

Scientific paper

Condensation Reaction between Carbohydrazide and Salicylaldehyde: *in-line* Vibrational Spectroscopy Monitoring and Characterization of the Reaction Products in Solution and Solid State

Tomislav Jednačak,¹ Predrag Novak,^{1*} Aden Hodzic,² Otto Scheibelhofer,² Johannes G. Khinast,² Janez Plavec,³ Primož Šket³ and Jelena Parlov Vuković⁴

¹ University of Zagreb, Faculty of Science, Department of Chemistry, HR-10000 Zagreb, Croatia

² Research Center Pharmaceutical Engineering GmbH, A-8010 Graz, Austria

³ National Institute of Chemistry, NMR Center, SI-1000 Ljubljana, Slovenia

⁴ INA-Industrija nafte d.d., Refining & marketing business division, Product development department, Lovinčičeva 4, HR-10002 Zagreb, Croatia

* Corresponding author: E-mail: pnovak@chem.pmf.hr
Tel.: +385 1 460 6184

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Abstract

The condensation reaction between carbohydrazide and salicylaldehyde was monitored *in-line* by using vibrational NIR and Raman spectroscopies and statistical methods. Prior to *in-line* data analysis the reaction products were fully characterized in solution and solid state in order to check the potential of the *in-line* approach as a tool for *in-process* Schiff bases reaction control. It was demonstrated that a combination of vibrational spectroscopy and principal component analysis made it possible to detect and identify the reaction products, e.g. mono(salicylidene)carbohydrazide (**1**) and bis(salicylidene)carbohydrazide (**2**) in different solvents, and to determine the reaction end points in real time. Owing to complexity of the reaction mixtures and band overlapping, it was not possible to determine the relative ratio of the reaction products *in-line*. The *off-line* analysis showed that **1** was predominant in methanol while the highest portion of **2** was obtained in ethanol.

Keywords: Carbohydrazide, salicylaldehyde, Schiff base condensation reaction, *in-line* vibrational spectroscopy, solid and solution state characterization

1. Introduction

In the last few decades, process analytical technology (PAT) has emerged as one of the key elements to fully implement advanced, sophisticated and efficient analytical methods for *in-line* process monitoring.^{1–3} Conventional monitoring procedures typically involve the analysis of samples taken out of the laboratory reactor and thereby do not provide information in real time. *In-line* vibrational spectroscopic techniques provide interesting alternatives for conventional monitoring procedures, as they

do not require transfer systems or sample preparation and offer a non-invasive, non-destructive and real-time monitoring of physical and chemical transformations during laboratory and industrial processes. Complimentary information on the investigated process can be obtained by combining *in-line* near-infrared (NIR) and Raman spectroscopies. Raman spectroscopy probes fundamental vibrations with high spectral resolution and provides unique fingerprint of the sample.^{4,5}

In-line NIR and Raman spectroscopies have already been used for continuous, *in situ* monitoring and control of chemical reactions, polymorphic transitions, crystalli-

zation, powder blending and polymer extrusion processes.^{6–19} We have recently shown that *in-line* Raman spectroscopy and PCA can successfully be used to monitor the formation reaction of drug entacapone.⁶ Hence, we were particularly interested to see whether a combination of *in-line* vibrational spectroscopy and statistics could also be applied to monitor the Schiff base condensation reactions *i.e.* to detect the reaction starting point, to estimate the reaction time and to identify the products. Condensation of carbohydrazide with aromatic carbonyl compounds can be used as a model system for the preparation of Schiff bases. These compounds are known to possess antimicrobial, anticonvulsant and antioxidative activity.^{20–23} Since the bioactivity of Schiff bases is greatly affected by their physico-chemical properties, it is of utmost importance to control and optimize the reaction parameters. The use of accurate, versatile and reliable *in-line* sensors enables reaction monitoring in real time and thus better control of process parameters in order to obtain the final product with desired properties. Successful application of *in-line* NIR and Raman spectroscopies for estimating the reaction end points relies on the interpretation of characteristic vibrational bands. However, in the case of band overlapping, low S/N ratio or strong background signal, a straightforward interpretation of the spectra is difficult without employing multivariate data analysis.^{2,7,24,25}

In this paper, *in-line* NIR and Raman spectroscopies in combination with multivariate analysis were used for *in situ* monitoring of the Schiff base condensation reaction between carbohydrazide and salicylaldehyde in the molar ratio 1:1. The reaction was performed in methanol, ethanol and propan-2-ol. Also the aim was to investigate the influence of solvent on the reaction selectivity and structure of the products in solution and solid state. In order to unambiguously identify and quantify the reaction products, the samples were first analyzed *off-line* using solution and solid-state nuclear magnetic resonance (NMR) spectroscopy, small- and wide-angle X-ray scattering (SWAXS) analysis and Raman spectroscopy.

2. Experimental

2.1. Reagents and Chemicals

Carbohydrazide (min. 98%), salicylaldehyde (min. 98%), and propan-2-ol (min. 99.9%) were obtained from Sigma-Aldrich (St. Louis, USA). Methanol (min. 99.95%) and ethanol (min. 99.8%) were purchased from Carl Roth GmbH (Karlsruhe, Germany). Piperidine (min. 99%) was purchased from Acros Organics (NJ, USA).

2.2. Synthesis Reaction

Carbohydrazide (0.60 g, 6.67 mmol) was dissolved in 40 mL of methanol. Salicylaldehyde (0.71 mL, 6.67

mmol) and a few drops of piperidine were added to this solution. The resulting reaction mixture was heated at 55 °C and stirred under reflux for 40 min.^{26,27} Yield: 0.92 g (71%). The reaction was repeated with ethanol and propan-2-ol as solvents. Yield: 0.96 g (75%) and 0.87 g (67%), respectively. The reaction temperatures were set according to physical properties of the solvents.

2.3. NMR Spectroscopy in Solution

NMR spectra were recorded on Bruker Avance 300 spectrometer from samples in 5 mm NMR tubes at 298 K. Sample concentration was 1.2×10^{-2} mol dm⁻³. DMSO-d₆ was used as solvent and TMS as internal standard.

Proton spectra with spectral width of 6200 Hz and a digital resolution of 0.25 Hz per point were measured with 16 scans.

¹³C proton decoupled, DEPT 90 and DEPT 135 spectra with spectral widths of 18030 Hz were collected with 256–1024 scans. Digital resolution was 1.00 Hz per point for ¹³C proton decoupled experiment and 0.55 Hz per point for DEPT experiments.

In the gCOSY experiment 2046 points in the *f*₂ dimension and 256 increments in the *f*₁ dimension were used. For each increment 4 scans and the spectral width of 3086 Hz were applied. Digital resolution was 3.01 and 24.11 Hz per point in *f*₂ and *f*₁ dimensions, respectively. Typical spectral conditions for gHSQC and gHMBC spectra were as follows. Spectral width was 2994 Hz in *f*₂ and 16605 Hz in *f*₁ dimension for both experiments. 2K data points were applied in the time domains and for each data set 256 and 128 increments were collected for gHSQC and gHMBC spectra, respectively. The resulting digital resolution was 2.92 Hz per point in *f*₂ dimension and 129.7 and 261.9 Hz per point in *f*₁ dimension in gHSQC and gHMBC spectra, respectively.

2.4. NMR Spectroscopy in Solid State

NMR spectra of solid samples were recorded on Varian NMR System 600 MHz NMR spectrometer equipped with 1.6 mm Fast MAS Solids Probe. Larmor frequencies of protons and carbon nuclei were 599.65 and 150.78 MHz, respectively. The ¹H MAS NMR spectra were externally referenced using adamantane. The ¹³C CP-MAS NMR spectra were externally referenced using glycine. All samples were spun at the magic angle with 20 kHz during ¹H measurements and 16 kHz during ¹³C measurements. The proton spectra were acquired using spin echo sequence. Repetition delay was 5 s. The number of scans was 16. The pulse sequence used for acquiring the carbon spectra was a standard cross-polarization MAS pulse sequence with high-power proton decoupling during acquisition. Repetition delay was 5 s and the number of scans was between 600 and 1600, depending on the sample.

2. 5. SWAXS Analysis

A high-flux laboratory SWAXS camera (Hecus S3-Micropix, Graz, Austria) equipped with a point-focus optics (FOX3D) and high-brilliance micro-beam delivery system operated at low power of 50 W (50 kV and 1 mA) was used in this study. The X-ray wavelength used in our experiments was 1.542 Å and the SAXS and WAXS spectra were recorded with two independent 1D-detectors (PSD-50, Hecus X-ray Systems, Graz, Austria) in the angular range of $0.06^\circ < 2\theta < 8^\circ$ and $17^\circ < 2\theta < 27^\circ$, respectively.

For the measurements, the powder samples (85 mg) were filled into glass capillaries with an inner diameter of 2 mm sealed with wax. The capillaries were rotated during the exposition to the X-ray beam to allow for angular averaging of the powder scattering patterns. Triplicate measurements were performed at room temperature by exposing the samples for 700 s to X-ray beam with a diameter of 200 µm. The obtained data were normalized by the sample with the highest value.

2. 6. Raman Spectroscopy

FT-Raman spectra were recorded using a Bruker Equinox interferometer equipped with a FRA 106/S Raman module (Bruker Optics GmbH, Ettlingen, Germany). The *in-line* spectral data were collected in real time using a fiber-optic immersion probe (Bruker Optics GmbH, Ettlingen, Germany) during the chemical reaction. To obtain the satisfactory S/N ratio at least 3.7 min per spectrum were needed. The experiments were repeated three times under the same conditions. *Off-line* Raman spectra of the filtered crystalline products were also recorded. Each spectrum was acquired by averaging 128 scans in the range 3500–100 cm⁻¹ with a resolution of 4 cm⁻¹.

2. 7. NIR Spectroscopy

FT-NIR spectra were recorded using a Perkin Elmer Spectrum 400 FT-IR/FT-NIR spectrometer (Perkin Elmer, Waltham, MA, USA). The *in-line* spectral data were collected continuously (1 min per spectrum) during the chemical reaction *via* a fiber-optic diffuse reflectance probe (Perkin Elmer, Waltham, MA, USA) with aluminum foil as reflector. The measurements were performed three times under the same conditions. Each spectrum was acquired in 1 min by averaging 20 scans with a resolution of 8 cm⁻¹ in the range 10000–4000 cm⁻¹.

2. 8. Multivariate Analysis of *in-line* NIR Spectra

The *in-line* NIR spectral data were recorded using the program Timebase by Perkin Elmer. The spectra were

treated using the software The Unscrambler 10.2 by Camo. In the next steps certain wavelength regions were chosen, in order to neglect regions, where the solvent was dominating the signal. The chosen regions were 10000–5000 cm⁻¹ in ethanol, 10000–6000 cm⁻¹ and 5500–5000 cm⁻¹ in propan-2-ol, 10000–7000 cm⁻¹ and 5800–5100 cm⁻¹ in methanol.

As it could not be guaranteed that the optical path length was always the same, SNV (standard normal variate) was performed across these regions, in order to normalize the spectra. PCA was performed with the normalized and mean-centered spectra.

2. 9. Multivariate Analysis of *in-line* Raman Spectra

The *in-line* Raman spectra were recorded using the program Opus by Bruker. The data were further processed using the software The Unscrambler 10.2 by Camo. A subset of spectral data in the range 1800–1000 cm⁻¹ was selected providing a data matrix (dimension 12 spectra × 1816 wavenumbers). The spectra were normalized by applying the SNV (standard normal variate) method. PCA was carried out with the normalized and mean-centred spectra.

3. Results and Discussion

3. 1. Identification of the Reaction Products in Solution

The condensation reaction between carbohydrazide and salicylaldehyde in methanol, ethanol and propan-2-ol gave a mixture of two carbohydrazide derivatives: mono(salicylidene)carbohydrazide (**1**) and bis(salicylidene)carbohydrazide (**2**) (Figure 1). The reaction products were characterized by NMR spectroscopy in solution. Proton and carbon chemical shifts of **1** and **2** in DMSO-d₆ were assigned by a combination of one- and two-dimensional NMR spectra (Table S1, Supporting Information).

It was observed that NMR spectra exhibited two sets of proton and carbon NMR signals corresponding to two different reaction products, *e.g.* **1** and **2**. Proton signal intensities indicated that the product content was dependent on the temperature and solvent used for the synthesis. The amount of **1** and **2** in methanol, ethanol and propan-2-ol was estimated from the intensity ratio of α(α') proton signals at 8.20 ppm and 8.48 ppm, respectively.

As estimated from the NMR spectra, the highest amount of **1** (58%) was present in the reaction product obtained in methanol. Significantly lower percentage of **1** (28%) was obtained in propan-2-ol. The reaction in ethanol gave the highest portion of **2** (90%).

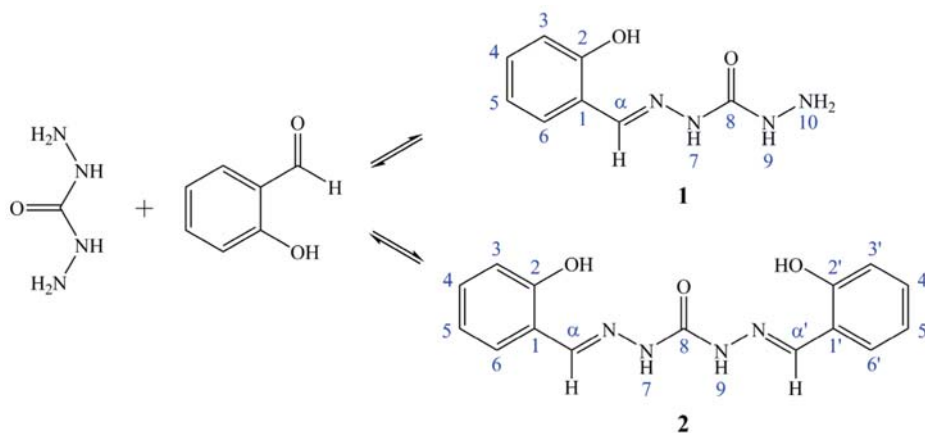


Figure 1. Condensation reaction between carbonylhydrazide and salicylaldehyde, chemical structures and atom numbering of the reaction products.

3. 2. Identification of the Reaction Products in Solid State

Powder samples of **1** and **2** were isolated and characterized in solid state. We have previously performed polymorphic screening and shown that **2** can crystallize in five different polymorphic forms, named I–V.²⁸ Hence, we applied here solid-state NMR and Raman spectroscopies together with SWAXS analysis to characterize the reaction products in various solvents. Solid-state NMR spectroscopy has been considered as one of the most powerful tools for the identification of solid forms.^{29–31} ¹H MAS

and ¹³C CP-MAS spectra of the reaction products were recorded and compared to the ¹H MAS (Figure S1, Supporting Information) and ¹³C CP-MAS spectra of compounds **1** and **2** (Figure 2). The reaction products exhibited three broad ¹H MAS signals whose signal shape and position revealed that they are mixtures of **1** and **2**. This was in accordance with the ¹H NMR spectra recorded in solution (Table S1, Supporting Information).

¹³C CP-MAS spectra were assigned based on the NMR data obtained in solution (Table S1, Supporting Information) and confirmed previous conclusion that the condensation reaction gave a mixture of **1** and **2**. As can be seen in Figure 2, the product synthesized in methanol showed signals at 158.02 ppm, 156.70 ppm and 143.69 ppm, corresponding to C-8, C-2 and C- α atom of **1**, respectively. Broad signals in the region 115–130 ppm were attributed to aromatic carbons of **1** and **2**. The presence of **2** was also confirmed by a weak C- α (α') signal at 149.76 ppm.²⁸ On the other hand, the products synthesized in ethanol and propan-2-ol exhibited high intensity signals at \approx 153 ppm and \approx 131 ppm, previously assigned to C-8 and C-4(4') atoms of **2**, respectively.²⁸ This finding provided evidence that the condensation reaction in ethanol and propan-2-ol yielded a higher amount of **2** than the reaction in methanol.

SWAXS analysis can give further insights both into the crystal content and structure by applying two independent detectors that simultaneously collect the scattered X-ray spectra from a single sample at small (SAXS) and wide (WAXS) angles.^{32,33} Figure 3 compares the SAXS spectra of **1** and five polymorphic forms of **2**²⁷ with the SAXS spectra of powder samples obtained by the condensation reaction in different solvents. The spectra show the logarithm of scattering intensity ($\log I$) versus the scattered vector (q), which is related to the scattering angle 2θ by $q = 4\pi \sin\theta/\lambda$. As shown in Figure 3, the SAXS peak characteristic of **1** was present at $q = 0.29$. In the SAXS spectra of the polymorphs of **2**, two distinct peaks were observed. The peak at $q = 0.45$ was common for poly-

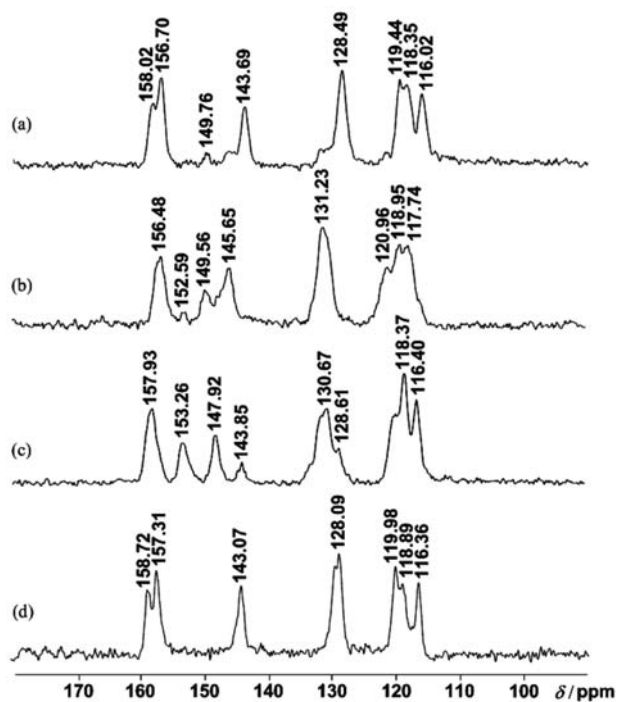


Figure 2. ¹³C CP-MAS spectra of the reaction product obtained in (a) methanol, (b) ethanol and (c) propan-2-ol and (d) pure compound **1**.

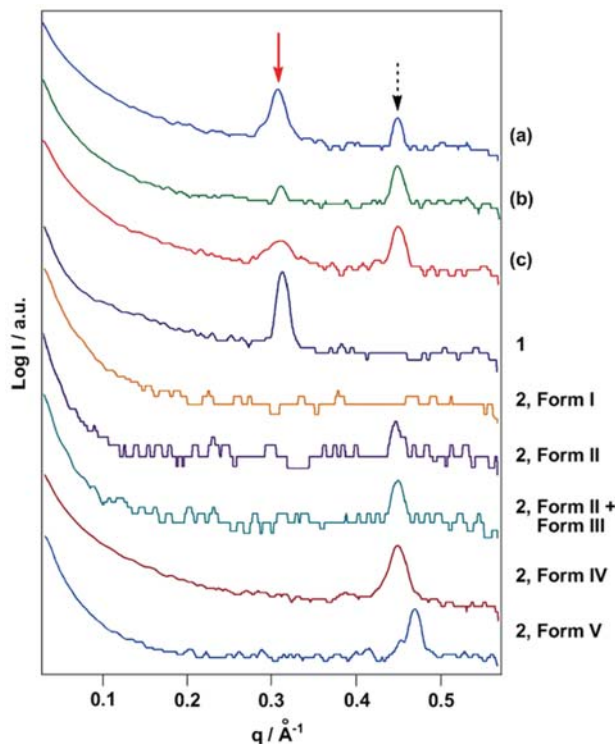


Figure 3. SAXS spectra (logarithmic scale) of the reaction product obtained in (a) methanol, (b) ethanol and (c) propan-2-ol, pure compound **1** and polymorphic forms of **2**. The grey arrow points to the peak characteristic of **1**. The peak corresponding to polymorphic forms II, III and IV is indicated by a dashed black arrow.

morphic forms II, III and IV, while the peak at $q = 0.47$ was characteristic of the form V (see Figure 3). On the other hand, the form I did not exhibit any peak in the SAXS region.

As shown in Figure 3, the reaction products obtained in methanol, ethanol and propan-2-ol exhibited the Bragg peaks characteristic of both **1** and **2** (see the grey and dashed black arrow, respectively). The peak intensity was proportional to the product content estimated from the NMR spectra. Furthermore, the SAXS peak present at $q = 0.45$ indicated that the samples contained at least one or a mixture of polymorphic forms II, III and IV of **2** (see Figure 3). No indication of polymorph V was found.

That finding was further supported by the analysis of WAXS fingerprints specific for the particular crystalline form (Figure 4). The WAXS spectra were recorded in parallel with the SAXS spectra and showed the scattering intensity (I) versus the scattering angle (2θ).

As indicated by grey arrows in Figure 4, the reaction products obtained in methanol, ethanol and propan-2-ol showed the crystallinity peaks at 24.25° , 25.25° and 26.06° which were characteristic of **1**. For the products obtained in methanol and ethanol additional peaks at 19.82° , 20.95° and 22.17° were observed (shown by dashed black arrows in Figure 4) which pointed towards the presence of polymorphic form II of **2**. However, the forms

III and IV could not be excluded. According to WAXS spectra, the form V is not likely to be present in any of the reaction products.

Moreover, the WAXS fingerprint of the product synthesized in propan-2-ol suggested the presence of **1** and the polymorphic form IV of **2** (Figure 4, marked by a dashed rectangle). Characteristic signals of the form II were not identified. Hence, the solvent used for the synthesis had a significant influence on the product ratio and the presence of a particular polymorph.

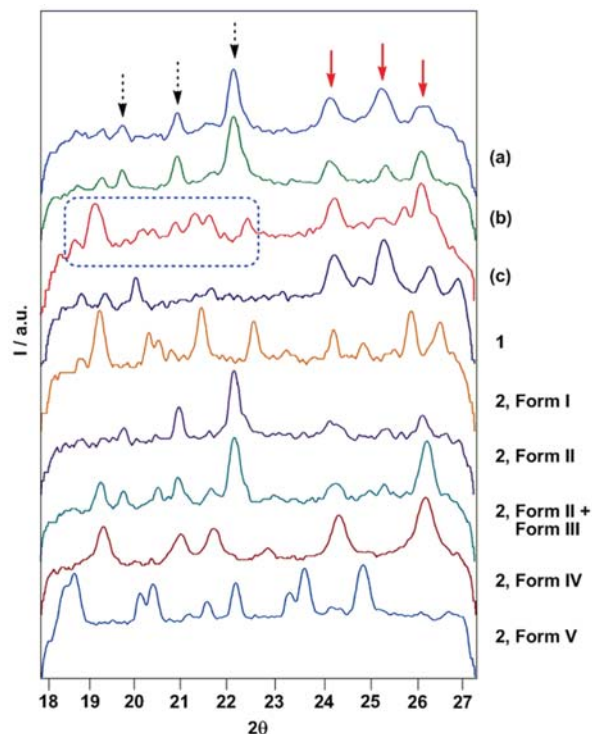


Figure 4. WAXS spectra of the reaction product obtained in (a) methanol, (b) ethanol and (c) propan-2-ol, pure compound **1** and polymorphic forms of **2**. The peaks corresponding to **1** are indicated by grey arrows. The WAXS fingerprint corresponding to polymorphic forms II and III of **2** is marked by dashed black arrows. The peak characteristic of polymorphic forms I, III and IV of **2** is marked by a dashed rectangle.

Prior to *in-line* reaction monitoring, the *off-line* Raman spectra of pure compound **1** and polymorphs of **2** were recorded and are displayed in Figure 5. The most pronounced differences were observed in the spectral region $1800\text{--}1000\text{ cm}^{-1}$.

Strong vibrational bands at 1607 cm^{-1} and 1610 cm^{-1} were assigned to C=N stretching modes of **1** and **2**, respectively (Figure 5). The relative intensity of vibrational band at $\approx 1575\text{ cm}^{-1}$, attributed to symmetric C=C stretching mode of benzene ring, was considerably higher in the Raman spectrum of **1** as compared to that of **2**.²⁸ The prominent feature in the Raman spectrum of **1** was the presence of NH₂ deformation mode at 1621 cm^{-1} . On the other hand, **2** exhibited a weak NH deformation band

at $\approx 1490\text{ cm}^{-1}$. Additional band in the range $1465\text{--}1450\text{ cm}^{-1}$ in the Raman spectra of **2** was assigned to asymmetric C=C stretching vibration of benzene ring. As shown in Figure 5, this band was shifted to higher wavenumbers (1476 cm^{-1}) in the Raman spectrum of **1**. Furthermore, weak C–H deformation bands occurred in the spectra of both derivatives in the range $1240\text{--}1249\text{ cm}^{-1}$. The C–O stretching bands were found in the region $1170\text{--}1191\text{ cm}^{-1}$.²⁸ Some of these bands might serve as indicators of the reaction progress in real time and can be used to differentiate between two derivatives. The recorded *off-line* Raman spectra of reaction products (Figure 5-a, b and c) corroborated the data obtained by SWAXS and solid state NMR. The analysis of spectral bands pointed towards the presence of both **1** and **2** in all solvents. It is seen in Figure 6 that the spectra of polymorphic forms of **2** are very similar and therefore could not be straightforwardly used for polymorph identification of the reaction products solely by Raman spectroscopy. However, it can be concluded that the form II could not be present in propan-2-ol being in agreement with SWAXS.

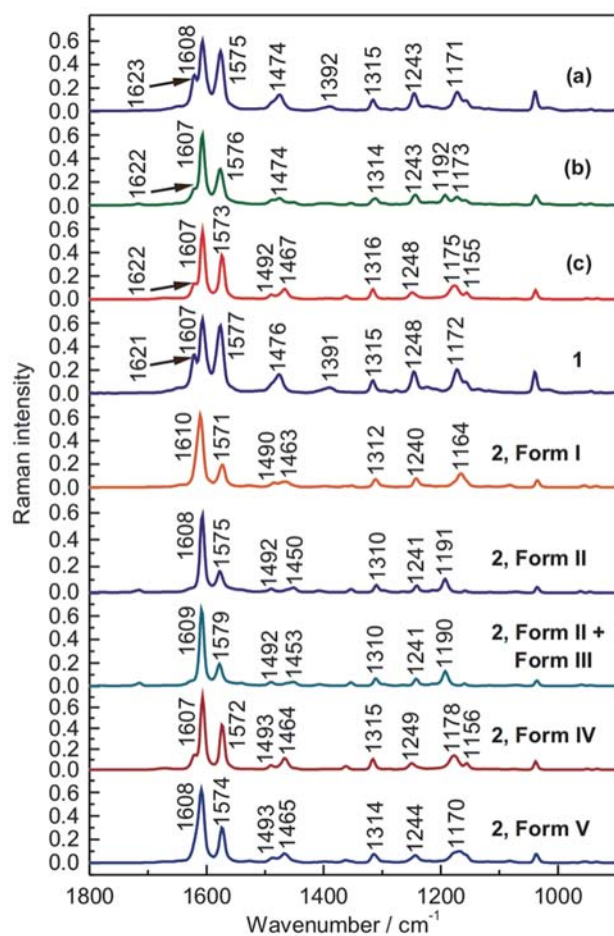


Figure 5. *Off-line* Raman spectra of the reaction product obtained in (a) methanol, (b) ethanol and (c) propan-2-ol, of pure compound **1** and of polymorphic forms of **2**.

The *off-line* NIR spectra of the compounds **1** and **2** exhibited low intensity overlapping bands which prevented an accurate spectral interpretation.

3.3. *In-line* Reaction Monitoring

In this work we were particularly interested to explore the possibilities of vibrational spectroscopy for *in-line* monitoring of the condensation reaction between carbohydrazide and salicylaldehyde as a model system for the preparation of Schiff bases and identification of the reaction products in real time. As already mentioned, NIR and Raman spectroscopies have proven useful for control of the chemical and physical processes. We applied here Raman spectroscopy to monitor the reaction products and NIR to monitor the reactants. The obtained *in-line* spectra were further processed by principal component analysis.

The *in-line* NIR and Raman spectra were collected continuously (1.0 min and 3.7 min per spectrum, respectively) during the reaction in methanol, ethanol and propan-2-ol. Figure 6 compares the representative spectra of the reaction in ethanol. Corresponding spectra in methanol and propan-2-ol are given as Supplementary Information.

As shown in Figure 6-a, the *in-line* NIR bands at 6559 cm^{-1} , 6437 cm^{-1} and 6397 cm^{-1} , attributed to a combination of the overtone NH and NH₂ stretching vibrations, disappeared during the progress of the reaction. The disappearance of these bands, characteristic for urea derivatives,⁴ indicated that the content of carbohydrazide was decreasing with time. That was further supported by a decrease in intensity of vibrational bands in the spectral region $5000\text{--}4500\text{ cm}^{-1}$ attributed to combinations of the overtone N–H and C–N stretching and deformation modes. The second reactant, salicylaldehyde, exhibited the combination band at 7078 cm^{-1} , which also decreased during the reaction course. Similar findings were observed in other solvents as well.

The representative *in-line* Raman spectra of the condensation reaction in ethanol are displayed in Figure 6-b. The presence of vibrational bands at $\approx 1610\text{ cm}^{-1}$ was assigned to amide C=N stretching mode and was characteristic of both carbohydrazide derivatives. Several weak bands in the spectral region $1400\text{--}1100\text{ cm}^{-1}$, attributed to C–H deformation and C–O stretching vibrational modes, further confirmed the formation of the reaction products.²⁸ The Raman band which occurred at $\approx 1454\text{ cm}^{-1}$ might be assigned to asymmetric C=C stretching vibrations. On the other hand, that observed at $\approx 1479\text{ cm}^{-1}$ is probably due to N–H deformation band of **2** overlapped with C=C of **1**. Strong symmetric C=C stretching band observed at $\approx 1580\text{ cm}^{-1}$ also indicated that both compounds might be present in the reaction mixture. The relative intensity of this band was lower in the *in-line* Raman spectra of the reaction in ethanol and propan-2-ol with respect to methanol (Figures S2 and S3, Supporting Information). Surprisingly, a characteristic Raman band at 1621 cm^{-1} observed for **1** in the *off-line* spectrum was

not observed in the *in-line* spectrum in methanol and in neither of the two other solvents. This might be accounted for by possible hydrogen bonding interactions between NH_2 group and solvent molecules which shifts this vibration to lower wavenumbers.

In order to estimate the reaction end points, we processed NIR and Raman spectral data using principal component analysis. The scores and loadings for *in-line* NIR and Raman spectra in methanol, ethanol and propan-2-ol are given as Supporting Information (Figures S4–S9). The greatest extent of variance for all solvents was described by the first principal component (PC1). It was observed that the PC1 scores for *in-line* NIR spectra were decreasing with time, indicating that the reactants were consumed during the course of the reaction. That was in accordance with the decrease in intensity of *in-line* NIR bands characteristic of carbohydrazide and salicylaldehyde (Figure 6-a). On the other hand, the PC1 scores for corresponding *in-line* Raman spectra in methanol and ethanol were increasing with time, as a consequence of the formation of the reaction products. In the case of propan-2-ol, the product loading peaks were negative, resulting with a slight decrease of the PC1 scores.

Furthermore, analysis of score plots made it possible to estimate the reaction end points in each solvent. Table 1 compares the reaction times estimated from the score plots of *in-line* NIR and Raman spectra with correspon-

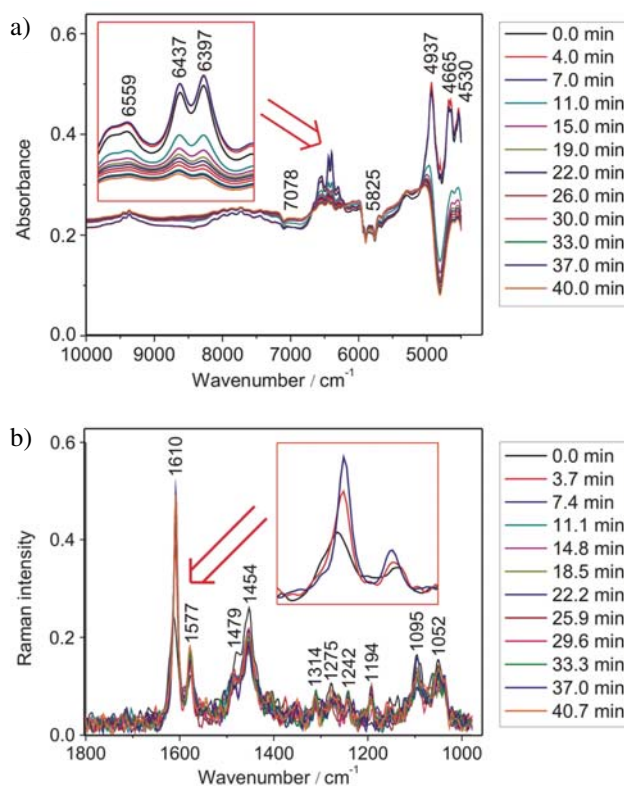


Figure 6. Representative (a) *in-line* NIR and (b) *in-line* Raman spectra of the condensation reaction between carbohydrazide and salicylaldehyde in ethanol. Significant vibrations are enlarged and indicated by arrows.

ding reaction temperatures. In ethanol and propan-2-ol, the reaction ended after approximately 12 minutes and 9 minutes, respectively. On the other hand, the reaction in methanol was slower than in the other solvents, reaching a final state after approximately 33 minutes. Similar results were obtained from the score plots of *in-line* Raman spectra. The data presented in Table 1 clearly indicates that the reaction rate is proportional to the temperature in the laboratory reactor. From the score plots (Figures S4–S9, Supporting Information) it is also clear the reaction starts immediately after the addition of salicylaldehyde to the solution of carbohydrazide.

In the PC1 and PC2 loadings for the reaction in ethanol, the positive peaks corresponding to *in-line* NIR

Table 1. The reaction times estimated from the score plots of *in-line* NIR and Raman spectra with corresponding reaction temperatures

Solvent	<i>In-line</i> NIR <i>t</i> / min	<i>In-line</i> Raman <i>t</i> / min	<i>T</i> / °C
Methanol	33	29	55
Ethanol	12	15	65
Propan-2-ol	9	7	75

bands characteristic of carbohydrazide were observed around 6550 cm^{-1} , 6440 cm^{-1} and 6400 cm^{-1} . Several weak bands which occurred in the region $8200\text{--}7500\text{ cm}^{-1}$ were also attributed to the combination vibrations characteristic of carbohydrazide. As the PC1 was decreasing with time, the positive peaks indicated that carbohydrazide was consumed during the progress of reaction. Furthermore, a broad band around 7000 cm^{-1} observed in the PC2 loadings pointed towards the presence of the second reactant, salicylaldehyde in the reaction mixture.

The PC1 and PC2 loadings for the reaction in methanol showed the carbohydrazide bands in the regions $9500\text{--}9300\text{ cm}^{-1}$, $8200\text{--}7500\text{ cm}^{-1}$ and $5400\text{--}5200\text{ cm}^{-1}$. Similar considerations were applied to the reaction in propan-2-ol. Hence, characteristic bands in the PC1 and PC2 loadings provided evidence that the content of carbohydrazide and salicylaldehyde was decreasing during the chemical reaction.

Vibrational bands characteristic of the reaction product were observed in the PC1 loadings for the *in-line* Raman spectra. Hence, the peaks at $\approx 1600\text{ cm}^{-1}$ and 1200 cm^{-1} , whose intensity increased during the reaction progress, were attributed to C=O and C–N stretching bands, respectively. Additional peak at $\approx 1580\text{ cm}^{-1}$, assigned to symmetric C=C stretching mode, further confirmed the product formation.

4. Conclusions

The combined use of *in-line* vibrational NIR and Raman spectroscopies together with principal component

analysis made it possible to monitor the condensation reaction between carbohydrazide and salicylaldehyde in different solvents, and to detect the reaction products, mono(salicylidene)carbohydrazide (**1**) and bis(salicylidene)carbohydrazide (**2**). The reaction end point in methanol, ethanol and propan-2-ol has been estimated as well. The reaction products have been characterized in solution and solid state by using NMR and Raman spectroscopies and SWAXS.

This study has demonstrated that the described approach can be useful for fast and non-destructive real-time monitoring of the Schiff base condensation reactions. However it has some drawbacks, such as complexity of the reaction system, sensitivity of the method and vibrational band overlapping. These issues should be taken into consideration when using *in-line* vibrational spectroscopy for Schiff base reaction monitoring in the future.

5. Acknowledgements

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Povzetek

S pomočjo NIR vibracijske in ramanske spektroskopije ter z uporabo statističnih metod smo spremljali reakcijo kondenzacije *in-line* med karbohidrazidom in salicilaldehidom. Pred tako imenovano *in-line* analizo podatkov, smo reakcijske produkte predhodno popolno okarakterizirali tako v raztopini kot v trdnem stanju. Namen tega je bil preveriti potencial *in-line* pristopa kot orodja za proces kontrole reakcije Schiffovih baz. Pokazali smo, da lahko s pomočjo kombinacije vibracijske spektroskopije in analize glavnih komponent zaznamo in določimo reakcijske produkte, kot so npr. mono(saliciliden)karbohidrazid (**1**) in bis(saliciliden)karbohidrazid (**2**) v različnih topilih ter določimo konec reakcije v realnem času. Zaradi kompleksnosti reakcijskih zmesi in posledično prekrivanja signalov, nismo mogli določiti relativnega razmerja reakcijskih produktov *in-line*. Tako imenovana *off-line* analiza je pokazala, da je spojina **1** prevladujoča zvrst v metanolu kot topilu, medtem ko je bil največji delež spojine **2** opažen v prisotnosti etanola.