

Analysis of Caffeine in Beverages Using Aspirin as a Fluorescent Chemosensor

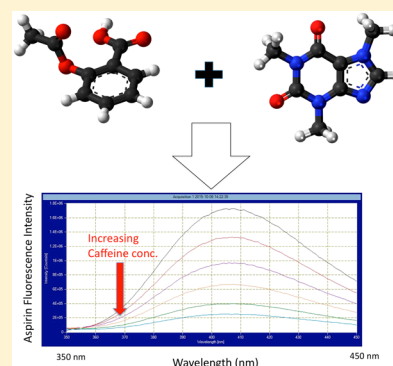
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S Supporting Information

ABSTRACT: Caffeine (1,3,7-trimethylxanthine) is an alkaloid stimulant that is popular in beverages. Fluorescence-coupled methods have been used to measure the caffeine content in coffee, tea, soft drinks, energy drinks, and cosmetics. In this experiment, we have developed a method for detecting caffeine in beverages utilizing the effect of the caffeine concentration on the fluorescence of acetylsalicylic acid (aspirin). Caffeine quenches the fluorescence of aspirin and the resulting Stern–Volmer plot from caffeine standard solutions can be used as a calibration curve. This method meets the criteria for fast, sensitive, and convenient caffeine detection. The caffeine concentration values in *avita* caffeine water samples and Ale-8-One ginger ale beverage were in quantitative agreement with the reported values, within experimental error. This method can be extended to other caffeinated beverages that are clear or have an overall light color. This experiment is appropriate for the physical chemistry laboratory, the analytical chemistry laboratory, and the medicinal chemistry laboratory. Instructors may find the framework useful for teaching concepts in fluorescence spectroscopy and quenching, as well as the analysis of active ingredients in food products and cosmetics.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Physical Chemistry, Hands-On Learning, Consumer Chemistry, Drugs/Pharmaceuticals, Fluorescence Spectroscopy, Medicinal Chemistry, Quantitative Analysis



Caffeine (1,3,7-trimethylxanthine) is an alkaloid stimulant that is popular in beverages. The worldwide daily use has been cited as 70–76 mg per person per day.¹ Caffeine is considered harmless for adults (at doses of less than 100 mg/day) but is of concern for young children and pregnant women.² Several experiments have been published in this Journal for the analysis of caffeine in coffee, tea, and carbonated beverages using high pressure liquid chromatography,^{3–5} thin-layer chromatography,^{6,7} capillary electrophoresis,^{5,8} solid-phase microextraction,⁹ electrospray ionization-mass spectrometry,¹⁰ and gas chromatography/mass spectrometry.^{7,9} The amount of caffeine and various phenols in tea leaves and green tea dietary supplements has also been reported.^{11,12}

The caffeine molecule, itself, does not exhibit fluorescence but experiments have been devised which measure the effect of caffeine concentration on Acridine Orange (AO) (a fluorescent chemosensor),² fluorescence polarization immunoassays (FPIA),¹ and competitive indicator displacement assays.¹³ A microfluidics device dubbed the “traffic light” detector has been developed using Caffeine Orange (CO), an aqueous phase fluorescence turn-on caffeine sensor with a 250-fold fluorescence enhancement with caffeine.¹⁴ In these fluorescence-coupled methods, the caffeine content in coffee, tea, soft drinks, energy drinks, beer, and shampoo have been measured and reported.

In this experiment, we have developed a method for detecting caffeine in beverages utilizing the effect of the caffeine concentration on the fluorescence of acetylsalicylic acid (aspirin).¹⁵ Caffeine quenches the fluorescence of aspirin and the resulting

Stern–Volmer plot from caffeine standard solutions can be used as a calibration curve. The photophysics of Stern–Volmer quenching has been reviewed previously in this Journal.¹⁶ Stern–Volmer kinetics have been used to measure quenching in the highly fluorescent compounds Rhodamine-6G,¹⁷ fluorescein,¹⁸ and quinine.¹⁹ The quenching of tryptophan fluorescence by urea in unfolded Cytochrome c has been employed to study protein folding in the physical chemistry laboratory.²⁰ Experiments have been developed to measure quenching in inorganic salts and complexes (including uranium,²¹ terbium,²² and ruthenium²³) and in benzene,²⁴ 2-naphthol,²⁵ pyrene,²⁶ and PAH-related compounds.²⁷ The quenching of iodine vapor with various gases and iodine self-quenching and polarization quenching have been reported.²⁸ The Stern–Volmer treatment has indeed proven to be extremely versatile in many different experimental designs.

This is the first time that an experiment utilizing the aspirin/caffeine bimolecular system for caffeine analysis has been proposed. This experiment is appropriate for the physical chemistry laboratory, the analytical chemistry laboratory, and the medicinal chemistry laboratory. Instructors may find the framework useful for teaching concepts in fluorescence spectroscopy and quenching, as well as the analysis of active ingredients in food products and cosmetics. One major benefit

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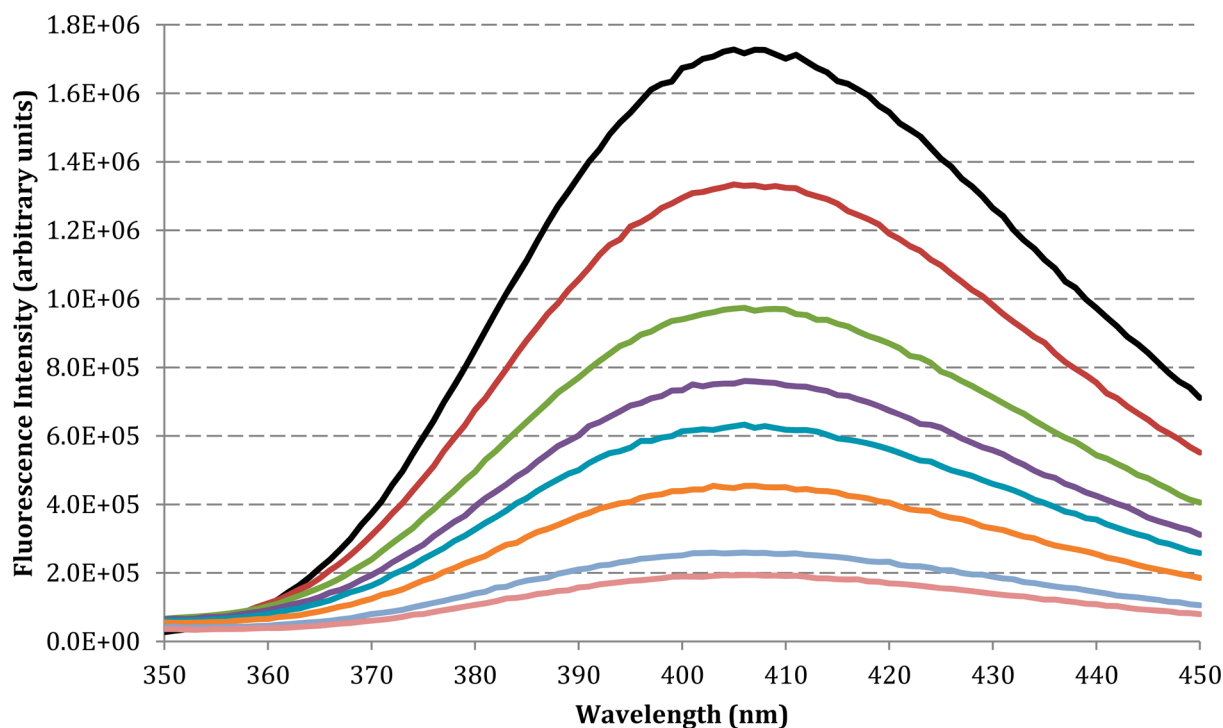


Figure 1. Fluorescence spectra for aspirin with no quencher (zero caffeine concentration—black line), and caffeine standards with increasing concentration (moving from top to bottom). The bottom line was measured with a caffeine concentration of 63 ppm.

of the method is the absence of lengthy or sophisticated sample preparation. Extraction or filtering of the beverage sample is not necessary. The demand for easier methods and shorter analysis time for caffeine detection has been the subject of recent research into alternative spectroscopic methods.^{2,14} This method meets the criteria for fast, sensitive, and convenient caffeine detection.

MATERIALS

Caffeine was obtained from Alfa Aesar and acetylsalicylic acid (aspirin) was obtained from Aldrich Chemical Co. Caffeine water was purchased at a grocery store (ávitāe U.S.A.) and the Ale-8-One ginger ale beverage was purchased in a vending machine. The analysis was performed using a Photon Technology International QuantaMaster QM-2000-4 spectrofluorometer, interfaced to a computer running Felix GX software. The excitation wavelength was 280 nm and the emission was measured from 350 to 450 nm. The bandwidth was set to 5 nm. The step size was 1 nm and each scan takes approximately 30 s; the sample is irradiated throughout the time of each scan. At zero aspirin concentration, the spectrometer registers approximately 20,000 counts.

EXPERIMENT

A caffeine stock solution was prepared by dissolving 25.0 mg of caffeine in a total volume of 250 mL of distilled water (100 ppm). A 40 ppm aspirin solution was prepared by dissolving 4.00 mg of aspirin in 100 mL total volume. A standard curve was prepared by measuring the fluorescence of the aspirin solution with varying caffeine concentrations. The caffeine concentration varied from 0 to 80 ppm; the aspirin concentration was held constant at 0.23 ppm. The Stern–Volmer plot constructed from the data served as the standard curve. Fluorescence spectra from the experiment, showing the fluorescence of aspirin with varying

caffeine concentrations are shown in Figure 1. A sample standard curve is shown in Figure 2.

A series of solutions with constant aspirin concentration and varying amounts of beverage (ávitāe caffeine water, Ale-8-One, etc.) were measured and a Stern–Volmer plot was constructed. Comparison of this plot with the standard curve was used to determine caffeine concentration of the beverages. Sample comparisons are shown in Figures 2 and 3. Sample results are shown in Table 1. The measurements were performed in triplicate and the errors represent the range of values obtained.

HAZARDS

Aspirin may cause skin irritation, eye irritation, respiratory tract irritation, and digestive tract irritation. Aspirin is toxic and harmful if swallowed. Caffeine may cause skin irritation, eye irritation, respiratory tract irritation, and digestive tract irritation. Students should wear goggles when preparing and handling the aspirin and caffeine standard solutions. Never eat while in the laboratory!

RESULTS AND DISCUSSION

The Stern–Volmer equation relating the ratio I_0/I to the concentration of the quenching agent $[Q]$ is as follows:

$$\frac{I_0}{I} = 1 + K_{SV}[Q]$$

where I_0 is the fluorescence intensity in the absence of the quencher, I is the measured fluorescence intensity, and K_{SV} is the Stern–Volmer constant. The Stern–Volmer constant is the product of the quencher rate coefficient k_q and the lifetime of the emissive excited state in the absence of the quencher, τ_0 .²⁹

The Stern–Volmer plots for aspirin with the caffeine standards and with the caffeinated beverages are curved upward

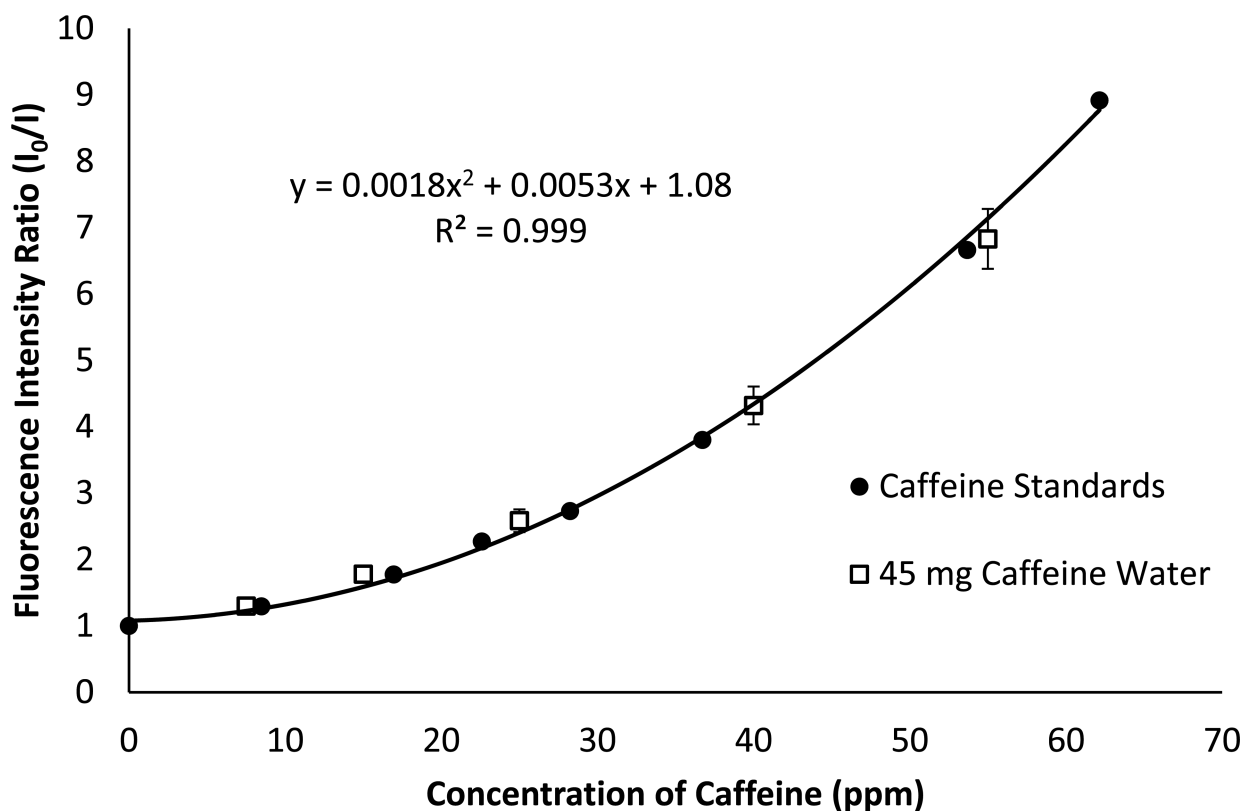


Figure 2. Stern–Volmer plot of aspirin with caffeine standards and 45 mg caffeine water.

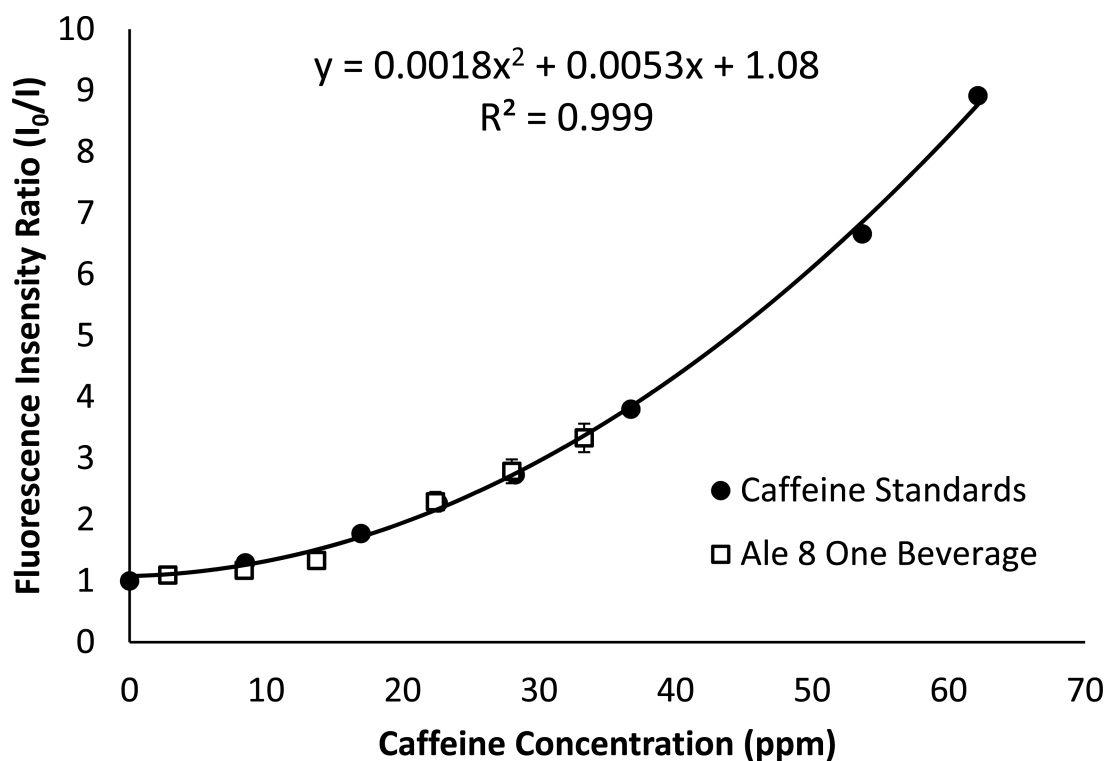


Figure 3. Stern–Volmer plot of aspirin with caffeine standards and Ale-8-One ginger ale beverage.

(Figures 2 and 3). A curved Stern–Volmer plot is indicative of static (complex formation through various intermolecular forces) and dynamic quenching (bimolecular collisions).^{30–32} The positive deviation from linearity in the Stern–Volmer plot has been modeled using a modified form

$$\frac{I_0}{I} = 1 + K_{app}[Q]$$

where

$$K_{app} = (K_D + K_S) + K_D K_S [Q]$$

Table 1. Reported and Measured Caffeine Content in Selected Caffeinated Beverages

| Product | Serving Size/ (oz/mL) | Caffeine Content/ mg/serving (reported) | Caffeine Content/ mg/serving (measured) |
|----------------------------------|--------------------------|---|---|
| ávitāe caffeine water | 16.9/500 | 45 | 45 ± 3 |
| ávitāe caffeine water | 16.9/500 | 90 | 91 ± 4 |
| Ale-8-One ginger ale beverage | 12/355 | 37 | 37 ± 4 |

and K_D and K_S are the Stern–Volmer constants for dynamic and static quenching, respectively.³¹ In our analysis, we were not concerned with finding the Stern–Volmer constants. An empirical equation of quadratic form was used to fit the data, using an Excel spreadsheet.

The caffeine content of the beverages was determined by comparing the Stern–Volmer curves for the caffeine standards and the caffeinated beverages. This can be accomplished using an overlay method or using the fit equation for the standards and inputting the measured fluorescence value for a given beverage concentration. The caffeine concentration in the ávitāe caffeine water samples and the Ale-8-One ginger ale beverage were in quantitative agreement with the reported values,³³ within experimental error. This method can be extended to other caffeinated beverages that are clear or have an overall light color. With darker caffeinated beverages, the color of the resulting solution interferes with the fluorescence measurement.

This experiment is intended for upper-level chemistry students who have learned about fluorescence spectroscopy in physical chemistry, analytical chemistry, or a spectroscopy-based chemistry course. The goal of this experiment is for students to experience first-hand the technique of fluorescence spectroscopy, as they use the method to analyze several caffeinated beverages and report the measured amount of caffeine in each beverage. After the experiment is completed, the students are expected to submit a laboratory report where they report their measurements and the associated error. Additional details are provided in the [Supporting Information](#).

The laboratory experiment was developed over a period of several months by an undergraduate researcher (the lead author on the paper, J.S.). This experiment was tested in our Physical Chemistry Laboratory course in two different terms by two teams of students. The experiment can be completed in a standard 3 h laboratory period; it works best with a smaller lab of 6–10 students, working in pairs. If only one spectrofluorometer is available, one team can measure the standard curve, a second team can measure one beverage, a third team another beverage sample, and so on.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available on the [ACS Publications website](#) at DOI: [10.1021/acs.jchemed.6b00303](https://doi.org/10.1021/acs.jchemed.6b00303).

Student handouts and instructor materials detailing the preparation of each solution, the creation of the Stern–Volmer plots, and the resulting data analysis are included. ([DOCX](#))

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Oberleitner, L.; Grandke, J.; Mallwitz, f.; Resch-Genger, U.; Garbe, L.; Schneider, R. J. Fluorescence Polarization Immunoassays for the Quantification of Caffeine in Beverages. *Agric. J. Agric. Food Chem.* **2014**, *62*, 2337–2343.
- (2) Ghosh, A.; Ghosh, C.; Gupta, A. A Simple Approach to Detect Caffeine in Tea Beverages. *J. Agric. Food Chem.* **2013**, *61*, 3814–3820.
- (3) DiNunzio, J. Determination of Caffeine in Beverages by High Performance Liquid Chromatography. *J. Chem. Educ.* **1985**, *62* (5), 446–447.
- (4) Delaney, M.; Pasko, K.; Mauro, D.; Gsell, D.; Korologos, P.; Morawski, J.; Krolkowski, L.; Warren, F., Jr. Determination of Aspartame, Caffeine, Saccharin, and Benzoic Acid in Beverages by High Performance Liquid Chromatography. *J. Chem. Educ.* **1985**, *62* (7), 618–620.
- (5) Beckers, J. The Determination of Caffeine in Coffee: Sense or Nonsense? *J. Chem. Educ.* **2004**, *81* (1), 90–93.
- (6) Ma, Y.; Yeung, E. Determination of Components in Beverages by Thin-Layer Chromatography. *J. Chem. Educ.* **1990**, *67* (5), 428–429.
- (7) Torres, J.; Hiley, S.; Lorimor, S.; Rhoad, J.; Caldwell, B.; Zweerink, G.; Ducey, M. Separation of Caffeine from Beverages and Analysis Using Thin-Layer Chromatography and Gas Chromatography-Mass Spectrometry. *J. Chem. Educ.* **2015**, *92* (5), 900–902.
- (8) Conte, E.; Barry, E.; Rubinstein, H. Determination of Caffeine in Beverages by Capillary Zone Electrophoresis. *J. Chem. Educ.* **1996**, *73* (12), 1169–1170.
- (9) Yang, M.; Orton, M.; Pawliszyn, J. Quantitative Determination of Caffeine in Beverages Using a Combined SPME-GC/MS Method. *J. Chem. Educ.* **1997**, *74* (9), 1130–1132.
- (10) Bergen, H., III; Benson, L.; Naylor, S. Determination of Aspartame and Caffeine in Carbonated Beverages Utilizing Electrospray Ionization-Mass Spectrometry. *J. Chem. Educ.* **2000**, *77* (10), 1325–1326.
- (11) Lin, Y.; Tsai, Y.; Tsay, J.; Lin, J. Factors affecting the Levels of Tea Polyphenols and Caffeine in Tea Leaves. *J. Agric. Food Chem.* **2003**, *51*, 1864–1873.
- (12) Seeram, N.; Henning, S.; Niu, Y.; Lee, R.; Scheuller, H.; Heber, D. Catechin and Caffeine Content of Green Tea Dietary Supplements and Correlation with Antioxidant Capacity. *J. Agric. Food Chem.* **2006**, *54*, 1599–1603.
- (13) Siering, C.; Kerschbaumer, H.; Nieger, M.; Waldvogel, S. A Supramolecular Fluorescence Probe for Caffeine. *Org. Lett.* **2006**, *8* (7), 1471–1474.
- (14) Xu, W.; Kim, T.; Zhai, D.; Er, J.; Zhang, L.; Kale, A.; Agrawalla, B.; Cho, Y.; Chang, Y. Make Caffeine Visible: a Fluorescent Caffeine “Traffic Light” Detector. *Sci. Rep.* **2013**, *3*, 2255.
- (15) Miles, C.; Schenk, G. Fluorescence of Acetylsalicylic Acid in Solution and Its Measurement in Presence of Salicylic Acid. *Anal. Chem.* **1970**, *42* (6), 656–659.
- (16) Bare, W. D.; Pham, C. V.; Cuber, M.; Demas, J. N. An Improved Method for Studying the Enzyme-Catalyzed Oxidation of Glucose Using Luminescent Probes. *J. Chem. Educ.* **2007**, *84* (9), 1511.
- (17) Cumberbatch, T.; Hanley, Q. Quantitative Imaging in the Laboratory: Fast Kinetics and Fluorescence Quenching. *J. Chem. Educ.* **2007**, *84* (8), 1319.
- (18) Koenig, M. H.; Yi, R. P.; Sandridge, M. J.; Mathew, A. S.; Demas, J. N. “Open-Box” Approach to Measuring Fluorescence Quenching Using

an iPad Screen and Digital SLR Camera. *J. Chem. Educ.* **2015**, *92* (2), 310–316.

(19) Sacksteder, L.; Ballew, R. M.; Brown, E. A.; Demas, J. N.; Nesselrodt, D.; DeGraff, B. A. Photophysics in a disco: Luminescence quenching of quinine. *J. Chem. Educ.* **1990**, *67* (12), 1065.

(20) Schlamadinger, D. E.; Kats, D. I.; Kim, J. E. Quenching of Tryptophan Fluorescence in Unfolded Cytochrome c: A Biophysics Experiment for Physical Chemistry Students. *J. Chem. Educ.* **2010**, *87* (9), 961–964.

(21) Burrows, H. D.; Formosinho, S. J. Uranyl luminescence quenching. An experiment in photochemistry and kinetics. *J. Chem. Educ.* **1978**, *55* (2), 125.

(22) Jenkins, J. L.; Welch, L. E. A Mechanistic Study of Terbium Phosphorescence Quenching. *J. Chem. Educ.* **2009**, *86* (5), 613. Ballew, R. M.; Demas, J. N.; Ayala, N. P.; Grubb, M.; Snyder, S. W. Computer interfaced fast kinetics luminescence decay experiment. *J. Chem. Educ.* **1991**, *68* (3), 222.

(23) Wilke, B. M.; Castellano, F. N. Photochemical Upconversion: A Physical or Inorganic Chemistry Experiment for Undergraduates Using a Conventional Fluorimeter. *J. Chem. Educ.* **2013**, *90* (6), 786–789. Watts, R. J. Ruthenium polypyridyls: A case study. *J. Chem. Educ.* **1983**, *60* (10), 834.

(24) Marciniak, B. Does Cu(acac)₂ quench benzene fluorescence?: A physical chemistry experiment. *J. Chem. Educ.* **1986**, *63* (11), 998. Churio, M. S.; Grela, M. A. Photochemistry of Benzophenone in 2-Propanol: An Easy Experiment for Undergraduate Physical Chemistry Courses. *J. Chem. Educ.* **1997**, *74* (4), 436.

(25) Boyer, R.; Deckey, G.; Marzzacco, C.; Mulvaney, M.; Schwab, C.; Halpern, A. M. The photophysical properties of 2-naphthol: A physical chemistry experiment. *J. Chem. Educ.* **1985**, *62* (7), 630.

(26) Shane, E. C.; Price-Everett, M.; Hanson, T. Fluorescence Measurement of Pyrene Wall Adsorption and Pyrene Association with Humic Acids. An Experiment for Physical Chemistry or Instrumental Methods. *J. Chem. Educ.* **2000**, *77* (12), 1617.

(27) Poulsen, L.; Ruiz, A. Z.; Pedersen, S. U.; Ogilby, P. R. Characterizing the Behavior and Properties of an Excited Electronic State: Electron-Transfer Mediated Quenching of Fluorescence. *J. Chem. Educ.* **2003**, *80* (7), 819. Rivera-Figueroa, A. M.; Ramazan, K. A.; Finlayson-Pitts, B. J. Fluorescence, Absorption, and Excitation Spectra of Polycyclic Aromatic Hydrocarbons as a Tool for Quantitative Analysis. *J. Chem. Educ.* **2004**, *81* (2), 242.

(28) Duchin, K. L.; Lee, Y. S.; Mills, J. W. Quenching of I₂ vapor fluorescence excited with He-Ne laser light. A kinetics-spectroscopy experiment. *J. Chem. Educ.* **1973**, *50* (12), 858. Masiello, T.; Vulpanovici, N.; Nibler, J. W. Fluorescence Lifetime and Quenching of Iodine Vapor. *J. Chem. Educ.* **2003**, *80* (8), 914.

(29) Permyakov, E. *Luminescent Spectroscopy of Proteins*; CRC Press: Boca Raton, FL, 1993.

(30) Scandola, F.; Balzani, V. Energy-transfer processes of excited states of coordination compounds. *J. Chem. Educ.* **1983**, *60* (10), 814.

(31) Fraiji, L. K.; Hayes, D. M.; Werner, T. C. Static and dynamic fluorescence quenching experiments for the physical chemistry laboratory. *J. Chem. Educ.* **1992**, *69* (5), 424.

(32) Ghosh, K.; Kar, D. Fluorometric Recognition of Both Dihydrogen Phosphate and Iodide by a New Flexible Anthracene Linked Benzimidazolium-Based Receptor. *Beilstein J. Org. Chem.* **2011**, *7*, 254–264.

(33) The concentration of caffeine in the caffeinated water is reported on the product label (ávitae U.S.A., LLC, PO Box 93686, Cleveland, OH 44101). The caffeine content for Ale-8-One ginger ale beverage is from <http://ale8one.com/productinfo/> (accessed August 29, 2016).