

EXPERIMENT 6

Molecular Fluorescence Spectroscopy: Quinine Assay

UNKNOWN

Submit a clean, labeled 500-mL volumetric flask to the instructor so that your unknown quinine solution may be issued. Your name, section number, and your locker number should be written legibly on this flask. The flask does not need to be dry on the inside, but needs to have been rinsed with distilled water after it has been cleaned. *The flask must be turned in at least 1 lab period before you plan to do the experiment* so that the Teaching Assistants will have time to prepare the unknown. Each student will have his or her own unknown to analyze even if you are working in pairs.

BACKGROUND

Quinine ($C_{20}H_{24}N_2O_2$, 324.43 g/mol) is an alkaloid extracted from the bark of the cinchona tree. It has been used for many years as an antimalarial agent. Although it does not cure malaria, it is effective in alleviating the symptoms of malarial attacks. The usual medicinal form is quinine dihydrochloride or quinine sulfate dihydrate, $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$, 782.97 g/mol.

Quinine is a very strongly fluorescing compound, especially in dilute acid solution, and thus can be detected in very trace amounts. In 0.05 M H_2SO_4 , quinine has two analytically useful *excitation wavelengths*: $\lambda_{ex} = 250$ and 350 nm. Regardless of which excitation wavelength is used, the wavelength of maximum *fluorescence emission* intensity, λ_{em} or λ_{fl} , is 450 nm. The basis for quantitation is that the intensity of fluorescence emission in very dilute solutions is directly proportional to the concentration of quinine – if the intensity of the excitation source and other experimental factors are kept constant. Because the absolute emission intensity can vary considerably with small differences in experimental conditions, a calibration curve is prepared by measuring the fluorescence-emission intensity of accurately known quinine standard solutions.

INSTRUMENTATION

Turner Quantech Digital Filter Fluorometer, Model FM 109525

The instrument used in this experiment uses glass filters to select wavelength ranges appropriate for quinine, rather than some type of expensive grating or prism monochromator to isolate narrow excitation and fluorescence wavelengths. The latter type may typically have a bandpass of 0.1 or 1 nm. A *bandpass* is defined as the width at half-height of the maximum intensity of light that passes through the filter. It is also called the *bandwidth*.

A monochromator-based instrument is much better for obtaining spectra, particularly those with fine structure, but a filter-based unit will often be better for routine quantitative analysis. The much larger bandpasses permit greater fractions of the excitation and fluorescent light to excite the sample and to reach the detector, respectively. This greatly increases the sensitivity of the instrument and can thus usually lower typical detection by an order of magnitude or more. Filter fluorimeters are also much less expensive.

Excitation Source: A 5-watt quartz-halogen lamp, which emits intense broadband radiation from 340 nm to 750 nm.

Excitation Wavelength Filter: A narrow-band 360-nm filter with a bandpass of 40 nm.

Emission Wavelength Filter: A sharp cut-in, long-wavelength-pass filter which transmits essentially all light with $\lambda > 415$ nm.

Detector: Photomultiplier tube, model 931B PMT.

Detection Limit. Stated in the manufacturer's literature as 30 ppt quinine sulfate, which is 30 ng/L or about 9×10^{-11} M.

Turn the instrument on at least 15 minutes before using to allow it to warm up and stabilize. The **ON-OFF switch** is on the back panel near the power cord. When turned on, the instrument runs a countdown timer during which it undergoes self tests.

PREPARATION OF SOLUTIONS

Preparation of Stock Sulfuric Acid Solutions

The two stock H_2SO_4 solutions should have already been prepared for you by the Teaching Assistants. However, if you use up all of the 0.05 M solution while doing your experiment, prepare another 2-L batch for those that follow. It is very straightforward.

1 M H_2SO_4 . Slowly and carefully add 56 mL conc. H_2SO_4 to about 500 mL distilled water in a 1-L beaker with stirring. This solution should have been already prepared for your use.

0.05 M H_2SO_4 . With a graduated cylinder, add 100 mL of 1 M H_2SO_4 with a graduated cylinder to about 500 mL of distilled water in the screw-capped acid reagent jug labeled for this solution. Mix and dilute to the 2.0-L mark on the bottle and mix thoroughly. This solution should already have been prepared for your use. If you use all of or most of what is there, prepare a new batch for the students who follow you.

Preparation of Quinine Stock Solution, 1000 ppm

1. Carefully weigh exactly 0.1207 g of quinine sulfate dihydrate onto a folded glassine weighing paper or into a small plastic weighing boat, and transfer this *quantitatively* into a 100-mL volumetric flask. A few squirts of distilled water from a wash bottle should help to wash the solid material from the weighing boat and the neck of the flask.

2. Pipet 5.00 mL of 1 M H₂SO₄ (located in hood #2) into the flask. Carefully dissolve all the quinine in this sulfuric acid solution by swirling *before* diluting to volume. *This is critically important.*
3. Carefully dilute to volume with distilled water and mix thoroughly.

Preparation of Intermediate Quinine Stock Solution, 10.0 ppm

1. Pipet 5.00 mL of the 1000-ppm solution into a 500-mL volumetric flask.
2. Add 25.0 mL of 1 M H₂SO₄, dilute carefully to volume with distilled water, and mix thoroughly.

Preparation of Quinine Standard Solutions

1. Using volumetric transfer pipets and/or a 10-mL graduated pipet, add 1.00, 3.00, 5.00, 7.50, and 10.00 mL of the 10-ppm intermediate stock solution into five properly labeled 100-mL volumetric flasks. This will result in standard solutions of 0.1, 0.3, 0.5, 0.75, and 1.0 ppm.
2. Carefully dilute to volume with 0.05 M H₂SO₄.
3. 0.05 M H₂SO₄ is used as the “blank.”

Preparation of Quinine Unknown

1. Your unknown solution is obtained from the teaching assistants in a 500-mL volumetric flask. Add 25.0 mL of 1 M H₂SO₄ to the flask.
2. Dilute to volume with distilled water and mix thoroughly.

PROCEDURE

Turn on the Quantec fluorometer at least 15-20 minutes before making measurements in order to let it warm up and stabilize.

Measurement of Emission Intensities

1. Carefully fill separate, clean, plastic fluorescence cuvettes (these have 4 clear sides) about $\frac{3}{4}$ full with the blank (0.05 M H₂SO₄), each of the five standards, and each unknown sample. Do not touch the optical surfaces with your fingers. Instead, handle with KimWipes. Be sure to wipe any smudges off the optical surfaces of the cuvettes.
2. On the main menu of the fluorometer, press **ENTER**.

3. Use the right cursor (®) to select “Quinine”. Press **RETURN**.
4. The instrument asks if you wish to change the name. Select **NO**.
5. The instrument asks if filters are correct. Select **YES**.
6. The instrument asks if you would like to you as standard curve from memory. Select **NO**.
7. The instrument asks for the number of points for the calibration curve. Use the **up-arrow (↑)** to increase the number to 5 in order to be able to use your five standards. Press **RETURN**.
8. Beginning with the highest concentration standard, enter the concentration using the **up-arrow** to change the values. Use (←) to move the cursor to the appropriate position. Use the **up-arrow** to set the appropriate units (**ppm**).
9. The instrument asks to insert the sample. Do so and press **RETURN**.
10. Repeat for other standards.
11. Instrument asks the operator to insert the **BLANK**. Do so and press **ZERO**.
12. The instrument gives a “Coefficient of Determination” value. If this value is less than 0.90, you must pour out your standard solutions and repipet them from the stock quinine solution. If this value is greater than 0.90, continue.
13. Insert the unknown and Press **RETURN**.
14. Readout shows **READ** and provides the value.
15. **Repeat the entire process at least two more times to obtain a set of three “good” replicate measurements:** If the values for a set of standards and the unknown do not “drift” over time, this would mean that the raw fluorescence data for your unknown are within a range of about 3-4% relative. If the values do appear to drift, then the ratios of the fluorescence intensities of the standard to those of a nearby standard are within a range of about 3-4% relative.

Comparison of Calibration Procedures

This procedure will compare values that are (a) calculated by the instrument based on its internal calibration procedure with (b) those obtained based on the calibration curve(s) that you prepare from the raw fluorescence-emission data obtained above.

1. At main menu, select **RAW FLUORESCENCE** as the units of measure.
2. The instrument requests a value for the standard. Enter the value and **RETURN**.

3. The instrument instructs you to insert the standard. **USE the 1.0 ppm STANDARD.** Do so and press **RETURN**. The instrument adjusts its internal gain.
4. The instrument asks “Insert Blank Sample?” Select **YES**, followed by **RETURN**.
5. Insert the blank and the instrument will take a reading.
6. Now insert each of the standard solutions and record the emission value for each.
7. Insert the unknown, and record the emission value.
8. Repeat the entire procedure at least twice more in order to obtain at least 3 “good” measurements for each sample.
9. Prepare calibration curves and calculate the average concentration of the unknown sample.

When done with the entire experiment, rinse out all the plastic cuvettes with distilled water, shake off excess water, and return them to the experiment drawer so they can be reused.

DATA ANALYSIS

Prepare appropriate calibration curve(s) from your replicate sets of data. Depending on the nature of the data and any drift in the instrument, it may be best to (a) average all the net emission intensities for a particular solution and to prepare one calibration curve or (b) prepare several calibration curves from the separate sets of data, obtain several values for the concentration of quinine in your unknown, then average these values. Determine which one is better or if both give equivalent results. Use linear least squares to fit each of the three sets of data, and the average of all three, in your analysis.

Report the “best value” for the concentration of quinine (in ppm) in your unknown and the estimated standard deviation of this best value.

Note: By definition, parts-per-million (ppm) is a mass-ratio unit – $\frac{g_{\text{analyte}}}{g_{\text{sample}}} \times 10^6$ or $\mu\text{g/g}$. In dilute aqueous solutions, 1 ppm is equivalent to 1 $\mu\text{g/mL}$ or 1 mg/L because the density of the solution is 1.00 g/mL . A 1000-ppm solution of *quinine sulfate* (1.000 g of quinine sulfate per liter of solution) would only be an 828.7 ppm of quinine itself. If you cannot weigh out exactly 0.1207 g of quinine sulfate, try to get it close, and simply calculate the correct values of the concentrations of your standards.

HAZARDOUS WASTE DISPOSAL

Dispose of ALL waste quinine and sulfuric acid solutions in the proper, labeled Hazardous Waste Container for this experiment, which is located in the hoods. 0.05 M H₂SO₄ has a pH

of about 1 and thus may NOT simply be run down the drain, even if you run large amounts of water with it. If you are unsure about the container, ASK.

TEXT REFERENCE

D. A. Skoog, D. M. West, F. J. Holler, and S. R. Crouch, *Analytical Chemistry: An Introduction*, 7th ed., Chapter 23, pp. 594-631.

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