

FLUORESCENCE SPECTROSCOPY OF QUININE

Basis of the Experiment

In this experiment, we will make use of a recording spectrofluorimeter to study the luminescence characteristics of the bicyclic alkaloid quinine, which is commonly used as a fluorescence standard. Quinine is also used for medicinal purposes, most notably in the treatment of malaria and also a common ingredient in tonic water.

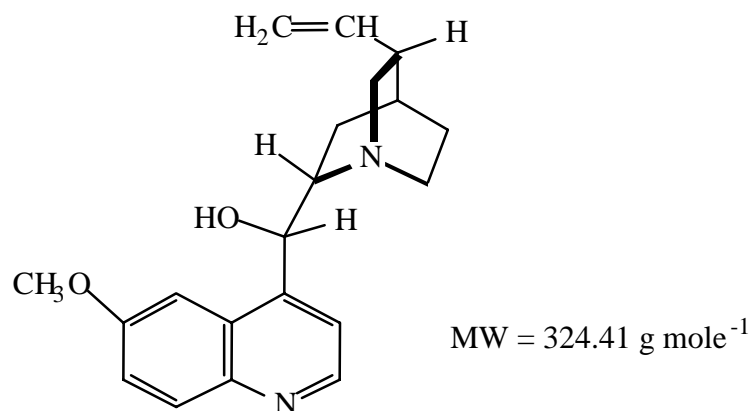


Figure 1. Molecular structure of quinine.

Different aspects of this experiment include recording the excitation and emission spectra of quinine, determining the concentration of quinine in a commercial tonic water, and comparing the influence of experimental parameters on the fluorescent intensity of quinine. Basic information on fluorescence theory and instrumentation can be found in Skoog and West or any advanced instrumental text.

Apparatus

Shimadzu 2401PC Spectrofluorimeter, 1 1-L volumetric flask, 8 25-ml volumetric flasks, microburet or various pipets, and any UV-Vis recording spectrometer.

Reagents & Solutions

1. The following reagents and solutions are available in the lab: sulfuric acid, quinine sulfate dihydrate, 2000 ppm NaCl in 0.05 M H_2SO_4 , ice, N_2 , O_2 , tonic water, 0.05 M sodium phosphate buffer, pH 7.4, 0.05 M sodium carbonate buffer, pH 9.6.
2. *Preparation of solutions.* Clean all glassware with cleaning solution and rinse with distilled water.
 - a. Prepare 1 liter of 0.05 M H_2SO_4 . Prepare this solution in the hood, wear gloves and remember that you should always add concentrated acids to water and **not** the water into acids. Use 2.75 mL concentrated H_2SO_4 for 1 L.
 - b. Prepare 500 mL of 10 ppm quinine in 0.05 M H_2SO_4 (use 6.1 mg of quinine sulfate dihydrate).
 - c. Prepare 25 ml each of 1.00, 0.500, 0.250, 0.100, 0.0500, 0.0250 and 0.0010 ppm quinine in 0.05 M H_2SO_4
 - d. Prepare 25 ml of 0.10 ppm quinine in 0.05 M H_2SO_4 with 1000 ppm NaCl.
 - e. Prepare 100 mL of the following solution: 0.1 ppm quinine in distilled H_2O (use the 10 ppm quinine in 0.05 M H_2SO_4 and dilute with distilled H_2O). Also prepare 25 mL of 0.1 ppm quinine in 0.05 M sodium phosphate buffer, pH 7.4, and 0.1 ppm quinine in 0.05 M sodium carbonate buffer, pH 9.6.

Waste: Sink except concentrated H_2SO_4 (inorganic)

Theory

The LS50B Fluorescence Spectrophotometer is designed for the measurement of fluorescence excitation and emission spectra. The fluorescence spectrophotometer includes two grating monochromators, one for irradiating a sample with monochromatic light in the 200 to 800 nm range (excitation monochromator), and the other for permitting selective measurement of the intensity of the light emitted by the sample in the 220 to 900 nm range (emission monochromator).

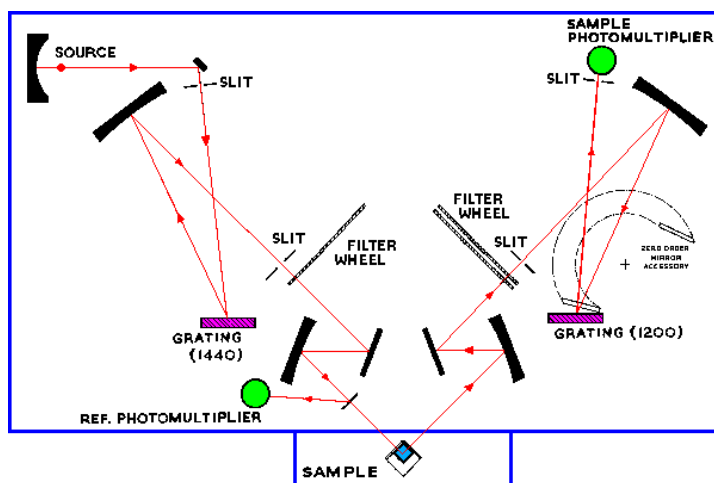


Fig. 1. Block diagram of the optical system of the LS50B

The LS50B consists of a spectrometer, a power supply for the lamp source, an amplifier, and computer. Light from the Xenon lamp passes into the excitation monochromator where it is dispersed, a narrow wavelength band is selected and then focused on the sample. Fluorescent light emitted by the sample is directed into the emission monochromator which is scanned to determine the wavelength of this radiation. The light from the emission monochromator strikes the sample photomultiplier, producing a signal proportional to the intensity of the fluorescence radiation. The signal passes through the amplifier and to the computer system. Scanning of the excitation and emission radiation is accomplished with monochromator cams which are motor-driven and linked to their respective gratings. Continuous scanning of either the excitation or the emission spectrum of a sample is possible.

The process of fluorescence depends on the molecule absorbing light energy of one wavelength (λ_1) and emission of light energy at a longer wavelength (λ_2). The difference between λ_1 and λ_2 depends on the amount of energy lost in the excited state which is dependent on the characteristics of the molecule. This difference is generally greater than 30 nm. Emission peaks less than 30 nm from the excitation wavelength are generally due to scattering.

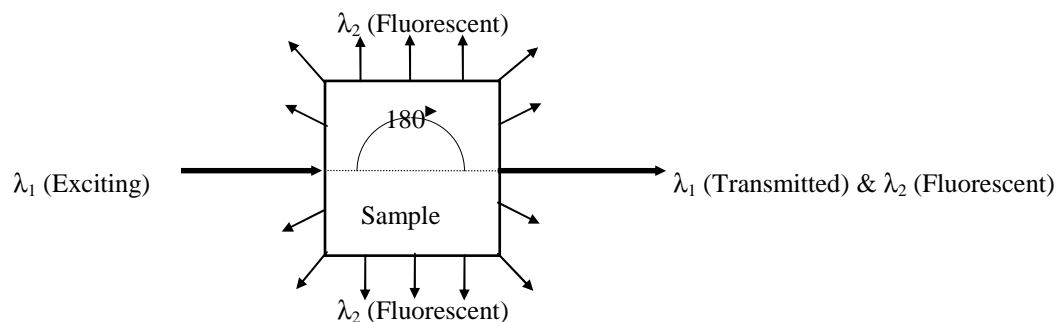


Fig. 2. Excitation and emission radiation from a sample cell.

Procedure

The experiment is divided into the following sections:

- A. Excitation spectrum of quinine
- B. Emission spectrum of quinine
- C. Quinine calibration curve
- D. Determination of quinine in tonic water
- E. Effect of experimental parameters on luminescence efficiency

General Operating Instructions

1. Uncover instrument and put on safety glasses.
2. Turn on spectrometer (switch on lower back of left side), computer, monitor, and printer.
3. Open the front door of the spectrometer and place the sample in cuvette holder.
4. At the Windows Desktop, Double click (with the left button of the mouse) on the "FL WinLab" icon.
5. Click on "Utilities", then "Configuration". Click on "Load", then highlight "scha-311.cfg" using the mouse. Click on "OK", then "OK" again.
6. Click on "Application", then "Scan". Click on either the emission or excitation tab. Type in the scan speed=240, excitation slit=#, and emission slit=#. Enter a file name, if desired.
7. To record a spectrum, click on "Instrument", then "Run".
8. To scale a spectrum, click on the red up and down arrows over the scan window.
9. You can obtain the maximum intensity by clicking the on the name of the scan.
10. To print the spectrum click on the printer icon at the top of the window.
11. Obtain from the A-Level Stockroom one 10 x 10 mm quartz cuvettes polished on four sides (approximately \$250.00 each,). These cuvettes require special care as they cannot be handled, as other cuvettes, on the frosted sides. They should be handled only by the corners and only near the top of the cell. Open the front door of the spectrometer and place the sample in cuvette holder.
12. Allow the instrument at least 15 minutes to warm up and stabilize.

A. Excitation Spectrum of Quinine

In this part of the experiment, we will investigate the variation of fluorescent intensity when the wavelength of excitation is varied with fixed emission monochromator wavelength.

1. Position cuvette with 1 ppm (or lower concentration if this keeps the spectrum on scale) solution in beam with cell positioning knob.
2. Click on "Application", then "Scan". Click on the excitation tab. Type in the scan speed=240, excitation slit=2.5, and emission slit=2.5. Enter a file name and select auto updating.
3. Set emission wavelength to 450 nm.
4. Set excitation wavelength range to 200-400 nm.
5. To record a spectrum, click on "Instrument", then "Run".

B. Emission Spectrum of Quinine

In this part, we will determine the variation of fluorescent intensity with variation in wavelength of emission. This study is carried out by leaving the excitation monochromator fixed while varying the wavelength of the emission monochromator.

1. Use the same sample from part A.
2. Click on "Application", then "Scan". Click on the emission tab. Type in the scan speed=240, excitation slit=2.5, and emission slit=2.5. Enter a file name.
3. Set the excitation wavelength to the wavelength of maximum response determined in Part A.
4. Set emission wavelength range to 380-540 nm.
5. To record a spectrum, click on "Instrument", then "Run".
6. Repeat 1-5 using an excitation wavelength of 250 nm.

C. Quinine Calibration Curve

1. Click on "Application", then "Scan". Click on the emission tab. Type in the scan speed=240, excitation slit=2.5, and emission slit=2.5. Enter a file name. It is suggested that you also select auto-incrementing.
2. Set excitation monochromator to 350 nm.
3. Set emission wavelength range to 380-540 nm.
4. Starting with the most dilute solution, record the emission spectra of the seven solutions from 0.001 to 1.0 ppm quinine. Repeat each point twice so you have a measure of data uncertainty.

D. Analysis of Tonic Water

1. Most commercial tonic waters contain 50-100 ppm quinine.
2. With this in mind, analyze a tonic sample for quinine concentration.
3. Choose the portion of your calibration curve you wish to employ (*why did you make your choice?*) and dilute the tonic water with 0.05 M H₂SO₄ so the quinine concentration falls within the chosen range.
4. Record an emission spectrum as in Part C, repeat twice so enhance the quality of your data.

E. Influence of Experimental Parameters on Quinine Luminescence

Using instrumental setting from C as a starting point, record the fluorescence intensity of the following solutions. Do one pair comparison at a time to overcome instrument fluctuation.

1. 0.1 ppm quinine cooled in ice water vs. 0.1 ppm quinine heated slightly under hot tap water.
2. 0.1 ppm quinine through which O₂ has been bubbled vs. 1 ppm quinine through which no O₂ has been bubbled. The oxygen should be bubbled through the sample for at least 10 minutes.
3. 0.1 ppm quinine through which N₂ has been bubbled vs. 0.1 ppm quinine through which no N₂ has been bubbled. The nitrogen should be bubbled through the sample for at least 10 minutes.
4. 0.1 ppm quinine vs. 0.1 ppm quinine with 1000 ppm NaCl added.
5. 0.1 ppm quinine in distilled H₂O vs. 0.1 ppm quinine in 0.05 M H₂SO₄.
6. 0.1 ppm quinine in 0.05 M sodium phosphate buffer, pH 7.4.
7. 0.1 ppm quinine in 0.05 M sodium carbonate buffer, pH 9.6.

F. Absorption Spectrum

1. Obtain an absorption spectrum of quinine from 200-400 nm using the 10 ppm quinine solution and an available UV-Visible spectrometer. *What should you place in your reference cell?*

Shutdown Procedures for LS50B

1. To turn off the spectrometer, click on "File", then "Exit". Shut down Windows 98, then turn off the computer, monitor, and spectrometer. Remove your sample.
2. Cover instrument.

Report

Tables.

1. Describe any solutions you prepared in a table format in your "Experiment and Instrument" section.
2. Tabulate the values from your calibration curve data. Remember you will be taking reading for emission intensity from the software.
3. Tabulate the effects of the different conditions on the signal for quinine.

Figures

1. Plot luminescence intensity vs. quinine conc. Plot a calibration curve and explain how it was obtained. Give your regression parameters with the associated errors you obtained. On the same figure, show how the concentration of quinine in tonic water was obtained. Give your result with its' associated standard deviation.
2. On a second figure plot the values obtained for the samples that were exposed to oxygen, heat, cold, and changes in solvent. Since you are comparing this to a solution on your calibration lines think about an appropriate way to present this data.
3. Your report should also include copies of the absorption, emission and excitation spectra of quinine. Remember to label these figures fully and correctly.

Questions to help you write your discussion.

1. What is the difference between an excitation and an emission spectrum?
2. What is concentration quenching?
3. Explain your results on the influence of experimental parameters (heat, cold, O₂, N₂, pH) on the fluorescence of quinine.
4. In section D, what portion of the calibration curve did you select for the determination of quinine in tonic water and why?
5. Compare the excitation and emission spectra you obtained.
6. Comment on the similarities between the excitation and absorption spectra of quinine. Why aren't these spectra identical?
7. Explain the similarity of the two emission spectra obtained using different excitation wavelengths. Why do the spectral intensities differ?