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A Fresh Look at the Crystal Violet Lab with Handheld Camera Colorimetry

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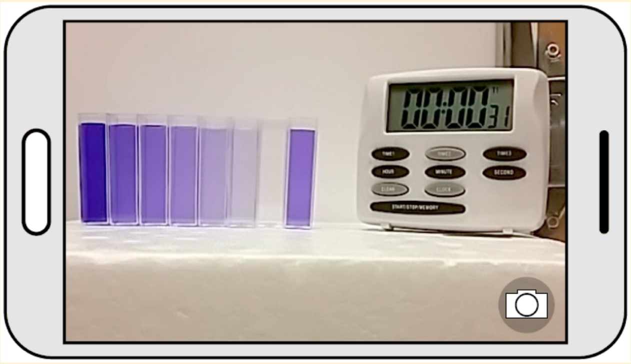
Supporting Information

ABSTRACT: Chemical kinetic experiments to determine rate laws are common in high school and college chemistry courses. For reactions involving a color change, rate laws can be determined experimentally using spectrophotometric or colorimetric equipment though this equipment can be cost prohibitive. Previous work demonstrated that inexpensive handheld camera devices can be used to quantify the concentration of a colored analyte in solution. This paper extends this approach to the kinetic study of the color fading of crystal violet upon reaction with sodium hydroxide. The results demonstrate accurate determination of the reaction order, with respect to crystal violet, using a method accessible in many high school and college laboratories.

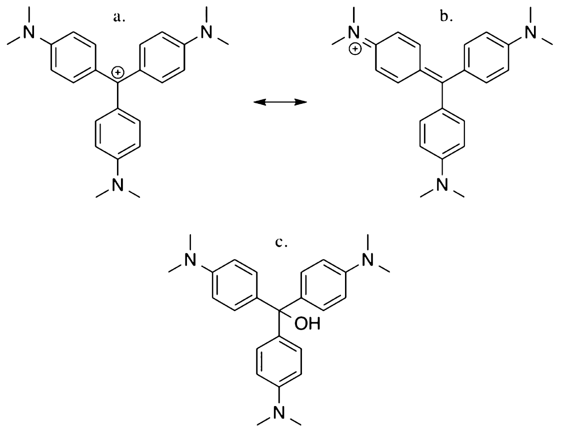


KEYWORDS: *High School/Introductory Chemistry, First-Year Undergraduate/General, Hands-On Learning/Manipulatives, Kinetics, Laboratory Equipment/Apparatus, Analytical Chemistry, Physical Chemistry, Dyes/Pigments*

Most high school and college students have some practical knowledge about speeds of reactions before taking a chemistry course. For example, students understand that foods cook faster at higher temperature. This knowledge is supported and extended by the study of chemical kinetics. While studying kinetics, students learn that rate laws for chemical reactions can only be determined experimentally. Experiments suitable for exploration of kinetic concepts are essential for building connections to the curriculum. Rate laws can be determined by measuring initial rates or monitoring concentration over time.

Monitoring changes in concentration of a colored analyte in solution can be accomplished through spectrophotometry or colorimetry. Traditional equipment used for these measurements can cost hundreds to thousands of dollars per instrument; many high schools do not have the financial means to purchase such instrumentation. Recently, Kehoe and Penn published a method for performing quantitative colorimetry using handheld camera devices.[1] Their work demonstrated suitable precision and accuracy; thus, quantitative colorimetry can be performed even in the absence of traditional equipment.

Scheme 1. Reaction Scheme between Crystal Violet and Hydroxide Ions[a]



[a]Structures (a) and (b) are two resonance structures of crystal violet before the reaction, and structure (c) is the colorless product of the reaction.

Crystal violet, an intensely violet-colored triphenylmethane dye, reacts with hydroxide ions in aqueous solution to form a colorless compound (Scheme 1). For years, this reaction has been successfully used as a lab exercise for the experimental determination of a rate law.[2,3] A large excess of sodium hydroxide relative to crystal violet is used, which means that the reaction's rate depends only on the concentration of crystal violet. Analytical spectrophotometry or colorimetry is performed at specified time intervals. Students monitor the concentration of crystal violet, which fades over time, by

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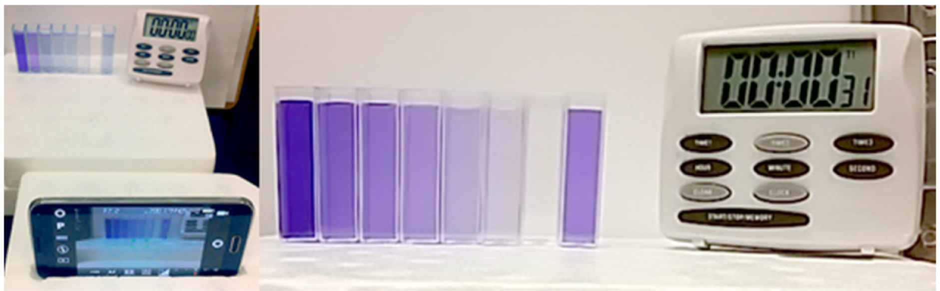

Figure 1. Photograph of the experimental setup (left image) for reaction and image acquisition. From left to right (right image), cuvettes containing 4 mL of 25.0, 12.5, 10.0, 7.50, 5.00, and 2.50 μM crystal violet solutions, Milli-Q water blank, reaction solution, and timer.

collecting absorbance or color intensity data. The integrated rate law is determined by plotting concentration vs time (zeroth order), natural log of concentration vs time (first order), and inverse concentration vs time (second order) and assessing the linearity of the plots. This reaction is known to be first order with respect to crystal violet concentration (eq 1); thus, a linear fit for the plot of the natural log of concentration vs time is expected with a slope of $-k$ in accordance with the integrated rate law for a first order reaction (eq 2), where k is the rate constant, CV^+ is the colored form of crystal violet, OH^- is the hydroxide ion, k' is the pseudo rate constant, and t is time.

$$\frac{-\mathrm{d}[\mathrm{CV}^+]}{\mathrm{d}t} = k[\mathrm{CV}^+][\mathrm{OH}^-] = k'[\mathrm{CV}^+] \quad \text{where} \quad k' = k[\mathrm{OH}^-] \tag{1}$$

$$\ln[\mathrm{CV}^+]_t = -kt + [\mathrm{CV}^+]_0 \tag{2}$$

Kinetic experimentation involving the fading of crystal violet is well documented and is a common lab activity in advanced high school courses. The Advanced Placement (AP) program provides a crystal violet fading experiment as one of 16 guided inquiry labs to meet the College Board standards.[4] Chemical supply companies have developed kits and guides to successfully complete this lab; however, without access to spectrophotometers or colorimeters, many teachers must choose alternative experiments. Here, we describe the use of smartphones or other camera capable devices combined with free applications to replace the need for expensive equipment. With 60% of high school students owning a smartphone, these devices are readily available in classrooms.[5] Image pixels are analyzed for color intensity, which is ultimately used to determine the concentration of crystal violet throughout the reaction.

■ MATERIALS AND METHODS

Preparation of Chemicals

Crystal violet (Flinn Scientific, Inc., reagent grade) and sodium hydroxide (Fluka, 0.1 M) were purchased and used as received. A 25.0 μM crystal violet solution was prepared by dissolving 0.0102 g in enough Milli-Q water (18.2 ΩM cm resistance) to produce 1.00 L solution using a volumetric flask. An approximate 0.040 M sodium hydroxide solution was prepared by adding 8.0 mL of a 0.100 M standard sodium hydroxide solution with 12 mL of Milli-Q water using graduated cylinders.

Preparation of Standard and Blank Solutions

Standard crystal violet solutions were prepared by diluting the 25.0 μM stock solution with Milli-Q water to yield 10.00 mL of 12.5, 10.0, 7.50, 5.00, and 2.50 μM crystal violet solutions. A volume of 4.00 mL of each solution, including the stock solution, was transferred to square plastic cuvettes (1 cm path length). Milli-Q water was transferred to a cuvette for a blank solution. 4.00 mL was used for consistency in volume.

Instrumentation and Reaction Setup

Cuvettes containing the standard and blank solutions, an empty cuvette, and timer were lined up under a diffuse light source with a matte white background and foreground, which minimizes reflections. A smartphone was mounted perpendicular to the lab bench in a styrofoam holder with the camera lens planar with and 12 in. away from the center of the cuvettes. Care was taken to minimize reflections on and shadows on or behind the cuvettes. Figure 1 shows a photograph of the experimental setup for the reaction and image acquisition.

Acquiring Images

Images were acquired using the back facing lens on a Samsung Galaxy S5 smartphone and an application that enables acquisition of images at timed intervals without touching the camera with simultaneous locking of white balance and focus. The specific results presented here were obtained using the free application Camera FV-5 Lite. Other camera capable devices and applications should produce similar results if these feature requirements are met. All images were collected using 640 × 480 image resolution and ISO 200. The camera application's auto white balance and focus were used to adjust base focus and contrast. Following auto adjustment, the white balance and focus were locked to minimize fluctuations in images that could influence results. Volumes of 4.0 mL of 25.0 μM crystal violet solution and 5.0 mL of 0.040 M sodium hydroxide solution were mixed. Upon mixing, the timer and image acquisition were started. An increased concentration of hydroxide ion was used, 1:2000 mol ratio of crystal violet to hydroxide ion in contrast to the 1:1000 mol ratio that other similar experiments suggest, which decreases the time of a typical reaction from 20 to 10 min.[6] A 4.0 mL aliquot of the reaction solution was quickly transferred to the empty cuvette next to the standard and blank solutions. Images were then acquired every 10 s for 10 min using the application's intervalometer feature.

Data Analysis

Red, green, and blue (RGB) color intensities for each solution in each image can be determined using a smartphone or computer. The first two images acquired were disregarded for analysis due to shadows from transferring the reaction mixture. The third image taken (time 31 s in this study) was chosen as the starting point for analysis and for preparing the standard calibration curve. Here, RGB color intensities were determined use the ColorPick Eyedropper extension for Google Chrome.

Measurements were performed at three locations for each of the standard and blank solutions. Average R, G, and B intensities were individually calculated and absorbance values were calculated using eq 3, where I is the intensity for the corresponding channel and I_0 is the intensity of the blank for the corresponding channel.

$$\text{Absorbance} = -\log(I/I_0) \tag{3}$$

The absorbance and concentration of each standard and blank solution were plotted to produce a standard calibration curve for each color channel and fitted linearly to yield an equation for determining concentration given the observed absorbance of the reaction solution. The R and G channels were chosen for further analysis of the crystal violet and sodium hydroxide reaction because the standard calibration curves for those channels had the highest slopes and correlation coefficient (R^2) values. This was anticipated as the most complementary color channels should yield the most quantitative results.[1] The R and G intensity values were measured at three locations for the reaction mixture at each time point. Again, an average was calculated and an absorbance value calculated using eq 3. The equation from the line of best fit from the corresponding standard calibration curve was used to calculate concentration for each time point. The natural log of concentration and inverse concentration were calculated for each time point. Concentration, natural log of concentration, and inverse concentration vs time were plotted and assessed for linearity to determine the order of the reaction with respect to crystal violet.

HAZARDS

Crystal violet is a strong dye and may stain clothes and skin. Crystal violet is moderately toxic by ingestion and is a body tissue irritant. Sodium hydroxide is caustic and may irritate the skin and eyes. Safety glasses and gloves should be worn at all times. Clean up spills immediately and consult SDS for complete safety information. Personal electronic devices, such as smartphones, may be placed inside clear plastic bags to limit contamination of devices with chemicals.

RESULTS

Standard calibration curves relating absorbance to concentration were prepared from the standards (Figure 2) in the image taken at 31 s for each channel. The red and green channels were used for the analysis of images as their standard calibration curves had high slopes and R^2 values. With the use of the equation obtained from the standard calibration curve, the concentration of CV^+ in the sample was calculated for each time point. Figure 3 shows plots of concentration, natural log of concentration, or inverse concentration versus time for data obtained using the R and G channels.

The first order plot was found to be the most linear, which is consistent with a rate law that is first order with respect to crystal violet. Both R and G channels yielded high quality plots, with standard calibration curves with high slopes and R^2 values.

Automatic image acquisition combined with locked focus and white balance means the standard calibration curve need only be determined from one image. Without those features, a standard calibration curve must be determined from each image, which required 21 additional measurements per image and did not substantively improve results (Figure 4). The average difference between creating a standard calibration curve for each image and using a single standard calibration curve was found to be 3.8% and 2.6% for the red and green channels, respectively. Further reduction in data analysis can be completed by analyzing every third image (one image every 30 s) while still visually and mathematically determining the order of the reaction to lessen the data analysis time while increasing lab efficiency (Figure 5). If analyzing every third image does not yield conclusive results, more images can be analyzed. The ideal time interval for data collection can be tuned directly to the initial conditions employed for the crystal violet fading experiment.

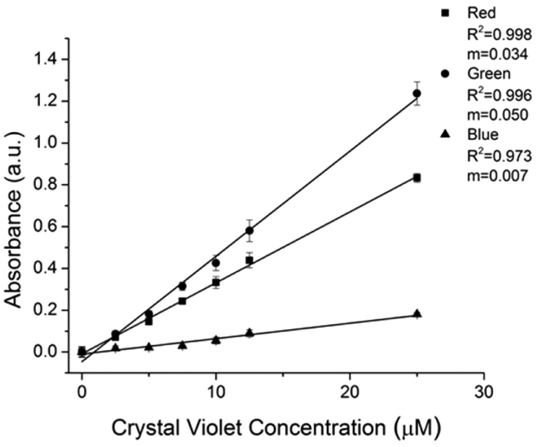

Figure 2. Calibration curves for the red, blue, and green channels. Data points represent the average ± standard deviation of three measurements. The coefficient of determination R^2 and slope (m) are given for each data set.

DISCUSSION

Smartphones and camera capable devices can be used for experimental determination of the rate law for a reaction that involves a color change, such as the fading purple color of the crystal violet lab exercise (Scheme 1). The results are suitably quantitative, and the reaction was successfully demonstrated to be first order with respect to crystal violet.

In the Kehoe and Penn paper,[1] a calibration curve was prepared for each individual image. Here, an application to lock the white balance and focus enabled analysis of the standards for the first time point, making the experiment realistic by decreasing the analyses required dramatically.

Important considerations that make the method robust are appropriate lighting that minimizes shadows on the cuvettes, the use of an application that can autocollect images and locks the white balance and focus, and a camera holder. This method allows a series of images to be analyzed that would otherwise be impractical and facilitates extension to other systems including the color fading, photobleaching, or catalytic degradation of colored species.

CONCLUSION

A smartphone or other camera capable devices have been successfully used to accurately determine the reaction order, with respect to crystal violet, of the fading of crystal violet by reaction with sodium hydroxide. The combination of a camera capable device and free applications enables a robust and accessible method for determining concentration of a colored species in solution. A single standard calibration curve can be applied to all images so long as imaging conditions and

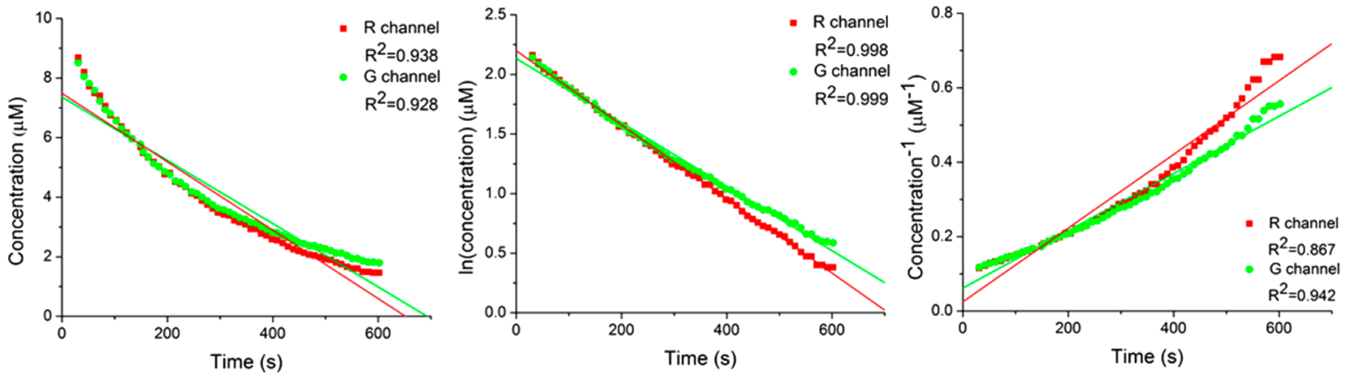

Figure 3. Plots of the integrated form of the rate laws for a zeroth (left), first (middle), or second (right) order reaction. The red and green channels and linear fits are plotted with correlation coefficients. Data points represent the average of three measurements.

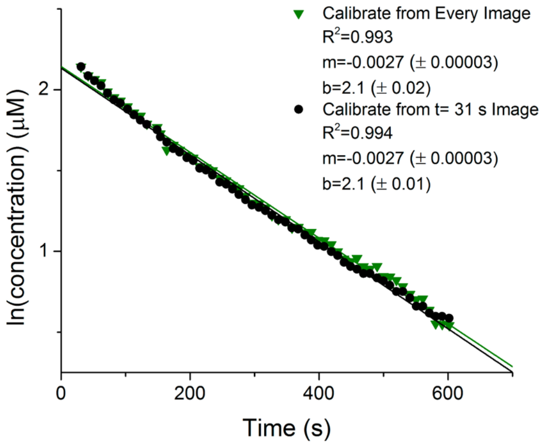

Figure 4. Plot comparing data from single image calibration curve and every image calibration of the green channel. Data points represent the average of three measurements. The coefficient of determination (R^2), slope (m), and intercept (b) are given.

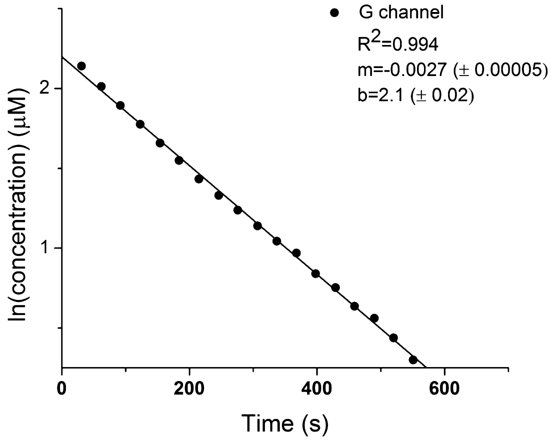

Figure 5. Plot of natural log of concentration as determined using the green channel versus time, with data collection every 30 s instead of 10 s. Data points represent the average of three measurements. The coefficient of determination (R^2), slope (m), and intercept (b) are given.

acquisition procedures are consistent, greatly reducing the data analysis time for the experiment. The developed method should allow for increased access to kinetic experimentation at the high school and college levels. A detailed introduction and procedure for the experiment can be found in the student guide produced for the experiment (Supporting Information).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The student lab guide contains a detailed introduction and procedure for the experiment with teacher notes and sample data. This material is available via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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