reflected by inorganic micro particles. With the increasing use of sunscreens, their environmental impact becomes an important issue. The sunscreen ingredients, which are applied to the skin, may enter the surface water directly (when released from the skin during water activities) or indirectly via wastewater treatment plants (when released during showering or washed from textiles).

Because of very low concentrations of UV filters in environmental water, pre-concentration has to be performed to allow a reliable analysis. Stir bar sorptive extraction (SBSE) is a suitable technique for collecting organic UV filters. Once the organic substances are collected, the stir bar is commonly placed into a thermodesorption unit of a GC-MS system<sup>1</sup>. Normally the GC-MS run can take more than 30 minutes depending on the complexity of the sample. A much more straightforward and less time-consuming technique is direct analysis in real time mass spectrometry (DART-MS). DART-MS allows the direct determination of UV filters, which are pre-concentrated on polydimethylsiloxane-coated stir bars (Twister) within only a few seconds without any further preparation.

To demonstrate the suitability of DART-MS for the determination of commonly employed UV filters, a test set of five organic filters was defined and subsequently measured in real samples.

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## P050-B2

### Environmental Behaviour Study of 4-*t*-Octylphenol Ethoxylates in Agricultural Soil Samples by GC-MS

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Alkylphenol ethoxylates (APEOs) are non-ionic surfactants widely used as industrial cleaning agents and in other applications such as emulsifiers, wetting agents, stabilizers, de-foaming agents and intermediates in the synthesis of anionic surfactants.

Although parent APEOs are not classified as highly toxic substances, their environmental acceptability is strongly disputed because of persistent metabolic products (alkylphenols) generated during wastewater treatment. The main environmental concern is not the toxicity of these compounds, but rather their estrogenic potential. Because of these findings, the use of APEOs in domestic products has been subject to a voluntary ban across Europe. Moreover their use in industrial products is planned to be phased out in the near future. However, mainly because of lower production costs, APEOs are still being used in substantial amounts in institutional and industrial applications. Hence, it is important to devise sensitive analytical methodology to determine these compounds and study their environmental behaviour.

This work presents an analytical method to determine 4-*t*-octylphenol ethoxylates from 1 to 7 ethoxy units in soil samples. It involves these steps: extraction of the analytes from the soil using Pressurized Liquid Extraction (PLE); preconcentration and purification by Solid Phase Extraction (SPE); derivatization with N,O bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane and lastly separation and identification by gas chromatography-mass spectrometry. The different variables affecting the PLE procedure, the SPE procedure and the derivatization reaction were optimized. Acenaphthene was used as internal standard. The detection limits found were between 1.0 and 38.0 ng g<sup>-1</sup> while inter- and intra-day variability was under 4 %. The method was validated using standard addition calibration and a recovery assay. Recoveries for spiked samples were over 97 % and under 104 %.

The proposed method was successfully applied to study the behaviour of 4-*t*-octylphenol ethoxylates in an agricultural soil of the fertile plain of Granada (Spain).



## P051-B2

### Liquid-Phase Microextraction Based on Solidification of a Floating Organic Droplet for Mercury Determination Using Electrothermal Atomic Absorption Spectrometry

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Liquid-phase microextraction (LPME) refers to a class of sample preparation procedures based on using very low volumes of extraction solvent [1,2]. Recently, a new type of LPME based on solidification of a floating organic droplet (LPME-SFO) has been introduced [3]. In this technique, a microdrop of the organic solvent is floated on the surface of an aqueous sample which is being stirred in such a way that the microdrop remains at the top-center position of the aqueous sample. After the completion of the extraction, the sample vial is cooled, the solidified organic solvent separated and next melted again in order to carry out the measurements in this analyte-enriched phase. More recently, another new procedure (dispersive liquid-liquid microextraction, DLLME) combined with solidification of a floating organic droplet (DLLME-SFO) has been proposed [4]. The large contact area between the organic droplets and sample solution is beneficial for the fast mass transfer from the aqueous to the organic phase and so the extraction time is shortened greatly.

In this communication, the preconcentration of mercury by using a very small volume of undecanoic acid (UA) that acts both as a complexing and extractant reagent is studied. Since the UA melting point is low (28-31 °C) this chemical is appropriate for the practice of LPME-SFO or DLLME-SFO. A study of both approaches has been carried out looking for the highest preconcentration factor and the shortest extraction time together with an easy phase separation. The measurement of the mercury preconcentrated in the organic droplet is carried out using electrothermal atomic absorption spectrometry. To obtain a suitable modification effect during the heating cycle, palladium is electrochemically deposited onto the surface of the pyrolytic atomizer. By extracting the aqueous phase (30 ml) with 50 µl UA a preconcentration factor as high as 350 is achieved. Low concentrations of mercury (0.1-3 µg/l) in the aqueous phase can be measured with relative standard deviations (five measurements) below 5%.

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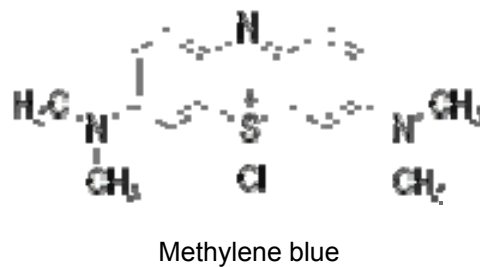
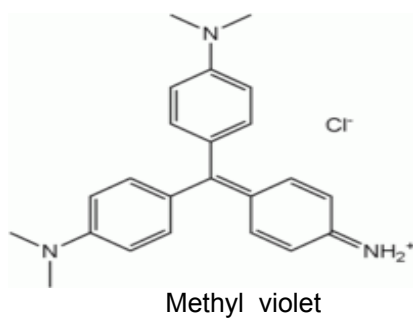
## P052-B2

### Competitive Adsorption of Methylene Blue and Methyl Violet Dyes from Aqueous Solution onto Rice Husk

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The removal of color from the aquatic systems caused by presence of synthetic dyes is extremely important for environmental view point due to these dyes is toxic and carcinogenic. Most of the work reported in literature involves single component adsorption of dyes onto different biomass. However, industrial effluents contain several dyes, therefore simultaneous removal of two or more dyes are necessary. In this study, competitive adsorption of methylene blue (MB, basic dye) and methyl violet (MV, basic dye) onto the rice husk were investigated. The effect of important parameters on the equilibrium, such as the value of initial pH, foreign ion, the flow rate, temperature, contact time, bed depth, the influent and adsorbent concentration of the studied dyes and particle size were examined using batch and column experiments. In the binary dyes mixtures, the affinity of the rice husk for methyl violet was found greater than that for methylene blue at the optimum pH of 6.5 and 30 min contact time. Depending on equilibrium data obtained in this study, thermodynamic parameters will be discussed in details.



## P053-B2

### Technological Conception of an Automated Biosystem for the Monitoring of Pollution in Water Samples

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In order to protect the public health and the environment, National and European authorities have published several legislative documents to improve the management of the environmental pollution. Currently, the high cost of the used methods limits the number of the analysis campaigns. The development of alternative, quick and cheap methods is thus necessary for the monitoring of environmental pollutions. Among them, bacterial biosensors are analytical devices which could be easily used for this purpose.

The aim of this work was to develop a biosensor (namely SENTINEL), based on the bioluminescence production emitted by the *Escherichia coli* DH1 pBZntlux strain in order to detect heavy metals. The bacterial cells are entrapped in a solid matrix of agarose and immobilized in the wells of a macro-chip. The polymer gel allows the chemical transfers between the sample and the cells. An internal canal, in the macro-chip, allows the fluidic circulation of the analyzed samples. A system of thermal regulation was designed to establish a stable and reproducible environment for the bacteria. The optical transducer (Charge-Coupled Device camera) were chosen according to the spectral emission of the bioluminescent bacteria.

The study proceeded in three phases: design and realization of the biosensor *Sentinel*, modeling of parameters of use in then biosensor and finally on-line detection of a model pollutant : Cadmium. The *Sentinel* biosensor appears as an additional alternative to the current methods of detection of the pollution in water samples and can be broaden to other applications.

## P054-B2

### Dimensional Characterization of Low-Z Elements in Airborne PM10 Samples Using $\mu$ -SRXRF

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Micro Synchrotron Radiation X-Ray Fluorescence ( $\mu$ -SRXRF) is a powerful spectroscopy technique that uses advanced light sources like synchrotron radiation to induce X-ray fluorescence in samples and provide exhaustive information on the micron and sub-micron scale. It is conducted by raster scanning of the sample with a focusing beam and measurement of the emitted x-ray fluorescence for each of the irradiated areas. One of the major advantages of synchrotron radiation  $\mu$ -XRF spectroscopy is its non-destructive nature and samples can usually be analysed with no pretreatment. At the ESRF (Grenoble, France) ID-21 beamline we have analysed a PM<sub>10</sub> sample collected at an urban-industrial site near a steel mill in the Province of Trieste (Italy), in order to determine possible spatial correlations among low-Z elements (S, Cl, K, Ca, Ti, V, Cr, Mn, Fe and Ba), as well as investigating the possibility of using imaging techniques as a way to determine the granulometry of PM<sub>10</sub> particles containing the various chemical elements. In the PM<sub>10</sub> sample we have found a consistent significant correlation between Ca and S.  $\mu$ -XANES analysis has shown that more than 98% of the sulphur was present in sulphate form. We can therefore suppose that a major part of the sulphate is present as CaSO<sub>4</sub>. Granulometry analysis via imaging techniques has shown that some elements like Fe, Ca and S are more suited to this type of analysis than others. Additionally we have investigated the spatial homogeneity of a PM<sub>2.5</sub> certified reference material (NIST SRM-2783) by analysing four adjacent areas on this sample (total area 1 mm<sup>2</sup>). The CRM has shown a %RSD less than 7% for Al, Si, P, S, Cl, K, Ca, V, Cr, Fe and Ba while Ti and Mn have shown a %RSD close to 17%.

## P055-B2

### Mass-Balanced Investigation of the Transport and Fate of Pharmaceuticals in Biosolid Enriched Topsoils

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Pharmaceuticals and personal care products (PPCPs) in the environment have generated increased interest recently, with research predominantly focusing on their occurrence in wastewater treatment effluents and downstream river catchments. However, their presence, mobility and fate within the soil environment have been left relatively unstudied. PPCPs present in sewage may partition onto sludge during treatment, which is often subsequently used as an agricultural fertiliser. Therefore, there exists the possibility for leaching of bioactive PPCPs to the soil environment over time. Cause for concern naturally lies in the possible development of pathogenic resistance to antibiotics.



This study focuses on the determination of the solid-water partition coefficients ( $K_d$ ) for a selection of pharmaceutical compounds from a variety of therapeutic classes in soil and biosolid suspensions. The consequent transport of pharmaceutical residues within the soil compartment is presented. Packed soil columns amended with sewage sludge were exposed to conditions of constant simulated rainfall typically observed for an average 6-month period. PPCPs and their distribution between solid and liquid phases were determined thereafter. Liquid leachates were analysed directly using liquid chromatography with tandem mass spectrometry (LC-MS/MS) in small volume intervals over the course of the percolation experiment to map leaching behaviour. For mass balancing, the sludge/soil analysis was performed using a previously validated pressurised liquid extraction, solid phase extraction and LC-MS/MS method [1]. Presented work here will indicate which pharmaceutical residues were retained by the topsoil compartment and, of these, which showed the potential for chemical or biological transformation even over an expedited time period. Similarly, those PPCPs showing the potential for transport to deeper soil strata or groundwater is shown.

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## P056-B2

### Determination of Bisphenol A (BPA) and its Chlorinated Derivatives by HPLC-MS/MS. Study of their Degradation in a Submerged Membrane Bioreactor (MBR)

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The ability of a MBR to remove BPA from wastewaters was evaluated, since this compound is potentially harmful because of its suspected carcinogenic and estrogenic properties. In order to prove this, an analytical method was developed, which was aimed at facilitating the determination of BPA in complex matrices, like urban wastewaters, without losing efficiency and sensitivity. The method includes a previous easier treatment, as far as the extraction of the analyte from the samples is concerned, followed by a preconcentration step using a solid-phase extraction procedure, and subsequent detection and quantification of BPA by LC-MS/MS using an ESI interface in negative mode and using bisphenol F as surrogate. The optimization of the parameters of the instruments reduced the retention times and the injection volume, compared to other methods.

The removal capacity of BPA by the MBR was evaluated by the direct addition of three increasing concentrations of this compound to the bioreactor. Each concentration was maintained during 24 hours. The results showed that MBR removed BPA with a low efficiency (between 30–50%). The contribution of sludge adsorption to BPA removal was quite low, so it was suggested that biodegradation dominated the BPA removal process. Since the physicochemical parameters and those for the control of the process in the MBR remained constant during the whole experiment, the observed increase of the elimination percentages every day, supports the idea that biomass adaptation by longer previous exposure and higher concentrations of BPA could stimulate the capacity of biodegradation of bacterial population in the bioreactor. At the same time this could explain the low overall efficiency of this MBR, since BPA was not detected in the so far analyzed wastewaters, unlike other studies, which could be an evidence that bacteria in this MBR were not adapted to the biodegradation of BPA.

## P057-B2

### Determination of Linear Alkylbenzene Sulfonate (LAS) Using Liquid Chromatography-Fluorescence Detection (LC-FLD). Study of its Removal in a Submerged Membrane Bioreactor (MBR)

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LAS is one of the major anionic surfactants used on the market. Important application products are household detergents, like laundry powders and liquids, dishwashing products and all-purpose cleaners, as well as in industrial processes<sup>1</sup>. The total tonnage consumption estimate of LAS in Europe was about 430Kt in 2005<sup>2</sup>. The extensive use of LAS results in thousands of tons of this pollutant being incorporated into wastewaters, which should be eliminated by wastewater treatment plants.

Nowadays, one of the most successful procedures to remove pollutants in wastewaters is the MBR<sup>3</sup>. Therefore, the removal of LAS in a MBR was studied, and for this purpose it was developed a new method that allows the quantification of this contaminant in complex matrices such as wastewaters without using a solid phase extraction procedure, because the detection of LAS is based on the specific fluorescence that it shows, while the other components of the wastewaters don't exhibit fluorescence at the used wavelength. Thus the time of analysis was significantly reduced, as well as it was maintained the high level of efficiency and accuracy of the analytical method.

A monitoring program of LAS in the MBR was carried out. The samples were taken twice per week during 3 months. The studied degree of resolution was the main homologues of LAS that are found in the commercial formulations (LAS-C<sub>10</sub>, LAS-C<sub>11</sub>, LAS-C<sub>12</sub>, LAS-C<sub>13</sub>). The results showed that all the homologues were removed almost completely.

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## P058-B2

### Characterization and Quantification of Alkyl Sulfates in River Sediments by Liquid Chromatography - Mass Spectrometry

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Detergents are manufactured in large quantities, used by many people, and disposed after household use in the environment. When the surfactants enter to the environment have the potential to influence microbial, plant and animal life. Thus, information on the possible impact on biota, is of a decisive importance to determining acceptable levels of use and release to the environment.

Fate and effects of surfactants in aquatic surface waters has been studied extensively, nevertheless the terrestrial environment (sediments, soils,..etc) has received considerably less attention.

Alkyl sulfates (AS) are a group of anionic surfactants, characterised by having both a hydrophobic and a hydrophilic group. AS is one of the major ingredients of synthetic detergents and surfactants and is used world-wide for both domestic and industrial applications.

This work presents a new and highly specific method for the determination of the following compounds: sodium n-dodecylsulfate (C<sub>12</sub>H<sub>25</sub>SO<sub>4</sub>Na), sodium 1-tetradecyl sulfate (C<sub>14</sub>H<sub>29</sub>SO<sub>4</sub>Na), sodium n-hexadecyl sulfate (C<sub>16</sub>H<sub>33</sub>SO<sub>4</sub>Na), and sodium n-octadecyl sulfate (C<sub>18</sub>H<sub>37</sub>SO<sub>4</sub>Na) in river sediments samples.

Based on pressurized liquid extraction (PLE) followed by direct identification and quantification by means of reversed-phase liquid chromatography - mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) operating in negative mode.

Quality parameters were determined and satisfactory results were obtained.

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## P059-B2

### Determination of Alkyl Sulfate Derivatives in Wastewater Treatment Plants by Gas Chromatography-Mass Spectrometry

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Synthetic surfactants are among the chemicals that are produced and consumed in the largest volumes in the world, due to the variety of their applications, mainly as key ingredients in detergents and cleaners. Particular attention has been given to anionic and non-ionic surfactants, which account for up to 90% of overall production of these chemicals, so understanding their distribution, behaviour and final fate once they reach aquatic environments is very important. Alkyl sulfates (AS) are a widely used class of anionic surfactants. They are used in household cleaning products and personal care products, etc. In spite of their great use, these compounds have received less attention and only a few data are available about the presence of residual AS in environmental samples.

After use, detergents are usually discharged down the drain into municipal sewer systems and afterward treated in wastewater treatment plants (WWTPs), where they are completely or partially removed by a combination of sorption and biodegradation. After wastewater treatment, non-degraded surfactants together with their biodegradation products (metabolites) are discharged by WWTP effluents into surface waters.

This work presents an accurate and reproducible gas chromatographic - mass spectrometry (GC-MS) method for the determination of the following compounds: sodium n-dodecylsulfate ( $C_{12}H_{25}SO_4Na$ ), sodium 1-tetradecyl sulfate ( $C_{14}H_{29}SO_4Na$ ), sodium n-hexadecyl sulfate ( $C_{16}H_{33}SO_4Na$ ), and sodium n-octadecyl sulfate ( $C_{18}H_{37}SO_4Na$ ).

The proposed method was performed using a solid-phase extraction (SPE) procedure followed by a reaction of acid hydrolysis/derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1 % TMCS) and piridine as solvent, and finally the silylated derivatives were identified and quantified by GC-MS.

Analytical and statistical parameters such as linear dynamic range detection and quantification limits, inter-day and intra-day repeatability and accuracy were established

## P060-B2

### Evaluation of Thermal and Chemical Stability of a High Alkalinity Romanian Waste Red Mud

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Aluminum Romanian industry holds great quantities of red mud wastes. High content of valuable elements, specific morphostructural characteristics and the imperativeness to solve the environmental problems coming from the high alkalinity impose studies for the valorization of red mud.

The study contains the results about the thermal and chemical stability of the red mud, resulted from the Romanian aluminum industry.

In order to evaluate the neutralization capacity and the alkalinity reduction, red mud samples, with pH = 12.3- 12.8, from different heaps, were processed by a systematic thermal treatment (oven air stream calcinations in controlled temperature at 105<sup>0</sup>C, 400<sup>0</sup>C, 600<sup>0</sup>C, 800<sup>0</sup>C) and by chemical treatment (batch and dynamic treatment with HCl, CH<sub>3</sub>COOH, CO<sub>2</sub> gaseous (g), 4h, 25<sup>0</sup>C).

The chemical characterization (pH, conductivity, atomic absorption spectroscopy), thermal characterization (TG, DTA and DSC) and morphostructural characterization (XRD, FTIR, SEM and BET) of the initial and treated red mud samples proved phase composition and textural modifications. TG and DTA showed mass lose of about 30% and phase modification of Al and Fe compounds. These modifications are supported also by FTIR and XRD. Thermal treatment at 600<sup>0</sup>C, produced an increase in the specific surface with 50 %, but over 800<sup>0</sup>C, the specific surface had the initial value.

The neutralization of the red mud, at pH= 6-7, can be performed with consumption of 0.24M HCl (1M)/100g or 0.05 M CO<sub>2</sub> (g, 100%)/100g. The alkalinity of the aqueous suspension (1:10) obtained from chemically treated material decreased in comparison with the untreated material suspensions from 12.30 to 8.58 (CO<sub>2</sub>) and respectively 8.39 (HCl).

FTIR spectra showed structure modification caused by treatment with HCl and CO<sub>2</sub>, zeolitic and carbonates structures disappearing.

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## P061-B2

### High-Performance Liquid Chromatographic Method for Simultaneous Determination of Antidepressants and Aromatase Inhibitors in Environmental Samples

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The occurrence of residues of pharmaceuticals in the aquatic environment has attracted considerable interest in recent years [1,2]. Thousands of tons of pharmacologically active substances are used yearly to treat human and animal illnesses, in farming and aquaculture. These substances can be excreted unmetabolized or as active metabolites; they can escape degradation in sewage treatment plants and enter the environment, and can be detected in sewage, surface, ground, and drinking waters [3-5]. Given, the occurrence of residues pharmaceuticals in the aquatic environment, it is very important to have a method that allows the determination of these substances in environmental samples.

In this work, a fast HPLC method with UV detection was developed for the simultaneous determination of thirteen drugs concretely being ten antidepressants (venlafaxine, trazodone, citalopram, doxepine, paroxetine, fluvoxamine, imipramine, fluoxetine, sertraline and clomipramine) and three aromatase inhibitors (anastrozole, letrozole and exemestane) in environmental samples (tap, sea, wastewater and ground). These compounds were separated on an ultrabase C<sub>18</sub> column with acetonitrile-pH 2.5 phosphate buffer, 35:65 (v/v) as mobile phase at a flow rate of 1.5 mL/min. All these drugs were simultaneously extracted and preconcentrated by a single solid phase or liquid-liquid extraction for water and ground samples respectively. An exhaustive assay validation for the analysis of these thirteen drugs was performed obtaining results very satisfactory. All the compounds show good reproducibility of both the retention times (RSD % < 0.89%) and peak areas (RSD % < 3.95). The limits of detection for the studied antidepressants were in the range of 1-40 ng/mL and for the aromatase inhibitors were in the range of 5-50 ng/mL. The recovery obtained over spiked water or ground samples were ranged between 85 to 100 %.




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## P062-B2

### Determination of Alcohol Sulfates (AS) Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS): Study of its Environmental Behaviour in Agricultural Soils

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AS are a widely used class of anionic surfactants<sup>1</sup>. They are used in household cleaning products, personal care products, institutional cleaners and industrial cleaning processes, and as industrial process aids in emulsion polymerisation and as additives during plastics and paint production. The European production<sup>2</sup> volume of AS surfactants on an active matter basis is estimated to be 114,000 tonnes/year. About 102,000 tonnes/year are estimated to remain in Europe, the remainder is exported. Based on a survey conducted among detergent formulator companies, and input from AS producers, the tonnage used in household detergents and cleaning products is estimated to be 65,000 tonnes/year.

Several studies of degradation<sup>3</sup> have been realized. However, and based on the importance of these surfactants, a study of degradation in agricultural soils has not been developed yet. The studied AS family encompasses commercial grades of linear-type primary AS in the C12 to C18 range. Taking into account these evidences, a modern method for the determination of AS in agricultural soils was developed. The main variables related to accelerated solvent extraction (ASE) and LC-MS-MS were studied and optimized. Validation parameters like linear dynamic range, detection and quantification limits and accuracy were satisfactorily established in soil samples. The proposed method was successfully applied to determination of AS in soil samples. In addition, an environmental behaviour, based on sorption-desorption isotherms and kinetics by batch and column study, was carried out. Finally, the study was completed through application and environmental monitoring of AS in agricultural soils. It has been obtained parameters like Freundlich constant, maximum adsorbed concentration and half life.

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## P063-B2

### Mercury Concentrations in the Mutum Paraná Watershed - Jirau Hydroelectric Complex, Brazilian Amazon

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The Mutum Paraná River Basin has a surface of 8.840 km<sup>2</sup> and is situated on the right shore of the Madeira River, State of Rondônia (Brazilian Amazon), in the area where the construction of the Jirau Hydroelectric Complex is under way.

Samples of superficial waters and bottom sediments were collected at nine sampling spots during different seasons, according to the regional hydrological cycle: rising water, rainy, descending water, and dry periods. Total and methyl mercury concentrations were determined by an Atomic Fluorescence Spectroscopy apparatus coupled to a system of cold vapor generation and pre-concentrations on a gold column. “*In loco*” analyses on physic and chemical parameters were carried out using a multi-parameter apparatus, which allows a differentiation between two systems: the lotic system, which was characterized by different sampling points throughout the Mutum Paraná Watershed (pH: 5,2±0,2; dissolved oxygen: 4,2±0,9 mg L<sup>-1</sup>; conductivity: 11,2±2,6 μS cm<sup>-1</sup>; temperature: 26,9±0,9 °C; turbidity: 31,5±8,2 NTU); the lentic system, characterized by sampling points in the lake of the Mutum Paraná (pH: 5,2±0,2; dissolved oxygen: 3,2±0,1 mg L<sup>-1</sup>; conductivity: 3,3±0,1 μS cm<sup>-1</sup>; temperature: 31,1±0,2 °C; turbidity: 3,0±0,1 NTU). Total and methyl mercury levels in samples of superficial waters collected in both lotic and lentic systems were bellow 4.2 and 0.3 ng L<sup>-1</sup>. Bottom sediment samples from those two systems showed total Hg concentrations in the range of 170,3±57,8 μg kg<sup>-1</sup> and 177,7±66,2 μg kg<sup>-1</sup>, while MeHg levels ranged from 0,76±0,26 μg kg<sup>-1</sup> to 1,0±0,21 μg kg<sup>-1</sup>.

The Amazonian region presents elevated background levels of naturally occurring mercury, which has been found in several environmental compartments, and makes extremely important the monitoring of mercury species in areas either directly or indirectly influenced by the Madeira River Hydroelectric Complex, in order to investigate if lentic systems can be favorable sites for mercury methylation, all this in a context of socio-environmental responsibility.

## **P064-B2**

### **Chemometrically Assisted Simultaneous Separation of the Pesticides Used in Crop and Sugar Beet Cultivation**

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Crop and sugar beet cultivations are frequently present adjoining or in turnation on the same field and are characterised by the wide use of herbicides of pre- and post-emergence.

Different commercial formulations of these pesticides are constituted by one or more active principles, characterised by different physical-chemical properties.

The present study investigated the active principles most used in Italy in crop and sugar beet cultivations with the addiction of atrazine (forbidden since 1992) as from the monitoring performed by the governmental laboratory devoted to the environmental monitoring (ARPA) it seems to be still present at stable concentrations in water and soil samples.

The aim of this work is the optimisation of a rapid and cheap HPLC method able to simultaneously detect and determine the above mentioned pesticides in water and soil. The reversed phase chromatography was selected as the analytical technique. The concentration of the organic modifier, pH and flow rate role was investigated by experimental design and the optimisation was performed by mean of the multicriteria target functions based on the desirability functions. The optimisation involved also parameters regarding the sample pre-treatment, in this case SPE (Solid Phase Extraction) technique was selected as it allows either the preconcentration necessary to determine trace concentrations of the analytes ( $0.1 \mu\text{g L}^{-1}$ ) and the clean-up needed for the analysis of such environmental samples.

## P065-B2

### Polyethylene Plastic Pellets as a Monitoring Tool for Fullerene C<sub>60</sub> Nanoparticles

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Plastic pellets are carriers of organic contaminants in the marine environment since intra-polymer sorption and surface sorption of these compounds may occur. Fullerene C<sub>60</sub> is a hydrophobic compound which industrial production is expected to increase in the next decades. Fullerenes form stable aggregates in aqueous systems ( $nC_{60}$ ) and may be deposited on surfaces of mobile particles. Thus, the present study attempted to investigate how different levels of C<sub>60</sub> and polyethylene plastic pellets affect sorption processes. Beakers containing 100mL of seawater (filtered at 0.45 $\mu$ m) were spiked (triplicate) with three different concentrations of C<sub>60</sub> (1, 10 and 100ng mL<sup>-1</sup>). The solutions were sonicated and two different volumes of pellets (50 and 500mg) were introduced. After 3 days, the pellets were sonicated with dichloromethane (3X). The extracts were analyzed by HPLC (Perkin Elmer Series 200) equipped with a C18 column (150x4.6mm) and an ultraviolet (UV) detector (330nm). Spiking tests showed recoveries between 74 and 104%. Two-way analysis of variance (ANOVA) test was performed to evaluate differences between C<sub>60</sub> concentrations and pellet volumes. Adsorbed C<sub>60</sub> varied up to 2 orders of magnitude for the volume containing 500mg of pellets, from 0.03 (1ng mL<sup>-1</sup> solution) to 5.42ng mg<sup>-1</sup> of pellets (100ng mL<sup>-1</sup> solution). Thus, C<sub>60</sub> concentration and pellet mass have significantly affected sorption kinetics ( $p < 0.05$ ). However, the molar volume of C<sub>60</sub> (480cm<sup>3</sup> mol<sup>-1</sup>) is greater than the pore spaces of polyethylene, indicating that the process is restrict to surface sorption. Plastic pellets analysis provides advantages in comparison with other monitoring matrix (e.g. water, sediment and biological samples) which are more complex, resulting in higher cost analysis. The accumulation potential of C<sub>60</sub> by polyethylene pellets draw attention to their practical use as a tool for monitoring or removing contaminants from the environment. Additionally, the ability of fullerene to associate with other contaminants must be further investigated.

## P066-B2

### Sequential Extraction of Cr, Cu and V in Soil and Determination Using ICP-MS

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Among the instrumental techniques that can be used for determination of trace elements in soils, inductively coupled plasma mass spectroscopy (ICP-MS) presents advantages such as multi-elemental character and superb sensitivity. However, aspects such as the content of total dissolved solids and the polyatomic ions formed in complex matrices may limit its application. The determination of Cr, Cu and V may suffer from polyatomic interferences either originated in the argon plasma or in the matrix. In the case of  $^{65}\text{Cu}^+$  the main interferences are  $^{23}\text{Na}^{40}\text{Ar}^+$  and  $^{23}\text{Na}^{40}\text{Ca}^+$ . The determination of  $^{52}\text{Cr}^+$  is affected by  $^{40}\text{Ar}^{12}\text{C}^+$  and  $^{51}\text{V}^+$  is affected by  $^{35}\text{Cl}^{16}\text{O}^+$ . Notwithstanding, all these elements also have less abundant isotopes not affected by severe interference processes. The goal of this study was to determine Cr, Cu, and V in soil sequential extraction fractions (BCR) using strategies such as the choice of less abundant isotopes and/or the introduction of  $\text{H}_2$  gas in the collision reaction interface (CRI - Varian 820-MS) when measuring the major isotope. Solutions containing  $5 \mu\text{g mL}^{-1}$  of Cr, Cu, and V were prepared in different media ( $\text{CH}_3\text{COOH } 0.11 \text{ mol L}^{-1}$ ,  $\text{NH}_2\text{OH.HCl } 0.05 \text{ mol L}^{-1}$  and  $\text{CH}_3\text{COONH}_4 0.05 \text{ mol L}^{-1}$ ) and addition and recovery studies were performed, evaluating the introduction or not of  $60 \text{ mL min}^{-1}$  of  $\text{H}_2$  in the skimmer cone. The results have shown that the determination of Cu and Cr can be performed using the less abundant isotopes of these elements, i.e.  $^{53}\text{Cr}^+$  and  $^{65}\text{Cu}^+$ , respectively, with recoveries around 120%. The determination of the  $^{50}\text{V}^+$  was not possible due to their low abundance (0.25%), leading to too high standard deviations. Otherwise, the  $^{51}\text{V}^+$  isotope can be properly determined using by adding  $\text{H}_2$  in the CRI for destroying  $^{35}\text{Cl}^{16}\text{O}^+$ .

FAPESP, CNPq



## P067-B2

### Multivariate Analysis of ICP OES Calibration Conditions for Sequential Extraction of Metals Ions

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Sequential extraction procedures are normally applied to evaluate sediments, soils and plants. The reagents are often selected with the intention that they should target well-defined minerals phases although specificity cannot be guaranteed. Inductively coupled plasma optical emission spectrometry (ICP OES) is an instrumental technique that can be used for inorganic determinations. The simultaneous, multi-element nature of the technique allows the determination of a large number of elements in a very brief period of time. However, to accuracy results it is necessary the preparation of multiple calibration curves nearest as possible of the sample preparation extraction solution. In this work some commonly solutions used in sequential analysis (nitric, acetic, hydrochloric and perchloric acid, water, aqua regia, ammonium acetate, DTPA, and hydroxylamine chloridrate) were tested for Al, B, Ba, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Sr and Zn determination in different wavelengths and in robust and non-robust ICP OES condition, aiming to evaluate their influence on results. Calibration curves were plotted to compare the extractants solutions effect in each analyte. With the obtained sensitivity, principal components analysis was performed. The results showed that when robust condition was used, most of the extractants solutions do not cause interference in the calibration. The intensity of emission is also a significant factor, the most intense lines showed better accordance among the extractors. Therefore, considering the robust conditions and the more intense spectral lines, the determination of analytes in the different evaluated extractions solutions can be performed with accuracy using only two calibration curves for a wide range of elements by ICP OES, considering that principal components analysis showed two distinct groups: one from water and nitric acid and other from all another evaluated solutions.

FAPESP, CNPq, CAPES

## P068-B2

### GC-MS Determination of Polycyclic Aromatic Hydrocarbons Evolved from Pyrolysis of Biomass

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Polycyclic aromatic hydrocarbons (PAHs) are recognised toxic and ubiquitous pyrolysis products of biomass. Understanding the pyrolytic formation of PAHs is important for assessing health and environmental hazards related to the thermochemical utilisation of biomass for energetic purposes (combustion, pyrolysis, gasification). Analysis of PAHs in biomass pyrolysate is difficult since they occur at trace levels into a complex mixture, so that cumbersome sample pre-treatments are required. Analytical pyrolysis based on a micro-scale pyrolysis apparatus can be adopted as a fast method to evaluate the propensity of organic materials to produce PAHs under controlled conditions. In this study, analytical (<10 mg sample) and preparative pyrolysis (< 10g) were applied to the analysis of PAHs in biomass pyrolysate. Analytical pyrolysis was utilised in the off-line configuration where pyrolysis products were trapped prior to GC-MS analysis. Two sampling procedures were investigated: 1) sorption onto a resin followed by elution with dichloromethane and liquid-liquid extraction (Py-SPE), 2) dynamic solid phase micro extraction followed by fibre clean-up with aqueous ammonia (Py-SPME). A method for the determination of PAHs in liquid pyrolysate (pyro-oil) was also developed. An aliquot of pyro-oil, obtained from poplar by preparative pyrolysis with a bench scale reactor, was dissolved into acetonitrile/water, extracted with hexane and subjected to silica gel SPE. PAHs were determined at  $\mu\text{g}/\text{ng g}^{-1}$  level with good reproducibility. Results obtained from the analysis of pyro-oil were used to evaluate the accuracy of analytical pyrolysis. Py-SPE was more accurate than Py-SPME, but the latter was faster. Both methods were adequate for screening purposes to assess PAH formation from different biomass types and the effect of catalysts. In particular, they agreed to show that the content of PAHs expected in pyro-oil was lower than in conventional fuels, but PAH emission increased dramatically in catalytic upgrading over zeolites resulting in highly hazardous pyro-oil.

## P069-B2

### Determination of Organophosphorus Plasticizers and Flame Retardants in Surface Waters by Liquid Chromatography - Tandem Mass Spectrometry

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To prevent or delay the initial phase of fire development organophosphorus compounds (OPs) are used. These chemicals are present in many products such as building materials, TV, computer and carpets; tripropyl phosphate (TPPrP), trimethyl phosphate (TMP), tributyl phosphate (TBP), triphenyl phosphate (TPP), tris(2-ethylhexyl) phosphate (TEHP) and tris(2-butoxyethyl) phosphate (TBEP) are retardants and plasticizers in products such as paints and glues; chlorinated, as tris (chloropropyl) phosphate (TCPP) and tris (2-chloroethyl) phosphate (TCEP) are added to polyurethane foam, rubber and textile goods, while TBP and TPP are also additives in lubricants products and hydraulic fluids. OPs occurrence in different environmental matrices can be due to their employment both as additives and as chemical reagents, at any time of manufacture, sale or destruction of products. Therefore, they are highly ubiquitous, being air and water the main mediators in their distribution.

Our research group has developed a method, based on liquid chromatography/tandem mass spectrometry (LC-MS/MS) with electrospray interface, for the analysis of 12 OPs which are widely used (some of them are toxic) in aqueous matrices from the three volcanic lakes characterized by a different anthropic impact (Vico, Martignano, Albano) and Tiber river. The extraction was achieved by solid phase using cartridges containing a hydrophobic-hydrophilic copolymer (OASIS HLB), that allowed the best results in terms of accuracy and precision. 90% recovery for almost all the analytes was obtained, but recovery of the most polar compound TMP was ca. 30%. In addition to sample preparation, both the chromatographic separation on C18 column, and the electrical parameters for the mass-spectrometric determination were optimized too. In this work, the selected substances were found at  $\text{ng L}^{-1}$  levels in all the water samples analyzed. In the two most anthropized lakes (Albano and Vico), the most abundant OPs were TPPrP and TBP, 784 and 951  $\text{ng L}^{-1}$ , respectively. In Tiber river, the presence of ten OPs, starting from a few  $\text{ng L}^{-1}$  up to 323  $\text{ng L}^{-1}$  of TBEP, was detected. The highest levels of OPs contamination was revealed on October and November, whereas the concentrations showed a minimum value on March and April. Chlorinated OPs also showed the same trend, but their concentrations were an order of magnitude lower than those of alkyl OPs. In contrast, concentrations of TBEP were quite similar among all collected samples, indicating that their source in nature were different.

## P070-B2

### Particulate Matter inside an Urban Tunnel in the City of Lisbon

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Most cities constructed road tunnels to reduce traffic congestions and improve their flow. However, when traffic jams are frequent and the air ventilation system is not very efficient, elevated concentrations of airborne pollutants are reached, representing a major threat for car occupants. This fact can assume a particular importance both at the beginning and at the end of working days, due to the intense circulation between the city centers and the suburban residential areas.

In Lisbon, a recently built tunnel with 1725 m long at Praça Marquês de Pombal, has three entrances and five exits, connecting the Lisbon city centre and the A5 motor-way to Estoril and Cascais, 30 km away. Due to Lisbon topology (city also known as the "City of Seven Hills"), the tunnel was constructed with an elevated slope (average of 9%), which lead the city's traffic administration to impose several traffic restrictions like a radar controlled speed limit of 50 km/h and the prohibition of circulation of trucks and transport of dangerous goods.

In this work, an account is given of an aerosol sampling campaign carried out in October 2008, aiming to have an insight at the tunnel's air quality. Two Hi-Vol samplers operating two hours per day, both in the morning and in the afternoon, were placed at the middle of the tunnel's extension, one in each direction of the traffic flow. Particulate matter in four size fractions (<0.5 µm, 0.5 - 1.0 µm, 1.0 - 2.5 µm, and 2.5 - 10.0 µm) was collected on quartz fiber filters. The smaller particles are known to have the most severe health effects due to their ability to penetrate lungs. Aerosol particles mass and chemical characteristics represent valuable information to support measures and regulations concerning ventilation, emissions control and access of private vehicles. Analyses were performed by Ion Chromatography for mineral composition and by Atomic Absorption for metals. Results are presented and discussed.

#### Acknowledgments

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## P071-B2

### **Development of Methodology for Determination of Sarin and Soman in the Atmospheric Air of Populated Areas by the Thermal Desorption/Gas Chromatography with Flame Photometric Detection**

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Determination of sarin and soman in the atmospheric air of populated areas represents a complicated analytical task. The complexity is resulted from both the extremely low values of the tentative safe exposure levels (TSEL) for these compounds, which provides the requirement of their determination at concentrations of  $2 \times 10^{-7}$  and  $1 \times 10^{-7}$  mg/m<sup>3</sup> (Russian standards) respectively, and its physicochemical properties (i.e. lability, high adsorption activity).

The report represents the results of studies on the development of analytical techniques for sarin and soman determination in the air of populated areas at TSEL levels by the thermal desorption/gas chromatography with flame photometric detection.

The developed analysis schemes include sarin and soman sampling by non-standard tubes with Tenax TA adsorbent and thermal desorption on a standard passivated Markes tubes with a less amount of Tenax TA adsorbent using a special device. The further procedures consist in two-stage low temperature focusing desorption followed by sarin and soman determination using the analytical system including Unity (Markes) thermal desorber coupled with Agilent 6890N chromatograph with enhanced flame photometric detector.

The assessment of these analysis schemes under various sampling time and rate was carried out.

In accordance with the developed analysis scheme, sarin and soman detection limits in the air are equal to  $2 \times 10^{-7}$  mg/m<sup>3</sup> and  $1 \times 10^{-7}$  mg/m<sup>3</sup>, respectively.

The work was performed with financial support from ISTC in the frame of Project #3320.

## **P072-B2**

### **Direct Optical Emission Spectrochemical Analysis of Topsoil**

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The potentials of two advanced laser sampling techniques for optical emission spectrochemical determination of contents of humus (total carbon) and other nutrients in topsoil have been examined. This problem is conventionally solved by routine chemical methods which are laborious and require a time- and material-consuming sample preparation procedure. Therefore, the new modern approaches are necessary which will ensure more rapid (on-line), cost-effective, and sensitive measurements. The first one is a double-pulse laser sampling. In this mode a Q-switched double-pulse Nd:YAG laser is used for sample ablation and analyte spectra excitation. Its optimized parameters are as follows: 1064 nm lasing wavelength; 50+50 mJ energy; 10 ns duration of each pulse. An optimized delay between the pulses of 8  $\mu$ s has been chosen. The second approach is the combination of a single-pulse laser sampling with a following spark excitation of expanding plasma plume triggering a gap between electrodes close to the target surface. In this case a single pulse from Q-switched Nd:YAG laser is used. Its main parameters chosen for the analytical measurements are as follows: 1064 nm, 180 mJ, 8 ns. Tungsten wire electrodes with 3 mm gap have been set at a distance of 1.5 mm from the sample surface to avoid surface self-breakdown at operating voltage. The laser ablation plume seals-in the electric circuit containing a low-inductive 1  $\mu$ F capacitor charged to 4 kV, which gives rise to a quasi-periodical (4  $\mu$ s period) damping electric discharge. In both modes the calibration graphs have a nonlinear trend in the actual range of carbon contents with a good  $R^2$  value (0.97). The limit of detection is about 0.07 % for both techniques. Certain regularities in the amount of some nutritious and toxic elements (K, Fe, Al, Mg, Mn, Ca, Si, Pb, Ni, Cu, Zn, Ti) in the topsoil have been revealed.

## P073-B2

### The Appreciation of Mineral Elements Accumulation Level in Some Herbaceous Plants Species by ICP-AES Method

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From the metallurgic industry zone of Dambovita County, with a high level of pollution, we sampled and analyzed seven herbaceous plants species (*Lolium perenne*, *Festuca pratensis*, *Stipa capillata*, *Agrostis alba*, *Cynodon dactylon*, *Luzula campestris* and *Agrostis tenuis*) to establish the heavy metals accumulation levels in this species. The heavy metals contents (for Cr, Mn, Zn, Sr, Cu, Ba and Sn) were determinate by analyzing the dry matter with an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES). This method has detection limits of 0.4-0.5 mg/kg for the analyzed metals. The dry matter for the herbaceous species is about 33% for *Festuca pratensis* to 50% for *Luzula campestris*.




The heavy metals concentrations in plants samples from the industrial area are in mg/kg of dry matter and ranged from 10.34 to 197.36 mg/kg of dry matter for Cr; 165.90 to 1351.89 mg/kg of dry matter for Mn; 59.94 to 1009.87 mg/kg of dry matter for Zn; 27.25 to 61.76 mg/kg of dry matter for Sr; not detected to 89.88 mg/kg of dry matter for Cu; 58.02 to 165.02 mg/kg of dry matter for Ba and 8.47 to 491.06 mg/kg of dry matter for Sn.

The heavy metals accumulation levels in the studied species of plants were calculated by the rapport between the level concentrations of the metal in plant samples and the level of the same metal in the soil, nearby of the radicular system for each species of plants. The highest accumulation levels were found in *Stipa capillata* for Cr (218%); in *Lolium perenne* for Mn (68%), Zn (165%), Sr (138%), Ba (75%) and in *Cynodon dactylon* for Cu (121%) and for Sn (621%).

## P074-B2

### The Fate of Soap in Agricultural Soils: Determination, Preliminary Studies and Seasonal Monitoring

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Soap is by far the most used surfactant worldwide, with an estimated consumption of nine million tons (1). Fatty acid salts (soap) are widely used in household cleaning products, cosmetics, lubricants (and other miscellaneous industrial applications) and coating. Use in household cleaning products, include fabric washing products, fabric conditioners, laundry additives, and surface and toilet cleaners. These uses cover chain lengths of C<sub>10</sub>-C<sub>22</sub> predominantly with counterions of sodium and potassium (2).

The fate of fatty acid salts in aqueous systems is strongly influenced by the poor water solubility of their calcium and magnesium salts. In practice, the use of Na salts are by far the most common use of soap in finished products. However, the predominance of calcium and magnesium ions in waste water lead to the rapid formation of their corresponding fatty acid salts. As a consequence, moderate/high concentration of insoluble Ca and Mg soap has been found in environment matrices (3).

Based on this fact, the present research work has been divided into three parts: First, determination of soap in agricultural soils by liquid chromatography, second, obtaining the sorption-desorption kinetic and isotherms curves in batch and column conditions, and finally, the seasonal monitoring study of soap in agricultural soil (Granada, Spain).

In order to evaluate the possible biodegradation of soap, several parameters has been determined such as half-life time or degradation constant.

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## P075-B2

### Determination of Linear Alkylbenzene Sulfonates in Marine Sediments: Seasonal Monitoring Study in Mediterranean Sea

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Linear alkylbenzene sulfonates (LAS) are the most commonly used anionic surfactants on the market with an annual production in Western Europe over 360.000 tons in 2005 (13,5% of total surfactants production) (1). Commercial LAS are made up of a linear alkyl chain consisting of 10–13 carbon atoms, a phenyl ring, which is randomly distributed in all possible positions (except 1-phenyl), and a sulfonic group in the para position. Most of LAS European consumption is in household detergency (>80%). Important application products are laundry powders, laundry liquids, dishwashing products and all purpose cleaners. The remainder of the LAS (<20%) is used in Industrial and Institutional cleaners (2).

After use and disposal, most of the LAS are discharged into sewage treatment plants, where they are effectively removed as a result of their biological degradation. Despite this, load concentration of LAS has been found in environmental compartments such as marine sediments (3). This fact, is due to its tendency to precipitate in hardness water and its high adsorption capacity in soils.

Taking into account these evidences, the present research work is focused on two parts: First, the determination of LAS in marine sediments samples, based on microwave assisted extraction (MAE) following by liquid chromatography detection, and second, the presentation of a seasonal monitoring study of LAS carried out in the Mediterranean sea (Almería, Spain).

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## P076-B2

### Evaluation of Some Mushrooms Absorbion Affinity for Heavy Metals and their Substrate Content by EDXRF and AAS Methods

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This work is part of one research project won by national competition which has the main aim to identify the mushroom species which can accumulate heavy metals in their body.

Biological samples consisted in some mushrooms species: *Coprinus picaceus*, *Coprinus cinereus*, *Pseudotrametes gibbosa*, *Lyophyllum crassifolium*, *Paxilus involutus*, and *Phellinus tremulae* which are growing in a forestry ecosystem in the neiborough of a army plant in Dambovita county. In the same time were prelevated substrate samples under each mushroom species. It were determinated heavy metals as iron, manganese, magnesium, chromium, copper, lead, titanium and others in both kinde of samples.

Biological samples and their substrate samples have been dried at 60<sup>0</sup>C some hours. After drying the solid samples have been grinded until to fine powder and weighed.

For the evaluation of EDXRF results was used a certified reference sample (NIST SRM 1571- Orchard leaves).

For calibration in AAS were used: Nickel Standard Solution (Merck) traceable to SRM from NIST Ni(NO<sub>3</sub>)<sub>2</sub> in HNO<sub>3</sub> 0.5 mol/L; Selenium Standard Solution (Merck) traceable to SRM from NIST SeO<sub>2</sub> in HNO<sub>3</sub> 0.5 mol/L; Chromium Standard Solution (Merck) traceable to SRM from NIST Cr(NO<sub>3</sub>)<sub>3</sub> in HNO<sub>3</sub> 0.5 mol/L and Lead Standard Solution (Merck) traceable to SRM from NIST Pb(NO<sub>3</sub>)<sub>2</sub> in HNO<sub>3</sub> 0.5 mol/L.

Dried mushrooms samples (500 mg) were introduced into digestion vessels and then 3 mL nitric acid and 5 mL hydrogen peroxide were added. The clear solution volume is made up to 50 mL for each sample using deionised water.

Dried solid substrates (500 mg) were introduced into digestion vessels and then 3 mL nitric acid and 9 mL hydrochloric acid (aqua regia) are added. For soil, EPA 3051A program was chosen. So, it were determinated the iron concentrations between 0,17 and 1,38% in mushrooms (minimum in *Phellinus tremulae* and maximum in *Lyophyllum crassifolium*) by EDXRF methods. The concentration of cadmium was obtained by AAS method, and was between minimum 0,02mg/kg d.w. in *Paxilus involutus* and maximum 0,14 mg/kg d.w. in *Pseudotrametes gibbosa*.

## P077-B2

### Uptake of Carbamazepine from Contaminated Waters by *Typha* spp. Potential Use for Phytoremediation

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The presence of pharmaceuticals in the aquatic environment is an emergent issue in analytical environmental chemistry. The frequent detection of these substances in effluents of wastewater treatment plants (WWTPs) is an evidence of the inadequacy of conventional treatment processes to remove these pollutants from wastewaters. One option for the removal of pharmaceuticals from WWTPs effluents is the implementation of phytoremediation technologies such as constructed wetlands systems. In this context, a study was conducted to assess the macrophyte *Typha* spp.'s ability to withstand and remove the anti-epileptic drug carbamazepine from water.

As an important preliminary step, analytical methodologies for quantification of carbamazepine in nutrient solutions and in plant tissues samples were developed and optimized for each type of sample. For nutrient solutions the methodology comprised, whenever necessary, an optimized step of pre-concentration with SPE, chromatographic separation with HPLC and detection/quantification by UV/Vis spectrometry. In plant tissues, a methodology was developed for the extraction of carbamazepine from leaf tissues by an optimized SSDM method, followed by chromatographic separation in HPLC and selective detection/quantification by quadrupole ion trap mass spectrometry. For an initial carbamazepine concentration of 20 µg/L in nutrient solution, *Typha* plants removed nearly 50% of the pharmaceutical within the first 48h, attaining over 98% removal by the end of the assay. Exposure to higher carbamazepine concentrations (up to 2000 µg/L) did affect *Typha*'s growth but, by the end of the assays, plant's growth as well as photosynthetic pigments approached normal values. An alteration in antioxidant enzymes' activities (superoxide dismutase, catalase, guaiacol peroxidase) indicated that leaves were affected by the xenobiotic.

Carbamazepine and some metabolites were detected in leaf tissues of *Typha* plants thus indicating carbamazepine translocation to aerial parts and its metabolization.

Eventually, *Typha* seemed able to cope with carbamazepine induced oxidative damage, suggesting its ability for phytotreatment of waters contaminated with carbamazepine.

## Removal of Arsenic from Water by Used Natural Waste Materials

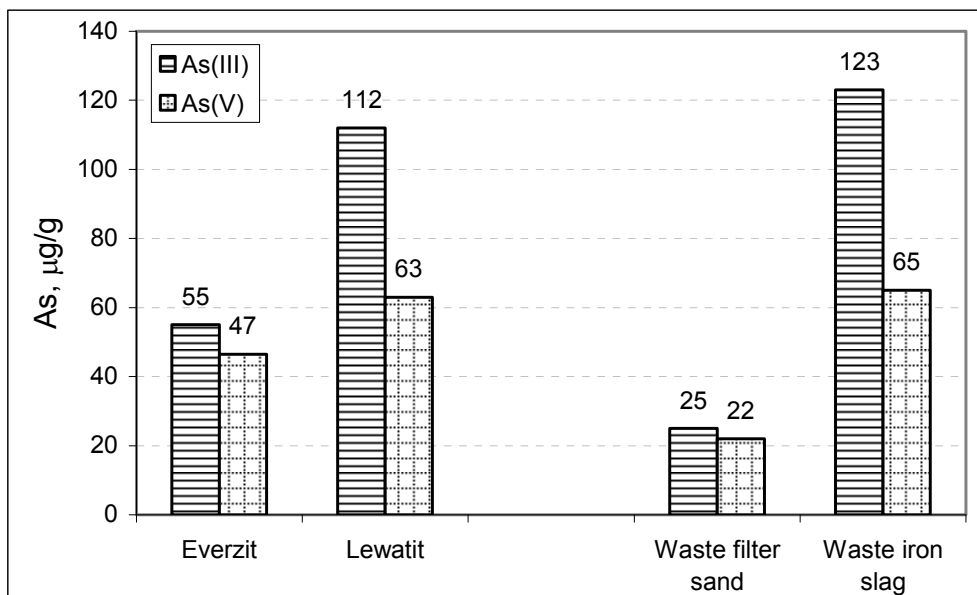
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This paper presents investigations of arsenic removal efficiency on waste materials and considers its application in water treatment. Two waste materials are examined: waste filter sand (from groundwater treatment plants) and waste iron slag from steel production industry. Two commercial materials (GFH and ion exchange resin) are examined as well for comparison purposes only. Arsenic removal efficiency was examined in batch test for two arsenic species: As(III) and As(V) and for different initial concentrations. On Figure comparison of removal for both arsenic species,  $\tau=2$  h,  $C_0=0.500$  mg/L is presented.



Examinations proved that waste materials can efficiently sorb both arsenic species. Arsenic removal efficiency is strongly influenced by initial arsenic concentration, arsenic specie applied and pH. Waste iron slag exhibited higher sorption efficiencies (600 µg As(III)/g and 800 µg As(V)/g) than waste filter sand (200 µg As(III)/g and 400 µg As(V)/g). However, waste filter sand performed significantly less impact on initial water quality than waste iron slag.

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## P079-B2

### Efficiency of Ion Exchange Resins for Arsenic Removal from Water

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Arsenic occurs in natural waters as As(III) and As(V) in both molecular and ionic forms. It is a specific toxicant which can be removed by sorption processes combined (adsorption, chemisorption and ion exchange) and with complex sorbents or exchangers which possess multifunctional features. This paper has been focused on efficiency of a simple and complex ion-exchange process (combined with sorption) for arsenic removal from water in batch system. Three types of resins were investigated: a strong base anion exchange resin (SBAR), a weak base anion resin (WBAR) and hybrid resin (HYR) for selective removal of arsenic. Efficiency of resins was determined by measuring the arsenic concentration before and after the exchange/sorption process. AAS-GH and ICP-MS methods have been applied as analytical methods for determination of arsenic concentration in water. Governing factors for ion-exchange/sorption efficiency were proposed, analysed and compared. WBAR is not efficient. SBAR is efficient. It was concluded that the removal process on SBAR is mostly affected by pH value of water. Depending on pH value SBAR separates As(V) and As(III) in water by retaining As(V) and allowing As(III) to pass through.

HYR is the most efficient for arsenic removal. HYR is complex ion-exchange resin which bonds arsenic also with sorption process on  $\text{Fe}(\text{OH})_3$  as a component of sorbent. Excellent feature of HYR is based on the fact that this sorbent covers a wide range of pH of water and it is efficient for both arsenic species As(III) and As(V) and for both ionic and molecular forms. Removal of As with HYR is a result of both ion exchange and sorption, sorption is physical adsorption and chemisorption on  $\text{Fe}(\text{OH})_3$ , physical adsorption is a favorable process (sorption efficiency decreases with increasing temperature). The following sorption capacity is obtained: 1.0 g of resin after 60 min at 20 °C and pH 7.5 bonds 90% of arsenic(III, V) from 100 mL of water polluted with 100 mg/L of arsenic.

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## P080-B2

### Total-Aldehyde Monitoring in Condensed Phases by Sensors Based on Electrodes Supported on Ion-Exchange Membranes

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Aldehydes are usually formed as products of the incomplete combustion of petroleum fuels and biomass, but they are also formed in human as metabolites after alcohol consumption and as leakage products of fermentation by yeast during alcoholic beverages production, as well as during lipid oxidation in food where they are adopted as valuable indicators to estimate the state of turning rancid of lipids. The ascertained toxic effects of these pollutants have prompted a marked public fear of cancer which has driven regulatory agencies worldwide to set very low tolerances for these species in various environmental settings, from food to drinking water to toxic waste sites. The worrying peculiarities of volatile aldehydes have prompted the development of several methods for their analysis also in condensed phases, such as drinking water, biological fluids and food samples, requiring almost always preconcentration and derivatization steps which make them in general unsuitable for on-line and in situ monitoring.

In this study, we propose a method for the determination of total volatile aldehydes based on the use of a gold electrode supported on an ion-exchange membrane (SPE sensor, allowing electroanalytical measurements to be performed in gas phases) adopted as a detector for a flow analysis apparatus where these compounds are injected after their suitable preconcentration from the head-space in equilibrium with either aqueous or lipidic real samples, by resorting to a solid phase microextraction (SPME).

The flow injection apparatus consists of a conventional GC injector where thermal desorption of preconcentrated aldehydes is rapidly achieved at 270 °C under a N<sub>2</sub> stream conveying analytes perpendicularly to the Au-sensing surface.

The performance of this approach was optimised for temperature and duration of the preconcentration step, desorption temperature, detection potential and distance of the carrier-gas outlet from the electrode, thus attaining FIA responses characterized by good repeatability ( $\pm 8\%$ ), wide linearity range (extending over ca. 4 orders of magnitude) and a detection limit of about 30 ppb, estimated for S/N = 3. Moreover, voltammetric findings point out that aldehyde monitoring is not affected by usual volatile interferences.

## P081-B2

### Investigations on the Electrocatalytic Assessment of Antioxidant Capacity Using a DNA-Modified Carbon Paste Electrode

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Reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism and are able to induce DNA oxidative damage. These lesions are the primary risk factor for gene mutations which might play a key role in a variety of human diseases, such as, atherosclerosis, diabetes mellitus, neurodegenerative disorders and certain types of cancer. Most living organisms have developed complex antioxidant systems (enzymatic and non enzymatic) to counteract reactive species and to prevent the deleterious effects of ROS. At the cellular level, the evaluation of the protective effect of antioxidants, molecules that slow a free radical chain reaction preventing damage to the DNA, can be achieved by examining the integrity of the DNA nucleobases using electrochemical techniques. It has been reported that the oxidation of both adenine and guanine homopolynucleotides in neutral or alkaline conditions lead to the formations of a common oxidized product that catalyzes the oxidation of NADH [1].

Herein we proposed an electrocatalytic voltammetric method to assess the antioxidant capacity using carbon paste electrodes (CPE). The generation of the hydroxyl radical is achieved by mimicking the *in vivo* metal ion-dependent breakdown of H<sub>2</sub>O<sub>2</sub> (Fenton's reaction) [2]. The oligo (dA<sub>21</sub>) adsorbed on the CPE is immersed in the Fenton mixture to generate the oxidative lesions that are indirectly quantified by the magnitude of the electrocatalytic current of NADH measured by CV after the electrochemical oxidation of the adenosines that remain unoxidized on the CPE. The increase of this electrocatalytic current in the presence of several antioxidant species is studied.

#### Acknowledgments

M. F. Barroso is grateful to Fundação para a Ciência e a Tecnologia for the Ph.D. Grant (SFRH/BD/29440/2006). N.S.Á thanks to MEC for a Ramón y Cajal Contract. This work has been financed by Gobierno del Principado de Asturias (PCTI 2006-2009) Project FICYT IB08-087.

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## P082-B2

### Voltammetric Determination of Ethiofencarb

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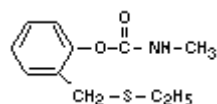
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Ethiofencarb (2- ethylthiomethyl-phenyl)-N-methyl carbamate) is a systemic insecticide widely used in agriculture, and their mode of action is as a cholinesterase inhibitor.

A method for the determination of ethiofencarb by electroanalytical techniques, has been developed on the basis that this pesticide is hydrolysed in alkaline media given rise to a compound that show a oxidation peak to  $E_p = 0.80$  V.

The instrumental parameters and different chemical conditions have been optimized. The parameters quality of the developed method has been established. The method is applied to the determination of the pesticide in river water samples.

On the other hand, the possibilities to develop a new method to analyze mixtures of pesticides belonging to the same family that ethiofencarb, based on their different speeds of hydrolysis have been assayed.



Ethiofencarb

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## P083-B2

### Square-Wave Voltammetric Determination of Ellagic Acid in Fruit Extracts and Juices

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Ellagic acid (EA) is a fused four-ring polyphenol found in numerous vegetables, fruits (including raspberries, strawberries, blackberries, cranberries and pomegranate) and some nuts (including pecans and walnuts). Interest in EA has increased during the past few years due to its possible antioxidant, chemopreventive and anticarcinogenic effects; what is more, EA has been marketed as a [dietary supplement](#) with a range of claimed benefits against cancer, heart disease, and other medical problems. Extracts from red raspberry leaves or seeds, pomegranates, or other sources containing high levels of EA and are available as dietary supplements and juices.

To our knowledge, there was no report about the square-wave voltammetric (SWV) determination of EA. We have found the EA provided a well-defined redox peak which is affected by pH. We have determined EA in a Ac<sup>-</sup>/HAc buffer solution of pH 5.5, at a glassy carbon electrode by SWV in a range between  $4 \times 10^{-8}$ - $2 \times 10^{-5}$  M, with a detection limit of  $1 \times 10^{-8}$  M (S/N=3). The method showed a good reproducibility and selectivity. Other substances, such as gallic acid, caffeic acid, ferulic acid, ascorbic acid, quercetin, coumaric acid and catechin did not interfere.

The proposed method has been applied to the determination of EA in many fruit extracts and juices with good analytical results.

The results in our work contribute to the potential application of electrochemical techniques in the detection of important substances within phytochemistry.

## P084-B2

### Optimization of Experimental Conditions for Bismuth Film Plating on Screen Printed Carbon Electrodes and its Applicability to Heavy Metal Monitoring in Water Samples

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Monitoring of relevant trace elements in environmental and waste water samples demands dedicated sensors capable of providing exact measurements with adequate spatial and temporal resolution. Voltammetric stripping methods based on glassy carbon working electrodes modified with metallic bismuth have become well established strategies for this purpose [1-2]. Modification protocols include Bi codeposition during the accumulation step, Bi preplating and a variety of Bi particles and nanoparticles arrangements. Screen printed carbon electrodes (SPCEs) have been only scarcely used as Bi supports, although they are promising base materials for cheap and portable heavy metal voltammetric sensors. In this work, we have studied chemical and instrumental parameters of Bi preplating on commercial ceramic strips with SPCEs working electrodes, to optimize signal stability and square wave voltammetric response of the heavy metals zinc, cadmium and lead. Optimization studies have been developed on two hydrodynamic configurations (convective and flow). Chemical variables considered were the effect of Bi(III) plating solution concentration and the presence of bromide ions. Stirring speed (convective cell), flow rate (flow cell) and deposition time were studied as instrumental variables. Linear calibration lines were found, with detection limits under the  $\mu\text{g/L}$  range. Results about the applicability of the methods developed to heavy metal pollution monitoring in water samples are presented and discussed.

Acknowledgements: Financial supports from Junta de Extremadura, Spain (project PDT07A016) and A.G. Siderurgia Balboa are gratefully acknowledged.

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**Mathematical Modeling, Numerical Simulation and Experimental Study of Metal Nanoparticle Electrooxidation**

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A mathematical model is proposed that describes the processes of electrooxidation of metal nanoparticles localized on the surface of an indifferent macroelectrode. In contrast to previously proposed models based on geometric factors (shapes of particles and diffusion zones), the proposed model has introduced thermodynamic considerations which take into account the energy differences between the nanoparticle ensembles from micro- and macroparticles.

A series of calculated voltammograms and experimental ones describing electrooxidation of gold nanoparticles localized on glassy carbon or carbon strip electrodes was obtained. Analysis of microscopic and electrochemical results have shown that particles size and structural organization of the electrode surface have a considerably influence on the electrooxidation process. Electrochemical activity of gold increases when transition from macro- to micro- and nano state occurs. Immobilization of mixture of different size gold nanoparticles on electrode surface leads to appearance a few peaks on the anodic voltammograms.

Good agreement between theory and experiment is observed. Results have thus shown an interrelationship between the size and surface energy effects and shapes of the experimental curves. This confirms that electrochemical methods may be used as the source of very valuable information both for analysis of thermodynamic and energy properties of nanomaterials, and the kinetics of redox reactions of nanoparticles.

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## P086-B2

### Development of Screen-Printed Enzyme Biosensors for the Analysis of Tyramine

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Tyramine (Tyr) formed by the decarboxylation of tyrosine is has one of the most harmful of the biogenic amines found in fermented foods and beverages, meat, fish, and dairy products. This biogenic amine is shown to be pathogenic neurotransmitter of hepatic encephalopathy and has been related to some general diseases of intoxication associated with migraines and hypertension. For these reasons, the development of a fast and accurate method to measure Tyr concentrations in foods would be valuable.

Most of the traditional methods developed to determine the Tyr content in foods involve conventional chromatographic separations with complex processes of sample processing [1-2]. Nowadays, the use of sensors based in enzymatic modified electrodes as transducers in amperometric and potentiometric techniques has opened important perspectives in the development of numerous biosensors for the determination of many kind of analytes [3]. The possibilities of the amperometric biosensors can be increased by means of replacing the classical electrodes by disposable screen-printed electrodes (SPEs). SPEs present important advantages such as the elimination of memory effects in the analysis at trace levels and they appear to be particularly attractive for *in situ* determinations.

In this study, the analysis of Tyr by means of different enzyme-based electrochemical biosensors is described. SPCEs were used as a support for the electropolymerisation of a polypyrrole film, in which the enzyme was immobilised. In this work, Horseradish Peroxidase (HRP) has been one of the selected enzymes. Amperometric experiments using the HRP immobilised electrode were performed at different pH and applied potential values. The biosensor gave a response by addition of hydrogen peroxide attributed to the reduction of some HRPox. Subsequent additions of Tyr produce a substantial signal decrease due to the oxidation of the Tyr oxidised species generated by HRPox present in the biosensor.

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## P087-B2

### Disposable Biosensors for Biogenic Amines Determination

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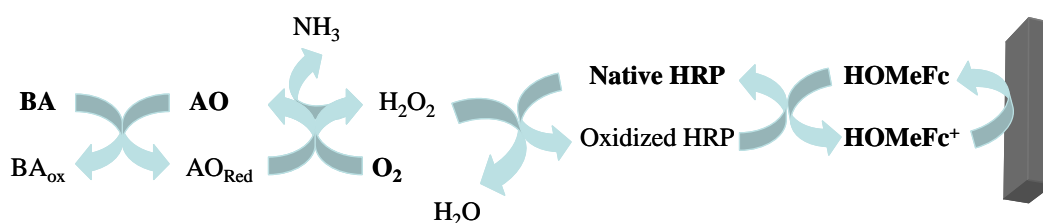
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Biogenic amines (BA) detection has been shown of great interest as biomarkers for various analytical purposes, such as freshness of fish, fruit and vegetables. Various methods have been developed for separation and quantification of biogenic amines and among them all, electrochemical biosensors. Biosensors, which combine biological recognition through enzyme specificity with construction simplicity, have been reported as a good and cheap alternative for the traditional ones.

The screen-printing technology had been shown as a method for mass production of biosensors at low cost. This technique offers design flexibility, process automatization and good reproducibility in the transducers fabrication, as well as the possibility of using a wide choice of materials.

In this work, Monoamine Oxidase (MAO)/Horseradish Peroxidase (HRP) and Diamine Oxidase (DAO)/HRP based biosensors using screen-printed carbon electrodes (SPCEs) have been attempted for the determination of BA. The enzymes have been immobilized onto the carbon working electrode, previously modified by an aryl diazonium salt. The formation of amide bonds between the amino and the carboxylic groups of the enzyme surface, catalyzed by hydroxysuccinimide and carbodiimide, leads to the electrode functionalization.

Amine Oxidases (AO) catalyze the oxidative deamination of BA to the corresponding aldehyde. Measurements of the oxygen consumption or the hydrogen peroxide production are commonly used for the quantification of BA in different samples. The quality of this kind of determination can be clearly improved reducing the high applied potentials, which can be reached using a second enzyme, HRP, and a mediator, ferrocene methanol (HOMeFc),



In order to perform a selective BA determination, experimental variables that can affect its chronoamperometric response have been optimized using the experimental design methodology. Under these optimum conditions, reproducibility, repeatability, limit of detection and interferences have been analyzed.

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## **P088-B2**

### **Electrochemical Polymerization of Proflavine from Aqueous Solutions and Analytical Applications of Synthesized Films**

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Proflavine is an acridine derivative with antiseptic properties, which does also possess native fluorescence and electroactivity. Correspondingly, it was sometimes used to develop sensitive and selective analytical methods of nitrite determination. Moreover, the planar structure of proflavine makes this dye an ideal candidate in biochemical studies, to easily intercalate between DNA-bases. Despite the attractive properties and proven versatility of proflavine, the literature is surprisingly scarce in the description of modified electrodes grounded on polymeric films synthesized electrochemically from the proflavine dye. Therefore, the present contribution reports the cyclic voltammetry polymerization of proflavine on glassy carbon electrodes, from aqueous solutions with pH-values spread over the range 2.1 – 7.1, with or without the presence of the SDS-tensioactive agent. In addition, the contribution proves the equivalence of the nonconductive films synthesized at different pH-values from the specified range and demonstrates the conductivity of the films in strong acidic conditions. Finally, the electroactivity of the polymeric films toward the nitrite oxidation has been revealed and a sensitive method of nitrite analysis has been developed by differential pulse voltammetry and applied on synthetic samples with high ionic strength.

## P089-B2

### Screen-Printed Sensor for Batch and Flow Injection Potentiometric Chromium (VI) Monitoring

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Chromium is one of the heavy metals frequently found in water and wastewater streams as a consequence of industrial and agricultural activities. It mainly exists as Cr (III) and Cr (VI) oxidation states which present different properties. Whereas Cr (III) is considered an essential microelement, Cr (VI) is a toxic pollutant. Therefore, the direct and accurate determination of this metal and its most toxic form in static and flow analysis is an important issue to analytical chemists. In fact, the analyses of pollutants in on-line systems are interesting options to easy and rapid control of contamination episodes.

Potentiometry is a simple, economic, selective, precise and rapid analytical technique which has found applications in different fields. The potentiometric sensors employed in on-line systems must present fast and accurate responses and must also be robust to avoid the loss of their potentiometric properties when they are directly used in analysis of complicated samples.

The aim of this work is to design and develop new chromium (VI) potentiometric sensor to use in chromium (VI) analysis in static and flow analysis. For this purpose carbon screen-printed electrodes are modified with composite materials which contain a selective compound to hexavalent chromium. The surfaces of these sensors are imaged using scanning electron microscopy to test the modification procedure. The principal analytical parameters of the sensors, including linear response range, pH effect, response time, detection limit and selectivity to other ions are evaluated. The parameters for flow measurements are also optimized. Hexavalent chromium can be accurately determined at low concentration level in both batch and flow injection potentiometry. These good characteristics make the sensor adequate to evaluate chromium toxicity level in wastes and other environmental samples. The sensor is employed to Cr (VI) determination in water samples and in extracts from soils used as barrier in landfills with successful results.

The authors acknowledge the financial support of this work from Ministerio de Ciencia y Tecnología (Project nº CTQ2008-06338/BQU).

## P090-B2

### Study of Structural Changes Accompanying Thermal Evolution of a Chromite Spinel Mineral Using Solid-State Electrocatalysis

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A wide range of complex exothermic and endothermic transformations take place during heating a South African chromite ore composed by spinels and silicates. The spinels of the chromite ore decompose in other spinels, with a partial change of the iron oxidation degree. From nearly 800°C, chrome oxide ( $\text{Cr}_2\text{O}_3$ ) comes off from the chromite forming another phase, and almost at 1000°C magnetite and other species are formed. Simultaneously, the silicates undergo significant modifications, including decompositions and incorporation of iron(II) in their structure and producing other silicates stable at high temperatures, which modify the behaviour of the pure spinels further (1200°C) decomposing to cristobalite ( $\text{SiO}_2$ ). Structural and oxidation state changes of iron and chromium minerals have been monitored by the electrocatalytic effect exerted by Fe-Cr spinels on  $\text{O}_2$  evolution reaction (OER) in alkaline aqueous media and the catalytic effect exerted by iron oxide minerals on the electrochemical reduction of dissolved  $\text{O}_2$  in aqueous media, in both cases using the voltammetry of microparticules approach (VMP).

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*Keywords:* solid state electrochemistry; electrocatalysis; chromite ores, thermal evolution.



## P091-B2

### Electrochemical Determination of Lactoferrin in Milk

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Lactoferrin is very important iron binding protein. Therefore, the biological function of lactoferrin is maintaining iron homeostasis. It is related to cell growth regulation, cell differentiation and it has an anti-inflammatory activity. Based on these functions possible relation to tumour diseases and metastases has been published. Lactoferrin is found in mucosal secretions, granules of neutrophils, milk and colostrum. The aim of this study was to investigate the electrochemical behaviour of lactoferrin by using of stationary electrochemical method. Further, lactoferrin was isolated from real sample (milk, colostrum) at first. For this purpose FPLC system coupled with monolithic column with catex -SO<sub>3</sub> groups was used. Subsequently 1 ml of the fraction of purified protein was collected and analyzed directly with UV (280 nm) using standard silica cuvette. The final step was measuring of lactoferrin electrochemically using carbon paste electrode and differential pulse voltammetry with scan from 0.1 to 1.1 V. Other parameters were as follows: accumulation time: 60 s, phosphate buffer at pH 7.5 (5 mM) as supporting electrolyte. In addition, we aimed at distinguishing of native and denatured structure of lactoferrin. The distinguishing was carried out via comparison of their signal heights at same concentration level of sample-isolated and referent-commercial lactoferrins.

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## P092-B2

### Study of Complexes of Metallothionein Fragment with Platinum Based Cytostatics

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Despite very intensive research in the synthesising of new cytostatics, cisplatin is still one of the most commonly used anticancer drugs. Therefore, an investigation of interactions of different forms of platinum based cytostatics with biologically important amino acids, peptides and proteins is very topical. In this study, we attempted to utilize flow injection analysis coupled with electrochemical detection for studying and characterizing the behaviour of various platinum based cytostatics (cisplatin, carboplatin and oxaliplatin). Primarily, we focused on studying their basic electrochemical properties on the surface of glassy carbon electrodes in FIA (flow injection analysis) arrangement. When we assessed that MTs (metallothionein) fragment could be analysed by a solid electrodes sensitively, the utilizing of flow injection analysis using glassy carbon electrode as working ones to analyse the compounds of interest followed. Optimized FIA technique was used for investigation of interactions between cisplatin drugs and fragment of metallothionein (MTs). It clearly follows from the results obtained that the optimized technique is suitable for investigation the interaction between MTs fragment and cisplatin. Moreover, we evaluated the formation of the complex by spectrometry. The spectrometric results obtained were in good agreement with electrochemical ones.

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## **P093-B2**

### **Modified Diamond Paste Microelectrodes for the Assay of Ascorbic Acid**

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Ascorbic acid is a well known antioxidant. Seven modified diamond paste microelectrodes based on porphyrins were proposed for the assay of ascorbic acid. Ascorbic acid was determined using differential pulse voltammetry (DPV) with very low detection limits. The working concentration ranges were up to  $10^{-12}$  mol/L. A comparison between the performances of the microelectrodes based on carbon and diamond paste will be shown. The advantages of utilization modified diamond paste microelectrodes over carbon paste microelectrodes were: lower detection limits, increased S/N ratio and higher selectivity. Ascorbic acid was determined accurately from pharmaceutical products and beverages, its recovery being higher than 90.00%.

## P094-B2

### Procedures Involving Reduction with Thiourea. Rapid Potentiometric Method for Determination of Vanadium Alone or in Mixtures, Alloys, Ores and Glass

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Thiourea used in analytical chemistry in different purposes firstly ,interaction with many heavy metals to form complexes eg. Hg, Cu, Ag, Bi, Cd and lead. Secondly, as masking agent especially to bind with Bi, Cu and Cd, finally, as a reducing agent with Mo, Rh, Pd, Pt, Se and Te. In our method thiourea could be used for vanadium determination, based on its reduction with excess of thiourea in slightly acidic medium with boiling for few minutes .

excess reductant was subsequently determined through oxidation with iodine dissolved in ethanol to produce iodide which is titrated with Hg(II) potentiometrically in presence of Ag / amalgam as an indicator coupled with S.C.E as the other half cell.

The reaction proceeds stoichiometrically according to

$$4\text{VO}_3^- + 2\text{N}_2\text{H}_4\text{CS} + 14\text{H}^+ = 4\text{VO}^{2+} + \text{C}_2\text{H}_6\text{N}_4\text{S}_2 + 8\text{H}_2\text{O}$$

The method finds wide applications to the determination of the element alone or in binary mixture containing Fe or ternary one containing Cr or Mn

this methods also applied successfully for determination of vanadium in some industrially important products such as an alloys, ores and a glass containing vanadium

## P095-B2

### Determination of Lead(II) by Chronopotentiometric Stripping Analysis after Extraction of the Bromide Complex

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Lead is one of toxic heavy metals, which can enter human bodies through drinking water, food chains and air. Lead tends to bioaccumulate and may cause long-term health problems. Monitoring trace lead ion in natural water, drinking water, biological and other real samples, therefore, is essential and meaningful. Lead determination by anodic stripping voltammetry (ASV) on different mercury electrodes and screen-printed carbon electrodes has been reported. Due to high toxicity of mercury, bismuth film electrode as an alternative has been developed for the determination of metal ions in aqueous medium. Stripping chronopotentiometric analysis (SCP) offers much less interference by the presence of closely related compounds and organic matters than ASV; it also enables determining traces of metals with a lower limit of detection. The purpose of this study is to develop a selective method for the determination of lead(II) in propylene carbonate extract by SCP following electrodeposition with an in-situ plated bismuth film electrode.

CSP studies indicate that Pb(II) gives an enhanced anodic peak at about -500 mV vs. Ag/AgCl with anodic scan. The Pb(II) response,  $dt/dE$  (s/V), was directly proportional to the initial Pb(II) concentration in the ranges of 1.0 - 20.0  $\mu\text{g L}^{-1}$  (correlation coefficient 0.9977) at the optimized parameters. A  $3\sigma$  detection limit of 0.088  $\mu\text{g L}^{-1}$  Pb(II) was obtained at 180 s deposition time. The relative standard deviation was 3.19% on replicate runs ( $n = 12$ ) for the determination of 1.0  $\mu\text{g L}^{-1}$  Pb(II). Analytical results of standard reference materials demonstrate that the proposed SCP method is applicable to the determination of Pb(II) in water samples.

## P097-B2

### Selective Recognition in Potentiometric Transduction of Amoxicillin by Molecularly-Imprinted Materials

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The indiscriminate use of antibiotics in food-producing animals has received increasing attention as a contributory factor in the international emergence of antibiotic-resistant bacteria [1]. Numerous analytical methods for quantifying antibacterial residues in edible animal products have been developed over years [1,2]. Being amoxicillin (AMX) one of these critical veterinary drugs, efforts have been made to develop simple and expeditious methods for its control in food samples. In literature, only one AMX-selective electrode has been reported so far. In that work, phosphotungstate:amoxycillinium ion exchanger was used as electroactive material [3]. Designing new materials based on molecularly-imprinted polymers (MIPs) which are complementary to the size and charge of AMX could lead to very selective interactions, thus enhancing the selectivity of the sensing unit.

AMX selective electrodes used imprinted polymers as electroactive materials having AMX as target molecule to design a biomimetic imprinted cavity. PVC sensors of methacrylic acid displayed Nernstian slopes ( $60.7 \text{ mV decade}^{-1}$ ) and small detection limits ( $3 \times 10^{-5} \text{ mol L}^{-1}$ ). The potentiometric responses were not affected by pH within 4-5 and showed good selectivity. The electrodes were applied successfully to the analysis of real samples.

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## P098-B2

### Novel Potentiometric Sensors of Ion-Imprinted Polymers for Specific Binding of Iron and Potentiometric Transduction

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An appropriate knowledge of iron levels in both environmental waters and water for human consumption is very desirable. Many works are reported for this purpose, but they may require complex and high cost procedures, providing highly disruptive wastes. Alternative and advantageous methods should rely on non-destructive and highly specific/sensitive measurements, such as those employing ion-selective electrodes. The most vital component of these sensors is the ionophore or the ion carrier, as its binding to the target ion is the sensed phenomenon [1]. Although ion exchangers and neutral macrocyclic compounds have been employed as sensing materials over the past decades, the design of new compounds that are complementary to the size and charge of a specific target analyte could lead to very selective interactions, thus enhancing the selectivity of the sensing unit. This could be achieved by ion-imprinted polymers (IIP). Only few works report the use of imprinted materials as potentiometric PVC sensors [2,3], and none of them is designed for Fe<sup>2+</sup> selective recognition. Electrodes of IIP displayed limits of detection of 8x10<sup>-8</sup> mol/L and linear ranges after 1x10<sup>-7</sup> mol/L.

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## P099-B2

### Evaluation of a Biosensor Made out of *Ulva Fasciata* Algae Supported on a Carbon Paste for Copper (II) Pre - Concentration

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Algae are photosynthetic aquatic organisms. They are generally found in sea waters, surface waters and mostly in soils. Algae can tolerate heavy metals in their environment keeping low concentrations of these metals in their intracellular space. Interaction of heavy metals with algae involves two basic processes. First, the metal is adsorbed on the external surface of the cellular membrane. This is a fast and reversible process. Second, the metal is absorbed and stored in the cytoplasm of the cells forming small vacuoles. This process is governed by the metabolism of the cell.

This work takes advantage of the absorption capacity of algae, particularly *Ulva fasciata*, to concentrate and determine low levels of heavy metals. A novel electrode based on a carbon paste containing *Ulva fasciata* was built for pre-concentration of Cu (II). The algae was dried at 60 °C in an oven, powdered to a small grain size and distributed in the carbon paste in concentrations ranging from 25 to 50 % m/m. performance of the electrode was evaluated using a 0,1 mg/L CuSO<sub>4</sub> solution and a 0,5 M H<sub>2</sub>SO<sub>4</sub> solution as a support electrolyte. Mechanical stirring of the CuSO<sub>4</sub> solution, after insertion of the biosensor electrode, allowed for optimization of its performance as a function of the peak current observed by means of cyclic voltammetry. Peak current was monitored during 14 hours. Peak current increased by a factor of 17 compared to the measurements after 5 min stirring. Effect of the acidity of the CuSO<sub>4</sub> solution (pH) was also monitored in a range between pH 1 and pH 6. This study indicated that pH = 5 was best option.



## P100-B2

### Study of Additive Behavior in Acrylic Artist's Paint Films by AFM, SEM-EDX and Spectroscopic Techniques

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Commercial acrylic artist's paints include a number of compounds in addition to the acrylic polymer and pigments to improve the physical and chemical properties of the resulting product. The behavior of these additives, specially the surfactants, during the drying and ageing processes influences not only the chemical and mechanical properties but also the visual appearance of the paint film. Conventional analytical methods, used separately, have proved to be unable to satisfactorily detect the presence and/or distribution of additives in the film. A multi-method approach is proposed combining microscopy (SEM/EDX, AFM) and spectroscopy (vis-UV, FTIR) techniques in order to characterize the overall behavior (migration, lixiviation) of the additives after the drying process. Seven different commercial acrylic paints have been selected for this study, namely, cadmium red, phthalocyanine blue and zinc oxide Liquitex® heavy body, burnt Sienna and phthalocyanine green Liquitex® soft body, raw Sienna Talens® and ultramarine blue Vallejo®. Comparison of vis-UV and IR spectra obtained in ATR mode from different areas of the films surface has evidenced that migration from the bulk and phase separation phenomena involving PEG type surfactants are notably taking place, at different extent, depending on the pigment and commercial brand. SEM examination of the films enabled the identification of microparticles selectively deposited in the different areas of the surface.

Nanoindentation carried out by AFM has provided load-unload curves and evidenced the change in the mechanical properties in the different areas of the paint films. *Acknowledgements:* Financial support is gratefully acknowledged from the Spanish "I+D+I MEC" project CTQ2008-06727-C03-01 and 02/BQU supported by ERDEF funds as well as the AP2006-3223 project ascribed to the program of predoctoral stages of university professors and researchers in Spanish universities and research centers from the Ministerio de Educación y Ciencia (MEC).

## P101-B2

### Identification of Additives in Poly(vinylacetate) Artist's Paints Using Py-GC-MS: Study of their Influence in the Mechanical Properties of Paint Films

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Commercial poly(vinyl acetate) (PVAc) paint formulations for artists include a number of compounds in addition to the PVAc polymer and pigments to improve the physical and chemical properties of the resulting product. Among the most common additives are surfactants, coalescing agents, defoamers, freeze-thaw agents, thickeners. These products significantly influence the behavior of the dried film. Nevertheless, they are usually difficult to detect with conventional analytical methods given their low concentration.

In order to identify these additives present in the dried film as minor components, a new analytical method is proposed based on the "on-line" silylation using hexamethyldisilazane (HMDS) as a derivatization reagent in Py-GC-MS. Five different commercial PVAc paints have been analyzed, namely, armour green, burnt umber, oriental red, raw umber and white from Flashe®. This new method improves the conventional GC-MS analysis performed by direct pyrolysis. Internal plasticizer VeoVa consisting of C<sub>10</sub> fatty acids with highly branched chains has been recognized from the MS spectra. On the other hand, the differences found in the additive content of the studied paints, in particular the PEG (polyethyleneglycol) type surfactant, are in good agreement with their mechanical properties. *Acknowledgements:* Financial support is gratefully acknowledged from the Spanish "I+D+I MEC" project CTQ2008-06727-C03-01/BQU supported by ERDEF funds as well as the AP2006-3223 project ascribed to the program of predoctoral stages of university professors and researchers in Spanish universities and research centers from the Ministerio de Educación y Ciencia (MEC).

## P102-B2

### Multi-Method Analysis of Iranian *Iljanato* Ceramics from the *Tajte-Soleiman* Palace

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Glazed ceramic has been traditionally used in Iran for decorating mosques and some civil historical building. In particular, *Iljanato* glazes have been extensively used in the indoor and outdoor decoration of Palaces in Iran in 13<sup>th</sup>-15<sup>th</sup> century. The pieces have a complex elaboration based on the application of a tin-opacified lead-alkali glaze decorated in cobalt and turquoise blue and mulberry colors which contain a number of areas covered with a thin gold sheet. This last, according to a number of historical writings, is fixed after the heating of the pieces with *Kaman* oil, consisting of cedar resin solved in linseed oil. Nevertheless, evidence of use of this adhesive in 15<sup>th</sup> century ceramics has not still been provided. The present work describes the analytical study performed on the glazes of several pieces of Iranian *Iljanato* ceramics from the *Tajte-Soleiman* Palace (Iran). Several advanced instrumental techniques including pyrolysis-gas chromatography-mass spectrometry, FTIR spectroscopy, light microscopy, x-ray diffraction, scanning electron microscopy-x-ray microanalysis and voltammetry of microparticles, have been used to perform the characterization of the glazes and the composition of the organic product used as adhesive of the gold film. Results obtained lead to identify the chemical and mineralogical composition of the pastes and glazes, the colouring agents and show that the glazes exhibit characteristic corrosion processes associated to the extreme burial conditions. Recognition of fatty acids suggests the use of organic natural products according to traditional recipes.

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## P103-B2

### Characterization of Raw Materials Used in the Encaustic Pictorial Technique

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Although the exact formula of the encaustic technique has not yet been determined, different studies indicate that Romans employed waxes, oils and probably soaps mixed with several pigments in mural paintings. Organic and inorganic analysis of these artworks may help to establish the composition of the paintings and the pictorial technique employed by the artists, thus allowing to determine the specific treatments for their preservation and restoration.

A study to characterize assumed natural binders (whitewashing beeswax<sup>1</sup>, linseed and walnut oil, pine resin, soap and lard) used in the encaustic pictorial works has been carried out by gas chromatography with flame ionization detection (GC-FID) and Fourier Transform Infrared Spectroscopy (FTIR). Pigments were analyzed by electron diffraction with high resolution transmission electron microscopy (HRTEM) and X-ray energy dispersive microanalysis (EDS) combined with scanning electron microscopy (SEM).

The inorganic analysis indicates the presence of hematite (iron oxide), umber earth (iron/manganese oxides), Egyptian blue (copper/calcium silicate) and natural cinnabar (mercury sulphide) pigments as well as their crystalline form in the artworks.

FTIR and chromatographic data from the analysis of the natural binders were modelled by principal component analysis (PCA) to find or identify clusters of raw materials used in the encaustic pictorial works. The second derivative of the original FTIR spectrum and the GC peak area data of the fatty acids and hydrocarbons of the samples were used as variables in the PCA. The ageing study of whitewashing beeswax showed a change of composition from the seventh day and a cluster between 21<sup>st</sup> and 82<sup>nd</sup> day. On the other hand, the score plot showed that the four samples of linseed oils have the same composition whereas the different composition of the soaps, pine resin and walnut oil samples was also apparent. Two or three principal components were necessary to explain more than 91% of the variability in the raw data and allowed to discriminate between the sample type and the treatment of beeswax.

<sup>1</sup>the samples were treated with UV radiation at 365 nm to simulate the composition changes produced by ageing.

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## P104-B2

### Electrochemical Characterization of Glass Materials with Ultrasonic Assisted Sampling. Application to Lead Determination in Historical Glasses

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Lead has been used in the fabrication of glasses since antiquity. The lead oxide gives attractive characteristics to glasses such as brightness. Glasses with high PbO content are currently classified as sonorous glass (~10 wt.% PbO), crystal glass (at least with 24 wt.% PbO) and superior crystal glass (at least with 30 wt.% PbO). For archaeologists and historians, the lead content is important to trace its origin and reasons for use in the general historical and technological development of glass.

The analysis of valuable materials of archaeological and historical interest requires the use of methodologies based on techniques sufficiently sensitive and selective and, at the same time, poorly invasive. It is therefore necessary to develop methods able to obtain relevant analytical information using very small samples or even with direct measurement on the piece of interest.

Electroanalytical techniques are very adequate for determination of metals and presents interesting advantages. They are easy to apply and instrumentation is not expensive.

In this work we propose a new voltammetric protocol for determination of lead in glasses, using a carbon screen printed electrode (SPE) that includes a carbon disk-shaped working electrode. The glass sample is suspended, as a fine powder, in 0.10% nitric acid using an ultrasonic probe. A small volume of the slurry is deposited on the SPE and quantification of lead is performed by Differential Pulse Voltammetry (DPV). Parameters that influence suspension stability and voltammetric measurements were optimized. The protocol has been proved in glasses with low, medium and high PbO content (0 to 30 wt.%), using 20 mg of glass sample. The design is simple and cheap, and is available to laboratories studying archaeological and historical glasses.

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## P105-B2

### Copper Bioactive Nanoparticles as a Strategy to Fight Microbial Communities on Stone Artworks

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Biological agents play an important role in deterioration of cultural heritage causing aesthetic, biogeophysical and biogeochemical damages. As a matter of fact, biodeterioration commonly results from the complex interaction established by the microorganisms co-existing simultaneously. It is mainly due to biofilm formation, biocorrosion caused by organic and inorganic acids, redox processes on cations from the mineral lattice, and physical penetration by microbial communities. Conservation is based on the use of preventive and remedial methods. The former aim at inhibiting biological attack, the latter aim at eradicating the biological agents responsible for biodeterioration. In our paper we propose a both remedial and preventive approach based on the use of bioactive copper nanoparticles (CuNPs) as antimicrobial agents capable to exert a marked biological activity over a long period of time. The CuNPs are prepared by means of the sacrificial anode technique [1,2]. The "core-shell" structure of CuNPs is capable to control the release of metal ions when exposed to an aqueous solution. Our approach is based on the mix of CuNPs with a state-of-the-art water-repellent/consolidant, to obtain a combined bioactive system to be applied on stone substrates. The nanocomposites have been fully characterized on the basis of a multi-technique analytical approach, involving both morphological and spectroscopic characterisations. The nanocomposites have been also applied to stone samples having different porosity and colour changes measurements have been performed. Preliminary results indicated a bactericidal activity on Gram-positive and Gram-negative viable cells. Moreover, it is found that the metal release and the resulting bioactivity can be easily tuned by changing the metal NPs concentration [3].

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## **P106-B2**

### **Optical Emission Spectrochemical Microanalysis of Unique Museum Samples**

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Detailed structural characterization and identification of key chemical constituents can uncover important information on historical and artistic significance of artworks and archaeological findings. When valuable objects such as museum exhibits are to be analyzed, the preservation of the integrity and aesthetic value of the object becomes a major concern. In this context, optical emission spectrometry with laser microsampling, a rapid elemental analysis technique, which is applicable *in situ* and is almost non-destructive, offers a potential alternative to other optical spectroscopic, mass spectrometric, or X-ray techniques.

As one of the last examples of a successful technological investigation of unique museum exhibit optical emission spectrochemical analysis with laser microsampling has been used for the examination of authenticity of a massive gold breast embellishment manufactured presumably in the early Middle Ages. Such embellishment has no analogues in Belarus as well as in Russia and presents a large historical interest.

The fundamental (1.06  $\mu\text{m}$ ) harmonic of a nanosecond Q-switched Nd:YAG laser with a pulse duration of 6-8 ns is employed in our experiments. The laser used is an improved version with variable reflectivity mirrors. The light emitted from the vertically expanding plasma plume is imaged (1:1) onto the horizontal entrance slit of a compact spectrograph. The spectrum is recorded by an optical multichannel analyzer with a linear CCD detector.

Microanalysis of the biggest central plate with lavalieres has showed that the major components are gold (91 %), silver (5 %), and copper (4 %). In turn, microanalysis of the plates from the chain has showed that their composition includes the same major components: gold (79 %), silver (12 %), and copper (9 %). Among small detectable admixtures, calcium has been detected and its distribution in alloy is very uneven for all examined plates. Besides, precious stones on the embellishment have been analyzed.

## **P107-B2**

### **Comparison of Trace Determination of Copper on Modified and Non Modified NANO Alumina by Factorial Design Optimization**

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A simple and rapid method is described for per concentration and determination of cu on a column packed with modified nano sized  $Al_2O_3$  (10 nm) and column of nano sized  $Al_2O_3$  (10 nm, non modified). Some variables such as sample flow rate, eluent flow rate, type of eluent, concentration of eluent and value of buffer on the columns were investigated. In this research 2 level factorial designs were used for optimization process and investigation of interaction effects. The optimum established conditions were applied to determination of cu by ICP-OES. Copper is quantitatively retained on the column in the PH range 3-11 at the flow rate of 1 ml/min , the copper were eluted with 3 ml of HCL 1M in nano sized  $Al_2O_3$  column and 3 ml of HCL 1M and thiourea on the modified nano sized  $Al_2O_3$  . The proposed method had a linear calibration range from at least 10 ppb to at 200 mg/l and 10 ppb to at least 140 mg/l for non modified and modified column respectively , also the enrichment factor of cu were , 14.55 and 15.88 for non modified and modified columns respectively . The detection limit of 0.2 ng/ml for non modified and 0.59 ng/ml for modified column were obtained. The comparison was done between modified and non modified columns. The optimized conditions developed were utilized for the trace determination of copper in various environmental samples.



## P108-B2

### Use of On-Column Scanning Contactless Conductivity Detection for the Direct Visualisation of Stationary Phase Gradients on Polymer Monoliths

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In HPLC, a separation column incorporating a gradient of functional groups along its length would theoretically lead to focussing of peaks as they traverse the column length, without the need for complex gradient pumping systems. Here we report a simple and repeatable method for fabrication of stationary phase gradients based on polymer monoliths in capillary formats, and the subsequent characterisation of these gradients using on-column scanning conductivity methods. A butyl methacrylate-co-ethylene dimethacrylate-co-vinyl azlactone monolith was first produced in a 100  $\mu\text{m}$  fused silica capillary wherein the concentration of vinyl azlactone was deliberately increased in a stepwise manner along the column length. This was achieved by sequentially filling the column housing with adjacent plugs of monomer mixture, each containing incrementally larger azlactone concentrations, prior to thermal polymerisation. The subsequent passage of iminodiacetic acid (IDA) through the column resulted in covalent immobilisation of this chelating ligand via stable dipeptide linkages with the exposed azlactone moieties at the stationary phase surface. The precise shape and slope of this gradient of IDA could be easily be visualised by scanning a capillary scale conductivity detector along the column length and plotting the increasing conductive response of the charged stationary phase versus the detector position on-column. On-column chelating events could also be directly visualised by the initial passage of copper across the column prior to scanning conductivity measurements. Chelation of copper by the immobilised IDA gradient resulted in a decreased stationary phase slope due to reduction of the charge of the ligand, which demonstrated that the monolith was suitable for protein separations in IMAC mode. The repeatability both of the stationary phase gradient fabrication procedure and the scanning conductivity based characterisation was studied in detail. Finally, the application of this monolith to the on-column focusing of selected proteins using an IMAC gradient stationary phase was demonstrated.

## **P109-B2**

### **Study on the Chromatographic Behavior of Some Nucleosides and Nucleobases on Immobilized Humic Acid Stationary Phase by HPLC**

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Chromatographic behavior of some nucleosides and nucleobases was investigated on humic acid-bonded 3-aminopropyl silica stationary phase. Immobilization of purified Aldrich humic (HA) on 3-aminopropyl silica (3-APS) was done through amide bond formation, and unreacted amino groups on 3-APS were deactivated through an end-capping step. Obtained material (EC-HA-APS) was converted into a ligand-exchange stationary phase by loading a suitable metal ion to the column packed with EC-HA-APS. Thus, metal loading step was performed under dynamic conditions, and the stability of fixed metal ions was evaluated in presence of ammonia. Chromatographic behavior of the studied compounds was investigated on both EC-HA-APS and copper-loaded EC-HA-APS (Cu-EC-HA-APS) to understand the role of metal ion in chromatographic behavior. All the experiments were performed on an HPLC system consisted of a quaternary pump with degasser, a thermostatted column compartment and a variable wavelength detector. Influence of some experimental variables in chromatographic separation was evaluated, and usability of the proposed concept was discussed in terms of various criteria used in chromatography.

## P110-B2

### Molecularly Imprinted Polymers: Pros and Cons

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Molecularly imprinted polymers (MIP) have recently become popular as selective sorbents. Some MIPs are already commercially available for SPE while others have been tested for industrial applications. MIPs may be used in chiral separations and they can also replace antibodies in binding assays. It is also relatively easy to synthesize MIPs. The adsorptive behaviour of MIPs is different, however, from the usual modern chromatographic sorbents. The adsorption isotherms of MIPs show non-linear behaviour already at low analytical concentrations and their adsorption kinetics may be slow. We have studied the consequences of the non-linear isotherms on the characterization and practical usefulness of the MIPs. Some of the results are:

- Non-linearity of the isotherm is a disadvantage in elution chromatography but a necessity in binding assays
- The typical assessment method of new MIPs by HPLC is not adequate
- Competition of analytes for the binding sites on the MIP is the basis of binding assays yet it cannot be observed in chromatography due to peculiarities of non-linear chromatography.

Finally, some novel technical developments which help to overcome the difficulties and speed up the work with MIPs are presented.

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Nonlinear adsorption isotherm as a tool for understanding and characterizing molecularly imprinted polymers

Journal of Chromatography A, 1119 (2006) 29-33

B. Tóth, T. Pap, V. Horvath, G. Horvai

Which molecularly imprinted polymer is better?

Analytica Chimica Acta, 591 (2007) 17-21

## P111-B2

### Silica-Alumina-Niobia (SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub>/Nb<sub>2</sub>O<sub>5</sub>) Matrix Obtained by the Sol-Gel Processing Method: New Material for On-Line Preconcentration and Determination of Trace Amounts of Zinc Ions in Water and Biological Samples

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The present work demonstrates a simple and sensitive flow injection spectrophotometric method for on-line preconcentration and determination of trace amounts of zinc. The preconcentration method is based on Zn<sup>2+</sup> ions adsorption under alkaline medium (pH 9.0) on the surface of new material SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub>/Nb<sub>2</sub>O<sub>5</sub> (designated as SiAlNb). This material SiAlNb was prepared by the sol-gel processing method and presented specific surface area of S<sub>BET</sub> = 309 m<sup>2</sup> g<sup>-1</sup>; pore diameter average of 3.38 nm; pore volume of 0.16 cm<sup>3</sup> g<sup>-1</sup> and 2.1 wt% of Al<sub>2</sub>O<sub>3</sub> and 33.6 wt% of Nb<sub>2</sub>O<sub>5</sub>. The elution step is carried out by using HNO<sub>3</sub> solution. After elution, Zn<sup>2+</sup> ions react with PAN (1-(2-piridylazo)-2-naphtol) in the presence of Tween-80 under amoniacal buffer solution (pH 9.3). The Zn(PAN)<sub>2</sub> complex formed is further determined at 560 nm. The optimal experimental conditions, including reaction conditions and preconcentration conditions, had been optimized. The optimized values are NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> buffer at pH 9.0, concentration of 0.05 mol L<sup>-1</sup>, preconcentration flow rate of 4.0 mL min<sup>-1</sup>, PAN concentration of 0.1 mmol L<sup>-1</sup>, reaction coil length of 2 m and 0.2 mol L<sup>-1</sup> eluent (HNO<sub>3</sub>) concentration. The method presented a linear range between 7.6 and 180.0 µg L<sup>-1</sup> (r = 0.9992) and limits of detection and quantification of 2.3 and 7.6 µg L<sup>-1</sup>, respectively. The precision (n=10) assessed as relative standard deviation (RSD) in terms of repeatability were found to be 3.2 and 1.7 % for the respective Zn<sup>2+</sup> concentrations 20.0 and 170.0 µg L<sup>-1</sup>. According to Langmuir linear model the adsorption maximum capacity was found to be 6.84 mg of Zn<sup>2+</sup>/g of SiAlNb. Interference study was conducted through the addition of metallic elements Ni<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, Ca<sup>2+</sup>, Sb<sup>3+</sup>, Hg<sup>2+</sup>, Pb<sup>2+</sup>, Fe<sup>2+</sup> and Mn<sup>2+</sup> during Zn<sup>2+</sup> analysis and no interference was noted. The proposed method was successfully applied for the Zn<sup>2+</sup> determination in lake water, mineral water, tap water and certified reference material (**TORT-2** Lobster Hepatopancreas).

## P112-B2

### Micro-Bore Titanium Housed Polymer Monoliths for High Temperature and Pressure Liquid Chromatography

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To date, the vast majority of polymeric monoliths have been housed within fused silica capillaries, due to availability, cost and ease of attaching/binding the monolith readily to the column wall. However, this approach limits the wider development and application of polymer monoliths, and comes with the inherent limitations of working at the capillary or nano-scale, including for example column capacity and loadability when dealing with complex samples. Titanium tubing is readily available in various dimensions, and can be used as an alternative to fused silica capillary as column housing for polymer monoliths. Titanium is a proven bio-compatibility material, temperature compatible, and suitable for use with typical RPLC and ion-exchange eluent systems.

In the research presented here, techniques for binding homogenous polymer monoliths to the inner wall of titanium columns, of internal dimensions of 0.8 mm for micro-bore liquid chromatography will be discussed. The controlled formation of butylmethacrylate - ethylene dimethacrylate based monolithic stationary phases with desired porous structure in titanium housing has been optimised for application to the reversed-phase separation of small molecules. The obtained titanium bound stationary phases were fully characterised with a number of studies, including column stability at high temperatures and backpressures, influence of sample injection volume on peak efficiency, and backpressure dependence on the eluent flow rate. Additionally, van-Deemter plots were constructed and optimal flow rates were found for a test mixture of small organic molecules separated under high-temperature conditions. Stability of the monolithic columns at 110 °C and at operating pressures of >280 bar was demonstrated. It was shown that the monoliths produced exhibited uniform, but very dense structure, which provided excellent small molecule efficiency, with up to 59000 theoretical plates per meter achieved under optimal conditions.

## P113-B2

### Isocratic or Gradient Separation?

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When starting RP-HPLC method development for a sample whose composition is undefined, an initial gradient elution is recommended. This allows a choice between isocratic and gradient elution for subsequent experiments. From this initial gradient a simple rule of thumb is applied. Once the difference in the retention time between last and first peaks ( $\Delta t$ ) and gradient time ( $t_G$ ) are known, calculation of  $\Delta t/t_G$  allows to decide between isocratic or gradient separation. If  $\Delta t/t_G > \textit{number}$  use gradient separation and if  $\Delta t/t_G < \textit{number}$  use isocratic separation. This topic is already considered in analytical chemistry textbooks though in some cases it is applied in a simplified form that can lead to an erroneous decision. The number that indicates the type of separation must be deduced according to the cosolvent used. The value of the *number* is obtained (1) from the following

equation:  $\frac{\Delta t}{t_G} < \frac{1}{S\Delta\Phi} \log\left(\frac{k_z}{k_a}\right)_{\max}$ .  $S$  is the slope of the retention equation ( $\log k = \log k_w - S\Phi$ )

for a series of solutes against the cosolvent volume fraction.  $\Delta\Phi$  is the change in  $\Phi$  during the

gradient.  $\frac{k_z}{k_a}$  is the maximum allowable value of the capacity factors ratio between the last and first

peaks. In this communication a worked example is used to show the relationship between the type of cosolvent and the number used in the rule to decide between isocratic or gradient elution.

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## P114-B2

### Robustness of the Co-Ion Transfer Ratio in Capillary Electrophoresis

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In capillary electrophoresis, indirect detection is used to detect analyte with low response factor to UV detection. In this mode, a UV absorbing ion, known as the probe, is used in the background electrolyte. A charged analyte will induce some variations in the probe concentration in respect to various conservation laws (mass and charge balance, Kohlrausch regulation function...) and thus a peak or a dip will be detected by the detector. The response of the probe is proportional to the analyte concentration of a constant known as the transfer ratio, depending on the analyte and background electrolyte characteristics. However, this mode often exhibits a lower precision than its direct counterpart. Various explanations have already been advanced, but in this work we aimed to investigate if this is due, in part, to problems of robustness of the co-ion transfer ratio, and thus being inherent to this particular detection scheme. This was investigated using simulation software that allows an accurate control of various parameters and validated using acetic acid as a test compound. It was conclusively demonstrated that the transfer ratio could vary by more than 6% when the concentration of one of the ions in the background electrolyte was changed by as few as 1%. The presence of a system peak seems to be particularly damaging as it has been shown that the transfer ratio of peaks whose mobilities differ by more than  $0.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$  from the one of the system peaks, still have a relatively low robustness.

## P115-B2

### Development of a New Chromatographic Response Function for Use in HPSEC Optimization Strategies: Application to Complex Organic Mixtures

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Chromatographic response functions (CRFs) are designed in order to provide effective means of comparison, differentiation and quantitative scaling of chromatogram quality. Over the past decades, many CRFs have been proposed and applied for high-performance liquid chromatography (HPLC) optimization and method development in different contexts (e.g. pharmaceutical analysis, biotechnology, and food quality control) [1-3]. Most of these objective functions are not of general validity for all chromatographic studies since they are usually designed to answer a particular separation problem.

In the present study we introduce a new CRF aiming at designing an optimal chromatographic separation protocol for accurately assess the molecular size distribution of complex organic mixtures, such as those of natural organic matter from different sources (aqueous, terrestrial and atmospheric (e.g. aerosols)), by means of high-pressure size exclusion chromatography (HPSEC). The new objective function includes the sum of the individual resolutions between pairs of peaks, which are derived from the peak and valley heights and their respective retention distances, being well-suited for peak pairs of highly unequal area and for asymmetric peaks. The number of peaks eluted and the total analysis time are also included in this new criteria function. The performance of the developed CRF was compared to some of the more frequently used criteria functions in chromatographic optimizations described in the literature [1,2], using simulated chromatograms. The new CRF was also used to quantitatively describe HPSEC analyses of a variety of different water-soluble organic compounds relevant to natural organic matter (mono- and dicarboxylic acids, saccharides, phenols, and aromatic acids).

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## P116-B2

### Selectivity Studies on a High-Capacity Macroporous Latex Agglomerated Anion Exchange Resin Incorporating Electrostatically Adsorbed Gold Nanoparticles

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Citrate stabilised gold nanoparticles of size 20 nm have been electrostatically adsorbed onto commercially available latex agglomerated anion exchange resins. Initial experiments involved adsorption of citrate gold onto batches of selected resins followed by recovery of the gold nanoparticle coated resin by simple centrifugation. A change in colour of the resin from white to pink was indicative of a successful immobilisation of gold as was the corresponding reduction/elimination of the gold plasmon absorption band in the supernatant. Selected resins were evaluated using FE-SEM imaging to readily visualise the adsorbed 20 nm gold nanoparticles on the resins. Adsorption was by a simple electrostatic mechanism between the negatively charged citrate stabilised gold and the positively charged quaternary amine functionalities on the agglomerated latex surface. The relative density of gold coverage on Dionex AS10, Dionex PAX-100, Dionex AS11 HC and Hamilton PRP-X100 resins was found to be a function both of inter-particle repulsive effects and also the ion-exchange capacity of the resin substrate. Washing the resin alternatively with 1 M solutions of HCl, NaOH, NaCl or 50 mM CTAB failed to remove the gold from the resin and was indicative of the significant strength of the electrostatic bond, presumably due to multiple attachment sites per gold nanoparticle. A Dionex AS10 anion exchange column was subsequently coated with 20 nm citrate gold in flow-through mode with a breakthrough curve acquired at 520 nm to indicate complete adsorption. Citrate eluents across a selected pH and concentration range were used to evaluate the repulsive effect of adsorbed negatively charged nano-gold upon retention and selectivity for inorganic anions. Additionally, the possibility for the gold coated column to allow simultaneous separation of anions (via latex quaternary ammonium groups) and cations (via deprotonated carboxyl groups of adsorbed citrate) was investigated.

## P117-B2

### Waste Management and Treatment Recovery of Acetonitrile

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The liquid chromatography (LC) is as widely used technique for the analysis of many compounds and those who work in LC in laboratories for research and analysis know the importance of acetonitrile as a mobile phase component or a solvent in various processes of extraction. LC produces, daily, large amounts of liquid wastes containing organic solvents that require appropriate storage and treatment since they have a negative impact in human health and environment due to their toxicity.

As currently there is a huge lack of acetonitrile in the world, this project proposes the recovery of LC acetonitrile wastes, which offers several advantages with important economic and environmental impacts and is an important contribution to a sustainable development.

Wastes were submitted to a fractional distillation using a column of 1 m length or a rotary evaporator (Buchi B-491) at a reduced pressure of 223 mbar (Buchi, V-700 vacuum pump). Several products of the distillation were subjected to a purity control that includes dry matter (ranged between  $3.0 \times 10^{-4}$  –  $9.0 \times 10^{-4}$ % for fractional distillation and  $1.0 \times 10^{-4}$  –  $7.0 \times 10^{-4}$ % for rotavapor), and water content ( $18.3 \pm 0.2$ % for the fractional distillation and  $15.2 \pm 0.2$ % for rotavapor). The impurities were checked using spectrometric techniques (Fluorescence, Ultra-violet-Visible and Attenuated Total Reflection Fourier Transform Infrared) and Liquid Chromatography (fluorescence, diode array and mass spectrometry detection).

These methodologies allowed recovering 78.8 litres of LC solvent wastes, corresponding to 33 x 2.5 litre bottles. 45 litres of a mixture of acetonitrile and water was obtained, with a quality analysis in a very good concordance with the specification of LiChrosolv grade quality of commercial solvents. Furthermore the recovered mixture of acetonitrile-water is already in use, in our laboratory, for LC isocratic analysis.

## P118-B2

### Development of a Capillary Ion Chromatography System for the Separation of Ions on Ion Exchange Polymer Monoliths

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Polymer monoliths have proven to be a useful tool in capillary chromatography due to their low cost, ease of manufacture and relatively low operating backpressures. Polymer monoliths have thus far mainly found application in the chromatographic separation of large bio-molecules due to their excellent convection-mediated mass transfer characteristics. More challenging is the chromatographic separation of small molecules, and particularly small inorganic ions due to the relatively low surface area of polymer monoliths relative to their silica-based counterparts. Previously, modification of polymer monoliths with ion-exchange functionalities has led to a stationary phase of prohibitively low ion-exchange capacity. In this work, a glycidyl methacrylate-co-ethylene dimethacrylate polymer monolith was fabricated using thermally initiated polymerisation. The surface of the polymer monolith was subsequently grafted with polymer chains of [2(methacryloyloxy) ethyl] trimethylammonium chloride (META) using UV irradiation, leading to an electrolyte-mediated swelling of the grafted chains which was indicative of a high ion-exchange capacity. This 100 µm x 10 cm ion-exchange polymer monolith was incorporated into a laboratory constructed capillary ion chromatography system consisting of an analytical pump, a 20 nL fixed loop injector valve and on-column capacitively coupled contactless conductivity (C<sup>4</sup>D) detection. Using a 2 mM sodium benzoate eluent at 1 µL/min and an injection volume of 20 nL, separation of a test mix of 5 anions (fluoride, chloride, chlorite, bromate and nitrite) was achieved in only 2 minutes with fluoride been significantly separated from the void. The selectivity of this column for a wide range of selected inorganic and organic anions was studied. Temperature studies were carried out using a polyimide flexible heater (1 cm x 10 cm). The flexible heater was placed inside a metal tube 10 cm in length and the grafted monolith placed in the centre of the tube. The temperature of the heater was controlled using a variable voltage controller. The work carried out so far uses most of the components that would be field deployable, which is one of the future directions of this project.

## P119-B2

### Development of LC-MS Method for Analysis of Convertible E/Z Isomers of Novel Anti-Cancer Agent Bp4eT

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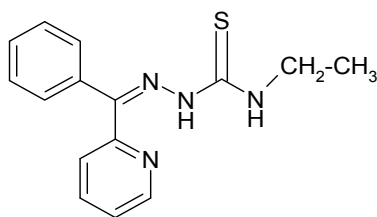
Selective targeting of the essential nutrient iron is a novel strategy in the treatment of cancer. 2-benzoylpyridine-4-ethyl-3-thiosemicarbazone (Bp4eT) is a newly developed thiosemicarbazone iron chelator, which has demonstrated an intriguing anti-proliferative activity both *in vitro* and *in vivo*. The crystalline structure of Bp4eT was found to be Z isomer, however the quick conversion to E isomer was observed in aqueous media.

The aim of this study was to develop sensitive, precise and accurate ion trap LC-MS method for determination of both isomeric forms of Bp4eT.

The best separation was achieved on Phenomenex Synergi 4u Polar RP column (150 × 3 mm, 4 μm) with a mobile phase consisted of 2 mM ammonium formate and an organic part (10% of methanol in acetonitrile) in the ratio of 30/70 (v/v). A flow rate of 0.25 mL/min, column temperature of 25 °C and 2-hydroxy-1-naphthylaldehyde-4-methyl-3-thiosemicarbazone (N4mT) as an internal standard were used for the analysis.

Since attempts to prepare the standards of each isomer failed, the aim was to develop such as MS settings, which provided the same ionization intensity for both isomers. This approach would enable to determine the whole content of Bp4eT as a simple summarization of peaks areas as well as evaluate each isomer individually without the need for the standards. Due to the unequal susceptibility of the isomers to sodium adduct formation, ESI was not suitable ionization technique. On the contrary, using APCI, no adducts were observed and the detector setting could be easily optimized to get the same ionization intensity for both Bp4eT isomers. Quantification was accomplished in selective reaction monitoring mode.

This method was validated in the range of 10-600 ng/mL and applied to investigation of the compound behavior in different physiologically relevant media.



Bp4eT

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## **P120-B2**

### **Study of Forced Degradation Behavior of Torsemide by LC/LC-MS**

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In the present study, comprehensive stress testing of torsemide was carried out according to ICH guideline Q1A(R2). The drug was subjected to acid (1N HCl), neutral and alkaline (1N NaOH) hydrolytic conditions with refluxing for definite time intervals, as well as to oxidative decomposition at room temperature. Additionally, the solid drug was also subjected to photo stability (sun light and UV light for seven days) and thermal stability at 50°C for 60 days. The products formed under different stress conditions were investigated by LC and LC-MS/MS. The LC method that could separate all degradation products formed under various stress conditions involved a C18 column and a mobile phase comprising of acetonitrile 40% and water (pH3.2 with glacial acetic acid) 60%. The flow rate and detection wavelength were 1.2 ml/min and 288nm respectively. Degradation was adequately modeled by specific equations for first order rate kinetics. The developed method was found to be precise, accurate, specific and selective. It was suitably subjected to LC-MS/MS studies with 0.2ml/min flow rate. The drug showed instability towards hydrolytic conditions (neutral, acidic and alkaline), but was found stable to oxidative, photolytic and thermal degradation. The degradation products formed via acid and alkaline hydrolysis were found to be same. The products were characterized through LC-MS/MS fragmentation studies.

## P121-B2

### HS-SPME and Hydrodistillation Analysis of *Ziziphora Persica* from Babaaman Mountain of Iran

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The genus *Ziziphora* L. consists of four species, *Z. clinopodioides* Lam., *Z. capitata* L., and *Z. persica* Bunge. and *Z. tenuior* L. Widespread all over of Iran. *Z. clinopodioides* Lam (1). In Iranian folk medicine, *Ziziphora* species have been used as infusion, decoction and maceration for various purposes such as sedative, stomach tonic, heart disorders, common cold, inflammation, depression, diarrhea, expectorant, coughing, antiseptic, migraine, fever and carminative (2-3). In Iranian folklore, the dried aerial parts of aforementioned species have been frequently used as culinary and spice in food. The antibacterial activity of the oil of *Z. taurica* subsp. *clenioides* and *Z. taurica* has already been studied. A preliminary study on the chemical compositions of essential oils of *Z. persica* growing in North Korassan, Iran( Babaaman Bojnourd) , was conducted. The yield of oil is quiet low, about 0.84 w/w%. The oil is yellow with odor characteristic of plant. The hydrodistillation time for 60g of dried *Z. persica* is about 4h. The most compositions of the oil will isolate around the first 20min of the analysis procedure and condition. A total of 22 components were identified by GC/MS, representing 98.61-99.71% of the components. Some parameters such as salt effect, extraction temperature, adsorption and desorption time were optimized. The main component was pulegone with 77.3% and 78.14% using HS-SPME and hydrodistillation procedures, respectively.

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## P122-B2

### Analytical Determination and Electrochemical Characterization of Oxazolidinones Antibiotics

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Oxazolidinones antibiotics, and linezolid in particular, are receiving great attention, due to their activity against multidrug resistant strain of Gram-positive and Gram-negative bacteria, and in particular to multidrug resistant *M. tuberculosis* (1).

For this reason, stability studies (2) and rapid method of determination of these antibiotics are required. Among the techniques that can be employed, electrochemical techniques offers the advantage of being rapid, economic and sufficiently selective.

In this work, a differential pulse voltammetric method for the determination of linezolid has been developed and optimized, which operates in aqueous medium (0.1M lithium perchlorate as supporting electrolyte) employing a glassy carbon electrode as working electrode and based on the oxidation process of the compound, with LOD of 50 ppb and a linearity up to 200 ppm.

The electrochemical characterization of the oxidation process was done using chronocoulombometry, voltammetric techniques and exhaustive coulombometry (3,4). In the same way, the electrochemical oxidation process of the electroactive moiety in the molecule (the phenylmorpholine ring) was studied both on the defluorinated analogue of linezolid and in phenylmorpholine itself. The oxidation process regards the anilinic N atom and resulted to be a one-electron reversible process coupled with an irreversible chemical reaction (EC mechanism). The electrochemistry in non-aqueous solvents was considered too.

Pre-concentration process of linezolid and its metabolites from highly diluted solution with SPE are in progress.

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## P123-B2

### Electrochemical Evaluation and Determination of Antiretroviral Drug Fosamprenavir in Pharmaceutical Dosage Forms and Biological Samples

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Fosamprenavir (FAMP) is a [pro-drug](#) of the [protease inhibitor](#) and [antiretroviral drug amprenavir](#). The human body metabolizes FAMP in order to form amprenavir, which is the active ingredient.

Despite the analytical importance of the electrochemical behavior and oxidation mechanism and of FAMP, the literature has no report on electrode processes of the drug. The goal of this work was the development of new voltammetric methods for the direct determination of FAMP in pharmaceutical dosage forms, raw materials and spiked biological samples without any time-consuming extraction or evaporation steps prior to drug assay.

FAMP is oxidizable at the boron-doped (BDD) electrode. In this study, the electrooxidative behavior of FAMP at BDD electrode was carried out using different electrochemical techniques such as LS, CV, DP and SW voltammetry. The effects of pH, nature of the supporting electrolyte and scan rate on the response of the electrode were investigated. The oxidation was irreversible and exhibited diffusion controlled process.

Validated voltammetric procedures are also described for the trace determination of the FAMP in bulk form, pharmaceutical formulation, and biological samples. According to the linear relation between the peak current and the concentration, differential pulse and square wave voltammetric methods for its quantitative determination in pharmaceutical dosage forms and biological fluids were developed. These two voltammetric techniques for the determination of FAMP in 0.1 M H<sub>2</sub>SO<sub>4</sub> which allows quantitation over the  $4 \times 10^{-6}$  –  $1 \times 10^{-4}$  M range in supporting electrolyte with a slope of  $2.22 \times 10^4$ ; intercept of 0.0499 and the limit of detection was  $1.23 \times 10^{-6}$  M for DPV and with a slope of  $2.57 \times 10^4$ ; intercept of 0.075 and the limit of detection was  $1.97 \times 10^{-7}$  M for SWV were proposed. The repeatability and reproducibility of the methods for all media were determined. Precision and accuracy were also checked in all media. No electroactive interferences were found in the biological fluids.



## P124-B2

### Electrochemical Oxidation of Zolmitriptan at Boron-Doped Diamond Electrode and its Direct Determination in Pharmaceuticals and Serum Samples

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Zolmitriptan is a selective serotonin receptor agonist of the acute treatment of migraine attacks with or without aura and cluster headaches. To our knowledge, no information about the electrochemical redox properties of zolmitriptan and its analytical application.

In this study the electrooxidation of zolmitriptan at boron-doped diamond electrode was carried out using cyclic, differential pulse (DP) and square wave (SW) voltammetric techniques. Boron-doped diamond electrodes have attracted much recent attention for electrochemical determination. The dependence of current intensities and potentials on pH, scan rate, nature of the buffer, concentration were investigated. The pH dependence studies were performed in the pH range 2.00-12.00 in various buffer solution. The oxidation of zolmitriptan was irreversible and exhibited a diffusion controlled process. The slope of  $\log I_p$ - $\log u$  linear plot was 0.48 indicating the diffusion control in pH 3.03 phosphate buffer. For analytical purposes a sharp peak was obtained at +0.875 V (vs Ag/AgCl) by DPV and +0.905 V (vs Ag/AgCl) by SWV in pH 3.03 phosphate buffer at boron-doped diamond electrode. The linear response was obtained in the two different ranges of  $8 \times 10^{-7}$ - $8 \times 10^{-6}$  M (part I) and  $1 \times 10^{-5}$ - $1 \times 10^{-4}$  M (part II);  $6 \times 10^{-7}$ - $8 \times 10^{-6}$  M (part I) and  $1 \times 10^{-5}$ - $1 \times 10^{-4}$  M (part II) for supporting electrolyte and spiked serum samples, respectively. For part I, the detection limit was found as  $7.3 \times 10^{-8}$  M and  $2.94 \times 10^{-7}$  M for DPV,  $2.63 \times 10^{-7}$  M and  $1.3 \times 10^{-5}$  M for SWV techniques for supporting electrolyte and spiked serum samples, respectively. The repeatability of the methods was found as 0.34 and 0.22 % for peak currents ( $6 \times 10^{-5}$  M) and 0.13 and 0.30 % for peak potential for DPV and SWV, respectively.

## P125-B2

### Identification of a New Cocrystal of Salicylic Acid with Benzamide of Pharmaceutical Relevance

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Modification of the physical properties of drug substances such as crystallinity, solubility, hygroscopicity, stability, particle size, flow, filterability and density, has been a focus of the pharmaceutical industry and the generation of new design elements that facilitate the generation of crystalline products having superior qualities represents an active area of research.<sup>(1),(2)</sup>

Salicylic acid was selected to examine the changes an intramolecular hydrogen bond would bring to a molecular scaffold matrix with a series of APIs which possess carboxylic acid and amide functionalities. Thereby new cocrystal systems have been identified and their crystal structures determined.

Here, the result for cocrystal formation between salicylic acid and benzamide are presented. The  $\nu(\text{C}=\text{O})$  band at  $1685\text{ cm}^{-1}$  in the Raman spectrum of benzamide disappears from the spectrum of the mixture and two new peaks at  $1657\text{ cm}^{-1}$  and  $1613\text{ cm}^{-1}$  appear in the cocrystal spectrum. The strong band in the salicylic acid spectrum at  $1636\text{ cm}^{-1}$  ( $\text{C}=\text{O}_{\text{str}}$ ) disappears and a new band at  $1586\text{ cm}^{-1}$  appears in the spectrum of the cocrystal. The band in salicylic acid at  $1430\text{ cm}^{-1}$ , assigned to an  $(\text{O}-\text{H})_{\text{c.i.p.bend}}$ , also disappears in the Raman spectrum of the mixture. The peak at  $1320\text{ cm}^{-1}$  ( $\text{C}-\text{O})_{\text{c.str}}$  in the spectrum of the salicylic acid appear a triplet at  $1331\text{ cm}^{-1}$ ,  $1322\text{ cm}^{-1}$  and  $1315\text{ cm}^{-1}$  in the spectrum of the cocrystal as shown in Fig.1. This study suggests that formation of a cocrystalline phase has occurred and that salicylic acid and benzamide are not merely in physical admixture. Confirmatory evidence of the new cocrystal is provided by powder XRD patterns, which show significant differences between the diffraction patterns of the raw material and product.

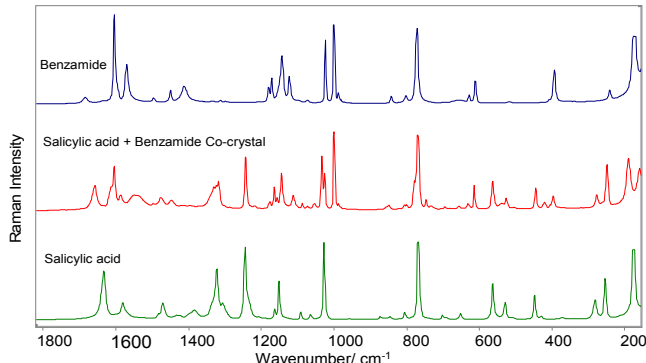


Fig-1- Raman spectra

Additionally, the melting point for the reported cocrystal determined from DSC measurements is significantly lower than that of either of the two components.

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## P126-B2

### The Triclosan Determination in Consumer Products with Visual Spectroscopy Using

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Key words: triclosan, reaction of azo coupling, spectrophotometry, hygienic objects

As one of potent wide spectrum antibacterial and antifungal agent 5-chloro-2-(2,4-dichlorophenoxy)-phenol (triclosan) is added to soaps (0.10-1.00%), deodorants, toothpastes, shaving creams, mouth washes, and cleaning supplies and is infused in an increasing number of consumer products, such as kitchen utensils, toys, bedding, socks, and trash bags. Triclosan is stable under normal condition, but on the environment, it may be degraded by microorganisms or react with sunlight forming other compounds which may include chlorophenols and dioxin [1]. Triclosan contents in household objects, waste and natural waters is regulated by both the U.S. Food and Drug Administration, the Environmental Protection Agency, and the European Union.

A number of sensitive techniques were developed for triclosan determination ([2], [3], [4]). These methods include the usage of gas or liquid chromatography and they are expensive and requiring a lot of time.

Triclosan is a chlorinated aromatic compound which has functional groups representative of both ethers and phenols. Reaction of azo coupling is used in well-known visual spectroscopic method of phenols determination. This reaction may be applicable to developing of new cheap, rapid and sensitive method of triclosan determination in consumer and natural objects.

2-aminonaftalin-4,8-disulfuric acid was decided to be used as amine, which form diazonium salt. The main advantage of this reagent is that background product of reaction dediazonation (phenol) can not form dye in reaction of azo coupling. Optimal conditions for reaction of azo coupling were found. The calibration graph was linear in the range  $5.0 \cdot 10^{-6} \text{ mol} \cdot \text{L}^{-1} - 1.0 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ . The detection limit was  $5.0 \text{ mkmol} \cdot \text{L}^{-1}$ .

Disturbing influence of matrix of hygienic objects, phenols, humic acids, macro- and micro-components of natural waters was studied. It was found that only twofold excess of phenol interfere with triclosan determination by proposed method.

The developed method was tested on toothpastes, liquids and gels for disinfection, etc.

The data obtained confirmed the usefulness of the proposed method for simple and rapid in procedure route to household objects analysis.

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## P127-B2

### Photochemical Degradation of Components in Pharmaceutical Formulations: an Approach of Degradation Studies Using Near Infrared Spectroscopy and Multivariate Control Charts

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It is well known that light can change the properties of different materials and products. Photostability has for many years been a main concern within several fields of industry, e.g., textile, paint, food, cosmetic, and agricultural, among others. In the pharmaceutical field, photostability has played a less important role. Meanwhile, the number of drugs found to be photochemically unstable is steadily increasing. The most obvious result of drug photodecomposition is a loss of potency of the product. As a consequence, this can result in a therapeutically inactive drug product. In this work, it was used multivariate control charts based on the principal component analysis- Hotelling's  $T^2$  and Q (residue) charts- and the near infrared spectroscopy to monitor the degradation of the Piroxicam under exposure to UV-VIS for different times. It was prepared 11 synthetic samples containing the active principle (Piroxicam) in the range of 20.0-40.0% (w/w) in excipient. After that, the samples were exposed to UV radiation. In each time interval, the near infrared spectra of the samples were collected. For the development of the multivariate charts, control samples not subjected to irradiation were used. Analyzing the results, all samples were inside the limits stipulated in the  $T^2$  chart using an exposure time of 54 hours. Although in the Q chart the samples 1, 3, 5, 6, 8 and 9 were out of the limit set. This condition is considered the minimum criteria to be observed drug degradation. In this way, is presented an approach to monitor the drug photodegradation based on statistical process control. This methodology is simple and easily implemented in quality assurance in the pharmaceutical industry.

## P128-B2

### A Novel Detection Approach Based on Coupled Redox-Complexation Reaction with 2,4,6-Trypyridyl-s-triazine (TPTZ) as an Chromogenic Reagent and its Application to Flow-Injection Spectrophotometric Determination of Tiopronin {N-(2-Mercaptopropionyl)-Glycine}

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A flow injection spectrophotometric procedure for *N*-(2-mercaptopropionyl)-glycine (MPG) determination is proposed. The determination is based on coupled redox-complexation reaction between MPG, Fe(III) and 2,4,6-Tripyridyl-s-triazine (TPTZ). Firstly MPG reduces Fe(III) to Fe(II) and the reduced Fe(II) is rapidly converted to the highly stable, deep-blue colored  $\text{Fe}(\text{TPTZ})_2^{2+}$ . This colored complex was monitored at 593 nm. Optimal conditions for determining MPG are achieved by univariate method. A linear calibration curve is established in a concentration range of  $4.0 \times 10^{-6}$  to  $2.0 \times 10^{-4}$  mol L<sup>-1</sup> MPG with regression equation  $y = 4704 x - 0.019$  ( $R^2 = 0.9998$ ) ( $n = 10$ ) and detection limit of  $2.35 \times 10^{-7}$ . The analytical frequency was 60 h<sup>-1</sup>. The proposed method is simple, rapid, sensitive and reproducible (RSD 0.5%,  $n = 10$ ), and can be applied for determination of MPG in pharmaceutical preparations up to nanomole quantity. Usual excipients used as additives in pharmaceuticals do not interfere with the proposed method.

Keywords: tiopronin; N-(2-Mercaptopropionyl)-Glycine; Flow-injection spectrophotometry; pharmaceuticals

## P129-B2

### Determination of D-Penicillamine in Pharmaceuticals by Indirect Atomic Absorption Spectrometry

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This work describes the development of indirect atomic absorption spectrometric method for determination of D-penicillamine (PEN) in pharmaceutical preparations. The method is based on the complexation of PEN with Ag(I) ion. The optimum conditions for complexation reaction were determined: 25 min at temperature 40°C and pH = 3.0. The unreacted Ag(I) ions from reaction solution were adsorbed on cation exchanger Amberlite IR 120 by batch process. The solution with retained complex, after required dilution in 0.2 % HNO<sub>3</sub>, was subjected to electrothermal atomic absorption spectrometer (ETAAS). The absorbance linearly increases with increasing PEN concentration in range 10-70 µg/mL ( $R^2 = 0.993$ ) with no need to use the standard addition method. Limit of detection (LOD) and limit of quantitation (LOQ) were obtained, 5.26 µg/mL and 7.19 µg/mL, respectively. The effect of resin dosage, pH, contact time and temperature on the adsorption of Ag(I) on ion exchange resin was studied. Also, graphite furnace temperature program and concentration of matrix modifier were optimized. Temperature of 800°C and 1600°C were selected for the pyrolysis and atomization steps, respectively, using a mixture of Pd and Mg(NO<sub>3</sub>)<sub>2</sub> as a matrix modifier (at concentrations of 1g/L and 0.001 g/L, respectively). After some preliminary experiments for standards and samples no interfering effects were observed from the sample matrix. The method developed was applied to the determination of PEN in commercial available pharmaceuticals with mean recovery 101.8 %.

## P130-B2

### Study of Different LC Columns and Separation Mechanisms in Metabonomics LC-MS Analysis

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Global metabolic profiling, as used in metabonomics and metabolomics, is increasingly being performed using chromatographic methods in connection to mass spectrometry detection and multivariate statistical analysis of the resulting datasets. The quality of the raw data is of great importance when multivariate statistical analysis is going to be used. The question is whether the global metabolite profiling is attainable from the chromatographic point of view using columns of different chemistry. Obviously no chromatographic method or material is capable of analysing all metabolites present in a sample as these differ greatly in terms of molecular weight, polarity, presence of active groups etc. Conventional reversed phase materials for example are incapable of retaining polar metabolites hence other alternative separation mechanisms are needed. Hence issues are remaining as to whether there is a preferable choice of chromatographic columns for targeted analysis of certain metabolite groups (a theme closer to so called "targeted metabolomics") or in general how different separation media perform in non-targeted (unbiased) metabolomics/metabonomics. In this study the performance of three different chromatographic columns was evaluated in a metabonomic study: a) Atlantis silica-based, RP C<sub>18</sub> column b) a Merck silica monolith and c) an RP-WAX column. Urine samples from 10 lean and 10 Zucker rats, along with the quality control samples were analyzed in a Waters ACQUITY UPLC system (Waters), using a Micromass Q-TOF Micro mass spectrometer (centroid +ES Mode for a range 100-800 m/z). Comparison was made on the basis of pure chromatographic criteria (such as analyte retention time, peak shape/asymmetry) but also on the basis of the number of ions that could be counted for each column as well as the quality of the separation that was seen using multivariate statistics (Simca-P). As a general conclusion we can say that all three columns can be used for a metabonomic study and that they provide different perspectives on the whole metabolome.

## P131-B2

### An NMR-based Metabonomic Exploration of Changes Induced by High-intensity Exercise in Human Urine

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Metabonomics represents a holistic, hypothesis-free, approach to the study of metabolic responses to various stimuli through powerful data acquisition and advanced data processing techniques that determine tens or hundreds of analytes simultaneously. Metabonomic data related to exercise are scarce.

Fourteen young, moderately trained, males were equally and randomly assigned to either of two exercise sessions commonly used in sprint training. Both sessions involved three sets of two 80 m sprints. The two sprints in each set were separated by either 10 s (in one session) or 1 min (in the other) of rest, and sets were separated by 20 min of rest in both sessions. Urine was collected before and 35 min after exercise and was subjected to proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy. Spectra were acquired on a 600 MHz spectrometer and were analysed using multivariate statistical methods including principal component analysis (PCA), partial least squares discriminant analysis (PLSDA), orthogonal partial least squares (OPLS), and principal component variable grouping (PCVG). In both PCA and PLSDA score plots, clear-cut grouping of the samples was afforded according to time (pre- vs. post-exercise) and rest interval (10 s vs. 1 min). Loading plots and further statistical treatment assigned the differences to lactate, compounds of the Krebs cycle (citrate, succinate), amino acids (alanine, glycine, histidine, phenylalanine, threonine), products of branched-chain amino acid catabolism (oxoisocaproate, isovalerate), short-chain carboxylic acids (butyrate, propionate, formate), 3-hydroxybutyrate (a ketone body), hippurate, and dimethylamine. The present study illustrates the utility of holistic analytical methods (rather than targeted analysis of a small number of analytes) in the study of exercise metabolism. Samples differing in as little as the rest interval between repeated sprints can be classified and predicted. In this way, important biomolecules involved in exercise biochemistry can be identified and further studied. The fact that such methodology can be applied to biological material obtained non-invasively (urine) is an added value.



## **P132-B2**

### **New Advances in Glycoprotein Analysis Addressing Structural Investigation of Complex Glycans by Capillary Gel Electrophoresis**

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Glycosylation is one of the most thoroughly studied co- and post-translational modifications of proteins. Investigation of the human serum glycoproteome, especially focusing on glycosylation changes due to various diseases, is of great recent interest, with particular respect to biomarker discovery. This presentation gives a thorough evaluation of various sample preparation methods for multicapillary gel electrophoresis based glycan analysis to support electrokinetic injection. First the removal of excess derivatization reagent is discussed. While the Sephadex G10 filled multiscreen 96 well filter plate and Sephadex G10 filled pipette tips enabled increased analysis sensitivity, polyamide DPA-6S pipette tips provided particularly good performance. In this latter case an automated liquid handling system was used to increase purification throughput, necessary to feed the multicapillary electrophoresis unit. Problems associated with the high glucose content of such biological samples as normal human serum were solved by ultrafiltration. Selective glycoprotein enrichment is a crucial issue in the analysis of complex biological samples. We also demonstrate the efficiency of boronic acid - lectin affinity chromatography to address this challenge. Finally, a volatile buffer system was developed for exoglycosidase digestion based structural analysis of carbohydrates.

## P133-B2

### Phosphopeptide Enrichment by Magnetic Beads with Radiate Microstructure Chip for MALDI-MS Analysis

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This paper is reported about a new method for phosphopeptide sample isolation and concentration using the micro scale radiate structure chip applied to matrix assisted laser desorption/ionization mass (MALDI-MS). Magnetic beads were used for phosphopeptides isolation and were enriched by external magnetic field, and finally concentrated within our chip. While mass spectrometry is nowadays an important tool for analyzing and characterizing large biomolecules of varying complexity, sample preparation has become a key point within the whole experiment.

Phosphorylated proteins play a critical role in regulating biological functions, especially in post translational modifications. Generally, phosphoproteins were characterized by MALDI-MS after enzyme digest, but the signals of the phosphopeptides are always suppressed by the presence of other non-phosphopeptides residues. Affinity-based methods such as immobilized metal ion affinity chromatography (IMAC) and metal oxide affinity chromatography (MOAC), are the most often used principles to enrich phosphopeptides from complex samples before MALDI-MS analysis. On-plate enrichment of phosphopeptide digests followed by MALDI-MS is more attractive for analyzing small quantities of phosphoproteins because of minimal sample handling and diminishing sample loss.

Here we demonstrated another simple magnetic method to concentrate phosphopeptides before MALDI-MS analysis by using the micro scale radiate structure chip. This silicon-based chip was manufactured by photolithography, which then serves as the MALDI sample plate. Samples applied around the chip could auto-orientate to the center and deposit on the central zone. In briefly, digested sample and magnetic beads mixture were applied to the chip, then all the binding and washing processes were performed on the chip. After that, phosphopeptides were re-suspended with DHB matrix and then concentrated within the central zone of the chip after dried. While all the experiment processes were operated on the chip, it would simplify the sample handling, eliminate the time consuming and sample loss. When the sample was analyzed by MALDI-MS, the phosphopeptide signals were significantly enhanced by concentrated in our chip compared to the original MALDI steel plate.

## P134-B2

### A Proteomic Identification Chip with Protein Digestion and Maldi-TOF MS

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We construct a droplet chip system with auto-positioning and enriching the sample for proteomic identification. Utilizing the droplet chip system, protein sample would be digested to smaller peptides on chips and directly analyzed with matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Comparing to the traditional methods, the proteomic identification chip has four advantages. The first is the shortening reaction and analysis time (total ~2.5 hours); 2. auto-positioning and enriching the sample concentration; 3. low volume reaction (1~5  $\mu$ L); 4. whole process sequentially on the chip. The proteomic identification includes three processes; i.e. the droplet chip making, protein digesting, and results analysis (MALDI-TOF MS). According to the view of the surface-structure of the proteomic identification chip, we observed that the reaction solution stood on the chip surface and the digestion products concentrated in the chip center during air-drying. And the dried powder of digestion products and CHCA matrix would congregate inside of the microstructure ring. The special design could assist the detection sensitivity of MALDI-TOF MS and enhance MS spectra signal. The molecular weight of insulin B chain, oxidized was 3493.65 Dalton and it would be digested into two polypeptides (858 Da. and 2585 Da.) with trypsin. The results of MALDI-TOF MS spectra of insulin B chain, oxidized digested on the droplet chip confirmed that insulin B chain, oxidized could be digested and detected on the proteomic identification chip. Thus, it would be convenient to identify and analyze the interesting protein by using the droplet chips

## **P135-B2**

### **An Investigation of Mass Spectrometry as a Specialist Tool to Enhance the Reach of Stability Indicating Methods for Active Pharmaceutical Ingredients with Poor UV Chromophores**

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Mass Spectrometry (MS) instrumentation is routinely used early on in Pharmaceutical R&D for the development of Stability Indicating (SI) methods. At this stage, Time-of-Flight (TOF) or single quad analysers are the tools of choice for establishing LC method selectivity e.g. impurity peak counting using orthogonal screens and molecular ion identification (m/z). A vast majority of SI methods for drug substance (DS) are then validated on a LC/UV or UPLC/UV platform. The SI method often becomes a one-size-fit-all solution for multiple applications including main band assay, stability, impurity quantification and often drug product (DP) potency as well. The objective of this poster is to explore the benefits of another model where the main SI chromatographic method is complemented by a suite of MS and MS/MS-based experiments finely-tuned for a particular application.

An example of a triple quad analyser used as an alternative detector for SI method validation of compounds with poor UV chromophores is discussed in this poster. In this particular case, the use of Corona Charged Aerosol Discharge Detector (cCAD) as a primary detector was pivotal to define stability and impurity profile for the active pharmaceutical ingredient (API). Once a high level of confidence was built around understanding API synthetic route and degradation pathways, using MS as a secondary detector, significantly accelerated specific applications such as specified impurities control and assay validation.

## P136-B2

### Resonance Rayleigh Scattering Method for the Assay of Streptomycin Using Ion Pair Formation with Congo Red

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In pharmaceutical analysis resonance Rayleigh scattering method has become known due to its simplicity, selectivity, sensibility and low costs.

The paper presents a resonance Rayleigh scattering (RRS) method for the assay of streptomycin based on the ion pair formation.

We studied the ability of the aminoglycoside antibiotic streptomycin to form ion pairs with sulfonated azo dyes. The preliminary results showed that Congo red is the appropriate reagent for this purpose. The spectral characteristics of the 1:1 ion pair formed between streptomycin and Congo red in acidic media were investigated by UV-Vis spectrometry and RRS. Resonance Rayleigh scattering spectra were recorded on a classic spectrofluorimeter. The excitation and emission monochromator wavelengths were coupled and adjusted to scan simultaneously through the range 200 to 600 nm. In acidic media the individual reagents streptomycin and Congo red produce very weak RRS signals. When the two agents react with each other to form ionic association complex a new RRS spectrum was obtained. A significant enhancement of the RRS intensity in the studied wavelength range has been observed. The maximum scattering peak was located at the wavelength 384 nm. The optimum reaction conditions were established. Thus the maximum intensity of RRS signal was obtained at pH 5,5 created with Britton-Robinson buffer and using a Congo red concentration of  $2 \cdot 10^{-8}$  M. The reaction time is 30 minutes. The linear relationship between the RRS intensity and the antibiotic concentration was found in the concentration range  $6.49 - 9.74 \mu\text{g} \cdot \text{mL}^{-1}$ .

Based on the formation of the ionic association complex between streptomycin and Congo red using resonance Rayleigh scattering we developed a sensitive, simple and rapid method for the assay of streptomycin. The method was successfully applied to the determination of streptomycin in pharmaceutical formulations.

## P137-B2

### **Analytical Characterization of Complex Combinations of Some Basic Drugs with Dodecaphosphowolframic Acid**

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The secondary aminic group and the pyrrolidinic nucleus confer the basic character of two studied drugs, timolol and clemastine.

Intrinsic basic function determined their tendency to form protonated cations. The analytical study performed reflected the capacity of the monoprotonated cations to form combinations with voluminous complex anions by ionic association, very slightly soluble in water.

The dodecaphosphowolframic acid was the reagent used. The low solubility complex combinations were prepared and their structures were confirmed from physical and physico-chemical viewpoint by IR spectra analysis and thermo gravimetric analysis coupled with differential calorimetric analysis. IR spectra shows important changes in the characteristic bands of functional groups which are involved in the complexation process,

Caloric effects were determined on the basis of the data supplied by thermal analysis. Thus the conditions for processing the complex combinations are established in order to further assay of drugs. The obtained data through thermal and IR spectra analysis were corroborated. The formulae mass and the solubility of the examined ion pairs were determined. Their structure formula was also established.

## P138-B2

### Flow Injection Amperometric Determination of Spiperone Using a Glassy Carbon Electrode

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Spiperone (8-[4-(4-fluorophenyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one) belongs to butyrophenone derivative group. The butyrophenones are a class of drugs employed in the treatment of psychiatric diseases [1]. A simple, sensitive flow injection method was developed for spiperone determination on a glassy carbon (GC) electrode at the working potential of +0.8 V versus Ag/AgCl reference electrode (3M NaCl). Cyclic voltammetry and amperometric measurements were performed on a electrochemical workstation CHI760 (CHI Instruments, USA). CHI 130 thin-layer flow cell was used, equipped with GC electrode as working electrode, Ag/AgCl as reference electrode and a stainless steel counter electrode, which also served as the outlet or inlet for the solution phase. A 0.5 mm thick silicon rubber gasket was used as a spacer in the cell. Injection valve volume was 100  $\mu$ L. Tris-HCl buffer at pH 7.30 at concentration of 0.1 mol/L acted as a sample carrier at a flow rate of 2.8 mL/min and supporting-electrolyte. All measurements were done at room temperature. Hydrodynamic voltammogram was also recorded. It showed that the highest current intensity was at +0.8 V which was chosen for further experiments. Cyclic voltammograms of  $1.0 \times 10^{-5}$  M spiperone were recorded to confirm results obtained by hydrodynamic voltammogram and to understand the mechanism of the reaction. Other parameters investigated were: length of mixing coils, flow rates, gasket thickness, effect of pH and sample volume. Method was proposed and successfully applied for the determination of spiperone in tablets with mean recovery and RSD of 100.35 and 0.49%, respectively.

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## P139-B2

### Simultaneous Electrochemical Determination of Paracetamol and Propranolol Using Modified Multi-Wall Carbon Nanotube Paste Electrode and Electrochemical Properties of a Nanogold Modified Carbon Paste Electrode for Atenolol Determination in Pharmaceutical Formulations

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Recent electrochemical studies have shown that carbon nanotubes (CNTs) and gold nanoparticles are capable to minimize electrode surface-fouling, and to enhance electrocatalytic activity [1,2]. A carbon paste electrode modified with 1,1-[1,2-ethanediy] bis (nitrido methylidyne)]-bis-2-naphtol and CNT was used for the simultaneous determination of paracetamol and propranolol. The study was carried out using CV, DPV and chronoamperometry techniques. Some kinetic parameters such as the electron transfer coefficient ( $\alpha$ ) and heterogeneous rate constant ( $K_s$ ) were also determined for the paracetamol oxidation. A dynamic range of  $8.0 \times 10^{-7}$  to  $1.0 \times 10^{-3}$  M, with the detection limit of  $4.0 \times 10^{-8}$  M for paracetamol was obtained using DPV ( pH=8 ). Also a gold nanoparticles modified carbon paste electrode (GN-CPE) has been used for the determination of atenolol (ATN) in drug formulations and urine. Results revealed that the modified electrode shows an electrocatalytic activity toward the anodic oxidation of atenolol by a marked enhancement in the current response in buffered solution at pH 10.0. A linear analytical curve was observed in the range of  $1.96 \times 10^{-6}$  to  $9.09 \times 10^{-4}$  M. The detection limit for this method was  $7.3 \times 10^{-8}$  M. The methods were then successfully applied to the determination of drugs in tablets and humans urine. The percent recoveries in urine ranged from 92.0 to 110.0%.

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## **P140-B2**

### **Azithromycin Impurities in Oral Solution: Comparison of High Performance (HPLC) to Ultra Performance (UPLC) Liquid Chromatography Techniques**

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The new technologies offer possibilities for development of analytical methods with far more efficient resolving characteristics. Main goal of Quality Control analyst is to develop a robust method maintaining the quality of obtained data: resolution, method accuracy, efficiency. Ultra Performance Liquid Chromatography (UPLC) offers a new concept of faster liquid chromatography. In this work, HPLC method for determination of Azithromycin impurities was transferred to UPLC, and subsequently validated. The critical points of this HPLC method were: long gradient run of 95 minutes, resolving and quantification of 12 known impurities, and very poor stability of samples. The HPLC method was used as a starting point for transfer to UPLC. Mobile phase and sample preparation remained the same, but UPLC BEH C18 column with sub-2micron particles was used instead of XTerra C18 MS. In order to reduce the run duration and optimize method parameters (temperature, flow rate, gradient scaling) for good peak resolution, numerous runs were performed. After method optimization, it was necessary to perform the full UPLC method validation in order to confirm that the new method is suitable for use in quantification of impurities in Azithromycin oral solution. The validation demonstrate that new UPLC method is specific, linear, precise and accurate over the working range of the method, with run 3 times shorter than using HPLC technique.

## P141-B2

### Determination of Pyrrolizidine Alkaloids in Comfrey by Liquid Chromatography- Electrospray Ionization Mass Spectrometry

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*Symphytum officinale* L. (comfrey) is a medicinal plant commonly used in decoctions and aliments. Besides therapeutic bioactive compounds present in the herb, it is found to contain hepatotoxic Pyrrolizidine Alkaloids (PAs), such as lycopsamine and others. In the present study, PAs such as lycopsamine, echimidine and lasiocarpine were separated using electrospray LC-MS with the method precision (RSD) <10%. Detection of lycopsamine, symviridine and their *N*-oxides could be confirmed with a newly elaborated HPLC ion-trap and orbitrap MS with electrospray ionization interface approach. With LC-MS, quantitative analysis of lycopsamine in the botanical extract was carried out. The effect of extraction solvent was optimized by sonication and methanol: H<sub>2</sub>O (50:50) was selected. Then a rapid method pressurized hot water extraction (PHWE) was employed for the extraction of lycopsamine from comfrey followed by the comparison with heating under reflux with the method precision (RSD) varied from 2.49% to 19.32% respectively. Our results showed a higher extraction efficiency with heating under reflux. It was proposed that the lower extraction efficiency with PHWE was associated with the dissolved nitrogen from air which caused the reduction in the solubility of lycopsamine in the compressed hot solvent. Though the aim of PAs quantitative analysis was achieved, the different phenomenon on extraction efficiency of PHWE compared to our earlier work demonstrated that the use of subcritical water for extractions depended on the physical properties of the dissolved solutes and their tendency to degrade under the chosen extraction conditions.

## P142-B2

### **Electroanalytical Behavior of Rosiglitazone and its Square-Wave Voltammetric Determination in Pharmaceutical Formulations**

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Rosiglitazone is a potent antihyperglycemic agent that reduces insulin resistance in patients with type 2 diabetes. In this study, the electrochemical reduction of rosiglitazone was investigated using cyclic and square-wave voltammetry at a hanging mercury drop electrode. Rosiglitazone showed a cathodic peak in the pH range from 2.0 to 9.0. The peak potentials were shifted to more negative values with increasing pH, implying the involvement of protons in the electrode reaction. Cyclic voltammetric measurements showed an irreversible and diffusion controlled nature of the reduction process. The possible reduction mechanism was also discussed. Based on the reduction peak at about -0.70 V versus Ag/AgCl, in a Britton Robinson buffer of pH 2.0, a square-wave voltammetric method was developed for the determination of rosiglitazone. The repeatability, reproducibility, selectivity, precision and accuracy of the proposed method were investigated and calculated. The procedure was successfully applied to the determination of rosiglitazone in pharmaceutical formulations. The results were compared with those obtained by a published spectrophotometric method. No difference was found statistically.

## P143-B2

### Development of t-ITP-CZE Method for the Analysis of Phenolic Compounds in Plant Extract of *Epilobium Parviflorum*

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*Epilobium Parviflorum* is commonly used in traditional herbal therapy of benignus prostate hyperplasia (BPH). The main components responsible for pharmacological effects are phenolic compounds. Some of them are derivatives of caffeic acid and flavonoids. In this study a transient isotachopheresis-capillary zone electrophoresis (tITP-CZE) method with UV detection at 214nm was developed and validated for pre-concentration and determination of 7 phenolic acids. The effects of several factors such as control of EOF, pH and concentration of the running buffer, addition of organic solvents and their concentrations, addition of cyclodextrins and conditions of injection were investigated to find the optimum conditions. The optimal operational CZE system consisted of 200 mM boric acid adjusted to pH 9.2 with addition of NH<sub>3</sub>, with 35 % (V/V) content of methanol. Concentration of transient leader Cl<sup>-</sup> and volume of injected sample was also tested with optimal concentration 200mM of Cl<sup>-</sup> and injection 30% of total capillary volume. Under the conditions, the analytes were separated within 15 min. Linearity was evaluated for concentration range 0.1-10µg/ml with R = 0.9924-0.9983; the detection limits (S/N 3:1) ranged from 23ng/ml to 56µg/ml. The relative standard deviations of the migration times (peak areas) were between 0.79 and 1.01% (1.34 and 2.13%). After validation, the developed method was used for analysis of methanolic extract of *Epilobium parviflorum*. The method should be considered suitable for identification and quantitative evaluation of crude drug.

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## P144-B2

### Volatile Composition and Antioxidant Capacity of Medicinal Plants: *Ruta Chalepensis*, *Thymus Vulgaris* and *Rosmarinus Officinalis*

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It is well known that several plants, especially those belonging to the Rutaceae and Lamiaceae families, possess a wide range of biological activity, particularly antibacterial, antitumoral, antifungal, insecticidal, cytostatic, therapeutic and antioxidant properties. Their volatile oils have potential uses in medical procedures and are used extensively to add a distinctive aroma and flavour to food, pharmaceuticals and cosmetics.

Volatile compounds in macerate extracts of *Ruta chalepensis* (ruta), *Thymus vulgaris* (thyme) and *Rosmarinus officinalis* (rosemary) plants, which belong to Lamiaceae and Rutaceae families, respectively were determined by dynamic headspace solid-phase microextraction (HS-SPME) methodology followed by gas chromatography–mass spectrometry (GC-qMS). The effects of some parameters in the extraction efficiency, including fibre coatings, extraction temperature and exposure time were evaluated. The highest recovery, based on the total peak areas, was achieved using a divinylbenzene-carboxen-poly(dimethylsiloxane) coating during 60 min at 40 °C under constant stirring (800 rpm), after saturating the samples with sodium chloride (NaCl 10 % w/v). The identified analytes included several terpenoids, carbonyl compounds, alcohols and esters. Thymol (65.7 %) followed by octan-3-one (8.5 %) were found the most abundant components detected in *Thymus vulgaris*. In *Ruta chalepensis* undecan-2-one (53.4 %) and non-1-ene (28.2 %) were found as the predominant volatiles, while in *Rosmarinus officinalis*, the main constituents were eucalyptol (40.1 %) and decan-2-one (20.3 %), respectively. The total phenolic contents of these plants were measured by the Folin-Ciocalteu method. The antioxidant activity was evaluated using different *in vitro* assays, ABTS\* and DDPH\* radicals. Among the studied plants, *Ruta chalepensis* resulted in the more active within all used methods.

## P145-B2

### Identification of the Phospholipase A<sub>2</sub> Component from Snake (*Bungarus Sindanus Sindanus*) Venom Using MALDI-TOF Mass Spectrometry

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Phospholipase A<sub>2</sub> (PLA<sub>2</sub>; EC 3.1.1.4) is a multifunctional enzyme involved in many cellular processes. Snake venom is a rich source of PLA<sub>2</sub> enzymes together with other functionally diverse proteins and peptides. The manifestation of envenomation is the collective as well as synergistic action of these structurally similar but functionally diverse components, leading to neurotoxic, cardiotoxic, haemorrhagic, myonecrotic, anticoagulant, convulsant, hypotensive and/or edema-inducing effects. Majority of envenomation cases in Pakistan (>20 000 bites per year) are caused by the highly neurotoxic venoms of Krait (*Bungarus* species) and Cobra (*Naja* species.) snakes. Phospholipase A<sub>2</sub> constitutes a major component of Krait venom. Functional diversity within this group of structurally similar proteins raises interest in the study of the snake venom proteins and peptides for understanding their molecular basis of toxicity, regulation and designing of drugs to modulate the pharmacological activity. In the present study, the phospholipase A<sub>2</sub> component was separated from the whole venom of the snake by activity guided separation on size exclusion chromatography using the synthetic substrate 4-nitro-3-(octanoyloxy) benzoic acid. Further separation on reversed-phase high pressure liquid chromatography (RP-HPLC) revealed more than five peaks with masses around 13 kDa together with ~26 kDa and ~21 kDa masses suggesting presence of homo or heterodimers on MALDI-TOF mass spectrometer. This homo or hetero dimeric nature of the proteins was confirmed by rechromatography on RP-HPLC in the presences of reducing agent, as well as with SDS PAGE gel electrophoresis. The purified proteins and peptides were digested with trypsin in solution for identification with MALDI-MS/MS, LC-MS/MS and data bank search and could be characterized for further molecular studies in order to understand the mechanism of biological effects.

## P146-B2

### New Dioxin Derivatives from the Aerial Parts of *Lawsonia Alba*

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*Lawsonia alba* Lam (synonym: *Lawsonia inermis* L.) belongs to the family Lythraceae, commonly known as Henna. This acrid and bitter plant is claimed as a refrigerant, an expectorant, an anti-inflammatory, a liver tonic, an antipyretic, an antiperspirant and a sedative as well as diuretic, emetic and haematic. Almost every part of the plant has been used in traditional system of medicine for the treatment of various ailments. About 1.0 % of 2-hydroxy-1, 4-naphthoquinone (lawsone) is present in the leaves which is responsible for dyeing [1-3]. Aerial parts collected were dried and extracted with dichloromethane at ambient temperature. The extract obtained under reduced pressure was subjected to separation through various chromatographic techniques. The structures of two new and six known constituents were elucidated with the help of <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, 2D-NMR spectral studies including COSY, NOESY, J-resolved, HMQC, HMBC and chemical transformations the name of new compounds are 1',4'-dioxan-2'-dodecanoic acid-5',6'-dioxo-(E)-8-undecenyl ester (1), and 5-[(2'E, 5'E)- 2', 5'- decosadienyl] -1, 4-dioxan- 2, 3-dione (2). Additional pure compounds obtained are under investigation for their chemical structures and biological activities [4].

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## P147-B2

### Study on Interaction between 4-Aminopyridine and Methacrylic Acid Using Two Dimensional FTIR Spectroscopy

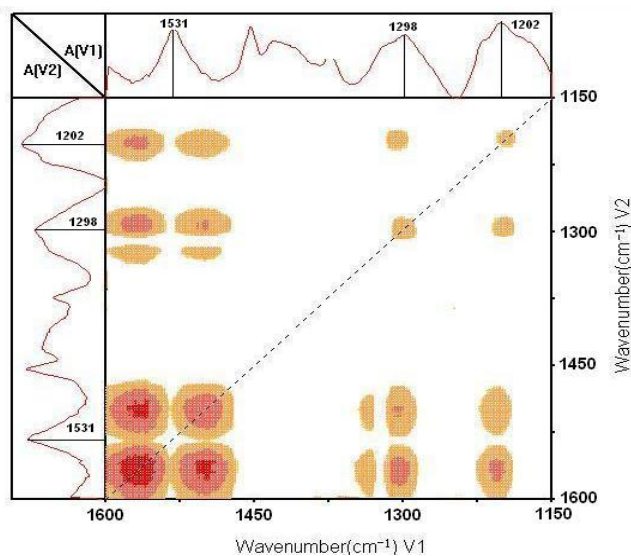
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A two-dimensional infrared (2D IR) correlation spectroscopy is an analytical technique based on time-resolution, which was proposed first by Noda<sup>1</sup>. During the spectroscopic measurement of samples, an additional external perturbation is applied to the system. This external perturbation stimulates the system to cause some selective changes in the state, order, surroundings, of system constituents. The overall response of the stimulated system to the applied external perturbation leads to distinctive changes in the measured spectrum, and a series of perturbation-induced dynamic spectra are collected. Such a set of dynamic spectra is then transformed into a set of 2D correlation spectra by cross-correlation analysis<sup>2-5</sup>.

A two-dimensional infrared (2D IR) correlation spectroscopy technique has been introduced to determine the intermolecular interaction between 4-aminopyridine (APY) and methacrylic acid (MAA). In this paper, temperature was chosen as external perturbation and a series of perturbation-induced dynamic spectra are collected and analyzed with 2D correlation. The synchronous cross peaks exist between stretching vibration of hydroxyl group of MAA at 1298 cm<sup>-1</sup> and 1202 cm<sup>-1</sup> and C-N group of APY at 1531 cm<sup>-1</sup>, between carbonyl group of MAA at 1705 cm<sup>-1</sup> and N-H group of APY at 3382 cm<sup>-1</sup> and 3212 cm<sup>-1</sup>, respectively. According to two-dimensional correlation rules, the interactions of static electricity and hydrogen bonding exist between APY and MAA molecule. Such results were verified by NMR. The successful applications demonstrate that two-dimensional infrared correlation spectroscopy may be a convenient and effective means in the study of the intermolecular and intramolecular interaction.



**Fig.1 Synchronous 2D IR correlation spectra of methacrylic acid—4-aminopyridine in 1600-1150cm<sup>-1</sup> region**

#### Acknowledgment

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## P148-B2

### Raman Spectroscopic Studies of Mixtures of Salicylic Acid and Benzamide in an Aqueous Solution as a Function of pH

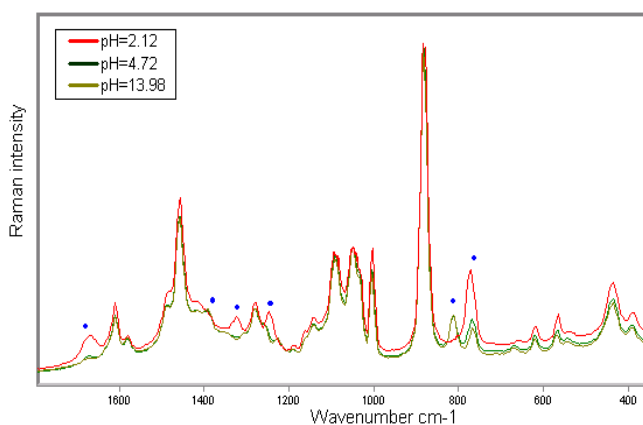
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Molecular speciation of organic compounds in solution is essential for understanding the ionic complexation of pharmaceutically relevant compounds for which the advantage of data collection in aqueous solutions by Raman spectroscopy has been demonstrated.<sup>(1)</sup> The aim of this study was to propose an analytical strategy for the *in-situ* monitoring of organic species in solution.

Figure.1. Raman spectra of salicylic acid and benzamide at different pHs.



Salicylic acid has a  $pK_1$  of 2.8 and  $pK_2$  of 13.4, whereas benzamide has a  $pK_a$  of 13-14.<sup>(2, 3)</sup> In the pH range 2.12 to 4.11 the Raman spectra show a broad band at  $1686\text{ cm}^{-1}$  [ $\nu(\text{C}=\text{O})_{\text{trans}}$ ] which becomes weaker in intensity with increasing pH. The peak at  $1322\text{ cm}^{-1}$ , assigned to  $\nu(\text{C}-\text{OH})$  disappears at pH 4.11. At pH 2.12 a shoulder is observed at  $1415\text{ cm}^{-1}$ ; this shoulder becomes weaker with increasing pH and a new band appears at  $1389\text{ cm}^{-1}$  assigned to [ $\nu_{14} + \nu_s(\text{COO}^-) + \delta(\text{C}_\phi-\text{OH}) + \nu(\text{C}_\phi-\text{OH})$ ] at pH 4.11. The peak at  $1245\text{ cm}^{-1}$  [ $\nu(\text{C}_\phi-\text{OH}) + \nu(\text{C}_\phi-\text{COOH})$ ] disappears at pH 4.11 and at the same time a new doublet appears at  $1258$  and  $1226\text{ cm}^{-1}$ . A new band is

observed at  $1031\text{ cm}^{-1}$  which does not appear in either the spectra of salicylic acid solution or benzamide solutions alone.

In the pH range 3.24 - 4.11, the spectra in the range  $770 - 820\text{ cm}^{-1}$  show two bands strongly dependent on the pH, at  $814\text{ cm}^{-1}$  ( $\nu(\text{C}_\phi-\text{COO}^-) + \delta(\text{C}_\phi-\text{OH}) + \delta_s(\text{COO}^-)$ ) for the salicylate monoanion ( $\text{Hsal}^-$ ), and at  $773\text{ cm}^{-1}$  ( $\nu(\text{C}_\phi-\text{COOH}) + \nu(\text{C}_\phi-\text{OH}) + \delta(\text{COOH})$ ). The peak at  $814\text{ cm}^{-1}$  first appears as weak peak at pH 3.03 and becomes of medium intensity with increase in pH; in contrast, the peak at  $773\text{ cm}^{-1}$  becomes weaker with increasing pH.

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## P149-B2

### Raman Spectroscopic Analysis of Minerals and PAHs of Relevance to Astrobiology

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Characteristic geological features and hydrated minerals recently found on the surface of Mars by the NASA planetary rovers Spirit and Opportunity suggest that a possible biosphere could have once existed there. Analytical instrumentation protocols for the unequivocal detection of biomarkers in suitable geological matrices are critical for future unmanned explorations, including the forthcoming ESA ExoMars mission scheduled for 2016.

Raman spectroscopy is currently a part of the Pasteur instrumentation suite of the Exomars mission for the remote detection of extant or extinct life signatures in the Martian surface and subsurface. Terrestrial analogues of Martian sites have been identified and the biogeological modifications incurred as a result of extremophilic survival activity have been studied. In this work, various concentrations of polyaromatic hydrocarbons in matrices of gypsum and quartz have been investigated by Raman microspectrometry to determine the lowest detectable levels of naphthalene, phenanthrene, triphenylene and perylene in quartz and gypsum under geobiological conditions in simulation of potential identification in Martian scenarios. Two laser source wavelengths, namely, 785 and 633 nm, were adopted to excite Raman spectra from the PAHs which represent degraded carbons and therefore potentially a key bimolecular Marker of ancient life.

The mixtures were ground and homogenized in an agate mortar. Powders of eight different concentrations of PAHs were prepared representing 0.1, 0.25, 0.50, 1, 2, 5, 10 and 25 mg kg<sup>-1</sup>. Spectra of the powdered mixtures were obtained directly at the surface and confocally subsurface through the crystal matrix. The number of observed PAHs Raman bands differed depending on the particular mineral matrix, the excitation wavelength and the concentration deployed. Estimates of the relative molecular scattering factors of the PAH components were evaluated, which informed the capability of detection of the PAH using Raman spectroscopy on the surface of Mars.

## P150-B2

### The Effect of Spectral Resolution on the Raman Spectra of Polyaromatic Hydrocarbons and Beta-Carotene Mixtures

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Raman spectroscopy has been proposed as part of an instrumentation suite for the remote detection of materials using robotic landers for planetary surface exploration<sup>1</sup> (ESA, ExoMars mission 2016) because of the ability to identify key organic biomaterials in their host mineralogical matrices as demonstrated for extremophiles in terrestrial niche scenarios. A fundamental process in the remote detection of extant or extinct life biomolecules using Raman spectroscopy is the detection of the changes in the band signatures<sup>2</sup> accompanied by molecular interactions in mixtures. In this work, three mixtures of polyaromatic hydrocarbons and beta-carotene, two groups of key biomaterials identified for detection on Mars, were analysed using laboratory instrumentation operating with 1064 nm laser excitation. The laser power was set at about 10  $\mu\text{W}$  and the samples run for co-accumulations of 500 scans with spectral resolutions of 4, 6, 8, 16, 32  $\text{cm}^{-1}$ . Initial experiments indicated that the molecular scattering factors for the Carotenoids, were about 20:1 compared with the PAHs, hence the three mixtures selected were of beta-carotene 5-95 naphthalene, beta-carotene 5-95 anthracene and beta-carotene 5-95 pyrene composition.

While no significant changes were observed between the FT-Raman spectra of polyaromatic hydrocarbons in beta-carotene collected at 4 and 6  $\text{cm}^{-1}$  spectral resolutions, the spectra collected at 8, 16, and 32  $\text{cm}^{-1}$  spectral resolution have a significantly different spectral appearance. The bands of the spectra collected at 8  $\text{cm}^{-1}$  are well-resolved whereas those collected at 16 and 32  $\text{cm}^{-1}$  have some overlapping features which could compromise the spectral identification of the biochemical composition.

These results will provide useful information to build up a database of simulated systems which can be used for the remote detection of possible life signatures in planetary exploration arising from PAHs and other biomolecules in crystalline mineral matrices.

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## P151-B2

### High Resolution PTR-TOF: A New On-Line Instrument for Organic Compound Measurements

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Over the last decade proton transfer reaction mass spectrometry (PTR-MS) has become very popular in many scientific fields. PTR-MS allows for the quantitative detection of volatile organic compounds (VOCs) at pptv level virtually in real time. Monitoring of VOCs with a time resolution of typically a second per compound has, for instance, enabled the tracking of pollution plumes by air-borne measurements, thus revealing the photo-chemical fate of pollutants. This rapidity, however, has been achieved at the cost of the number of compounds to be analyzed and compound selectivity. Conventional PTR-MS can, for example, not distinguish between isobaric species, e.g. between glyoxal and acetone. The use of a time of flight (TOF) instead of a quadrupole mass analyzer in PTR-MS provides a sufficient high mass resolution to identify the atomic composition of product ions by their exact mass and their characteristic isotope patterns. In addition PTR-TOF can record full mass spectra within a fraction of a second which is a dramatic increase in duty cycle. At the University of Innsbruck a field portable high resolution PTR-TOF has recently been developed, coupling a PTR ion source and a high resolution TOF. We achieved a mass resolving power of 6000 (FWHM), and a detection limit of tens to a few hundreds of pptv if integrating mass spectra for one minute. First results from field and laboratory experiments will be presented.

#### **Acknowledgments:**

The TOF-MS system was funded by the University of Innsbruck (Uni Infrastruktur II). The PTR-TOF was developed in collaboration with Ionicon Analytik GmbH and with assistance from TOFWERK AG. The development project is financially supported by the Austrian Research Funding Association (FFG; project 810074).

## P152-B2

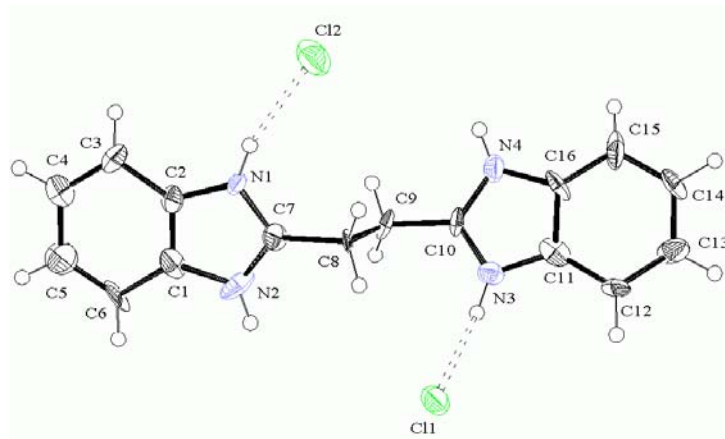
### Spectral Characterization and Crystal Structure of 1,2-bis-(1*H*-Benzimidazol-2-yl)-Ethane Dihydrochloride

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Bis-benzimidazoles give metallic or non-metallic thermal stable polymer structures [1,2]. Also, they have received much attention for their wide-ranging biological activity [3,4] and their importance in selective ion-exchange resins [5].

In this study, 1,2-bis-(1*H*-benzimidazol-2-yl)-ethane dihydrochloride (**1**) has been synthesized from 2:1 molar ratio of the *o*-phenylenediamine and the succinic acid in 5.5 N HCl. The crystal structure of **1** was determined by X-ray diffraction at room temperature (Fig. 1). The structure of **1** also was characterized by mass, elemental analysis, FT-IR and NMR techniques. The title compound crystallizes in triclinic system, and space group P-1,  $a = 7.1350$ ,  $b = 9.6299(1)$ ,  $c = 15.3340(7)$  Å,  $\alpha = 80.67(2)$ ,  $\beta = 79.66(2)$ ,  $\gamma = 68.395(11)^\circ$ ,  $V = 958.33(10)$  Å<sup>3</sup>,  $Z = 2$ . Owing to the *anti* conformations of –CH<sub>2</sub>– groups, the entire molecule is relatively flat. <sup>1</sup>H-NMR spectra of **1** shows AA'XX' system characteristic.



**Fig. 1.** The molecular structure of compound **1**. Displacement ellipsoids are plotted at the 50% probability level. Hydrogen bonds are shown as dashed lines.

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## P153-B2

### Determination of Th, U, Pb in the Accessory Minerals by Electron Probe X-Ray Microanalysis

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The contents of Th, U and Pb in accessory minerals (monazite, zircon, xenotime etc) vary from ppb, ppm to 12-15% (Th in monazite). Pb is generated consequently radioactive decay of Th and U. They content are usually from ppb to 1%. Th, U, Pb has inhomogeneous distribution in accessory minerals. The contents and distributions Th, U, Pb in minerals are required to study time and thermodynamically conditions of minerals and rocks formation.

The aim of this work was development of techniques local determinations of Th, U, Pb in accessory minerals by electron probe microanalysis (EPMA).

In this work present 2 technique determinations of Th, U, Pb in monazites and zircons. The determination was carried out by scanning electron microscope (Tescan Vega II XMU) with energy dispersive (INCAx-sight) and wave (INCA wave 700) X-ray spectrometers.

The conditions of determination Th, U, Pb in monazite was acceleration voltage 15 kV, absorption electron current 150 - 200 nA on Faraday cup. Analytical line was ThM $\alpha$ , UM $\beta$ , PbM $\alpha$ .

Determination limit for Pb is 0.06 % (weight percent), for Th, U - 0.08 % (weight percent), relative standard deviation is founded in region from 1 to 4% subject to content of determination element.

For determination Th, U, Pb in zircon was used acceleration voltage 25 kV, absorption electron current 300 nA on Faraday cup. Analytical line was ThM $\alpha$ , UM $\beta$ , PbM $\beta$ .

Determination limit for Pb is 0.008 % (weight percent), for Th, U - 0.01 % (weight percent), relative standard deviation is founded in region from 1 to 6% subject to content of determination element.

As standards for determinations of Th, U, Pb was used ThO<sub>2</sub>, UO<sub>2</sub>, PbTe respectively.

## P154-B2

### Chemically Modified Porous Silicon for Laser Desorption/Ionization Mass Spectrometry

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Laser desorption/ionization mass spectrometry (LDI MS) is one of the most popular soft ionization techniques in mass spectrometry. Application of porous silicon (PS) as an ionization platform in this technique is applicable for the analysis of relatively small biomolecules. Chemical modification of the PS surface with organic groups allows combining the selective solid-phase extraction of studied compounds followed by highly sensitive DIOS MS analysis. However, chemical modification of the PS surface influences significantly on the mechanisms of organic molecules desorption/ionization; also undesirable background ions can be formed from chemically grafted organic groups.

Processes of ion formation from different chemically modified PS platforms were investigated. In particular as-prepared p<sup>+</sup> type derived PS, oxidized PS, PS with chemically grafted hydrophobic groups, ion-exchanging groups and fragments of nonionic surfactant Triton X-100 were studied. Negatively charged silicon clusters Si<sub>n</sub><sup>-</sup> and Si<sub>n</sub>H<sup>-</sup> (n = 4÷10) were detected in the spectra of initial PS. Organic fragments observed in the spectra of chemically modified PS samples are significantly less fragmented in compare with the ions, usually observed in temperature programmed desorption MS, which is widely used for the characterization of modified surfaces [1]. Hence the LDI MS gives finer characterization of modified PS surface in compare with TPD MS method.

Studied materials were successfully applied for preconcentration and detection of model ionic dyes (methylene blue and methyl orange) [2], pharmaceuticals (gramicidin and tetracycline antibiotics) and biomolecules (tauroholic acid).

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## P155-B2

### Time Resolved FTIR Spectroscopy Using a Four-Layer Lamination Micro Mixing Device

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Time resolved measurements are one of *the* hot topics in IR spectroscopy. Step-scanning FTIR techniques allow following fast (bio)chemical reactions at time resolutions on the ns- $\mu$ s timescale; slower reactions can be monitored by rapid scanning instruments. With these, however, the time resolution in rapid scanning mode is dependent on the speed of the moving mirror in the spectrometer, limiting the achievable time resolution to around low ms. Both techniques share the need for a triggerable reaction. One highly promising possibility to close the ensuing gap between these two techniques, without the need for triggering, is the use of micro-mixing devices.

The basic principle of this technique is to mix two liquids through diffusion in a mixing channel also serving as measurement area. The actual measurements take place at well defined spots along this channel, corresponding to specific reaction times, moving the measurement spot towards the entry yields shorter, moving it towards the channel's end gives longer reaction times. The achievable time resolution with this technique is dependent on the spot size of the IR beam, with modern IR microscopes, it is typically in the range of 1 ms.

Kaun et al. [1] have presented IR-spectroscopic measurements with a lamination mixer using two vertically stacked fluid layers. Kauffmann et al. [2] used three layers, which shortens the mixing time but has the disadvantage of requiring three fluid connectors. Our newly developed mixer stacks four fluid layers, while requiring only two inlet channels [3]. The two inlet channels each split into two streams and end in wedge-shaped inlet channels. The wedged form of the channels yields an optimised flow pattern over the width of the channel, thus warranting homogenous, reproducible diffusion mixing.

The performance of our mixer was evaluated using the reaction of  $\text{Na}_2\text{SO}_3$  with HCOH forming  $\text{CH}_2(\text{OH})\text{SO}_3^-$

With the experimental setup of Kaun et al. [1] it took 540 ms for the reaction to start. In our newly developed mixer the product starts forming after only 20 ms, proving a significant improvement in mixing time.

This significant improvement in mixing time ensures a well defined starting point of the chemical reaction and thus proved to be crucial for accurate results for time resolved monitoring. Consequently, our new mixer is a momentous step forward in enabling reliable time resolved FTIR measurements in the low ms time range.

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## P156-B2

### Simulation of Platinum Surface Complexes Using NMR Spectroscopy

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Interpretation of phenomena associated with heterogeneous catalysis is feasible using liquid NMR spectroscopy on a suitable system which mimics adsorption on a catalytic surface but in a homogeneous phase. Homogeneous complexes of (olefin)Pt(0)(PPh<sub>3</sub>)<sub>2</sub>-type appear to be appropriate for the simulation of surface complexes formed in a course of hydrogenation on platinum catalysts. NMR spectroscopy, corroborated by the molecular modeling of these complexes, offers a versatile approach to study phenomena proceeding on the atomic level and thus acquiring very precious information applicable to heterogeneous catalysis. The formation of complexes with dimethyl fumarate and diethyl maleate via substitution of ethylene in the complex (ethylene)Pt(PPh<sub>3</sub>)<sub>2</sub> were verified experimentally. The system was studied in the individual as well as in the competitive arrangement. The values of relative stability of the complexes were obtained and compared with results obtained from heterogeneously catalyzed hydrogenations over Pt/supported catalysts. NMR experimental results were also correlated with the results of molecular modeling using DFT level of theory. The results of the molecular modeling were in good agreement with the results of the NMR experiments. The method was experimentally tested on the wide group of alkenic substrates and validated.

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## P157-B2

### Advantages of Proton Transfer Reaction - Mass Spectrometry (PTR-MS) in the Analysis of Potentially Dangerous Substances

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We present first results of measurements on explosives and chemical warfare agents utilizing a high mass-resolution and high-sensitivity PTR-TOF-MS instrument as well as a quadrupole based PTR-Quad-MS instrument. For details about the technique please see [1], [2] and very recently [3]. The high mass-resolution of the TOF instrument makes it possible to distinguish between "real" signal from explosives and (isobaric) background contributions at the same nominal mass; e.g., protonated TNT (228.026amu) can be well separated from background contaminations at 228.23amu. This means a huge step towards unambiguous identification.

On the other hand quadrupole mass filters are compact, lightweight, rather inexpensive and therefore probably the preferred choice for a portable detector for explosives and other critical substances. To ensure a sufficient detection capability we developed a prototype of an explosive "inlet booster" that (i) increased the sensitivity in first preliminary tests by at least two orders of magnitude and (ii) showed the potential to reach for the first time the low ppqv concentration range. Sampling times used for these booster tests were in the range of a few seconds, which means that the improved detection limit can be reached in a total time well below one minute (including adsorption, desorption and measurement).

In summary it can be said that PTR-MS fulfills the demands of up-to-date detection of illicit substances, like extremely high sensitivity, very short response times and unambiguous identification, while being a reliable and rugged instrument. In contrast to others, the PTR technique is not limited to a single class of substances (in particular in its newest mode where besides  $H_3O^+$  also reagent ions such as  $NO^+$  and  $O_2^+$  can be used) but can analyze various molecules no matter if they are explosives, CWAs, TICs, etc. and distinguish them from harmless everyday compounds.

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## P158-B2

### Switchable Reagent Ions (SRI) and Ultra-Low Detection Limits - Latest Developments in PTR-MS Instruments

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Proton Transfer Reaction – Mass Spectrometry (PTR-MS) is an innovative technology invented and developed by scientists of the "Institut für Ionenphysik" at the Leopold-Franzens University in Innsbruck and made commercially available by the spin-off company IONICON Analytik GmbH. (for details see [1] and very recently [2]). In short, in a hollow cathode ion source reagent ions are produced at very high purity levels (up to 99.5%) and afterwards injected into a drift tube where the actual ionization process takes place. Finally either a quadrupole or a time-of-flight mass spectrometer analyzes the product ions according to their masses and yields.

Here we report on the latest instrumental developments [3], namely i) the improvement of the detection limit that now allows for measuring trace gas compounds in a concentration range from several ppmv down to the ppqv (parts-per-quadrillion) region with a typical response time well below 100ms and, in case a TOF mass analyzer is used, a mass resolution better than 5.000 m/Δm and ii) the possibility to switch between H<sub>3</sub>O<sup>+</sup>, NO<sup>+</sup> and O<sub>2</sub><sup>+</sup> as reagent ions. We show, that the sensitivities obtained with NO<sup>+</sup> and O<sub>2</sub><sup>+</sup> are comparable to the outstanding sensitivity of the established PTR-MS instruments and therefore well above those from e.g. SIFT-MS instruments.

To demonstrate the advantages of the new setup we e.g. measured acetone and propanal (isomeric molecules at nominal mass 58amu) utilizing NO<sup>+</sup> as the precursor ion. According to Spanel et al. [4] NO<sup>+</sup> interactions with aldehydes follow the reaction: NO<sup>+</sup> + XH → X<sup>+</sup> + NOH whereas ketones follow: NO<sup>+</sup> + XH → XH<sup>+</sup> + NO (and clustering). This means that we see isomeric compounds on different nominal masses and can identify them unambiguously. Furthermore, by using O<sub>2</sub><sup>+</sup> precursor ions we are able to ionize molecules that cannot be measured via hydronium proton transfer reaction.

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## P159-B2

### **A Comparison of Atmospheric Pressure Photoionization (APPI), Atmospheric Pressure Chemical Ionization (APCI) and Electrospray Ionization (ESI) for the Determination of Polymer Additives by Liquid Chromatography Coupled with Mass Spectrometry**

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The quantification of polymer additives is important for quality control and trouble shooting. Identification of polymer additives is desired if products of unknown origin are investigated or degradation processes are studied. Quantification and identification are difficult tasks because there is a wide variety of different additives. Most commonly liquid chromatography (LC) in combination with UV detection is used. UV detection may be sufficient for the quantitative analysis of known additives but does not provide enough specific information about the molecular structure of the separated compounds if identification is needed or degradation products are investigated. Mass spectrometric (MS) detection can solve this problem because it is highly sensitive and structural information can be derived from the mass spectra.

Although ESI and APCI are the most frequently used ionization techniques in LC-MS analysis, atmospheric pressure photoionization (APPI) has recently expanded the range of compounds that are accessible to LC-MS.

A method for the determination of polymer additives including antioxidants, UV absorbers, and processing stabilizers using liquid chromatography coupled with mass spectrometry is presented. Detection limits were determined using APCI, APPI with or without dopant and were compared with ESI. APPI ion source parameters were optimized regarding temperatures, gas flow rates and voltages applied. Differences between APCI, APPI and ESI are pointed out and the effect of dopants is discussed. Linear calibration plots could be obtained for all solutes over a wide concentration range showing satisfying repeatability. The developed method was applied to the analysis of polypropylene samples and for the investigation of degradation products.

## P160-B2

### Spectroscopic Characterization of Oxovanadium(IV)-Rutin Chelates

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Flavonoids are a large group of polyphenolic phytochemicals with antioxidant properties; they also exhibit a chelating effect on transitional metals, which results in major biomedical active compounds with enhanced solubility.

Complex formation between vanadyl and rutin in methanol or mixtures of water and methanol was studied, with special interest focused on the composition of the complexes, the role of pH on complex formation, stoichiometry and stability. Based on our studies, only a mononuclear complex is obtained in alcoholic or water-alcoholic solutions. The stoichiometric composition of the complex was determined using the method of continual variation of equimolar and non-equimolar solutions and the Harvey and Manning slope ratio method. The results indicate that the 1:1 complex is predominant in methanol containing solution.

The reaction between vanadyl ion and rutin was investigated in the pH range 5.5-6.0 with respect to the stability constant and the spectrometric data show that it is a moderately stable chelate ( $\log \beta \sim 4$ ). Based on this features, a solid complex of oxovanadium (IV) with rutin has been synthesized and characterized by elemental analysis, thermal analysis (TG, DTG, DTA) and spectroscopic techniques (IR, UV-Vis, <sup>1</sup>H-NMR and fluorescence spectra).

## P161-B2

### X-Ray Shift Identification by Energy Dispersive X-Ray Fluorescence

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The influence of different oxidation states as well as of the solvation effect in the characteristic spectra of the Energy Dispersive X-Ray Fluorescence spectrometry was studied. For this reason vanadium, chromium and manganese compounds at various oxidation states were measured and the energy shifts of the emitted characteristic X-rays were measured. A Si(Li) X-ray detector with energy resolution 175 eV at 5.9 keV was used, and energy shifts of the order of a few hundreds of meV were measured for K-L<sub>2,3</sub> (K $\alpha$ ) and K-M<sub>2,3</sub> (K $\beta$ ) transitions, between salt, oxide and metallic targets. The excitation was performed by a <sup>109</sup>Cd radioactive irradiation source.

Chloride ion solvation effect was studied with water and glycerine mixtures. Sodium chloride samples with various water and glycerine content were irradiated by a <sup>55</sup>Fe radioactive source. X-ray spectra were collected with the necessary statistics (high count number). Measurable energy shifts were determined in the centroids of the K-L<sub>2,3</sub> and K-M<sub>2,3</sub> characteristic x-rays peaks of chloride. The dependence of the energy shifts on the chlorine to solvent molar ratio was determined.

## P162-B2

### Fluorescence Quenching of Fulvic Acid by Iridium(IV)

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Fulvic acid belongs to the group of humic substances. It can be considered a macromolecular polymer with structure and characteristics that change along with its origins and humification processes. The binding of metal ions to humic substances is of great interest in the understanding of metal ion transport and speciation in natural waters and soils.

Fulvic acid from the humus obtained in the Zagreb (Croatia) area was isolated by a previously described procedure [1].

The fluorescence of the fulvic acid was strongly quenched by iridium(IV), and the quenching was greatest at pH=4 (controlled by universal buffer) at  $\lambda_{\text{ex}}=340$  nm,  $\lambda_{\text{em}}=430$  nm. The fluorescence quenching of fulvic acid by iridium (IV) in water has been carried out at room temperature with a view to understand the quenching mechanisms. The quenching was found to be appreciable and showed positive deviation in the Stern-Volmer plots. This positive deviation was attributed to the static and dynamic quenching.

According to the obtained results, it can be concluded that the reaction of iridium(IV) with fulvic acid can be applied for the detection of the low  $\text{Ir}^{\text{IV}}$  concentrations in aqueous solutions. Decrease in the emission intensity was linearly proportional to the iridium(IV) concentration in the range 0.96–15.38  $\mu\text{g cm}^{-3}$  of iridium, if the concentration of fulvic acid was 31,22  $\mu\text{g cm}^{-3}$ . The relative standard deviations estimated from six independent determinations in samples of 4,81 and 9.62  $\mu\text{g cm}^{-3}$  of iridium(IV) were 1,35 and 1,21 %, respectively.

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**P163-B2**

**Effect of Coupling and Absorption Coefficients on Optimum Crystal Thickness in Two Beam Coupling in Photorefractive Materials**

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Nonlinear two-beam coupling between a pump beam and a signal beam in photorefractive materials has been considered. Wave equations describing the non-linear two beam coupling were solved and the expressions for the intensities of the two beams in the photorefractive crystals with the absorbing and non-absorbing properties have been derived. The intensity of the signal beam increases with the increasing crystal thickness, reaches a maximum and then decreases. The influence of coupling coefficient, absorption coefficient and the input intensity ratio on the crystal length and peak height corresponding to the maxima signal beam intensity has been studied in details. The effect of the negative coupling coefficient of the materials on the intensities of the two beams in both the co-directional as well as contra-directional two beam coupling cases has been studied.



## P164-B2

### Prostate Cancer: Direct Tissue Characterization by FTIR-Imaging

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Prostate cancer has become one of the most common malignancies worldwide and early diagnosis is still based on the serum test for prostate-specific antigen, a test with limited disease specificity<sup>1</sup>. Therefore Fourier transform infrared (FTIR) spectroscopic imaging has become an essential tool for the detection and characterization of the molecular components of biological processes, such as those responsible for the dynamic properties of tumor progression. Major advantage of this new technique is the acquisition of local molecular expression profiles, while maintaining the topographic integrity of the tissue and avoiding time-consuming extraction, purification and separation steps. Using this method it is possible to investigate the spatial distribution of proteins and small molecules within biological systems by in-situ analysis of tissue sections<sup>2</sup>. In our work we established this method for the characterization of prostate cancer tissue. For the interpretation of the FTIR results we correlated the FTIR-images with the histopathological information.

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## LP01

### Trace Metals Speciation in Water Samples by Sequential Injection Anodic Stripping Voltammetry with Monosegmented Flow and On-Line UV Digestion

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A cost-effective sequential injection system incorporating with an on-line UV digestion for decomposing of organic matters before the determination of Zn(II), Cd(II), Pb(II) and Cu(II) by anodic stripping voltammetry (ASV) on a hanging mercury drop electrode (HMDE) using a small scale voltammetric cell was developed. The simplest voltammetric speciation was investigated by separation of the metal species into two groups: labile and inert. The labile species can be detected by direct ASV. The total metal was measured after destruction of dissolved organic matter by on-line UV digestion. A low-cost small scale voltammetric cell using a HMDE as a working electrode was fabricated from disposable pipet tip and microcentrifuge tube. A home-made UV digestion unit was fabricated employing a small size and low wattage UV lamps and flow reactor made from PTFE tubing coiled around the UV lamp. An on-line calibration or a standard addition procedure was developed employing a monosegmented flow technique. Performance of the proposed system was tested for on-line digestion of model water samples to release metal ions from organic complexes such as strong organic ligand (EDTA) or intermediate organic ligand (humic acid). The wet acid digestion method (USEPA 3010a) was used as a standard digestion method for comparison. Under the optimum conditions, deposition time of 180 s, linear calibration graphs in range of 10-300 µg/l Zn(II), 5-200 µg/l Cd(II), 10-200 µg/l Pb(II), 20-400 µg/l Cu(II) were obtained with detection limit of 7.4, 2.7, 5.2 and 3.8 µg/l of Zn(II), Cd(II), Pb(II) and Cu(II), respectively. Relative standard deviation were 4.2, 2.6, 3.1 and 4.7% for 7 replicate analyses of 27 µg/l Zn(II), 13 µg/l Cd(II), 13 µg/l Pb(II) and 27 µg/l Cu(II), respectively. The system was validated by the analysis of certified reference material of trace metals in natural water (SRM 1640 NIST). The developed system was successfully applied for the determination of Zn(II), Cd(II) Pb(II) and Cu(II) in ground water samples collected from the nearby zinc mining area.

## LP02

### Sequential Injection Anodic Stripping Voltammetry with Monosegmented Flow for Determination of Cadmium and Lead Using a Bismuth Film Electrode

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A cost-effective sequential injection monosegmented flow analysis (SI-MSFA) with anodic stripping voltammetric (ASV) detection has been developed for determination of Cd(II) and Pb(II). The system consisted of a syringe pump, an injection valve and a voltammograph equipped with a flow through electrochemical cell. A software for control the system was written in-house based on Visual Basic 6.0. A bismuth film (BiFE) coated on glassy carbon electrode was employed as an environmental friendly working electrode. The SI-MSFA provides a convenient means for preparation of a homogeneous solution zone containing sample in an acetate buffer electrolyte solution and Bi(III) solution for in situ plating of BiFE, ready for ASV measurement at a flow through thin layer electrochemical cell. Under the optimum conditions, linear calibration graphs in range of 10–100 µg/l of both Cd(II) and Pb(II) were obtained with detection limits of 1.4 and 6.9 µg/l of Cd(II) and Pb(II), respectively. Relative standard deviations were 2.7 and 3.1%, for 11 replicate analyses of 25 µg/l Cd(II) and 25 µg/l Pb(II), respectively. A sample throughput of 12 h<sup>-1</sup> was achieved with low consumption of reagent and sample solutions. The system was successfully applied for analysis of water samples collected from a draining pond of zinc mining, validating by inductively coupled plasma-optical emission spectroscopy (ICP-OES) method. The proposed method provided high degrees of automation, low consumption of chemicals and low waste production.

## LP03

### **Multi-Modal Peptide Separations (RP, AEX, Ion-Exclusion, HIC, HILIC Modes) with a Single Column Containing a Mixed-Mode Reversed-Phase/Weak-Anion Exchange Material**

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Analytical and preparative scale separation of peptide has become an important application area of liquid chromatography in fields like proteomics, synthetic peptide purification and purity control, and many more. The most frequent technique utilized for chromatographic separation of peptides is gradient reversed-phase HPLC. On contrary, ion-exchange chromatography alone is seldom used, but became an important separation mode in 2-dimensional concepts in proteomic research.

We recently presented mixed-mode separation materials, based on particulate and monolithic silica support, that were modified chemically with a chromatographic ligand consisting of a hydrophobic strand and a weak anion-exchange moiety. Besides, polar embedded groups such as amide and thioether functionalities were incorporated in the chromatographic ligand as well. These mixed-mode materials turned out to be highly useful for separation of synthetic peptides by a separation mechanism that is complementary to that of gradient RP-HPLC that is commonly adopted as the state-of-the-art technique in the field.

Depending on the employed conditions, a column packed with this material can exploit hydrophobic interaction, anion-exchange, ion-exclusion, and hydrophilic interaction as retention and selectivity principles. As a consequence, the column can be operated in the RP mode (neutral compounds), anion-exchange mode (AEX) (acidic compounds), ion-exclusion chromatography mode, hydrophobic interaction chromatography (HIC) mode and hydrophilic interaction chromatography (HILIC) mode as well. This allows a flexible adjustment of selectivity by tuning mobile phase conditions. These distinct separation mechanisms will be outlined by selected examples of peptide separations.

For some synthetic peptides the column showed excellent loading capacity in the RP/WAX separation mode and outperformed gradient RP-HPLC by factor of 10-100.

## LP04

### Cloud Point Extraction of Cadmium in Soft Drinks by Thermospray Flame Furnace Atomic Absorption Spectrometry

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The dietary exposure to cadmium can lead to damage in vital organs such as kidneys, liver and lungs; due to, determination of this element is very important. However due low concentration of this analyte in most samples is necessary the use of techniques with high sensitivity and selectivity. The use of cloud point extraction/preconcentration is an alternative to conventional solvent extraction because it produces high extraction efficiencies, good concentration factors and uses inexpensive and non-toxic reagents. In addition, the sensitivity could be improved employing the thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) technique, which consists of a Ni tube positioned on the burner head of an air/acetylene flame with a ceramic capillary tube connected to it and heated by a flame. This way a method based on cloud point extraction and TS-FF-AAS was developed for determination of cadmium in soft drinks. The cadmium reacted with pyridyl-azo-naphthol (PAN) to form hydrophobic chelates, which were extracted into the micelles of Triton X-114 in a solution buffered at pH 9. For phases separation, NaCl was used. The variables which affect the preconcentration were optimized: PAN, Triton X-114 and NaCl concentrations and reaction time. After extraction and preconcentration steps, 100  $\mu\text{L}$  of sample were introduced into the hot Ni tube using water as carrier at a flow-rate of 0.4  $\text{mL min}^{-1}$ . Employing 0.12  $\text{mmol L}^{-1}$  PAN, 0.06 % m/v Triton X-114, 3.2 % m/v NaCl and reaction time of 20 min, the linear range was from 0 to 5.0  $\mu\text{g L}^{-1}$ . The limit of quantification obtained was 0.036  $\mu\text{g L}^{-1}$  and the relative standard deviations varied from 1.5 to 4.4 % ( $n=7$ ). Accuracy was checked by performing addition-recovery experiments. Recoveries varied from 95 to 104 %. Commercial samples analyzed presents Cd from 36 to 66  $\text{ng L}^{-1}$ .

## **LP05**

### **Simplification of Sample Cleanup for Difficult Matrices Using Molecularly Imprinted Polymers for Analysis of Antibiotics**

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Extraction of trace residues in sample pre-treatment is often elaborate and time consuming. Solid phase extraction (SPE) phases based on molecularly imprinted polymers (MIP) eliminate the need for multiple extraction steps, simplifying the pre-treatment procedure. MIP phases in most cases reduce the sample handling time, yield cleaner extracts, lower detection limits and improve MS compatibility. MIP phases contain pre-formed cavities that are complimentary in shape and chemical properties to the target analytes. It allows selective extraction of either single molecular species or 'classes' of molecules containing the same functional groups.

Analysis of antibiotics in food is important for monitoring food quality. In this presentation, we will report using MIP SPE to clean up complex food matrices to achieve lower detection limit of chloramphenicol and fluoroquinolones, two widely used antibiotic classes found in food products, such as milk and honey. Two MIP phases have been developed specifically for the extraction of chloramphenicol and fluoroquinolones. The extracted samples were analyzed by LC/MS/MS without the need for matrix-matched standards. The presentation will present the method performance data.

## LP06

### Selective Depletion of Phospholipid Interference Utilizing Hybrid SPE Technology

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Analysis of biological samples is often hindered due to interferences carried through the sample preparation technique. Protein precipitation is a widely accepted sample preparation method for biological plasma samples due to its simplicity. Though widely used, protein precipitation methods often result in chromatographic irregularities due to co-extracted endogenous species such as phospholipids that negatively affect chromatographic analysis. A more thorough sample clean up can be achieved using solid phase extraction (SPE), but at a cost of time and method complexity. In this presentation a new platform was developed to process various plasma samples using a simplified two-step procedure to produce biological samples depleted of phospholipids prior to LC-MS/MS analysis.

In these experiments, the chromatographic impact of phospholipids is evaluated. The co-retention of phospholipids from a standard protein precipitation of rat plasma was observed on the LC/MS separation using generic conditions. A method was then developed to measure the impact of phospholipids on the ionization of analytes. Plasma samples were then processed using the Hybrid SPE platform and a comparison was made in regards to phospholipid content and response of acidic, basic and neutral compounds. The Hybrid SPE platform employs the simplicity of standard protein precipitation with the added selectivity of SPE. The platform exhibits a high affinity towards phospholipids while remaining non-selective towards a broad range of basic, neutral and acidic compounds

Due to the hydrophobic nature of phospholipids, these species often remain on the analytical column after sample analysis. Chromatographic build up of phospholipids from multiple injections of standard protein precipitated plasma samples on a conventional C18 column were demonstrated. Ion suppression was observed on samples processed by standard protein precipitation due to coelution of phospholipids. Samples processed using the Hybrid SPE platform were depleted of phospholipids and showed no ion suppression nor chromatographic buildup of phospholipids. The sample preparation technique enabled faster sample preparation and more consistent analysis by depletion of phospholipids from biological samples resulting in cleaner extracts with high recovery of analytes of interest.

## LP07

### Method Optimisation Using Alternative Selectivities in Fused-Core™ Particle HPLC Column Technology

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Increasing the speed and resolution of HPLC analyses are drivers for innovation and cost reduction in both HPLC column and hardware design. The recent development of Fused Core technology has provided such improvements in terms of speed, efficiency and robustness. These particles have a solid core and a porous outer layer bonded with C18 and C8 on the surface that results in a highly ordered packed column bed and very significantly less diffusion within the analytical column that results in twice the efficiency compared to 3µm columns. The flatter van Deemter curves of Fused Core particles also allow for higher flow rates while still maintaining near maximum efficiencies. Further, they provide all the benefits of the high speed and high efficiency of sub-2 µm particles but at a much lower backpressure, making these advances realisable for conventional HPLC instrumentation and UHPLC alike.

For polar molecules such as metabolites, peptides and natural products, traditional C18 chemistries can be insufficiently selective, such that the separation requires a gradient or mobile phase additives. Orthogonal selectivity differences may be observed when using alternative chemistries that incorporate different mechanisms such as dipole, pi-donor-acceptor, hydrogen donor-acceptor or dispersive interactions. For the first time such chemistries have been applied to the Fused Core technology, enabling the advantages of the speed and efficiency of the Fused Core to be extended.

In this presentation, we discuss the mechanisms, method development and applications of the different chemistries that have been developed for the Fused-Core particle technology. This will include the applicability of this technology to pharmaceutical analysis, bioanalysis and environmental applications.



## LP08

### Prediction of Retention and Elution Profiles for Anion Analysis in High Performance Ion Chromatography

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The aim of this work was the development of theoretical and practical methodology in order to plan effectively and optimize the ion-chromatographic analysis of complex environmental samples. The stochastic theory of chromatography and an equilibrium based approach were used for the prediction of peak shape and retention data of anions. This attempt incorporating the potential advantages of two different chromatographic phenomena for analytical purposes [1,2]. It is a new integrated method to estimate kinetic and thermodynamic properties for the same chromatographic run of ions.

The stochastic parameters of eluted anions, such as the residence time of the molecule on the surface of the stationary phase, and the average number of adsorption steps were determined on the basis of a retention database of organic and inorganic anions of great importance in water analysis (formate, chloride, bromide, nitrate, sulphate, oxalate, phosphate) obtained by using carbonate/bicarbonate eluent system at different pHs (9–11) and concentrations (7–13 mM). In the investigated IC system the residence times are much higher and the average number of sorption steps is somewhat smaller than in RP-HPLC. The simultaneous application of the stochastic and the multispecies eluent/analyte model was utilized to peak shape simulation and the retention controlling of various anions under elution conditions of practical importance. The similarities between the measured and the calculated chromatograms indicates the predictive and simulation power of the combined application of the stochastic theory and the multiple species eluent/analyte retention model using in simultaneous anion analysis.

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### **The Application of Chromatographic Method in Quercetin Derivatives Determination of Botanical Extracts Come from Asia**

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The use of herbs and other plants as dietary supplements and as over-the-counter (OTC) drugs has increased dramatically in the past few years in Europe because of thinking that "natural" is better and lower cost in comparison to most commercially available drugs. A lot of botanical products originated from Asiatic countries, which are components of dietary supplements and OTC drugs, are not well known in Poland and in Europe. Their compositions and effects on human body are unknown. These components connected with other botanical products or botanical drugs could cause real health hazard. For potential consumers, every qualitative and quantitative analysis of botanical dietary supplements is grounded. Content of toxic and harmful components should be monitoring.

However, a lot of phenolic compounds found in plant kingdom have positive impact for health (e.g. flavonols, flavones, isoflavones, flavanones, phenolic acids, anthocyanins, chalcones, stilbenes). They have antioxidant properties. Epidemiological studies showed that diets rich in fruits and vegetables were correlated with a reduced risk of chronic diseases.

In the present work, a method involving solvent extraction, HPLC – RP- C18 column chromatography with photodiode array detection is developed for determining the level of quercetin derivatives (e.g., quercitrin, isoquercitrin, hyperoside, rutin, rhamnetin) in botanical products from Asia (e.g. *Embllica Officinalis*, *Centella Asiatica*, Chinese Ivy, *Hoodia Gordonii*, *Pueraria Lobata* Wild). The total amount of studied flavonols was compared with total content of polyphenols using Folin-Ciocalteu's method. The obtained results were compared with those achieved by pharmacopoeian method. The spectrophotometric method for qualitative and quantitative analysis of polyphenols in dry plant extracts was applied.

The developed method was validated for specificity, repeatability, recovery and accuracy. The results demonstrate that HPLC-PDA method can be suitable for routine analysis of quality control and quantity evaluation of botanical products and extracts from plants containing phenolic compounds.

## LP10

### High-Throughput Analysis of Cancerostatic Platinum Compounds in Clinical Samples via Flow Injection and Ultra-Fast HPLC Combined with ICP-MS

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The cancerostatic platinum compounds (CPC) cisplatin, carboplatin and oxaliplatin are successfully applied against various kinds of cancer like lung, cervical, testicular, head and neck, bladder and ovarian cancer. The drugs are administered to the patient at doses of 75 to 400 mg m<sup>-2</sup> body surface. However, the individual therapeutic window of CPC is generally narrow and accurate monitoring of the drugs in patient plasma and urine is a prerequisite for studies aiming at the optimization of drug dosage in terms of improved response and reduction of unwanted side effects. In the present work two different methodologies for high-throughput analysis of clinical samples from platinum based chemotherapy have been developed. The first part aimed at the rapid quantification of total platinum in clinical samples combining flow-injection analysis and ICP-MS. The second part concerned the development and assessment of a high-throughput HPLC method for speciation of intact oxaliplatin in patient urine. The implementation of sub-2 µm particles as stationary HPLC phase led to an immense improvement of both chromatographic and economic efficiency due to the higher chromatographic resolution and the possibility of shorter cycle times.

Acknowledgement: ThermoFisher is acknowledged for supplying a HTC PAL autosampler.

## LP11

### High Throughput Automation of Solid Phase Extraction in the Field of Phytochemistry

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Plants are a rich source of active pharmaceutical ingredients (APIs). Their qualitative and quantitative characterisations are difficult tasks for every analytical scientist due to the complexity of the samples and their concentrations which often occur in very small amounts. As a result, sample concentration and purification are crucial steps for structure elucidation and quantification. The most common way to achieve this is Solid Phase Extraction (SPE) next to Liquid-Liquid Extraction (LLE). There exists a broad range of SPE materials, neither based on silica or on polymeric particles with different surface modifications [1]. A major drawback of these methods is time-consuming sample preparations which have to be done manually under light. Additional biases are occurring due to the manual handling causing difficulties in reproducibility and an increase in standard deviation of quantitative results. To overcome these problems a Phynexus (San Jose, CA) MEA™ Personal Purification System was adapted for the automation of sample preparation. This system contains an automated 12-channel pipette and allows to condition, equilibrate, load, wash and elute in one go. Additionally, the robotic system offers a range of flow rates that maximizes the purification and enrichment efficiency. Different SPE materials, e.g. NP-Silica, C-18 Silica, Cellulose beads, were filled in 1.2 ml tips and sealed with frits on both sides. The automation showed to be a reliable and a repeatable tool for extraction and purification.

1 Vallant R. M. et al., 2007, *Anal. Chem.* 79(21), 8144-8153.

## LP12

### Determination of Thymol in Different Medicinal Products

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The German scientific forum "Development of Medicinal Plant Lore" named the original thyme (*Thymus vulgaris* L.) medicinal plant of the year 2006. Its leaves contain 0.3 – 3.4 % of an essential oil consisting of 30 – 70 % thymol and its isomer carvacrol and up to 20 % p-cymene as well as cineole, pinene, borneol and linalool [1,2]. Thymol is a strong antibacterial agent and is used as a remedy for digestive disorders, bronchitis, pertussis, helminthic parasites, as well as a fungicide, in ointments and in toothpaste [2, 3]. On the market there are a lot of different antibronchitic medicinal products containing extracts of Thyme with varying concentrations of thymol. Many of these products have different formulations like tablets, film coated tablets, lozenges, syrups, soft and hard capsules or ethanolic solutions. These complex matrices make the analysis of thymol content a different but interesting task for analytical chemists. Thin Layer Chromatography (TLC), High Performance Liquid Chromatography with UV-detection (HPLC-UV), Gas Chromatography coupled to Mass Spectrometry (GC-MS) and Headspace-GC-MS (HS-GC-MS) were used to determine the concentration of the active ingredient.

- [1] Wolfgang Franke, *Nutzpflanzenkunde*, 5th edition, Georg Thieme Verlag Stuttgart, 1992
- [2] Eisenbrand, Schreier, *Römpp Lexikon Lebensmittelchemie*, 2nd edition, Georg Thieme Verlag Stuttgart, 2006
- [3] Atul Bhattaram et al. (2002). "Pharmacokinetics and Bioavailability of Herbal Medicinal Products." *Phytomedicine* **9** (Supplement 3): 1-33
- [4] Radoslaw Kowalski, Jacek Wawrzykowski (2009). "Essential oils analysis in dried materials and granulates obtained from *Thymus vulgaris* L., *Salvia officinalis* L., *Mentha piperita* L. and *Chamomilla recutita* L." *Flavour and Fragrance Journal* **24**(1): 31-35

## LP13

### An Innovative Organic Phase Enzyme Electrode (OPEE) for Ethanol Determination in Unleaded Fuel

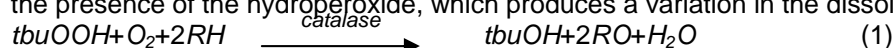
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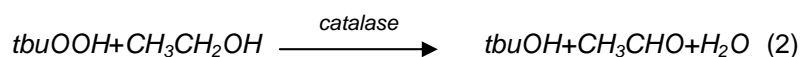
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Unleaded fuel used in many countries in the world contains ethanol, together with other oxygenated products. Oxygenated substances are added mainly to increase octane number, and replace more toxic and polluting substances such as tetraethyl lead, as well as to reduce carbon monoxide emissions. Propanol and butanol may instead be added mainly as solvents, in order to prevent phase separation. It thus becomes necessary to assess the presence of these oxygenated species, since they can cause corrosion problems in internal combustion engines, as well as being implicated in pollutant emission (O<sub>3</sub>, nitrogen oxides, aldehydes).

The aim of the present research work is to fabricate a new biosensor for the determination of the ethanol content of liquids or organic solvents, such as unleaded fuel and biofuels. The new sensor is also quite peculiar: it is a 'substrate competition' OPEE (Organic Phase Enzyme Electrode), in which the enzyme, catalase, is coupled to an amperometric gaseous diffusion Clark type oxygen electrode. This innovative biosensor is based on two parallel oxidation reactions, both of which catalysed by the same enzyme in the presence of the same hydroperoxide (tertbutylhydroperoxide or cumene hydroperoxide). In the first reaction the catalase enzyme in decanol catalyses an oxidation reaction in the presence of the hydroperoxide, which produces a variation in the dissolved oxygen concentration.



In the second reaction, catalase catalyses a reaction in which the hydroperoxide oxidizes the ethanol (the analyte to be determined) to acetaldehyde, but no change in dissolved oxygen concentration occurs.



As this second reaction competes with the first one for the substrate, this gives rise to a partial restoration of the initial O<sub>2</sub> concentration since the hydroperoxide present is now taking part in two reactions simultaneously; this therefore slows down the rate at which O<sub>2</sub> concentration varies under the effect of the first reaction alone. The extent of this "restoration" is measured using the Clark electrode and can be linked to the concentration of the ethanol present by constructing a suitable calibration straight line.

The catalase biosensor was optimized and characterized, then used to determine ethanol concentration in unleaded fuel samples purchased from service stations belonging to three different companies. The concentration values obtained were very low, consistently with the fact that the presence of ethanol in current unleaded fuels is very low, at "trace" level as stated in the literature. In order to assess any interference due to the complexity of the matrix, recovery tests were performed using the standard addition method.

In conclusion, the biosensor thus developed was found to be highly sensitive and relatively selective towards ethanol. The results obtained show that this biosensor method may be used to determine the ethanol content of green petrol. The analytical data obtained using these samples confirm that the measures performed are comparatively rapid and precise and that moreover the real samples tested do not need to be pretreated. Simple dilution is sufficient using the same solvent (decane) as that used as the medium in the biosensor analysis.

## LP14

### How to Make Styrene-Based Monolithic Chromatographic Supports Applicable for the Rapid and High-Resolution Separation of Low-Molecular-Weight Compounds?

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In this contribution we introduce an organic monolith based on poly(1,2-bis(p-vinylphenyl)ethane) (BVPE). The polymeric support material was prepared in situ into fused silica capillaries in the confines of 100  $\mu\text{m}$  and 200  $\mu\text{m}$  I.D and 8 cm length, applying a thermally initiated free radical polymerisation. The porous properties of the polymer fulfil the requirements for the high-efficient and rapid separation of small molecules (e.g. alkylbenzenes, alkylphenones, phenols, parabens), since the mesoporosity (pores with radius of 2 to 50 nm) and the specific surface area of the support is tremendously enhanced.

By additionally optimising the polymerisation time as a novel polymerisation parameter beyond well-studied parameters like porogen to crosslinker ratio, porogen nature and composition, polymerisation temperature, the polymeric support experienced a beneficial broadening of the pore-size-distribution. A high fraction of mesopores and small macropores was obtained while keeping the amount of macropores and throughpores high. Due to this exceptional pores-size-profile it was possible to create organic monoliths with high resolution power for low-molecular-weight compounds. The porous support material based on BVPE was characterised by BET measurements, Scanning Electron Microscopy (SEM), Mercury Intrusion Porosimetry (MIP) and NIR studies. High mechanical robustness and low swelling properties in organic solvents ensure chromatographic applicability of the developed stationary phase. Furthermore, the polymerisation process as well as the separation of small molecules offers highly reproducible results.

Due to the steadily growing interest in the fields of proteomics and metabolomics, the new styrene-based monolithic column supports can be an attractive tool for the fractionation complex samples, as they combine high chromatographic efficiency and short analysis time.