Absorption Spectroscopy Experiment:

Experimental Goal:

Take a sample of fluorescein dye of unknown concentration and determine its concentration. Using this stock solution make a 50nM sample of fluorescein and verify its concentration using absorption.

Theoretical Background:

The absorption of photons by a molecule follows the Beer-Lambert equation:

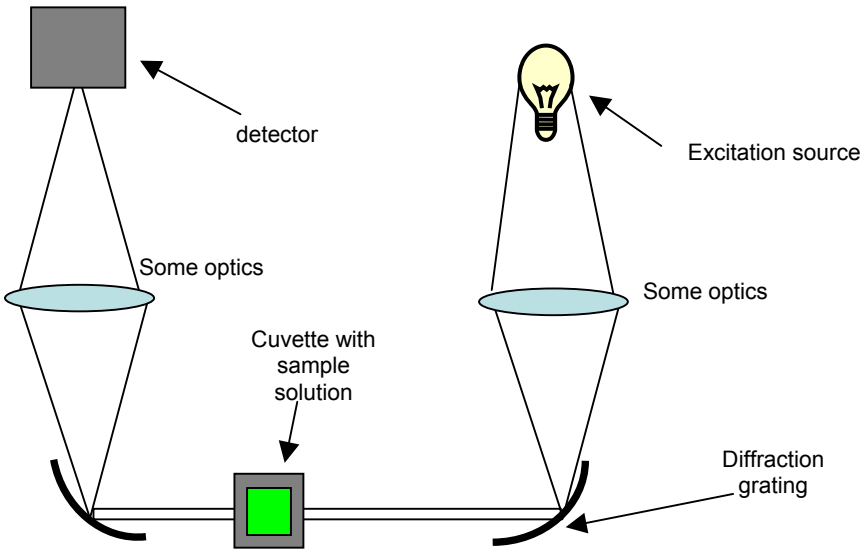
$$\log \frac{I_0}{I} = \varepsilon c d$$

Where I_0 is the initial intensity and I is the intensity after passing through a distance d of the solution. c is the molar concentration of the solution and ε is called the molar extinction coefficient and is typically measured in units of M^{-1} cm^{-1}.

Fluorescein's extinction coefficient provided by the manufacturer is 80,000 M^{-1} cm^{-1} at a wavelength of 490nm in a pH 8 buffer. We will use this to determine the concentration of our samples.

The Absorption Apparatus:

The sketch below shows a very simplified layout of an absorption spectrometer. Briefly, light from an excitation lamp is shone onto a mirrored diffraction grating which is scanned in order to select wavelengths. The light passes through the sample and the emitted light that passes through the sample at that wavelength is measured relative to a calibration sample. The log of the ratio of this intensity is the absorbance.



Experimental Procedures:

i) Determine concentration of fluorescein stock solution.

1) On the software of the computer select "SETUP". Select appropriate wavelength for fluorescein for the "start" and "stop" values. These determine the spectral region over which the scan is performed.
2) Click the "BASELINE" tab and make sure "baseline correction" is checked. Click "OK" when finished.
3) Fill a cuvette with 1mL of the buffer solution that has already been prepared. This will be our reference solution used to take the baseline calibration scan. Wear gloves when handling the cuvette to prevent fingerprints from biasing the measurement.
4) Open the compartment on the spectrometer and put the cuvette into the holder.
5) Shut the compartment and click "BASELINE". Click "OK". The scanner will now start and determine the throughput of the selected wavelengths through this blank sample.
6) After the scan has completed remove the cuvette from the spectrometer.
7) Now, removed 10uL of this buffer solution from the cuvette.
8) Using a new pipette, put 10uL of the stock fluorescein in the cuvette and mix.
9) Place this sample into the Spectrometer. Click "START"
10) The absorbance is given as $\log\frac{I_o}{I}$. The labeled peak is the value we will use in the above equation. Therefore you can calculate the concentration of this sample. By taking into account the dilution of the initial stock we will know its concentration as well.

ii) Make a 200nM solution of fluorescein and check.

1) Using the appropriate amounts of buffer and stock, make a sample of 200nM fluorescein.

2) Measure to ensure that this is indeed 200nM using absorption.