Experiment 4
Operation and Limitations of a Fluorescence Spectrometer

Objectives:
1. Learn how to operate the instrument.
2. Gain experience working with low (ppb and ppt) concentrations.
3. Determine quinine concentration.

Background:
The principles of molecular fluorescence are covered in your text. In brief, a high-energy photon (the excitation frequency) is absorbed and it raises the molecule to an excited state. As the excited molecule relaxes to a lower excited level or to the ground state, the excess energy is released to the molecular environment by a variety of mechanisms. One of these relaxation processes is fluorescence. With fluorescence, light is emitted (the emission frequency) at the same or at a lower frequency than the excitation frequency. The other relaxation mechanisms are frequently more efficient than fluorescence so only a few molecules fluoresce. However, for those molecules that do fluoresce, one usually obtains very low detection limits. Progress is being made in attaching fluorescence tags to molecules that do not fluoresce.
The quantum yield (Φ_o), sometimes called the quantum efficiency, is the ratio of the molecules that fluoresce to those that are excited.
QUANTUM YIELD

$$\Phi_0 = \frac{photons - emitted - per - \mathbf{sec}ond}{photons - absorbed - per - \mathbf{sec}ond} = \frac{emission - rate}{absorption - rate}$$

in steady state
absorption rate = disappearance rate
emission rate = $k_e[M^*]$, where M^* is the concentration of *M* in excited state
disappearance rate = $k_e[M^*] + k_d[M^*]$

$$\Phi_0 = \frac{k_e[M^*]}{k_e[M^*] + k_d[M^*]} = \frac{k_e}{k_e + k_d}$$

$\Phi_0 = I_f / (I_o - I)$ where I_f is the intensity (power) of the fluorescence beam, I_o is the intensity (power) of the excitation beam and **I** is the intensity of the beam after it is some distance into the sample.
Therefore, $I_f = \Phi_0 (I_o - I)$.
And from the Beer law,

$$A = \log I_o/I = \varepsilon Cl$$

or $10^A = I_o/I$ or $I = I_o/10^A = I_o 10^{-A}$ so that

$$I_f = \Phi_0 (I_o - I_o 10^{-A})$$

and $$I_f = \Phi_0 I_o(1-10^{-A}) = \Phi_0 I_o(1-10^{-\varepsilon Cl})$$

which is the working equation for fluorescence spectroscopy.

Note that the equation is not linear so you should not expect a plot of **fluorescence** *versus* **concentration** to be a straight line. However, at very low concentrations (when A is less than 0.05), the equation approaches a straight line and at even lower concentrations the degree of curvature is negligible.
The emitted light (fluorescence) is incoherent and is emitted in all directions. With the spectrofluorimeter, you will monitor the intensity of the emitted light at 90 degrees to the incident light. This greatly reduces the probability of the light from the excitation source from striking the detector.

Sample compartment of PerkinElmer LS-55 spectrofluorimeter

In this experiment, you will determine the exact amount of **quinine** in various liquids (tonic water and colas). The approximate concentration is 1**00 ppm**.

Preparation of solutions:
1.00 mM quinine in 0.05 M sulfuric acid is a standard solution
0.000100 mM (1.00×10^{-7} M) quinine in 0.05 M sulfuric acid is a good starting point for measurements.

Instrument Operation:
Turn the instrument on and wait 15 min. Open the **FLWinLab** program (just click on icon). Load **quinine** method. Enter the file name yo suggest for you data set (no more than 4 symbols!). Run the blank (0.05 M sulfuric acid).

Procedure for Quinine Determination:

By trial and error, dilute your stock solution to get a linear calibration curve (fluorescence intensity *versus* concentration). Start in the vicinity of 10^{-7} M and use the linear part of your curve to estimate detection limit of method and sensitivity. Include both the calibration curve and the spectra in your report.
Concentrations of $1x10^{-9}$, $2x10^{-9}$, $4x10^{-9}$, $1x10^{-8}$, and 2×10^{-8} M usually are suitable calibration points. To make those solutions, dilute an appropriate amount of your $1.0x10^{-7}$M stock solution in a 25 mL volumetric flask with 0.05 M sulfuric acid. Measure intensities of this solutions as well as intensity of a blank solution at the maximum of emission.
Build a calibration plot. If it looks satisfactory, analyze your sample.
Usually quinine-containing drinks require 1000-10,000 fold dilution with 0.05 M sulfuric acid before measurements. Dilute the sample using 100 mL volumetric flasks, and measure the intensity at emission maximum in triplicate
Analyze your results using the calibration. Process results: calculate amount of quinine in your starting sample in mg/mL.

Report:

1. Emission spectra of quinine.
2. Calibration curve for quinine determination.
Be sure your calibration curve shows the origin and the upper limit of the linear region of the curve. Include a rigorous determination of the reliability of your data and an estimate of the lowest detection limit for quinine you can obtain using this general fluorescence method and after optimizing all instrument variables to enable you to measure the lowest possible concentration of quinine.
3. Detection limit and determination limit for quinine in ng/mL.
4. Your estimate of quinine concentration in your sample in mg/mL.