

Flash Photolysis Experiment of *o*-Methyl Red as a Function of pH: A Low-Cost Experiment for the Undergraduate Physical Chemistry Lab

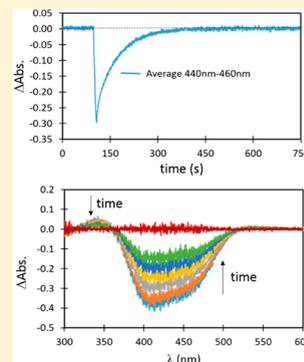
Molly C. Larsen* and Russell J. Perkins

Department of Chemistry and Biochemistry, University of Colorado—Boulder, UCB 215, Boulder, Colorado 80309, United States

S Supporting Information

ABSTRACT: A low-cost, time-resolved spectroscopy experiment appropriate for third year physical chemistry students is presented. Students excite *o*-methyl red in basic solutions with a laser pointer and use a modular spectrometer with a CCD array detector to monitor the transient spectra as the higher-energy *cis* conformer of the molecule converts back to the thermodynamically more stable *trans* form. The transient absorption dynamics are monitored as a function of time averaged between 340 and 360 nm, and the transient bleach dynamics are monitored as a function of time averaged between 440 and 460 nm. The bleach dynamics are monitored in basic solutions at several values of pH, and the observed rate constants are used to extract the rate constant for the isomerization of the protonated form and the deprotonated form of the molecule.

KEYWORDS: Hands-On Learning/Manipulatives, Laboratory Instruction, Upper-Division Undergraduate, Physical Chemistry, Kinetics, Spectroscopy, Photochemistry, Lasers, pH, UV-vis Spectroscopy



INTRODUCTION

In this paper, an experiment is presented in which students excite the azobenzene derivative *o*-methyl red with a laser pointer and monitor the spectral dynamics using a UV-vis spectrometer with a CCD array detector. Flash photolysis experiments have been proposed for use in teaching laboratories before,^{1–7} but this experiment takes advantage of the slow isomerization reaction rate of *o*-methyl red in basic solutions and the ability of a spectrometer with a silicon CCD array detector to quickly collect spectra to examine the spectrum as a function of time.^{8,9} Azobenzene derivatives are ideal for introducing kinetics concepts to undergraduate physical chemistry students because the rate to re-form the *trans* conformer from the photoexcited *cis* conformer is very molecule, solvent, and temperature dependent.^{6,10–14} In this experiment, students observe how transient spectra change when the solvent is used as a reference and when the solution is used as a reference. Transient absorption dynamics, transient bleach dynamics, and an isosbestic point are observed. The spectrometer's software is then used to collect the average absorbance (over a specified wavelength range) as a function of time, and the first-order rate constant for the isomerization process is extracted. The reaction is also done as a function of hydroxide concentration, which allows the isomerization rate constant of the protonated and deprotonated form of *o*-methyl red to be extracted.

The more thermodynamically stable isomer of *o*-methyl red is the *trans* conformer. Upon photoexcitation, *cis*-*o*-methyl red is preferentially formed. The mechanism of photoisomerization in azobenzene dyes has been the subject of significant

study.^{10,15–18} The observed reaction rate for the isomerization to re-form the *trans* isomer has been shown to have the following form:^{8,9}

$$k_{\text{obs}} = \frac{k_1}{(K_A/K_W)[\text{OH}^-]} + k_2 \quad (1)$$

where k_{obs} is the observed first-order rate constant, k_1 is the isomerization reaction rate of the protonated form of *cis*-*o*-methyl red, and k_2 is the isomerization reaction rate of the deprotonated form of *cis*-*o*-methyl red. K_w is $10^{-14.1669}$ at 20°C ,¹⁹ and students calculate k_1 using two different values of K_A because K_A for the *cis* conformer is not known.^{8,9} Students use $K_A = 10^{-3.9}$, assuming the *cis* conformer has the same K_A as the *cis* conformer of methyl yellow,⁸ and $K_A = 10^{-5}$, assuming the *cis* conformer has the same K_A as the *trans* isomer.⁹ A derivation of eq 1 is shown in the student handout. Students plot the observed rate constant as a function of the inverse hydroxide concentration to obtain k_1 and k_2 . Students find that $k_1 \gg k_2$, and many students find that k_2 is smaller than they are able to quantitatively measure in this experimental setup. This has led to fruitful discussions with students about the limits of the experimental setup and the meaning of the extracted confidence intervals. It has also led to discussions about the difference between random and systematic errors in the experiment.

Received: June 2, 2016

Revised: September 7, 2016

EXPERIMENTAL OVERVIEW

A schematic of the experimental setup is shown in Figure 1. A 405 nm laser pointer is used to excite the sample 90° from the

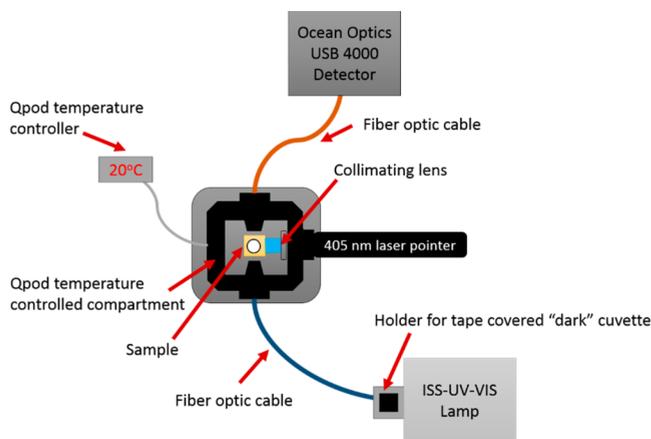


Figure 1. Schematic of the experimental setup as viewed from the top.

monitoring beam. The sample is excited with the laser pointer until the signal is maximized. Solutions are monitored after excitation using an Ocean Optics modular spectrometer with a USB 4000 detector and an ISS-UV-vis lamp. The lamp and the detector are coupled to the cell holder with fiber optics. A cuvette covered in electrical tape is used to block the light when the dark scan is taken. The software used to control the Ocean Optics spectrometer was SpectraSuite,²⁰ and the integration time is set in the program to 500 ms. The cell holder is a qpod temperature-controlled sample compartment which allows for cooling and stirring of the sample. The qpod has an extra adapter inserted into one of the blank ports, 90° from the probe beam, to hold the excitation laser pointer in place with a set screw. The laser pointer is also supported by an optical mount for stability. The temperature of the solutions was held constant at 20 °C, and the samples were being continuously stirred during the measurements. Samples were tested with and without stirring, and no effect was found. Glass cuvettes with two optically clear windows and two frosted windows were used. A collimating lens was used to disperse the excitation beam, and the samples were excited through the frosted windows to better disperse the laser light in the sample. For experiments done by the students, solutions were made using the mass of a 50% sodium hydroxide solution instead of starting with a stock solution of sodium hydroxide and adding the stock volumetrically. A better way to make the solutions is presented in the Supporting Information. More experimental details are provided in the Supporting Information. All error bars and errors reported in the Results section and in the Supporting Information are 95% confidence intervals.

HAZARDS

Students are given filled, sealed cuvettes to work with so student risk of chemical exposure is minimal. The solutions are very basic; proper protective gear should be used when preparing the solutions. The sale of laser pointers is not well regulated, and because laser pointers can be more intense than advertised, **light from the laser pointer should be completely contained, and care should be taken so that students have no ability to expose their eyes to the light.**

RESULTS

This experiment was done with CU chemistry majors at the beginning of their second semester of physical chemistry lab. The class was divided into groups of two or three students. The lab class is 3 h long with 2 lab sections being allotted to each experiment. Students did the experiment as a rotation with each group having access to the equipment for two lab periods. Lab reports were due for the experiment one week after the second lab period. A single student did the experiment in the fall 2015 semester as an elective and the entire class, 8 groups, did the experiment in the spring 2016 semester. Most students finished the experiment in the first lab period, but a couple of groups had to come back the following week to finish the experiment or to reanalyze their data. Six of the eight groups in the fall semester used the same solutions, and those are the results presented in this section. Much of the data analysis was done in class, which allowed students to repeat experiments if necessary. This allowed the instructor and TAs the chance to address any misconceptions the students had about the experiment. Students found the lab report to be manageable in difficulty; however, several groups did the calculations incorrectly. Several groups did do the experiment and calculations correctly, however, with some groups initiating some interesting discussions with the instructor about the limitations of the experiment and how to understand the calculated error on their rate constants. All the students seemed to get something meaningful out of the spectral traces and the difference between using water and the solution as a reference when measuring spectra.

The highest hydroxide concentration used in this experiment was ~0.14 M NaOH, which gave a cis/trans isomerization rate constant of ~0.01 s⁻¹. This rate is slow enough that students are able to observe the formation of the cis isomer and the depletion of the trans isomer in the spectrum. Students manually measure, save, and overlay the complete visible spectra after excitation and print them out during the class time. Students did this both using water as a reference and using the sample solution as a reference. Spectra taken by students during the isomerization of a 0.14 M NaOH sample are shown in the upper panel of Figure 2 (with water used as a reference) and in the lower panel of Figure 2 (with the solution used as a reference). The transient bleach with a maximum at ~410 nm, the transient absorption with a maximum at ~340 nm, and the isosbestic point at ~360 nm are all clearly observed in this data. This part of the lab is qualitative, and students do not know or record what the time is after excitation. If the sample decays before students are able to save enough data, they can re-excite the sample and overlay data from multiple excitations. Because the idea of a bleach signal, or negative absorbance, tends to be difficult for students, this part of the experiment provides an excellent opportunity for discussions with the students. For some groups, seeing both the absorbance change with the water used as a reference and the absorbance change with the solution used as a reference seemed to help them clarify this concept.

Students then monitor the transient absorption and transient bleach dynamics as a function of time for the highest-concentration sodium hydroxide solution. Students do this at two wavelength regions: the first, averaging between 340 and 360 nm, where a transient absorption is observed, and then averaging between 440 and 460 nm, where a transient bleach is observed. Students also monitor the bleach dynamics between

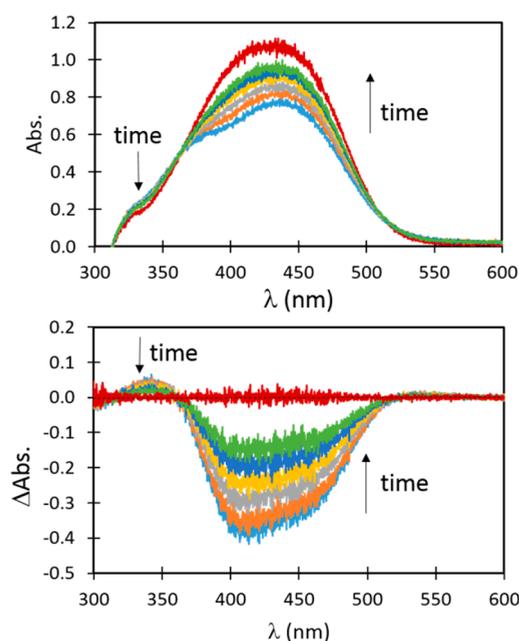


Figure 2. Upper panel: Transient spectra obtained when students used a cell with water as a reference.²¹ Lower panel: Transient spectra obtained when students used the *o*-methyl red solution as a reference. For each scan, the time is not known. Red lines are the absorbances at long times after the isomerization is complete.

440 and 460 nm for solutions where the hydroxide concentration has been varied. A plot of the bleach dynamics for a 0.10 M sodium hydroxide solution, obtained by students, is shown in Figure 3 (left side) along with a plot of $-\ln(-\Delta\text{Abs})$ used to obtain the k_{obs} for this sample (right side). For all the solutions examined, the plot of $-\ln(-\Delta\text{Abs})$ vs time were very linear, as expected from the literature,⁸ with typical R^2 reported by the students of 0.98–0.99 for the fit. A plot of average k_{obs} as a function of $1/[\text{OH}^-]$ obtained from six student groups who did this experiment on the same sets of solutions is shown in Figure 4. As can be seen from this graph, the data fits very well to a line. Individual plots of student data were also very linear with plots of k_{obs} vs $1/[\text{OH}^-]$ producing fits with R^2 values ranging between 0.96 and 0.99.

To account for calculation errors made by students in determining k_1 and k_2 , the student data was analyzed using the values of k_{obs} that were reported in students' lab reports. The average value for k_1 calculated from the reported values of k_{obs} for the 8 groups who did the experiment in the spring 2016 semester was $k_1 = 3.1 \pm 0.3 \times 10^7 \text{ s}^{-1}$ (using $\text{p}K_{\text{A}} 3.9$) and $k_1 =$

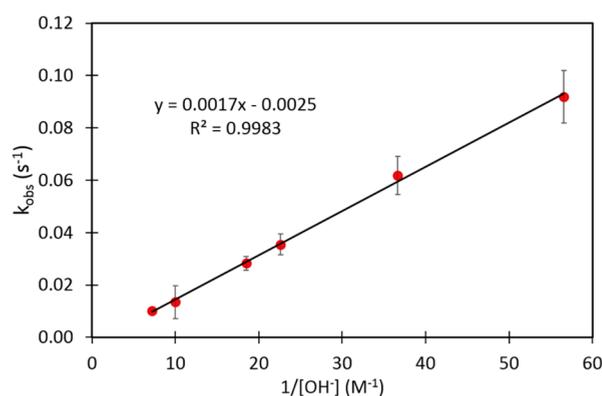


Figure 4. Average of k_{obs} as a function of $1/[\text{OH}^-]$ taken from student data. A linear fit to the data along with the R^2 value is also shown.

$2.5 \pm 0.3 \times 10^6 \text{ s}^{-1}$ (using $\text{p}K_{\text{A}} 5.0$). Error bars on k_1 extracted from the error in the slope of the linear fit are smaller than the variations in the value of k_1 obtained when the two values of K_{A} are used. This provides a good opportunity to discuss the difference between systematic and random error with the students. Students should recognize that even though their error bars are small, their certainty in k_1 is limited because they do not have an accurate value for K_{A} . The average k_2 value calculated from the reported values of k_{obs} was $k_2 = -0.004 \pm 0.004 \text{ s}^{-1}$. The average of k_2 is negative, and several students obtained negative rates for their value of k_2 . For all of the experiments done by the students, the limits on the 95% confidence intervals range from negative to positive. However, when repeating experiments using solutions made with the method presented in the Supporting Information, it was found that the values for k_2 were consistently negative, but that the error in the values was as large as the values. The negative values for k_2 likely indicates either an uncontrolled experimental condition or a breakdown in the simplistic model used to extract these parameters at 20 °C. This breakdown again provides an interesting discussion point about the difference between systematic and random error with some of the more advanced students in the class. Students can compare the random error in k_2 obtained from their fit, and if the error is negative within the 95% confidence interval, they can discuss how this indicates a source of systematic error that is not being accounted for in the experiment. In the Supporting Information, data is also presented where the experiment was done at 25 °C to compare to literature values. The data for this experiment was noisier than the data collected at 20 °C (because of the difficulty of maintaining the temperature at 25

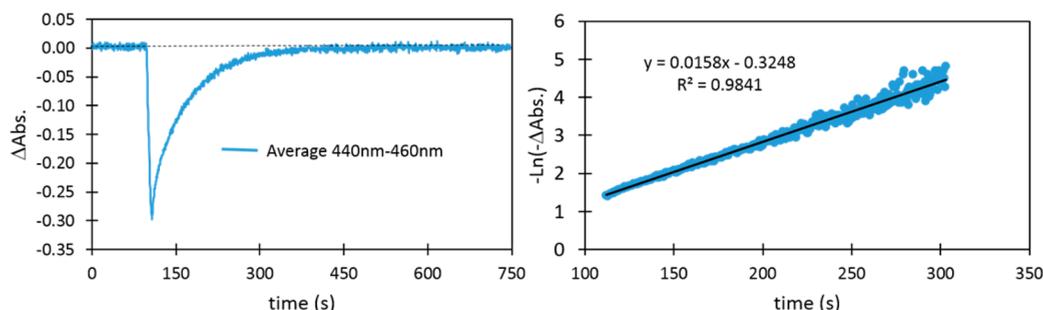


Figure 3. Change in absorbance dynamics trace (left) and linear fit to $-\ln(-\Delta\text{Abs})$ (right) for a *o*-methyl red in 0.10 M sodium hydroxide solution. The first-order rate constant, k_{obs} , comes from the slope of the linear fit shown in the right panel.

°C), but the values obtained are similar to what was reported in the literature.⁸

CONCLUSIONS

All of the students who performed this experiment seemed to grasp new concepts, and the students seemed to enjoy it. Having the transient spectra available during the experiment provided an excellent opportunity to discuss the spectral kinetics with the students in a qualitative way before they began measuring their time traces. This allowed any misconceptions the students had about the data to be addressed before they wrote their reports. There were many levels of difficulty in this experiment which allowed the most advanced students in the class to be challenged but which also allowed all the students in the class to get something meaningful out of the data. This experiment provides a low cost way to present a complicated experimental setup to students in a way that teaches the basics of pump–probe spectroscopy and which also provides a good kinetic problem for students to work with.

The use of spectrometers with CCD array detectors has been proposed for many experiments in undergraduate chemistry laboratories.^{22,23} As many of these spectrometers are capable of measuring kinetics, this experiment is easy and inexpensive for many schools to adopt. Ref 6 presents a method for making a temperature controlled sample holder for a flash photolysis experiment using a three-dimensional printer. Because no effect on k_{obs} was observed while stirring the samples, the sample holder from that experiment could be modified to couple to the spectrometer and the laser pointer and used in this experiment. The experiment proposed here could also be done after the experiment in ref 6 as another application for the sample holder built in that experiment.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.6b00403.

Instructor data and instructions for experimental setup and making solutions (PDF, DOCX)

Experimental handout and instructions (PDF, DOCX)

Example data set (XLSX)

AUTHOR INFORMATION

Corresponding Author

*E-mail: Molly.larsen@colorado.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Thanks to Professor J. M. Weber for help in editing this experiment. Russell Perkins would like to acknowledge funding from NSF Grant CHE 1306386.

REFERENCES

- (1) Clark, L. M.; Hayes, S. E.; Hayes, D. M.; McFarland, J. M.; Miller, R. L.; Shalmi, C. L.; Soltis, M. G.; Susnow, R.; Strong, R. L. Flash Photochemical Measurements in the Physical Chemistry Laboratory: Kinetics of Deactivation of Electronically Excited Aromatic Molecules by Oxygen. *J. Chem. Educ.* **1992**, *69* (4), 336.
- (2) Krasnoperov, L. N.; Stepanov, V. Introduction of Laser Photolysis- Transient Spectroscopy in an Undergraduate Physical

Chemistry Laboratory: Kinetics of Ozone Formation. *J. Chem. Educ.* **1999**, *76* (9), 1182.

- (3) Chambers, K. W.; Smith, I. M. An Inexpensive Flash Photolysis Apparatus and Demonstration Experiment. *J. Chem. Educ.* **1974**, *51* (5), 354.

- (4) Bigger, S. W. FlashPhotol: Using a Flash Photolysis Apparatus Simulator To Introduce Students to the Kinetics of Transient Species and Fast Reactions. *J. Chem. Educ.* **2016**, *93* (8), 1475–1477.

- (5) Maestri, M.; Ballardini, R.; Pina, F. J. S.; Melo, M. J. An Easy and Cheap Flash Spectroscopy Experiment. *J. Chem. Educ.* **1997**, *74* (11), 1314.

- (6) Kosenkov, D.; Shaw, J.; Zuczek, J.; Kholod, Y. Transient-Absorption Spectroscopy of Cis–Trans Isomerization of N,N-Dimethyl-4,4'-Azodianiline with 3D-Printed Temperature-Controlled Sample Holder. *J. Chem. Educ.* **2016**, *93* (7), 1299–1304.

- (7) Kozubek, H.; Marciniak, B.; Paszyc, S. Kinetics of Reactions of Monomeric Nitrosomethane Induced by Flash Photolysis. *J. Chem. Educ.* **1984**, *61* (9), 835.

- (8) Sanchez, A. M.; de Rossi, R. H. Strong Inhibition of Cis-Trans Isomerization of Azo Compounds by Hydroxide Ion. *J. Org. Chem.* **1993**, *58* (8), 2094–2096.

- (9) Sanchez, A. M.; de Rossi, R. H. Effect of Hydroxide Ion on the Cis-Trans Thermal Isomerization of Azobenzene Derivatives. *J. Org. Chem.* **1995**, *60* (10), 2974–2976.

- (10) Kobayashi, S.; Yokoyama, H.; Kamei, H. Substituent and Solvent Effects on Electronic Absorption Spectra and Thermal Isomerization of Push-Pull-Substituted Cis-Azobenzenes. *Chem. Phys. Lett.* **1987**, *138* (4), 333–338.

- (11) Hair, S. R.; Taylor, G. A.; Schultz, L. W. An Easily Implemented Flash Photolysis Experiment for the Physical Chemistry Laboratory: The Isomerization of 4-Anilino-4'-nitroazobenzene. *J. Chem. Educ.* **1990**, *67* (8), 709.

- (12) Whitten, D. G.; Wildes, P. D.; Pacifici, J. G.; Irick, G. Solvent and Substituent on the Thermal Isomerization of Substituted Azobenzenes. Flash Spectroscopic Study. *J. Am. Chem. Soc.* **1971**, *93* (8), 2004–2008.

- (13) Yang, S. Y.; Kim, J. G.; Huh, Y. D.; Choi, Y. S. Study of the Isomerization Reaction Rates of Azobenzene Derivs. *J. Korean Chem. Soc.* **1994**, *38* (8), 552–561.

- (14) Gille, K.; Knoll, H.; Quitzsch, K. Rate Constants of the Thermal Cis-Trans Isomerization of Azobenzene Dyes in Solvents, Acetone/Water Mixtures, and in Microheterogeneous Surfactant Solutions. *Int. J. Chem. Kinet.* **1999**, *31* (5), 337–350.

- (15) Hagiri, M.; Ichinose, N.; Zhao, C.; Horiuchi, H.; Hiratsuka, H.; Nakayama, T. Sub-Picosecond Time-Resolved Absorption Spectroscopy of a Push–pull Type P,p'-Substituted Trans-Azobenzene. *Chem. Phys. Lett.* **2004**, *391* (4–6), 297–301.

- (16) De Maria, P.; Fontana, A.; Gasbarri, C.; Siani, G.; Zanirato, P. Kinetics of the Z-E Isomerization of Monosubstituted Azobenzenes in Polar Organic and Aqueous Micellar Solvents. *ARKIVOC* **2009**, No. viii, 16–29.

- (17) Tiberio, G.; Muccioli, L.; Berardi, R.; Zannoni, C. How Does the Trans–Cis Photoisomerization of Azobenzene Take Place in Organic Solvents? *ChemPhysChem* **2010**, *11* (5), 1018–1028.

- (18) Sanchez, A. M.; de Rossi, R. H. Effect of β -Cyclodextrin on the Thermal Cis–Trans Isomerization of Azobenzenes. *J. Org. Chem.* **1996**, *61* (10), 3446–3451.

- (19) Weast, R. C. *Handbook of Chemistry and Physics*, 65th ed.; CRC Press: Boca Raton, FL, 1984.

- (20) Ocean Optics no longer sells or supports SpectraSuite software. The current version of the software, OceanView software, can also be used.

- (21) The negative values at wavelengths below 312 nm are due to the glass cells used for the reference and the sample not being matched.

- (22) Bernazzani, P.; Paquin, F. Modular Spectrometers in the Undergraduate Chemistry Laboratory. *J. Chem. Educ.* **2001**, *78* (6), 796.

(23) Czegan, D. A. C.; Hoover, D. K. UV-Visible Spectrometers: Versatile Instruments across the Chemistry Curriculum. *J. Chem. Educ.* **2012**, *89* (3), 304–309.