Analysis of Caffeine in Beverages Using Aspirin as a Fluorescent Chemosensor

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Supporting Information

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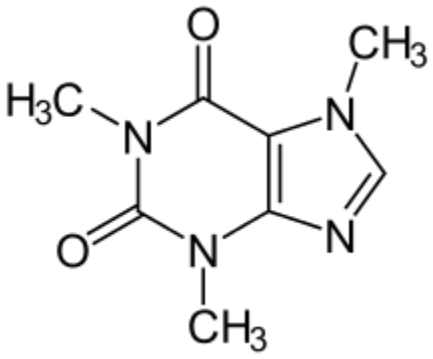
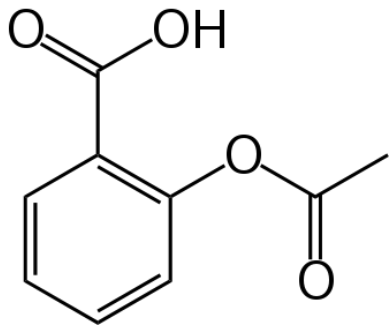
STUDENT HANDOUT

In this experiment, you will use emission fluorescence spectroscopy to determine the concentration of caffeine in beverages. Aspirin (acetylsalicylic acid) solutions exhibit fluorescence and the presence of caffeine quenches that fluorescence. You will construct a Stern-Volmer plot with a fixed concentration of aspirin and caffeine standard solutions of varying concentration. Using this plot as a standard curve, you will analyze beverage solutions (with fixed aspirin concentration) and varying caffeine concentrations. By making a careful comparison, you will determine the caffeine concentration in the original caffeinated beverage.



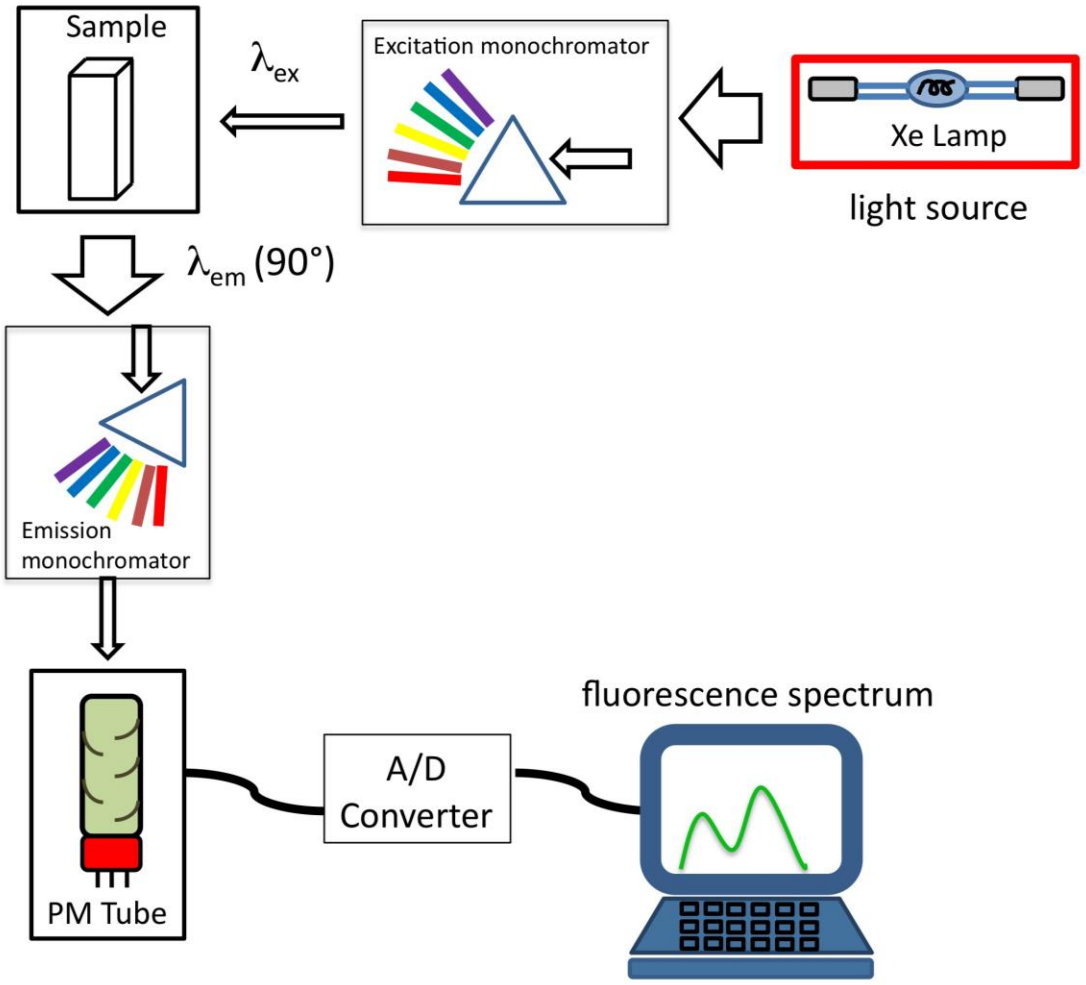
Caffeine (1,3,7-trimethylxanthine)



Aspirin (acetylsalicylic acid)

The Experimental Technique

Fluorescence spectroscopy is a very sensitive technique because the emitted light is measured at a 90-degree angle relative to the excitation light. This results in high signal to noise ratios and detection limits in the picomolar range. The observed fluorescence is proportional to the concentration of the analyte (at low concentrations). A schematic of the fluorescence instrument is shown below:



Light in the UV-Visible range is emitted by the xenon (Xe) lamp. A monochromator selects a specific excitation wavelength (typically with single nanometer resolution). The sample absorbs the excitation photons and the absorbing species is promoted from the ground electronic state to various vibrational states in the excited electronic state. The excited molecule collides with other molecules in the sample losing vibrational energy in the process. The net result is that when the molecule drops to the ground electronic state, it emits light of a longer wavelength (lower energy) than the excitation wavelength.

The emitted photons can be resolved into a narrow wavelength range using a second monochromator, again typically with single nanometer resolution, situated 90-degrees relative to the excitation source. The photons can be detected using a photomultiplier tube detector that counts the number of emitted photons (i.e., produces an electrical signal proportional to the number of detected photons). The detection of photons of a longer wavelength and at 90-degrees to the incident light greatly reduces the background, resulting in a much more sensitive technique than absorption spectroscopy. The resulting emission spectrum can be used to identify the absorbing species and determine its concentration in the sample.

Aspirin in solution fluoresces with an excitation wavelength of 280 nm.[1] Caffeine quenches this fluorescence. In quenching, the presence of another species can result in nonradiative de-excitation and therefore a decrease in the quantum yield (fewer photons are emitted in the de-

excitation process). These effects decrease the intensity of the observed fluorescence. A plot of the fluorescence intensity ratio (original intensity/quenched intensity or I_0/I) versus the quencher concentration is called a Stern-Volmer plot.

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

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

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.[2]

The Stern-Volmer plots for aspirin with the caffeine standards and with the caffeinated beverages are curved upward. A curved Stern-Volmer plot is indicative of static (complex formation through various intermolecular forces) and dynamic quenching (bimolecular collisions).[3-5] The positive deviation from linearity in the Stern-Volmer plot has been modeled using a modified form:

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

where

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

and K_D and K_s are the Stern-Volmer constants for dynamic and static quenching, respectively.[4] In our analysis, we are not concerned with finding the Stern-Volmer constants. An empirical equation of quadratic form can be used to fit the data, using an Excel spreadsheet.

REFERENCES

1. C. Miles; G. Schenk, Fluorescence of Acetylsalicylic Acid in Solution and Its Measurement in Presence of Salicylic Acid. *Anal. Chem.*, **1970**, 42 (6), 656-659.
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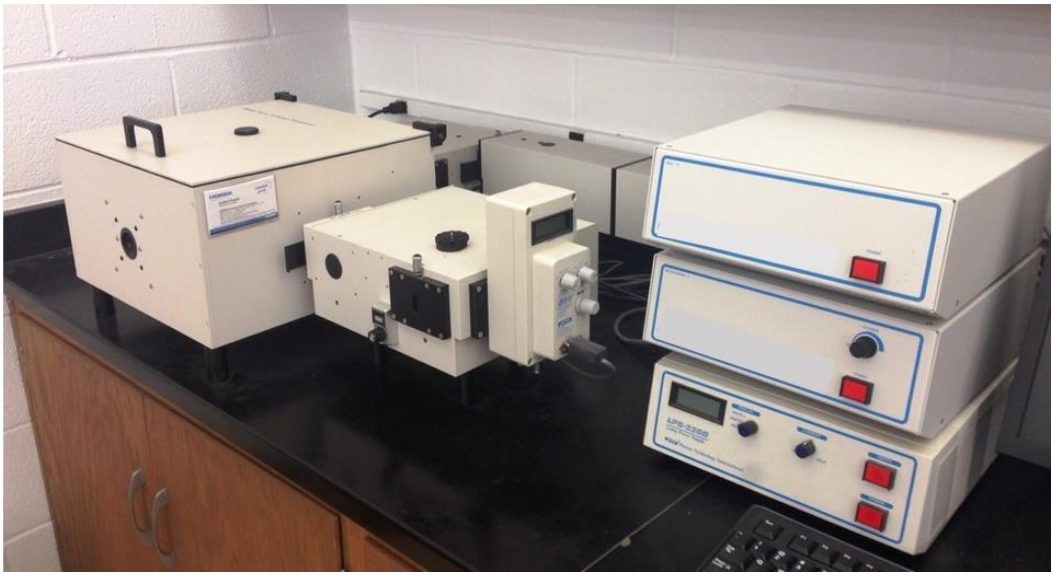
3. F. Scandola; V. Balzani, Energy-transfer processes of excited states of coordination compounds. J. Chem. Educ. **1983** 60 (10), 814.
4. L. K. Fraiji; D. M. Hayes; T. C. Werner, Static and dynamic fluorescence quenching experiments for the physical chemistry laboratory. J. Chem. Educ. 1992 69 (5), 424.
5. K. Ghosh; D. Kar, 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

Experimental Details

I. Instrument Setup:

1. Turn on the Lamp Power Supply on the PTI QuantMaster fluorescence instrument. The left knob should be set to watts. The reading should be in the 65-70 W range.
2. Turn on the other two power buttons.
3. Turn on the photomultiplier tube. The reading should be around 1050 V.
4. Turn on the computer after a few minutes.
5. Double click the Felix icon on the desktop. Click "ok" (to confirm license).
6. Click on the Setup button and select Emission Scan.
7. Click on the Acquisition Settings tab. The Excitation wavelength is 280 nm. The Emission wavelength range is 350-450 nm. The step size is 1 nm, and the integration time is 0.1 seconds.



Photon Technology International QuantaMaster QM-2000-4 spectrofluorometer

II. Preparation of the stock solutions:

8. Preparation of the caffeine stock solution (100 ppm)
    a. Weigh out 25 mg of caffeine and transfer this to a 250 mL volumetric flask.
    b. Fill the flask half-full with distilled water and invert the flask until the caffeine is completely dissolved.
    c. Fill with distilled water to the mark, cover it, and invert the flask several times to mix completely.
9. Preparation of the aspirin (acetylsalicylic acid) stock solution (40 ppm)
    a. Weigh out 4 mg of aspirin and transfer this to a 150 mL beaker.

b. Add approximately 50 mL of distilled water to the beaker. Gently heat the beaker; the solute needs to be completely dissolved. Once dissolved, transfer the solution to a 100 mL volumetric flask.
c. Fill with distilled water to the mark, cover it, and invert the flask several times to mix completely.

III. Experimental procedure:

10. For the standard curve, begin by placing 3.5 mL of distilled water into a quartz cuvette and add 0.020 mL (20 μL) of the 40 ppm aspirin stock. Invert the quartz cuvette three times before placing in the fluorimeter. Measure the fluorescence of this sample – record the number of counts at the peak maximum. Make sure that the fluorescence is close to full scale, but not saturating the instrument.

11. Rinse the cuvette with distilled water and add the following amounts to the quartz cuvette, measuring the fluorescence each time. Remember to clean the cuvette thoroughly after each measurement with distilled water.

Caffeine (100 ppm)	Aspirin (40 ppm)	Distilled Water	Concentration of Caffeine (ppm)	Fluorescence Intensity (a.u.)
0 mL	20 μL	3.5 mL	0	
0.3 mL	20 μL	3.2 mL	8.5	
0.6 mL	20 μL	2.9 mL	17.0	
0.8 mL	20 μL	2.7 mL	22.7	
1.0 mL	20 μL	2.5 mL	28.4	
1.3 mL	20 μL	2.2 mL	36.9	
1.9 mL	20 μL	1.6 mL	54.0	
2.2 mL	20 μL	1.3 mL	62.5	

To calculate the concentration of the caffeine:

[Amount of caffeine (mL) X Concentration of caffeine (100 ppm)] / Total Volume (3.52 mL)

For example: (0.3 mL X 100 ppm) / 3.52 mL = 8.5 ppm

12. Measurement of the Fluorescence Intensity of the Caffeinated Water Samples:

45 mg caffeinated water (90 ppm)	Aspirin (40 ppm)	Distilled water	Concentration of Caffeine (ppm)	Fluorescence Intensity (a.u.)
0.3 mL	20 μL	3.4 mL	7.3	
0.6 mL	20 μL	3.1 mL	14.5	
1.0 mL	20 μL	2.7 mL	24.2	
1.6 mL	20 μL	2.1 mL	38.7	
2.2 mL	20 μL	1.5 mL	53.2	

90 mg caffeinated water (180 ppm)	Aspirin (40 ppm)	Distilled water	Concentration of Caffeine (ppm)	Fluorescence Intensity (a.u.)
0.3 mL	20 μL	3.4 mL	14.5	
0.5 mL	20 μL	3.2 mL	24.2	
0.8 mL	20 μL	2.9 mL	38.7	
1.0 mL	20 μL	2.7 mL	48.4	
1.2 mL	20 μL	2.5 mL	58.1	

Note: the 45 mg caffeinated water contains 45 mg of caffeine per 500 mL, so the caffeine concentration is 90 ppm (45 mg per 0.5 L = 90 mg/L). The same is true for the 90 mg caffeinated water; the concentration in ppm is twice the amount in mg/500 mL.

13. Measurement of the Fluorescence Intensity of the Ale 8 One Ginger Ale Beverage:

Ale 8 One (104 ppm)	Aspirin (40 ppm)	Distilled water	Concentration of Caffeine (ppm)	Fluorescence Intensity (a.u.)
0.1 mL	20 μL	3.6 mL	2.8	
0.3 mL	20 μL	3.4 mL	8.4	
0.5 mL	20 μL	3.2 mL	14.0	
0.8 mL	20 μL	2.9 mL	22.4	
1.0 mL	20 μL	2.7 mL	28.0	
1.2 mL	20 μL	2.5 mL	33.5	

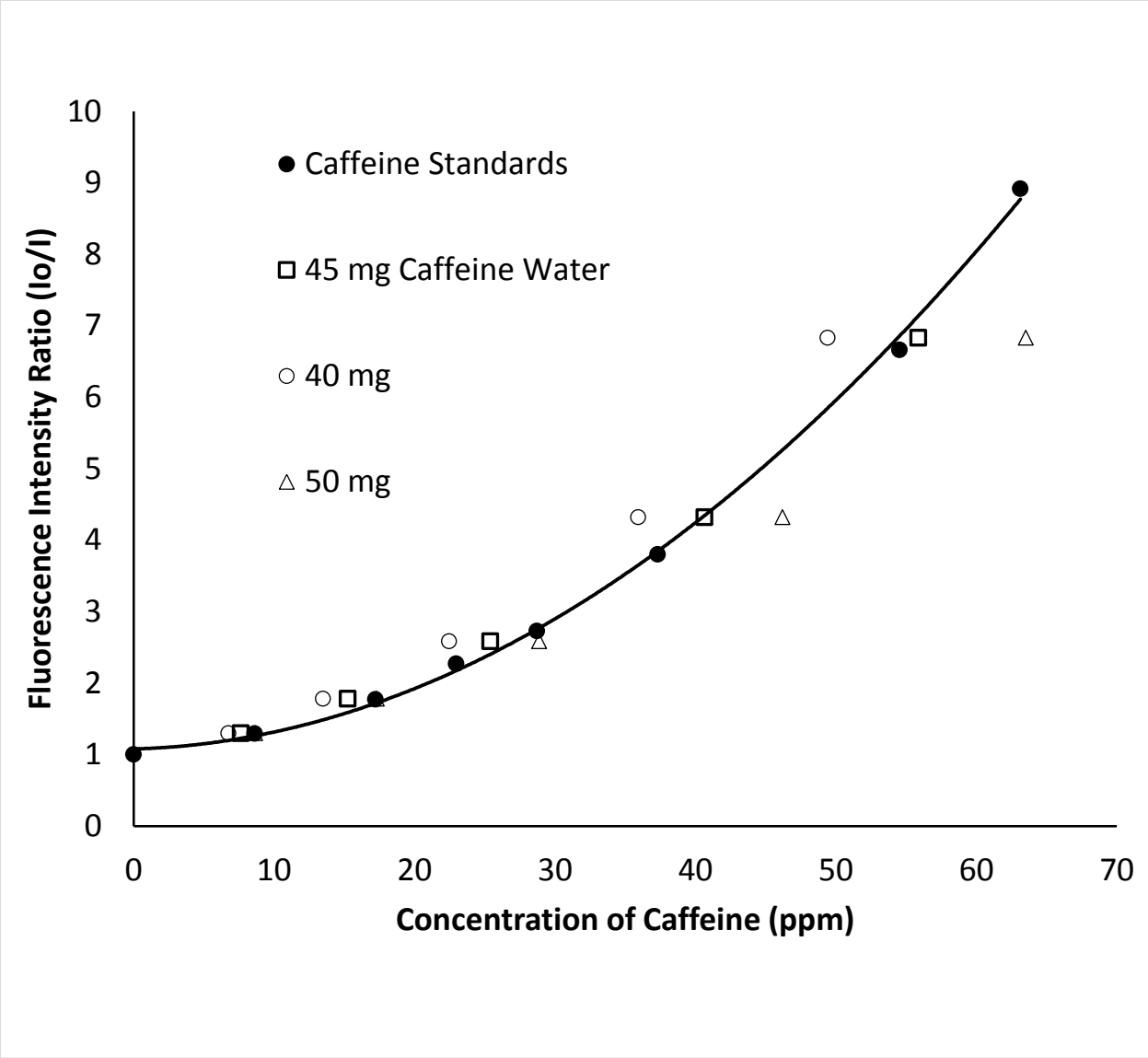
14. On Excel, plot (I_0 / I) vs. the concentration of caffeine for each series. Use the fluorescence intensity of aspirin solution with no quencher (0 ppm caffeine) as I_0.

15. The amount of caffeine in the beverages can be determined two ways:

    a. Each point in the Stern-Volmer plot can be used to determine the caffeine concentration. For example, a given fluorescence measurement of Ale 8 One yields an I_0/I value of 5.94. Using the fit to the standard curve, $y = 0.00305x^2 + 0.0218x + 1.03$, a caffeine concentration of 36.7 ppm is found. Adjusting for dilution (this particular point corresponded to 1.1 mL of Ale 8 One beverage in 3.12 mL total volume), yields a caffeine concentration of 104 ppm. This corresponds to 37 mg/12 oz. serving.

    b. If the Stern-Volmer plot (I_0/I versus caffeine concentration) for the caffeinated beverage overlaps the Stern-Volmer plot for the caffeine standards, then the measured caffeine is close to the expected amount. If the two plots do not overlap, the predicted caffeine concentration can be adjusted up or down. For example, instead of 45 mg/serving for the caffeine water, try 40 mg or 50 mg, and observe how the different curves line up. An example plot is shown below. This less rigorous approach gives more of a qualitative understanding of the effect of the quencher on the fluorescence.

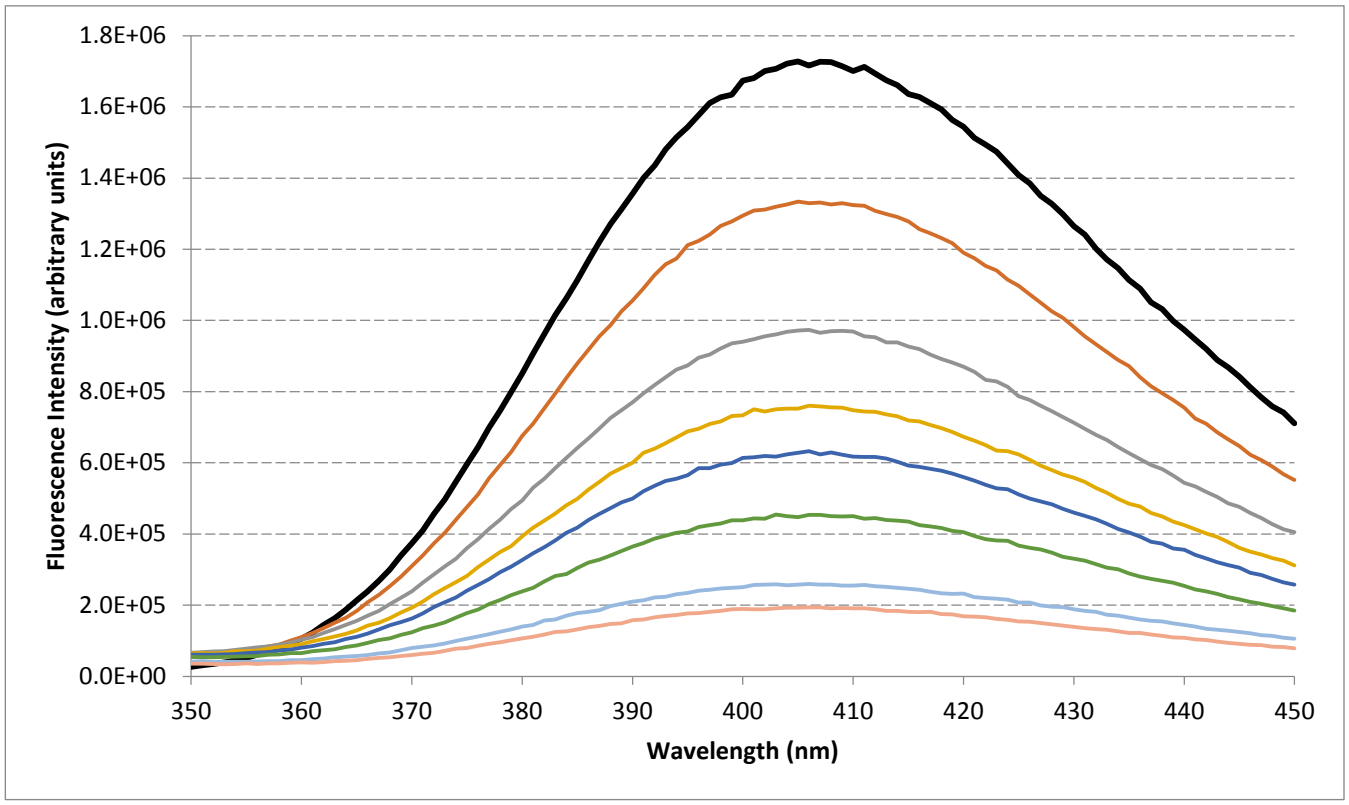
16. Optional: Run a standard curve for aspirin (measure the fluorescence for solutions with varying aspirin concentration, and no quencher).



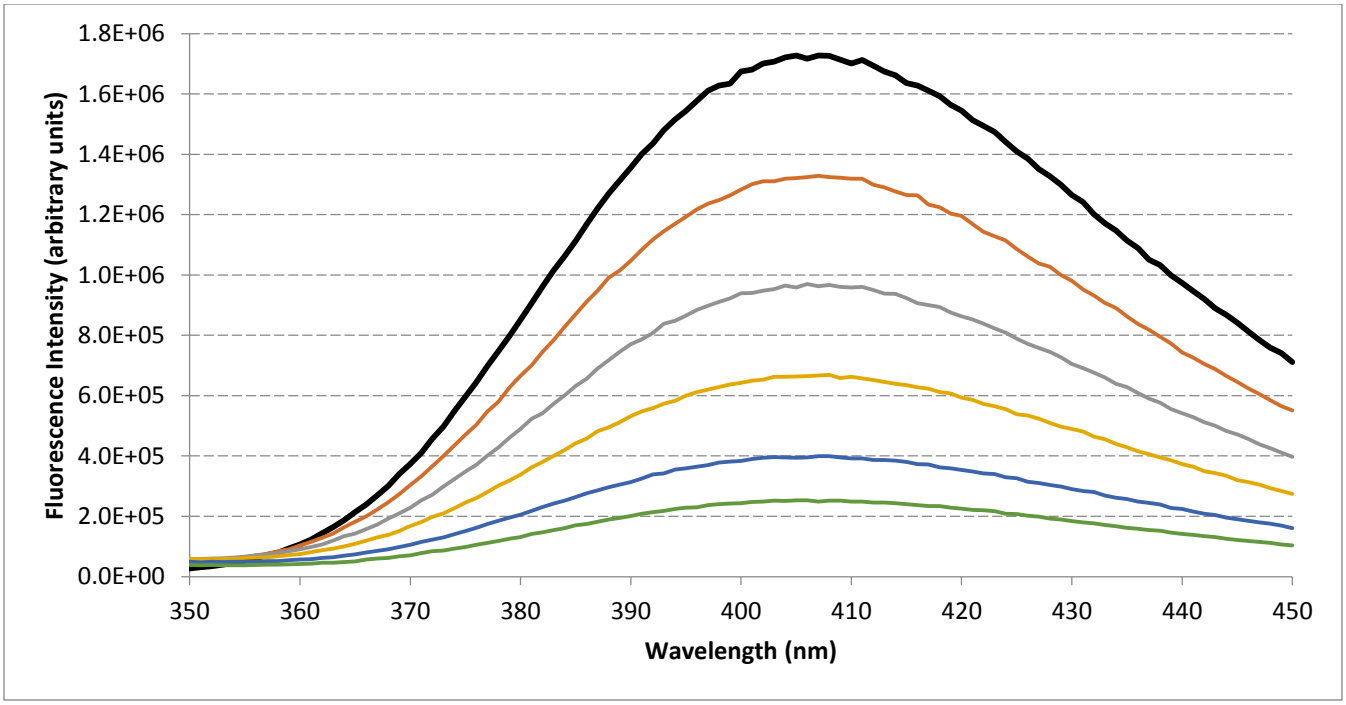
Stern-Volmer plot for aspirin with caffeine standards and with caffeinated water (45 mg/serving). The data points for the caffeinated water are also shown with different assumed caffeine concentrations (40, 45, and 50 mg per serving) to show the effect of the caffeine concentration on the Stern-Volmer plot. *This figure needs to be included in the Student Handout.

Instructor Notes

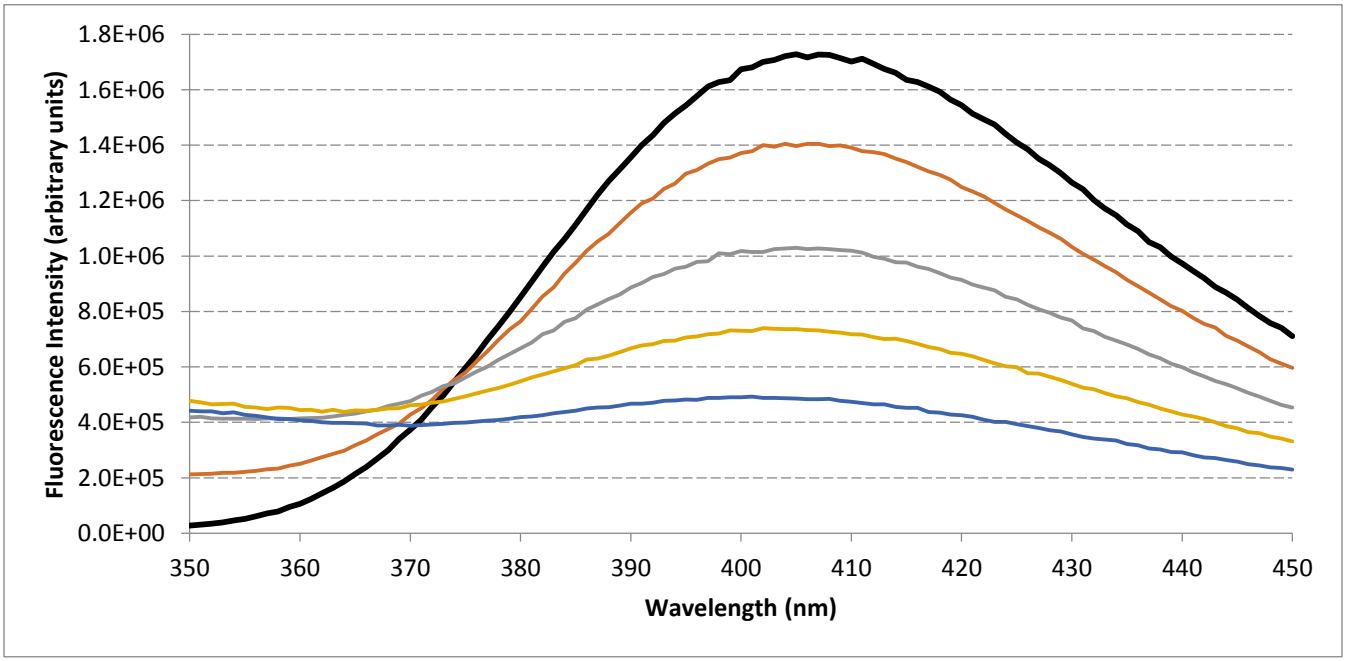
Sample Fluorescence Spectra – aspirin fluorescence with caffeine standards and beverages.



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.



Fluorescence Spectra for aspirin with no quencher (black line) and caffeinated water solutions (45 mg/serving) with increasing concentration (moving top to bottom). The bottom line was measured with a caffeine concentration of 53 ppm.



Fluorescence Spectra for aspirin with no quencher (black line) and Ale 8 One solutions with increasing concentration (moving top to bottom). The bottom line was measured with a caffeine concentration of 34 ppm.

Information about implementation, course format, and assessment:

This experiment is intended for upper-level chemistry and biochemistry majors who have learned about fluorescence spectroscopy in physical chemistry lecture, analytical chemistry, or a spectroscopy-based chemistry course. Prior to the experiment, the concepts of fluorescence and quenching are presented in a pre-lab lecture. After the experiment, the students complete the data analysis (constructing the standard curve and determining the amount of caffeine in the measured beverages). Experiments are usually completed in one week (in a conventional 3-hour laboratory period), and the data analysis is performed the following week. We all work together on the data analysis, in the model of a research team working on a common problem.

After the data analysis, the students write up a laboratory report. The laboratory report includes an introduction (where students would describe fluorescence spectroscopy), a procedure (written in the student's own words in paragraph form), results (tables, graphs, data analysis details), discussion (including error analysis), and references. The report is due the following week. I can determine from the quality of the laboratory report, specifically the introduction and discussion sections, the level of quality of the students' work and their level of mastery of the material studied. We typically perform six-to-eight experiments in a semester, in this fashion.

The laboratory experiment was developed over a period of several months by an undergraduate researcher (the lead author). 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-hour laboratory period; it works best with a smaller lab of 6-10 students, working in pairs. If only one fluorimeter 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.

Pre-Test and Post-Test Questions

The answers are in italics and can be removed before distributing to the students.

1. What is fluorescence?

   Fluorescence occurs when a molecule in solution absorbs light and is promoted to an excited state, loses some energy while undergoing collisions with other molecules in the sample, then emits light in a relatively short timescale (10^{-9} to 10^{-7} seconds) as the molecule returns to the electronic ground state.

2. Name the various parts of a fluorescence spectrometer and write a brief description of each part's function.

   a. Light source – Tungsten lamp that emits light for exciting molecules in the sample

   b. Excitation monochromator – selects a specific wavelength from the light source, in order to excite the molecules in the sample at a specific wavelength

   c. Sample – cuvette, usually quartz to allow passage of UV light, clear on all four sides to allow emission of light at 90°to incident to excitation light

   d. Emission monochromator – selects a specific wavelength of emitted light (from the fluorescing sample)

   e. Photomultiplier (PM) Tube – counts the number of emitted photons (i.e., produces an electrical signal proportional to the number of detected photons).

   f. Analog-to-Digital (A/D) Convertor/Computer – Converts the electrical signals from the PM Tube into a fluorescence spectrum (fluorescence intensity at each measured wavelength).

3. Explain the relationship between the wavelength of absorbed light and emitted light that is measured in fluorescence spectroscopy.

   The emitted fluorescence is measured at a 90-degree angle to the absorbed light (the excitation radiation). The emitted light is at a longer wavelength (lower energy) than the absorbed light, because the molecule has lost some of its excitation energy in collisions with other molecules in the sample.

4. Why is fluorescence spectroscopy such a sensitive technique?

   The emitted light is at a longer wavelength than the excitation light and is measured at a 90-degree angle relative to the excitation light. A blank or background measurement is not needed.

5. What is quenching in fluorescence spectroscopy?

   Quenching occurs when the fluorescence emitted by the excited sample molecule is decreased in the presence of another molecule. This may be due to the second molecule colliding with the excited molecule (collisional or "dynamic" quenching) or forming a complex with the excited molecule (static quenching).

6. What is the effect of caffeine concentration on aspirin fluorescence?

   Caffeine quenches the fluorescence of the aspirin fluorescence. The degree of quenching increases as the concentration of caffeine in the sample increases.

7. What is a Stern-Volmer plot?

   A plot of the fluorescence intensity ratio (original intensity/quenched intensity or I_0/I) versus the quencher concentration is called a Stern-Volmer plot.

8. How can a Stern-Volmer plot be used to measure the concentration of caffeine in beverages?

   Caffeine standards added to an aspirin solution (of fixed concentration) can be measured to create a standard curve (in the form of a Stern-Volmer plot). Caffeine solutions prepared from caffeinated beverages and added to an aspirin solution (of fixed concentration) can be measured and compared to the standard curve to find the caffeine concentration in the original beverage.

Historical note:

Ale 8 One ginger ale is a popular soft drink in Central Kentucky. It has been bottled in Winchester, KY since 1926. Founder and inventor G. L. Wainscott began bottling soda water and flavored drinks in 1902. The name Ale 8 One ("A Late One") was the winning entry in a slogan contest at the Clark County Fair, and was a pun describing the latest thing in soft drinks.

Caffeine Content – of Selected Beverages

Beverage	**Serving Size**	**Caffeine Content (mg/serving)***
Ale 8 One ginger ale	12 oz (355 mL)	37
Ávitāe caffeine water (45 mg)	16.9 oz (500 mL)	45
Ávitāe caffeine water (90 mg)	16.9 oz (500 mL)	90
Barq's Root Beer	12 oz (355 mL)	22
Coca Cola Classic	12 oz (355 mL)	34
Coffee (Brewed)	8 oz (237 mL)	163
Coffee (Instant)	8 oz (237 mL)	57
Diet Coke	12 oz (355 mL)	46
Diet Pepsi	12 oz (355 mL)	34
Dr. Pepper	12 oz (355 mL)	41
Mountain Dew	12 oz (355 mL)	54
Pepsi Cola	12 oz (355 mL)	38
Tea (Black)	8 oz (237 mL)	42
Tea (Green)	8 oz (237 mL)	25
Tea (White)	8 oz (237 mL)	28

*Ted Kallmyer. Caffeine Informer. http://www.caffeineinformer.com/the-caffeine-database (accessed June 23, 2016).

CAS Numbers

50-78-2 aspirin

58-08-2 caffeine