Determination of Quinine in Tonic Water by Fluorescence

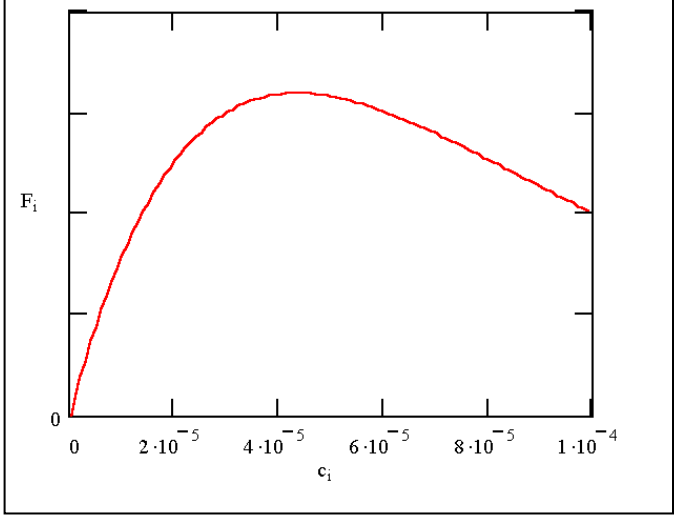
Introduction. In another lab, you determine the concentration of quinine and sodium benzoate in tonic water using UV-Visible absorbance spectroscopy. The benzoate ion absorbs strongly in the UV wavelength range, especially in neutral or basic solutions. Quinine, another component of tonic water, fluoresces strongly, allowing it to be detected at very low concentrations, but detecting quinine at low concentrations is not a goal in this experiment because the concentration of quinine is not especially low in tonic water. Benzoate also fluoresces, and its fluorescence response is similar to that of toluene; the fluorescence emission spectrum of toluene is attached for comparison with that of quinine, which is provided below. By judicious selection of the wavelength used for excitation, λ_{ex}, and the wavelength used to monitor the fluorescence emission, λ_{em} , you should be able to avoid any significant contribution from benzoate fluorescence when trying to excite and detect quinine fluorescence in this experiment. This ability to focus on one component of the sample simplifies the experiment as compared to a measurement made by absorption, because now it is not necessary to independently determine the benzoate concentration. Fluorescence is often very selective to only one or two compounds in a mixture, and using fluorescence can save time needed to separate those compounds from the mixture (by chromatography, for example). Fluorescence is, in general, also a more sensitive analytical technique than absorbance, as you should see when you compare detection limits for the two techniques.

Fluorescence. Ideally, in a fluorescence measurement the emission intensity F (labeled F_i in Fig. 1) is proportional to the incident power, P_0, of the excitation source, as well as the molar absorptivity ε and the concentration of the analyte c:

$$F = P_0 \Omega \varepsilon c \qquad \text{(eq 1)}$$

The parameter Ω represents a collection of terms, including the *quantum yield* and the collection and detection efficiencies. As you know from the theory of absorbance spectroscopy, when there are many absorbers in the light path, the power of the excitation light source propagating through the sample will decrease over the path length of the cell because absorption of the excitation wavelength occurs. In fact, the excitation source power P propagating through the sample decreases exponentially with c in accordance with Beer's Law, so we expect a nonlinear relation, as shown in Eq. 2.

Figure 1



$$F = P_0 10^{-\varepsilon b c} \Omega \varepsilon c \qquad \text{(eq 2)}$$

The collection efficiency term contained in Ω is not strictly constant but will decrease somewhat as *c* increases due to the sample reabsorbing its own fluorescence emission photons at λ_{em} (in addition to absorbing the incident power photons at λ_{ex}). We will neglect this effect (known as *self-absorption* or *inner filter* effects) because it is not large unless the emission and excitation spectra overlap significantly and the emission wavelength used is near a strong absorption.

A plot of Eq. 2 for simulated data is shown below, using $\varepsilon = 10{,}000\ cm^{-1}\ M^{-1}$. The calibration curve becomes noticeably nonlinear when analyte concentration becomes "high." For example, when $c > 1/(10 \times \varepsilon)$, P is noticeably less than P_0. Notice that nonlinearity in the F vs. c curve sets in at relatively low concentrations, and that F actually *decreases* for concentrations larger than about 5×10^{-5} M in this example.

PRE-LAB ASSIGNMENT

Write your answers to the following questions in your laboratory notebook. Be prepared to show these to your TA at the beginning of your lab period. Also be prepared to discuss the issues with your partners and your TA.

1. The fluorescence emission spectrum of quinine is provided below. For maximizing sensitivity and restricting the measured fluorescence emission signal to just that of quinine, choose the excitation λ_{ex} and emission λ_{em} wavelengths you should use for this lab. Explain how you arrived at these values.
2. Using Excel or other plotting software, plot Eq. 2 for quinine using the correct molar absorptivity and the same range of concentrations for studying the limiting high value for the absorbance spectrometer. Use any arbitrary, fixed values for Ω and P_0 because these will not affect the shape of the curve. Plot Eq. 2 again for a range of concentrations that is ten-fold lower. Based on these plots of Eq. 2, decide whether you should dilute your quinine sample to get it on the linear range of the calibration curve.
3. If you conclude that a diluted sample of tonic water must be used to achieve linearity in the relation between *c* and *F*, decide what dilution factor ought to be used. To support your idea, make a new plot of *c* and *F* for the new range of concentrations of standards that bracket that of the diluted tonic water sample to show that these are expected to be linear.
4. Explain how you will estimate the detection limit for the fluorescence determination of quinine. What will you need to measure, and how can you make the needed measurements?

EXPERIMENTAL

1. Using your arguments in the pre-lab and an iterative experimental process in the lab if necessary, choose the excitation λ_{ex} and emission λ_{em} wavelengths you should use. *To do this:*
   (1) Use the absorbance spectrum as a guess for the excitation spectrum, and set the excitation wavelength to the wavelength with highest absorbance – but away from that for the benzoate.
   (2) *Scan the emission spectrum, and find the wavelength of maximum emission for quinine.*

(3) *Using the emission spectrum, set the wavelength of the emission maximum as the emission wavelength and scan the excitation spectrum, then adjust the excitation wavelength as needed, based on your results*.

2. When collecting the spectra, be sure to set the non-scanned (excitation) wavelength to an appropriate value for λ_{ex}. Measure the fluorescence excitation spectrum of quinine ***once***, using the highest concentration, using your predicted value for the emission wavelength λ_{em} and scanning the excitation wavelengths. Then, measure the fluorescence emission spectra of quinine at the appropriate concentrations by fixing the excitation wavelength λ_{ex} appropriately, based on your spectral results, and scanning the response over the emission wavelengths.
3. Prepare, record in your notebook, and use your set of fluorescence calibration standards to determine the concentration of quinine in the supplied diluted tonic water "unknown". You will want to take a sample of the tonic water unknown and dilute it appropriately. Your dilutions should be with 0.05M H_2SO_4, so that the quinine remains in the acidic form throughout. ***When taking your liquids from the tonic water unknown, be careful not to contaminate it. Always pour out from the original containers into a clean, dry container that you will use for pipetting. Never pipette directly from a communal container!*** Take care in making these diluted solutions – most of the systematic error that occurs in this experiment arises from careless technique in pipetting, dilutions and from incomplete mixing!
4. Make the necessary fluorescence measurements for your determination of the fluorescence detection limit for quinine.

SAFETY AND DISPOSAL

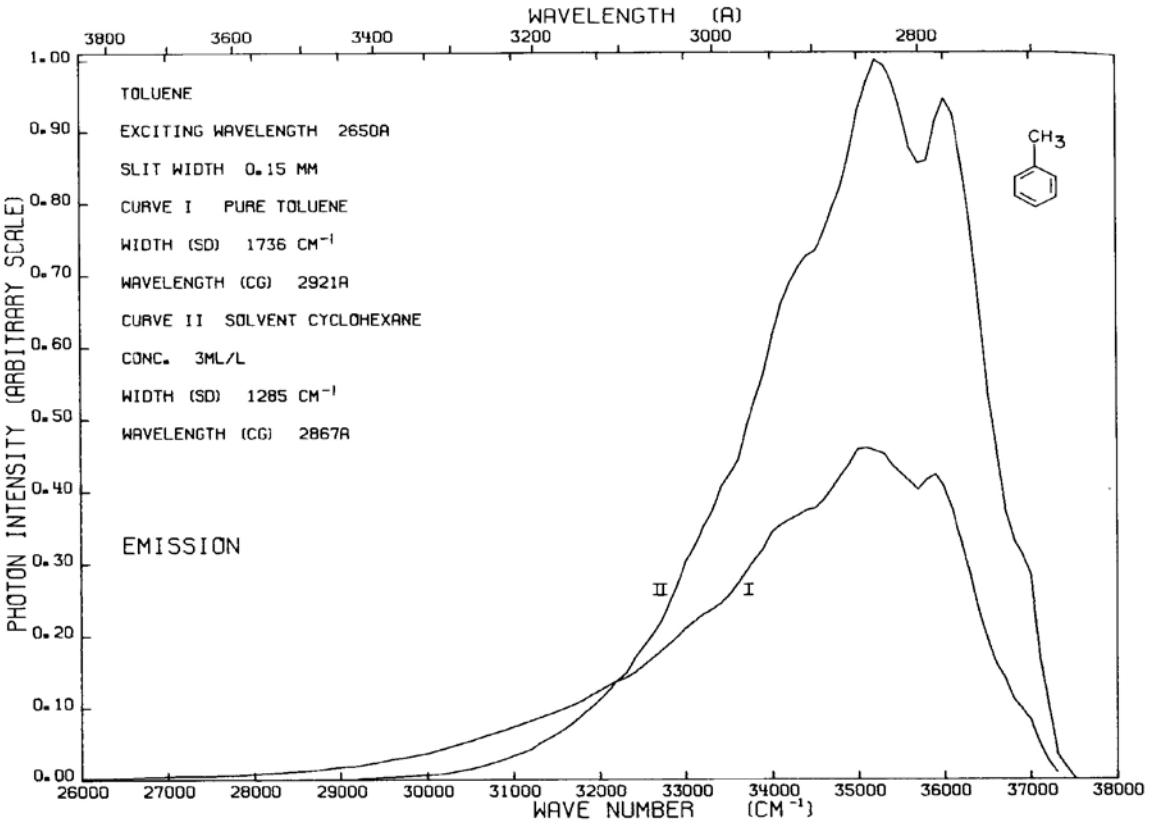
Warning: ***Though dilute, sulfuric acid is corrosive. Handle solutions with gloves.***

Dispose of all standards and the unknown solutions in the non-organic chemical waste jug in the hood.

WRITTEN REPORT

1. Discuss whether the experimental excitation and emission wavelengths you found in lab agreed reasonably well with your predictions made in the pre-lab.
2. Plot the calibration curve for quinine. Does it behave as you had predicted?
3. Calculate the concentration of quinine in tonic water and its 95% confidence interval. Calculate the detection limit (in molar concentration units) for the fluorescence determination of quinine in tonic water. Explain briefly why fluorescence detection limits should be smaller than those obtained from absorbance measurements.

Figure 2. Emission spectrum of toluene. This is provided as an example of a compound with an emission spectrum that should be similar to the emission spectrum of benzoic acid/benzoate. There are some important features to note here. Notice that the upper horizontal axis is in wavelength units of Angstroms, labeled as "A", where 10 Å equals 1 nm. Notice also that the top axis is increasing nonlinearly and in a different direction than the output you will generate in the lab.



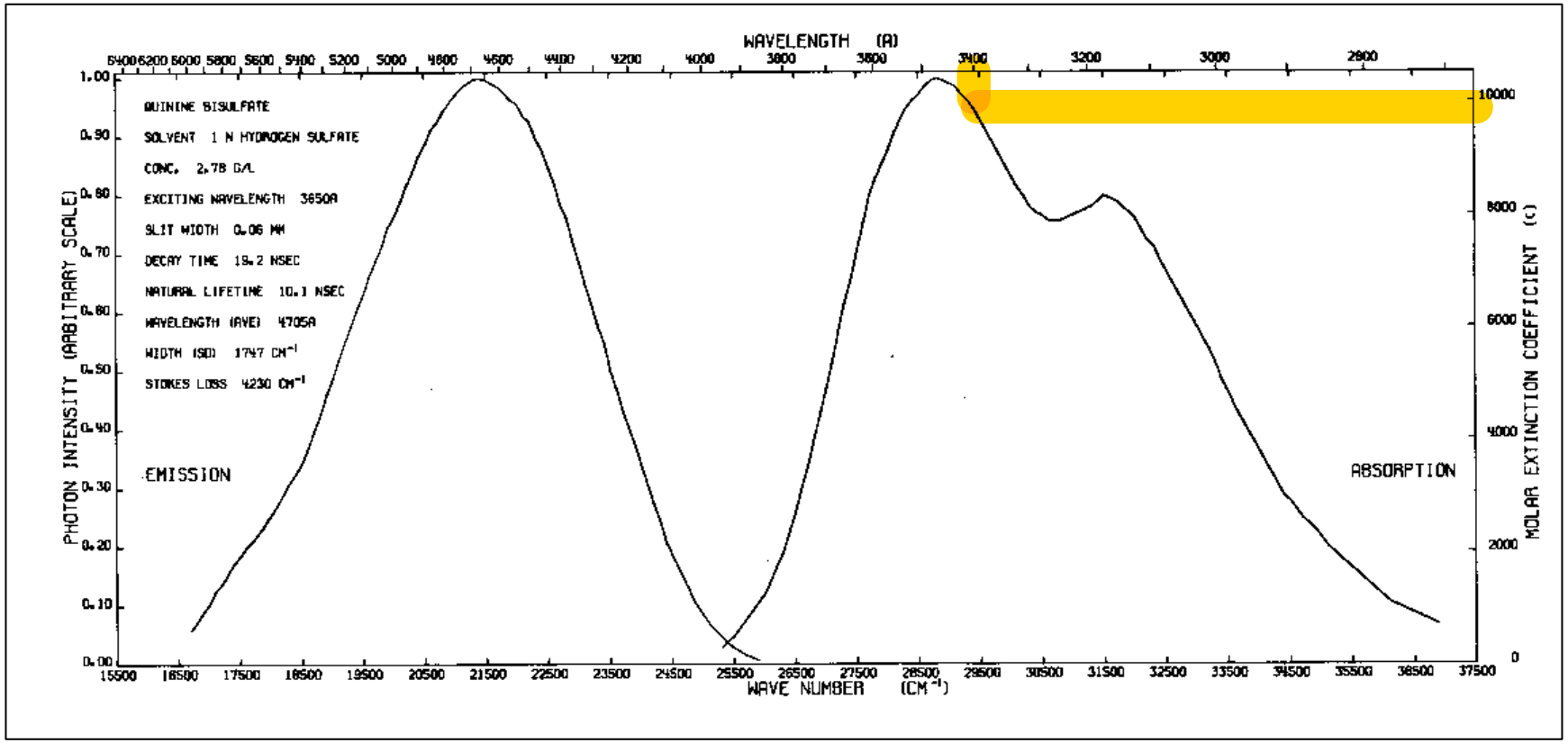


Figure 3. Excitation and Emission Spectra for quinine sulfate, from "Handbook of Fluorescence spectra of Aromatic Molecules", I.B. Berlman, Academic Press, 1971.