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# Designing ColorX, Image Processing Software for Colorimetric Determination of Concentration, To Facilitate Students' Investigation of Analytical Chemistry Concepts Using Digital Imaging Technology

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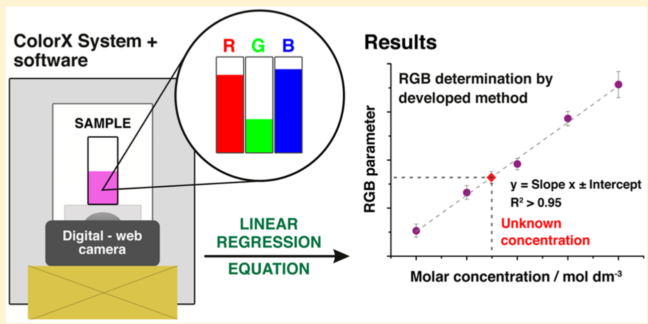
Supporting Information






**ABSTRACT:** This work combines laboratory quantitative analysis of colored solutions and common devices for digital imaging (digital or web cameras or mobile phones, i.e., smartphones). ColorX software, specially designed for this study, was used for data collection and analysis in order to calculate concentrations of colored solutions from measured RGB values. Three different custom methods for determination of concentration have been developed: (i) RGB value measurement by pixel, (ii) RGB value measurement by pixel and Gaussian blur, and (iii) calculation of average RGB value of the selected image area. The performance of the developed software, ColorX, is demonstrated using different colored solutions, $KMnO_4$ (purple), $CoSO_4$ (red), $NiSO_4$ (green), and $CuSO_4$ (blue) solutions, as well as the Lowry protein assay (blue) in terms of its determination of the concentrations of unknown samples. The most suitable and effective method for studying the mentioned solutions was the calculation of an average RGB value for a selected image area. ColorX software is primarily designed for accessibility and simplicity, with the aim of promoting and encouraging students to explore and discover potential applications for digital imaging technology in basic analytical chemistry concepts.

**KEYWORDS:** *High School/Introductory Chemistry, Analytical Chemistry, Computer-Based Learning, Laboratory Equipment/Apparatus, Quantitative Analysis*

## ■ INTRODUCTION

Colorimetric methods are commonly used for quantitative chemical analysis. To perform accurate colorimetric measurements, many instruments can be used, such as UV−visible (UV−vis) spectrophotometers or colorimeters, which can measure the absorbance of incident light. UV−vis absorption spectroscopy is based on measuring the absorbance of light that passes through an analyte and is absorbed by the analyte at specific wavelengths. As described by Beer's law, there is a linear correlation between absorbance and the concentration of analyte: $A = \varepsilon bc$, where $A$ is absorbance, $\varepsilon$ is the molar absorptivity, $b$ is the path length of the sample, and $c$ is the concentration of the compound present in the solution. Students are introduced to Beer's law in the early stages of their education, usually through laboratory practice. Because the measured absorbance is directly proportional to the concentration of the analyte, the amount of light absorbed by sample, and the path length of the sample (i.e., the parameters stated in Beer's law), students are able to measure and perform calculations independently, even in high schools.

Adequate access to commercial spectrophotometers can be an issue in many high school laboratories. Most high school students have access to electronic devices and are familiar with digital and web cameras and camera-enabled hand-held devices such as mobile phones, technology that can eventually be used as a source for quantitative analysis of colored compounds with sufficient precision and accuracy.

Analytical systems are interesting topics to study, exhibiting characteristics such as cost-effectiveness, simplicity, and portability. Because many chemical and analytical procedures are based on visual detection approaches, the accuracy and precision of analyses have been improved through the development of more advanced instrumentation, such as spectrophotometers or cameras.[1] However, commercial spectrophotometers are costly and bulky. Development of portable analytical devices and instrumentation has allowed the

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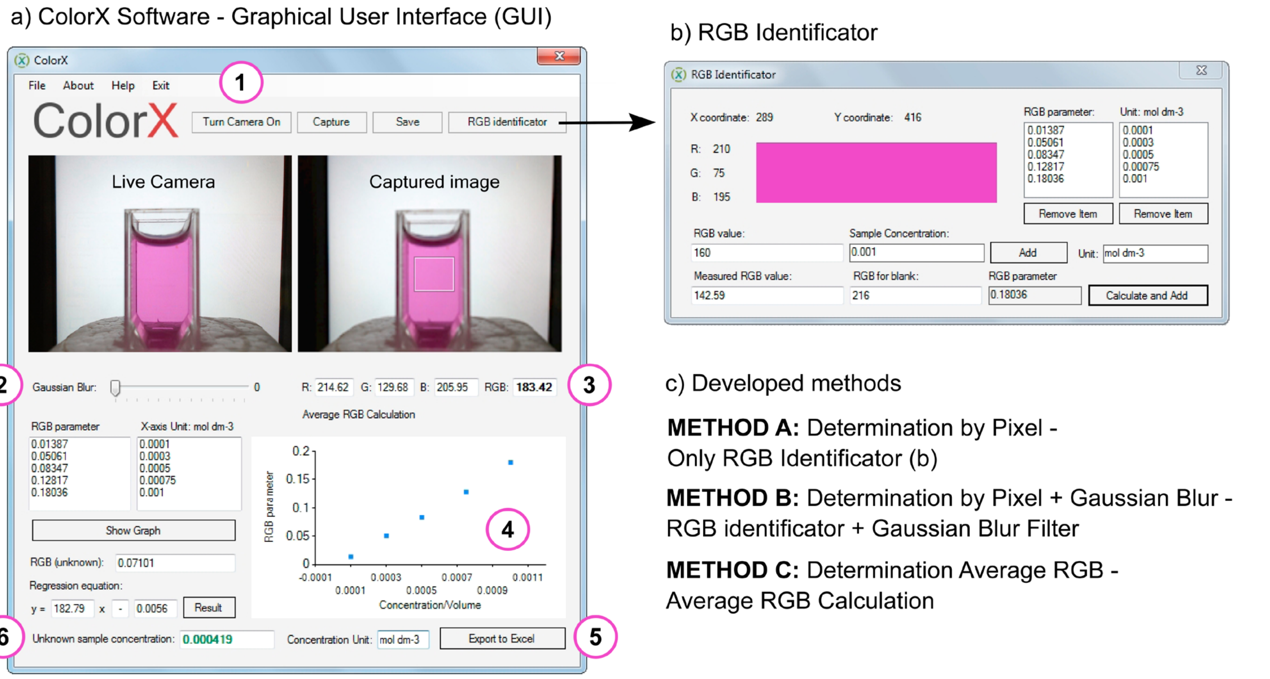


**Figure 1.** Developed graphical user interface (GUI) for the ColorX system. (a) Main interface (form) that includes six elements: (1) camera control, used to turn on the camera (live video), take pictures, and save images to the computer's desktop; (2) Gaussian blur filter, which blurs images by an adjustable amount (0, no blur effect is applied; 12, maximum blurring applied to an image); (3) average RGB values obtained by calculation of the image area selection; (4) data structured in a graph for initial insight into the results, expressed as concentration or volume; (5) button for data export to Excel through Library extension; and (6) final result displayed within a textbox. (b) GUI for the RGB Identificator, which is used to the determine RGB values at the mouse cursor position and to calculate the RGB parameters. (c) Three developed methods with their individual characteristics.

use of built-in camera detectors, which are widely used in chemical imaging analysis; therefore, image documentation is interesting and beneficial for additional studies. Colorimetric calibration prior to image processing and interpretation has gained increasing attention in some fields, such as biology,[2,3] food science,[4,5] medicine,[6] and environmental monitoring.[7] The images can be used for many biological applications[8−10] and determination in analytical fields.[11−13]

As a physical phenomenon, color can be defined as the visible electromagnetic spectrum reflected by an object. This spectrum is then perceived by a sensor within its detection range, conveying the most important layout attributes of the object. Each color is defined by the type of emission source that irradiates on an object, by the physical properties of the object itself (radiation reflected by an object that is detectable), and by the medium in-between the object and source (e.g., air or water).[14,15] In general, color information can be described by the color spaces of the object, most commonly defined by the three chromaticities of the standard red−green−blue (RGB) components.[16]

The digital-image information can be obtained from various types of cameras (TV cameras,[17] digital cameras,[18] webcams,[1] and mobile phone cameras[11,19,20]). The camera detector can be used instead of more expensive spectrophotometers, colorimeters, or fluorescence or chemiluminescence spectrophotometers.[21−24] Cameras have also been applied as alternative detector units for chemical and biochemical analysis systems.

A common 24 bit RGB image consists of three 8 bit color channels, namely, the red (R), green (G), and blue (B) channels, forming an additive color space. Different combinations of R, G, and B intensities can produce a wide spectrum of different colors and 256 variations in color intensity (from 0 to 255). In this color scheme, it is assumed that model could obtain 16,777,216 different colors ($256^3$ or $2^{24}$). For example, the RGB combination R = 0, G = 0, and B = 0 refers to pure black, whereas R = 255, G = 255, and B = 255 produces pure white.[25] This system provides unique combinations of R, G, and B values, producing lots of different colors, intensities, and lightness shades.[26] These dynamic colors of images provide the theoretical basis for quantitative analysis.

The most common image processing technique is undoubtedly grayscale mode.[27] For learning image processing, it is better to understand the complexity of colors. Processing of raw data is possible, though quite difficult even with modern technology. "Averaging" is the most common grayscale mode using one color channel instead of the three-channel RGB image mode. This provides faster image processing with the ability to differentiate gray intensity levels. Thus, grayscale mode could be useful in the determination of colored standard solution concentrations within optimized concentration ranges, if the collected data provided a good linear model or by selecting the "best-fitting" model.

Although several proposals based on RGB analysis have been previously described, most of the literature refers to the determination of RGB values using open-source or commercial software, such as ImageJ,[2,28,29] Photoshop,[2,29] PowerPoint,[28] or Google applications[20] (for result plotting). This potentially requires the use of multiple software applications for a single analytical procedure. Here we suggest an all-in-one approach to data collection, interpretation, and visualization, which is more convenient for teaching purposes. Thus, our software includes all the necessary components and algorithms to perform measurements simply and rapidly, and neither



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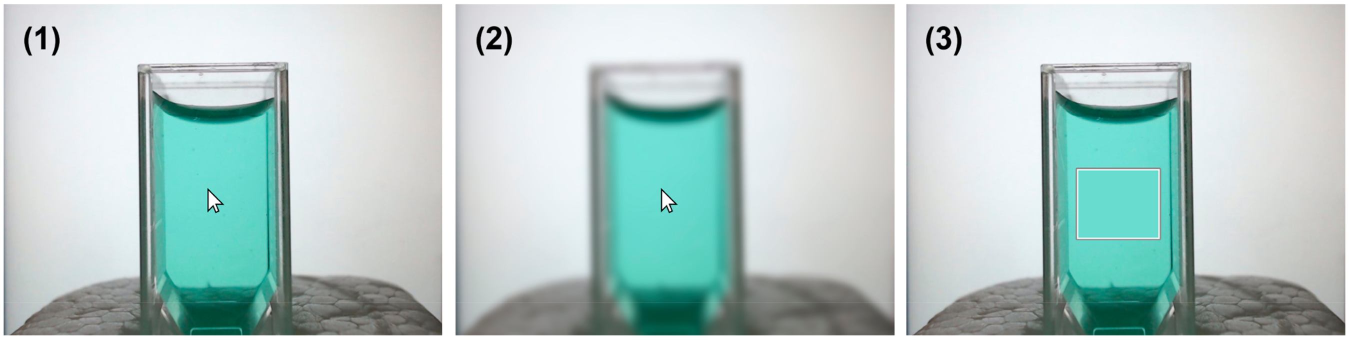


**Figure 2.** Graphical representation of the developed methods: (1) determination by pixel, (2) determination by pixel with Gaussian blur, and (3) average RGB.

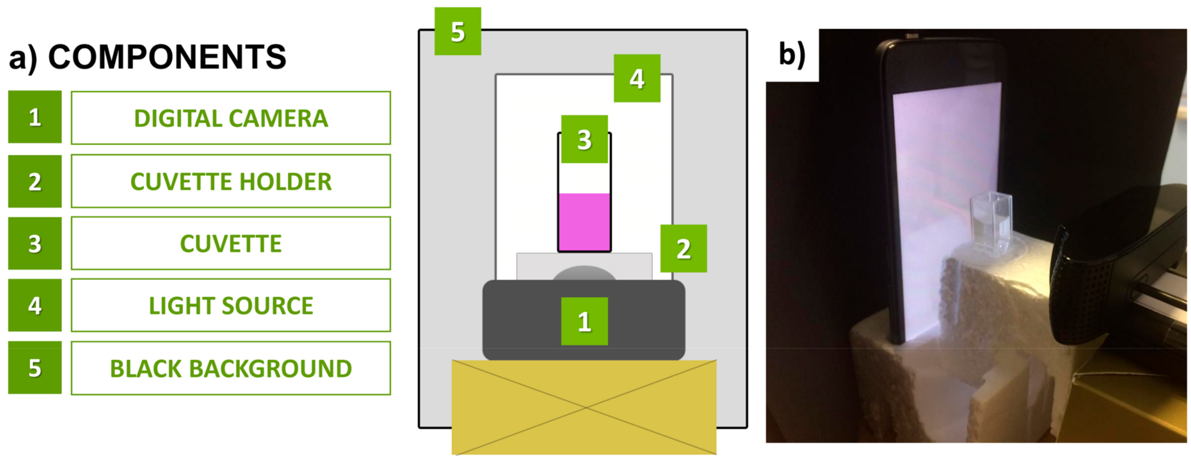


**Figure 3.** (a) Schematic figure and (b) photograph of the developed ColorX system with the main components included.

programming nor imaging experience is required, making it more user-friendly for students and teachers. Also, some authors[12,20,29] make an analogy between RGB mode and fundamentals stated in Beer's law; however, our main idea was to enhance interdisciplinary awareness (computer science and programming, chemistry, and mathematics, all STEM-related disciplines) with minimal changes to the image processing and mathematical concepts.

This work combines simple laboratory quantitative analysis and a common digital camera as a detector in one unit. Digital cameras have been used for color detection and are also sensitive to changes in color intensity in solutions. The developed software, ColorX, specially designed for this study, could be used for data collection and analysis through an algorithm for calculation of the specific parameters from measured RGB values.

ColorX integrates different methods for RGB−grayscale-based measurements, allows analysis of collected data with an Excel library extension, and solves mathematical models with simple linear regression all within one Windows application. The software is primarily designed for accessibility and simplicity, with the aim of promoting and encouraging individuals or students to explore and discover potential applications of technology in basic analytical chemistry concepts. This study could be also an example of how STEM fields can be interrelated and used to create interesting ideas that can be employed in educational environments.

## ■ EXPERIMENTAL SECTION

### Concept and Data Analysis

A Windows-based software used for measurement and data collection, ColorX, was developed using the Visual Basic programming language and can be downloaded at https://app-colorx.com/. ColorX has a user-friendly graphical user interface, as shown in Figure 1. The image acquisition is enabled directly from a connected digital (USB) camera with the option of automatic camera device detection, and the option of importing images acquired by other camera devices is also included in the software as an alternative.

Moreover, three different methods have been developed (see Figure 2), and their measurement results have been compared in order to find the most suitable method.

The first method uses one pixel from the captured or imported image that had been previously determined through the function Bitmap. GetPixel, which retrieves the RGB color values of the pixel at the specified coordinates ($x$−$y$ coordinate of the mouse cursor position). It is very important to choose the same sampling point for each sample, and that point needs to be truly representative to obtain accurate and precise measurements. By combining the first method with an imaging filter, the second method was developed. Added Gaussian blur filter significantly affects the image quality and removes outlier pixels that may be noise in the image.

One additional method was developed later in the research process, and it was expected to obtain the most reproducible and precise results from a statistical approach. The created algorithm provides area selection and calculation of the average



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RGB values of the rectangle shape defined by the image coordinates or, more precisely, the PictureBox control used for displaying images.

Therefore, in the first and second methods, a single pixel is used, and in the third method, the image area is selected for RGB value calculation. As a result, these methods allow measurement and calculation of initial RGB values, as described by eq 1:

$$\langle I_{\mathrm{RGB}}\rangle_{\mathrm{std/unknown}} = \frac{R_{\mathrm{pixel}} + G_{\mathrm{pixel}} + B_{\mathrm{pixel}}}{3} \quad (1)$$

where $\langle I_{\mathrm{RGB}}\rangle_{\mathrm{std/unknown}}$ is the product of averaging the initial values of the red ($R_{\mathrm{pixel}}$), green ($G_{\mathrm{pixel}}$), and blue ($B_{\mathrm{pixel}}$) channel intensities for the standard or unknown solutions. For convenience, data was linearized using logarithmic scaling by following eq 2. In the averaging method, the grayscale level intensity is produced.

It is well-known that an increase in an RGB value does not give a proportional change in color intensity (i.e., concentration of solution); thus, a logarithmic scale was used for convenience and ease of interpretation of the results, as given by eq 2:

$$\langle I_{\mathrm{RGB}}\rangle_{\mathrm{p}} = \log\left(\frac{\langle I_{\mathrm{RGB}}\rangle_{\mathrm{blank}}}{\langle I_{\mathrm{RGB}}\rangle_{\mathrm{std/unknown}}}\right) \quad (2)$$

where $\langle I_{\mathrm{RGB}}\rangle_{\mathrm{blank}}$ is the measured RGB value for the blank sample. Measurement of a blank sample in the absence of color allows the noise and background to be reduced and optimized. The obtained results with the RGB parameters (denoted by $\langle I_{\mathrm{RGB}}\rangle_{\mathrm{p}}$) and the corresponding sample concentrations need to be exported to the Excel extension to perform linear regression exclusively. The final result is calculated using a regression equation within our Windows application.

### Design and Construction

For the system shown below in Figure 3a, a small piece of polystyrene foam was used to hold the cuvette and a white screen firmly in place. A fixed camera position is also very important when taking sample images and measuring RGB values. A black background positioned directly behind the white screen and cuvette is considered to be an important element in reducing external light, which interferes with the measurement process. A white screen is simply used for sample illumination and improvement of color detection. Figure 3b shows an example of the overall system with the positioned components and equipment used for measurement. Complete instructions for installing and using the software and its features are included in the user's guide (Supporting Information).

### Materials and Equipment

All solutions used for experiments were prepared from analytical grade chemicals. Potassium permanganate ($KMnO_4$), copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), nickel sulfate heptahydrate ($NiSO_4{\cdot}7H_2O$), and cobalt sulfate heptahydrate ($CoSO_4{\cdot}7H_2O$) were purchased from Kemika. Deionized water (conductivity $\leq 0.055$ $\mu$S/cm) was used for dilutions. Bovine serum albumin (BSA) was purchased from VWR, and commercial Folin−Ciocalteu phenol reagent was supplied by Reagecon and prepared by 1:1 dilution in water.

**Preparation of Chemicals and Calibration.** Stock solutions of $KMnO_4$($c(KMnO_4)$ = 0.02 mol dm$^{-3}$) and of the copper, nickel, and cobalt salts ($c$(salt) = 1.00 mol dm$^{-3}$) were prepared by weighing the corresponding masses of solid chemicals. Calibration standard solutions and samples with unknown concentrations were prepared by diluting stock solutions to different concentration levels, covering concentration of the unknown samples, as follows: (1) For $KMnO_4$, the dilution series covered the range $1 \times 10^{-4}$ to $1 \times 10^{-3}$ mol dm$^{-3}$, and the unknown concentration was $4 \times 10^{-4}$ mol dm$^{-3}$. (2) For the copper, nickel, and cobalt salts, the dilution series covered the range 0.10 to 0.50 mol dm$^{-3}$, and the unknown concentration was 0.25 mol dm$^{-3}$. The solutions were appropriately diluted for accurate and precise UV−vis measurements. Laboratory documentation for additional software testing is included in the Supporting Information.

Alkaline copper reagent for the Lowry method was prepared on the day of the experiment according to the following protocol: (1) $Na_2CO_3$ (50 mL, 2%) was dissolved in NaOH (0.1 mol dm$^{-3}$). (2) $CuSO_4{\cdot}5H_2O$ solution (0.5 mL) and KNa tartrate (0.5 mL, 2%) were added to the previously prepared solution. (3) Standard solutions (10, 20, 40, 60, 80, and 100 $\mu$g per 100 mL of BSA protein solution; $\gamma$(BSA) = 1 mg/mL) were prepared in 1.5 mL test tubes with 1 mL of the alkaline copper reagent and 100 $\mu$L of the Folin−Ciocalteu phenol reagent. The blank sample was prepared by mixing 1 mL of the alkaline copper reagent and 100 $\mu$g of the Folin−Ciocalteu phenol reagent with 100 $\mu$L of deionized water. The total volume of the mixture (BSA and deionized water) per reaction was expected to be equal to 100 $\mu$L after addition of the appropriate quantities of BSA and deionized water.

**Equipment.** Samples were analyzed using our software with the developed methods, and the results were compared with those obtained by UV−vis spectrophotometry. Our software meets the minimum hardware requirements for optimal and high performance. Experiment testing was carried out using the equipment listed in the laboratory documentation included in the Supporting Information. Absorption spectra were recorded by means of a Shimadzu 1700 UV−vis spectrophotometer, and data analysis was performed with the UVProbe software package.

## ■ HAZARDS

Protective gloves and safety glasses are highly recommended when working with chemicals. Potassium permanganate is a strong oxidizing agent, and it can cause irritation of the skin and discoloration of the skin and clothes. Copper(II), nickel(II), and cobalt(II) salts are harmful if swallowed or inhaled. Folin−Ciocalteu phenol reagent (a mixture of phosphomolybdic and phosphotungstic acids) is corrosive and should be handled with care. Special caution is needed in preparing sodium hydroxide solution. The mixing of solid sodium hydroxide pellets with water should be done slowly to prevent the excess evolution of heat.

## ■ RESULTS AND DISCUSSION

### Software Testing on Potassium Permanganate, an Example of a Model System

The laboratory experiment was conducted with 20 volunteer students in their first and second years of undergraduate chemistry studies. The students were divided into four small groups of five students each. During a 60 min class, five series of experiments were conducted, one for each working group of students, in which the concentration of potassium permanga-



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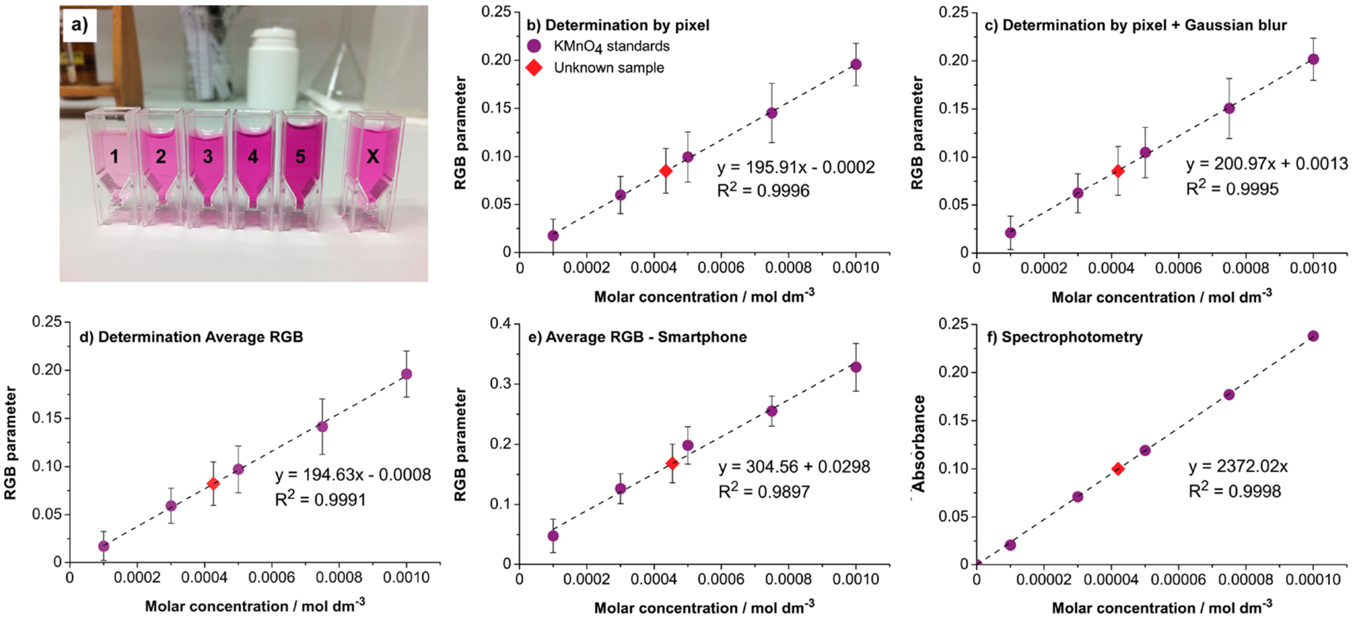


**Figure 4.** Comparison of the results obtained via RGB determination and absorbance measurement of $KMnO_4$ solutions as an example of a model system. (a) Photograph of the cuvettes with the prepared standard solutions and the sample with the unknown concentration. (b–d) Graphs of RGB parameters vs molar concentrations of the samples with corresponding $R^2$ coefficients. All images were obtained with a digital camera. (e) Test measurement with images obtained with a smartphone. (f) Absorbance vs molar concentration data measured by UV–vis spectrophotometry. Measurement was performed with a UV–vis spectrophotometer at $\lambda_{max}$ = 550 nm after dilution.

nate was determined with both a digital camera and a smartphone camera.

A calibration curve was obtained by plotting the calculated RGB values, denoted as RGB parameters, versus the concentrations of the five measured standard solutions for each tested analyte. First, the developed software was tested using potassium permanganate as a model system in order to determine the most adequate and accurate method for concentration determination. The results indicated that there were no significant differences in data obtained by our developed methods. The data shown in Figure 4 are the means of quintuplicate ($n$ = 5 series) measurements with standard error as indicated by the error bars. The results show good linearity for the calibration curves produced by all three developed methods over the entire investigated concentration range for potassium permanganate ($1 \times 10^{-4}$ to $1 \times 10^{-3}$ mol $dm^{-3}$), with $R^2$ ranging from 0.9897 to 0.9996. Using the equation for the standard curve calculated with an Excel extension by means of linear regression, the concentrations of unknown samples were calculated for each method as follows: (1) Determination by pixel (see Figure 4b) yielded a concentration of $(4.4 \pm 0.4) \times 10^{-4}$ mol $dm^{-3}$. (2) Determination by pixel with Gaussian blur (see Figure 4c) yielded an estimated concentration of $(4.2 \pm 0.6) \times 10^{-4}$ mol $dm^{-3}$. (3) The unknown concentration of potassium permanganate measured by method denoted as determination of average RGB (see Figure 4d) was $(4.3 \pm 0.3) \times 10^{-4}$ mol $dm^{-3}$. For the purpose of software testing, the concentration of the potassium permanganate solution was $4.00 \times 10^{-4}$ mol $dm^{-3}$ for the unknown sample.

It was found that the determination of average RGB method (see Figure 4d) produced more reliable and precise results, as seen in the lower standard deviations obtained. The experimental findings showed lower reproducibility and higher sensitivity to large movements of the mouse cursor in the first and second methods (determination by pixel and determination by pixel with Gaussian blur), in comparison with the determination of average RGB method, which was developed to improve the accuracy of the measurement from a statistical approach. Hence, the third developed method, based on calculation of average RGB values in a selected image area, was selected for additional testing and analysis of several analytes. Measurements from images acquired using a smartphone (see Figure 4e) were also performed with the selected method, and for the example of potassium permanganate, the concentration of the unknown sample was determined to be $(4.5 \pm 0.7) \times 10^{-4}$ mol $dm^{-3}$. Our findings indicate that the increase in RGB parameter deviations could be due to the higher sensitivity of potassium permanganate to illumination conditions and angle changes when the images of the samples were being taken with the camera devices; this phenomenon was particularly noticeable in the results obtained from the analysis of smartphone images. A detailed step-by-step measurement procedure for the experiment is provided in the Supporting Information as Microsoft Excel workbook.

Moreover, consistent results were also obtained with respect to those obtained by the standard spectrophotometric method (see Figure 4f) after the $KMnO_4$ solutions were appropriately diluted. A UV–vis absorption measurement at $\lambda_{max}$ = 550 nm yielded an estimated concentration of $4.2 \times 10^{-5}$ mol $dm^{-3}$ for the unknown sample, indicating that our methods are not only simple to use but also functional within the optimal accuracy ranges.

### Additional Software Testing on Copper(II), Nickel(II), and Cobalt(II) Solutions

Standard solutions of several analytes were studied by the selected method, and images were acquired using both a digital camera and a smartphone. The results obtained by measuring the average RGB values of the $CuSO_4$ solution images were compared with the standard UV–vis absorption measurements at $\lambda_{max}$ = 790 nm in order to evaluate the method. The



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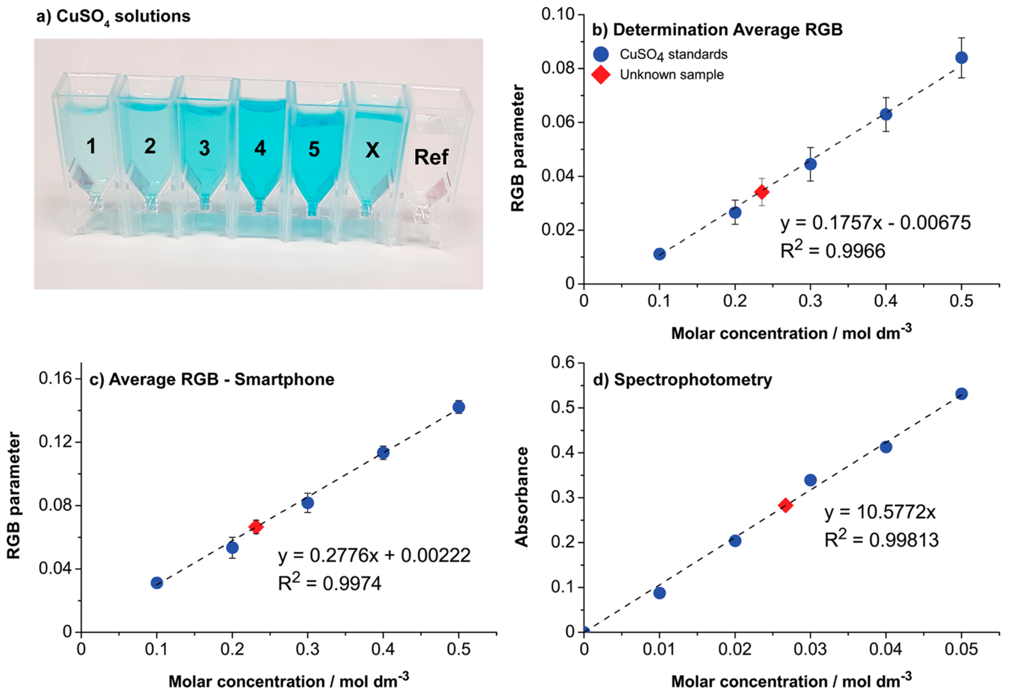


**Figure 5.** Comparison of the results obtained via RGB determination and absorbance measurement of $CuSO_4$ solutions for additional software testing. (a) Photograph of the cuvettes with the prepared standard solutions and the sample with the unknown concentration. (b,c) Graphs plotting RGB parameters vs molar concentrations of the samples analyzed by the selected method. Images were obtained by using both a digital camera (b) and a smartphone (c). (d) Absorbance vs molar concentration data measured by UV−vis spectrophotometry. Measurement was performed with a UV−vis spectrophotometer at $\lambda_{max}$ = 790 nm.

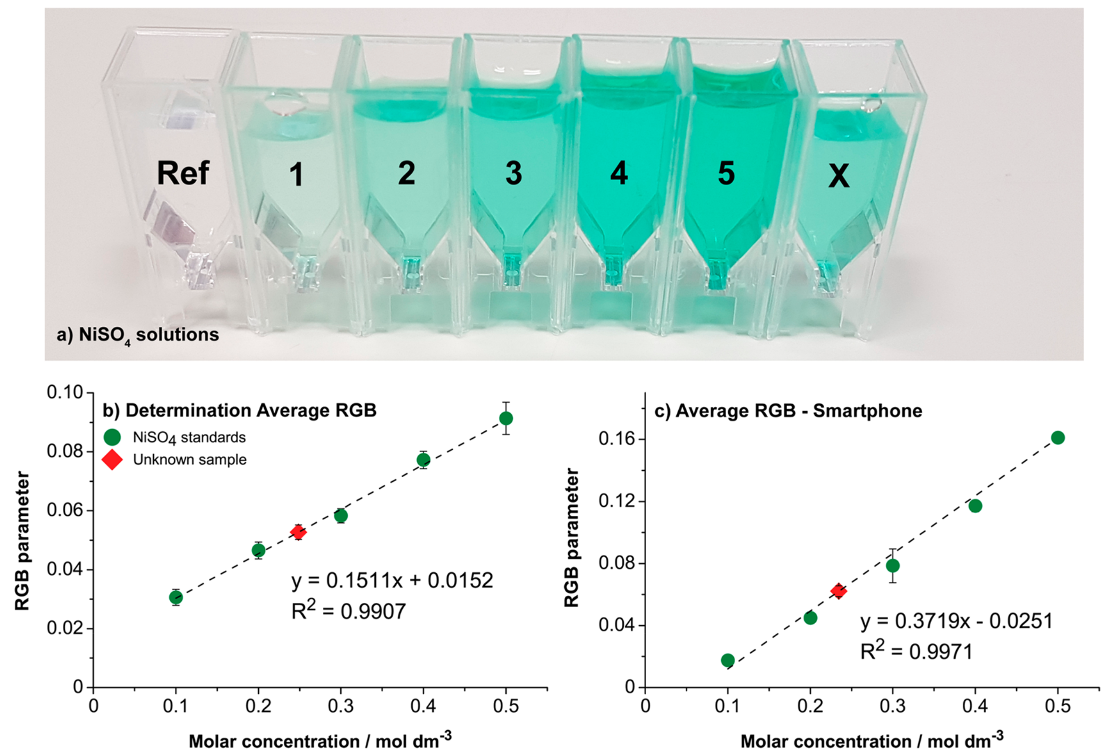


**Figure 6.** Comparison of the results obtained via RGB determination and absorbance measurement of $NiSO_4$ solutions for additional software testing. (a) Photograph of the cuvettes with the prepared standard solutions and the sample with the unknown concentration. (b,c) Graphs plotting RGB parameters vs molar concentrations of the samples analyzed by the selected method. Images were obtained by using both a digital camera (b) and a smartphone (c).

calibration curves for all the analytes showed good linearity ($R^2$ ≈ 0.99) within the test ranges. The unknown concentrations of all the prepared salt solutions were expected to be near 0.25 mol dm$^{-3}$. For the $CuSO_4$ solution, analysis with the



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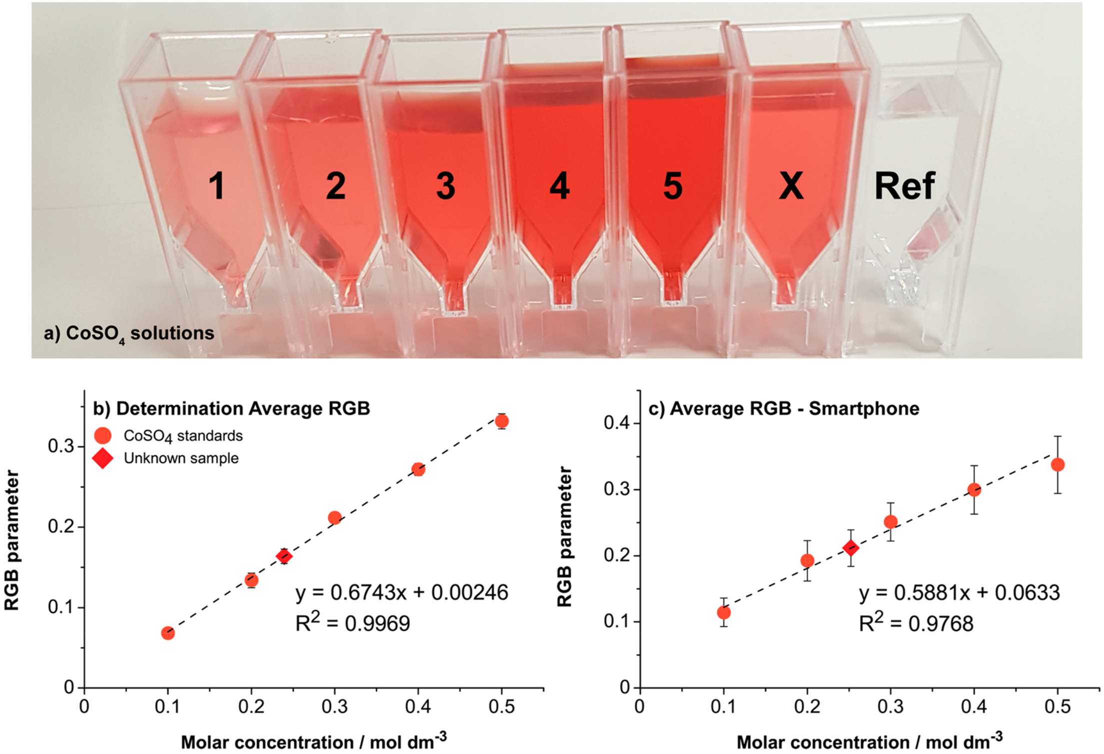


**Figure 7.** Comparison of the results obtained via RGB determination and absorbance measurement of $CoSO_4$ solutions for additional software testing. (a) Photograph of the cuvettes with the prepared standard solutions and the sample with the unknown concentration. (b,c) Graphs plotting RGB parameters vs molar concentrations of the samples analyzed by the selected method. Images were obtained by using both a digital camera (b) and a smartphone (c).

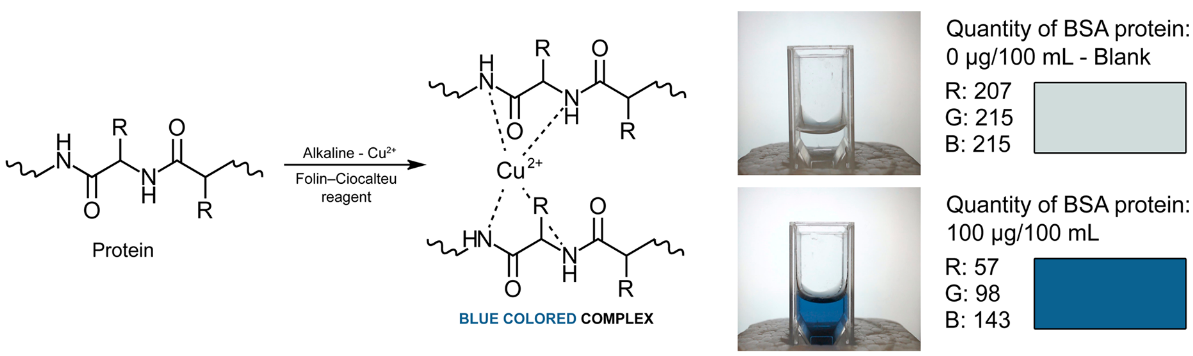


**Figure 8.** First step in the reaction mechanism (Biuret test) of the Lowry method (left) and corresponding colors and measured RGB values of the blank sample and the sample with the highest amount of added protein (right).

developed and selected method (determination of average RGB) yielded a concentration of 0.24 ± 0.01 mol dm$^{-3}$ from the images obtained with a digital camera (see Figure 5b) and a concentration of 0.23 ± 0.01 mol dm$^{-3}$ from the images obtained with a smartphone (see Figure 5c). These results are consistent with those obtained using a commercial spectrophotometer, as the UV−vis absorption measurement yielded a concentration of 0.027 mol dm$^{-3}$ (see Figure 5d) after the $CuSO_4$ solutions were appropriately diluted.

In addition, measurements with the $NiSO_4$ and $CoSO_4$ solutions showed good consistency in terms of curve linearity and getting results within optimal accuracy ranges. By using the selected method, the unknown concentration of the $NiSO_4$ solution was calculated to be 0.25 ± 0.01 mol dm$^{-3}$ for the images obtained by a digital camera (see Figure 6b) and 0.23 ± 0.02 mol dm$^{-3}$ for the images from a smartphone camera (see Figure 6c).

Similar results were obtained for the analysis of $CoSO_4$ solutions. Once a calibration curve had been constructed, the unknown concentration was calculated using a regression equation. The selected method was also applied to the determination of an unknown concentration of $CoSO_4$ solution, which was found to be 0.24 ± 0.01 mol dm$^{-3}$ for the digital camera images (see Figure 7b); an unknown concentration of 0.25 ± 0.01 mol dm$^{-3}$ was calculated for the smartphone camera images (see Figure 7c).

This study demonstrated satisfactory results for our selection of the most appropriate method for further investigation of analytes. It is also noteworthy that all the results overlapped within the optimal experimental error range of less than 10%,


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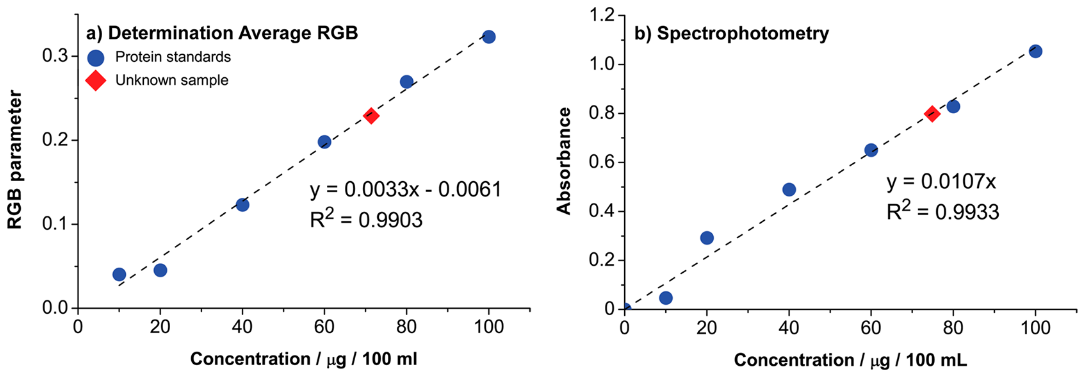


**Figure 9.** Graphical representations of the Lowry method as an example of a complex system. Plots of (a) RGB parameters vs protein concentration and (b) absorbance vs protein concentration with corresponding line equations and $R^2$ coefficients. Absorbance was measured using a UV−vis spectrophotometer at $\lambda_{max}$ = 660 nm.

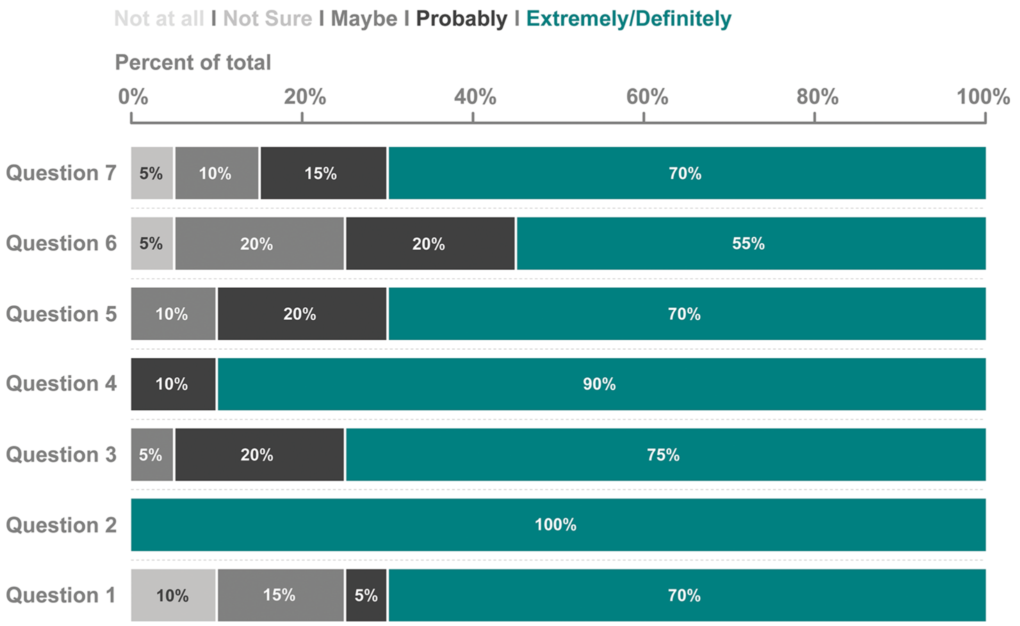


**Figure 10.** Graphical representation of the student survey responses. The questions asked in the survey were following: Question 1: "Do you consider idea of ColorX innovative, interesting and applicable in teaching purposes?" Question 2: "Are the graphical user interface, navigation and directions clear and logical?" Question 3: "How satisfied are you with ColorX Software's ease of use?" Question 4: "Is an informative User Guide for students and teachers provided with the software written in comprehensible way?" Question 5: "Would you describe overall performance of the experiment (preparation of the solutions + managing of the software) as simple and rapid?" Question 6: "Would you like to be informed about similar project in another development of custom analytical method?" Question 7: "Do you think that ColorX could contribute to quality of learning and enhance interest in chemistry and technology?"

which was especially pronounced in the analysis of the images obtained by the digital camera.

## Lowry Method for Protein Quantification, an Example of a Complex System

Finally, the developed and selected method was used to determine an unknown concentration of BSA protein in a prepared sample. The Lowry method, an example of a copper-based protein assay, uses copper ions, which coordinate to the amino groups of a peptide in an alkaline environment (see Figure 8) and oxidize aromatic amino acids, such as tryptophan, in the second step of the mechanism reaction.

On the basis of the results obtained by the selected method (see Figure 9a), it may be assumed that our method is even applicable to systems with a certain degree of complexity. The unknown concentration of BSA protein in the sample was estimated to be near 72 $\mu$g per 100 mL. Calibration curves constructed for the developed method and the standard spectrophotometric method (measurement carried out at $\lambda_{max}$ = 660 nm) showed good linearity, with a correlation coefficient of $R^2 \approx 0.99$.

The unknown concentration of BSA protein in the sample, determined by our developed method, was 71.25 $\mu$g per 100 mL for the images obtained using a digital camera (see Figure 9a). This indicates that the results overlap within the optimal accuracy range (<10%), which is comparable with the previous testing results.

The resulting calibration curve of measured absorbance of BSA protein versus protein concentration are shown in Figure 9b. The UV−vis absorption measurements (see Figure 9b) yielded an estimated concentration of 74.58 $\mu$g per 100 mL. This experiment was carried out by a group of students as an example of a laboratory experiment. Because of the time required to prepare the solutions and perform the measure-



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ments with the developed ColorX system, our method may be applicable not only to teaching and learning but also to practical purposes.

### Learning Objective and Student Perspective

The main aim of this research was to develop user-friendly software intended to facilitate learning and enhance student interest in analytical and instrumental chemistry and also to show that chemistry is closely related to other STEM fields (mathematics, programming, and engineering).

Encouraging schools and students to participate in STEM programs is of great importance, as STEM-based activities pervade every part of our lives. Nowadays, students are introduced to programming and informatics in early stages of their education, resulting in interdisciplinarity and allowing them to combine the skills and perspectives of multiple disciplines. This study is an example of how chemistry and programming can cooperate (with the basics of mathematics) to develop creative ideas that can be at the same time interesting and beneficial to educational environments. This commentary section is based on students' reflections and their interactive engagement while conducting the experiments and testing the software.

We have noticed a positive impact on student interest in the overall software development process and potential applications in education. Their developed ability to analyze the problem and discuss it indicated positive effects on student problem-solving skills as well as promotion of cooperative learning. During software testing, a working group of students was able to perform measurements independently after reading instructions in the provided User Guide or after a brief introduction by the assistant. Students showed improved confidence in software and computer handling, as well as interest in and understanding of functional concepts of the ColorX system. It was evident that student comfort also improved, as students were more confident in the lab after performing experiments using both the spectrophotometer and the ColorX system. Because it was easy to use, testing on the ColorX system also allowed students to prepare and repeat experiments, which we consider one of the most efficient ways to learn chemistry and gain practical skills and confidence in the lab. A working group of 20 students in their first and second years of undergraduate chemistry study were asked to participate in a small and brief survey in order to rate their experiences and give feedback on ColorX's performance and applicability. Figure 10 represents a summary of the survey results, giving insight into the students' perspectives on using ColorX software. Analysis of student responses showed that the majority of students were satisfied with the overall software performance and that they considered ColorX to be applicable to teaching purposes. Pleasingly, all of the surveyed students considered the software navigations and GUI logical, and most of them (75%) thought it was rather easy to use. More than half (55%) of the surveyed students indicated that they would like to take part in further improvements of ColorX system, and a majority of the students (70%) thought that ColorX could contribute to the quality of learning and enhance interest in chemistry and technology. The main complaint was about technical issues related to environmental design (more precisely the lighting), and their suggestions will contribute to improvement of the overall system. After the experiments were carried out and results were analyzed, it was concluded that this new approach to standard spectrophotometric and colorimetric methods had great potential to become a cost-effective alternative to the conventional, quite expensive techniques commonly used in chemistry teaching and research.

Student feedback on the software was positive, which is of great importance, and the survey clearly showed that the developed software was well-accepted by the students. Complete analysis of the student evaluation survey study and an example survey template can be found in the Supporting Information.

## ■ CONCLUSION

ColorX, the custom software developed for this study, is a simple, rapid, applicable, and functional tool used for determining the concentrations of different analytes. It is an image processing software based on RGB measurement and calculation, using images from digital or smartphone cameras. Three methods have been developed and tested: (a) determination by pixel, (b) determination by pixel with Gaussian blur, and (c) determination of average RGB, and the most suitable and accurate method for RGB-based concentration determination was determined. The best results were obtained using the third method, which was based on calculation of average RGB values of a selected image area and gave lower standard deviations compared with the other two methods. However, it is noteworthy that the results from all the developed and tested methods showed good linearity with optimal $R^2$ coefficients within test ranges, although the described method has been found to be slightly more accurate and less sensitive to large changes due to mouse cursor position. Finally, it should be also noted that the concentration ranges of the samples and testing environment play important roles in measurement performance; hence, further optimization of special surroundings may contribute to improvement of the ColorX system. This approach could be useful to students as an educational tool and learning model, and it can be adapted to certain aspects of chemistry-based classes.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.8b00920.

- Laboratory documentation with experiment handout for additional software testing (PDF)
- Microsoft Excel workbook with instruction notes for data analysis of the $KMnO_4$ solution measurements (XLSX)
- Student evaluation survey and survey results (PDF)
- ColorX User Guide providing step-by-step instructions notes, theoretical overview, and Visual Basic pseudocode for the developed methods (PDF)

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### Notes

ColorX can be freely downloaded from the official website at https://app-colorx.com/.


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