

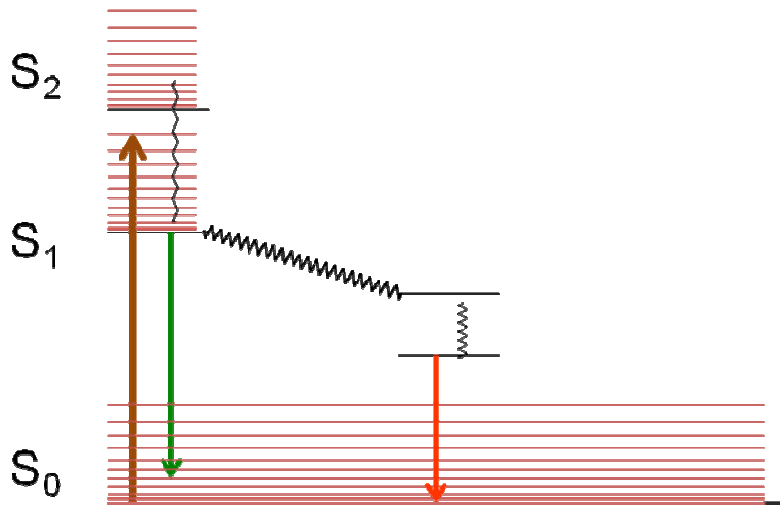
Quantification of Quinine by Fluorimetry

Objective

The purpose of this lab is to quantify of a fluorophore in solution.

Background

Once a molecule is electronically excited it could relax via photon emission. If the transition to low energy states occurs between states of the same spin state (e.g. $S_1 \rightarrow S_0$) this is termed fluorescence (green arrow). If the spin state of the initial and final energy levels are different (e.g. $T_1 \rightarrow S_0$), the emission is called phosphorescence (red arrow). Fluorescence is statistically much more likely than phosphorescence, the lifetimes of fluorescent states are very short (1×10^{-5} to 1×10^{-8} seconds) and phosphorescence lifetimes are longer (1×10^{-4} seconds to minutes or even hours).



Three non-radiative relaxation processes are also of significance:

- internal conversion (IC),
- intersystem crossing (ISC) and
- vibrational relaxation (not shown in the diagram)

Examples of the first two can be seen in the diagram. Internal conversion is the **radiation less** transition between electronic energy states of the same spin state. Intersystem crossing is a radiation less transition between different electronic spin states. Vibrational relaxation, the most common of the three—for most molecules, occurs very fast and is enhanced by collisions of the excited molecule with molecules wherein energy, in the form of vibrations and rotations, can be transferred. Thus, most excited state molecules never emit any energy because of these collisional deactivation processes.

To quantify the analyte in question at **low** concentrations, using either phosphorescence or fluorescence the following equation

$$I = 2.303 k' \epsilon bc P_0$$

is appropriate, where I is the fluorescent or phosphorescent signal, k' the product of a geometry factor and the quantum efficiency of the fluorescent process, ϵ is the molar absorptivity of the molecule at the excitation wavelength, b the path length, c the concentration and P_0 the radiant power of the excitation radiation.

Thus, provided low enough concentrations are measured, a plot of I versus [analyte] should be linear. The geometry factor and radiant power can be combined as a measured instrumental constant.

$$I = 2.303 k' \epsilon bc P_0 = k c$$

In this experiment a *synchronous* experiment is performed because such a mode of the experiment yields the lowest overlapping of the output signals. The functional form of the relationship between the signal and the concentration of analyte remains linear.

Procedure

A stock solution (*ca.* 100 ppm) Quinine sulfate in ethanol is provided. Make six standards, suggested concentrations 1ppm thro' 10 ppm, the concentration of lowest standard may be as low as 60 ppb. Make the most concentrated standard first and the other standards later. Use a four window cuvette for the experiment.

Collecting spectra: Take the UV spectrum of the solution from 220-600nm. Determine the λ_{\max} ($\cong \lambda_{\text{ex}}$) values. With a reasonable value for λ_{\max} proceed to determine a suitable λ_{ex} and λ_{em} for the experiment** using the fluorimeter. (See below - Measurements).

Set up the parameters to collect the synchronous spectra with proper λ_{ex} and λ_{em} wavelengths. Keep other parameters as default values. Check the Detector; both S and R must be active.

At this point, collect the *synchronous spectrum* of the most concentrated standard by placing the cuvette containing the standard in the sample holder, record the peak maximum and save the resulting spectra (real-time data is shown on the screen). It is recommended that you start with your most concentrated standard so as to have an idea of what the spectrum looks like.

Note: the highest intensity in counts per second (cps) must be less than two million to be quantitative for the detector of this instrument. If the intensity of the signal is too high, dilute the most concentrated solution appropriately, and record the dilution factor. Make the rest of the standards (five) by serial dilution and obtain the synchronous spectra of each of the six standards, needed to generate a calibration plot. Record the peak values of the outputs, because the system is not on line connected to a printer.

Obtain the *synchronous spectrum* of the 'unknown' (triplicate) using the same experimental parameters as for the standards. If necessary, make the appropriate dilution of the 'unknown' (and record the dilution factor) so that the signal falls within the calibration limits of the calibration plot. Using the calibration plot and any dilution factors used quantify the quinine in your unknown.

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Measurements: Turn ON the switches for the instrument, lamp controlling computer and the interfacing computer, in that order. Click on the 'Fluorescence' shortcut, wait for the initialization process to complete. Your work may be saved in the CHEM380 subdirectory

(1) Determine a suitable λ_{em} (Emission scan)

(In the following measurements make sure the intensity of the emission spectra do not exceed 2×10^6 cps, the end of the linear range of the instrument, for quantitative experiments).

Click [Experiment] Menu button (**M** button). From the next window select the choice [Spectra]; click Spectra>Experiment Type>**Emission**>Next

Experiment set up window for Emission experiment opens opens.

In the Experiment File field, enter a new filename or open a previous file ([Load] Quinine_Em.xml - experimental parameters recorded in this file – see below) set up and verify parameters.

Click (Monos) – view monochromator settings

Set Ex. Mono; λ_{max} , (found from the UV spectrum) Slit=2nm

Set Em. Mono; Start=($\lambda_{max}+5$) nm, End=650nm, Inc=1nm, Slit=1nm

Click (Detectors) – verify S channel detector is active (\checkmark).

Fill the Comment field and Data Identifier. [SaveAs] or [Save in Data] > CHEM380 subdirectory.

Insert the sample into the sample compartment, close cover.

Click [Run] – emission spectrum generated and eventually added to the origin window. Make sure the maximum signal does not exceed 2×10^6 cps.

At this stage a project name is sought; Give a name like Quinine Mon PM (if first time) and save it in CHEM380 subdirectory. Once the spectrum is generated,

Expand the data window followed by;

Click [Zoom] – enlarge the peak region, top bar right side

Click [Cursor] – select peak(s), note current cursor position ($X = \lambda_{em}$)

(2) Determine a suitable λ_{ex} (Excitation scan)

Click [Experiment] menu button

Click Spectra>Experiment Type>Excitation>Next

Experiment set up window for Emission experiment opens opens.

In the Experiment File field, enter a new filename or open a previous file – ([Load] Quinine_Ex.xml - and verify the parameters. .

Click (Monos) – view monochromator settings

Set Ex. Mono; Start=300 nm, End= λ_{em} nm , (determined in the first part of the experiment), Inc=1nm, Slit=1nm

Set Em. Mono; λ_{em} Slit=2nm

Click (Detectors) – verify detectors S and R are active, and add S1/R1 to the formula window.

Fill the Comment field and Data Identifier. SaveAs or Save in Data > CHEM380 subdirectory

Insert the sample into the sample compartment, close cover.

Click [Run] – *excitation spectrum generated and eventually added to the origin window.*

Expand the data window, top bar right side;

Click [Zoom] – enlarge the peak region

Click [Cursor] – select (a) peak (usually the one with the largest wavelength), note the current cursor position ($X = \lambda_{ex}$)

(3) Synchronous Run.

Once the λ_{ex} and λ_{ex} are determined set up for a *synchronous run*.

Click [Experiment] menu button

Spectra>Experiment Type>Synchronous>Next

Spectral acquisition window opens.

In the Experiment File field, enter a new filename or open a previous file – ([Load] Quinine_Synchronous.xml - verify parameters.

Click (Monos) – view monochromator settings

Set Ex. Mono; Start= λ_{ex} -50 nm, End=650 nm, Inc=1nm, Slit=1nm

Set the offset of emission monochromator $|\lambda_{em} - \lambda_{ex}|$ nm

Click (Detectors) – verify detectors S and R are active.

SaveAs or Save in Data > CHEM380 subdirectory.

Determine the peak height (signal) of the synchronous run peak.

Repeat the synchronous experiment for all solutions standards and the unknown (diluted solution, if a dilution was made); Click \triangleright to repeat ‘last run/experiment’.