Introduction to Spectroscopy

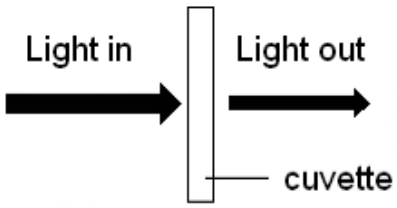
Aims:

- To prove that the absorbance of a solution is proportional to the concentration of the absorbing substance
- To measure the concentration of an unknown dye solution
- To learn to work carefully and accurately

Introduction

In this morning's test, the ink started blue and turned clear. But how much ink was left when the colour was somewhere in between?

When light falls on a material, some of it is absorbed, while the remainder either passes through unchanged or is reflected or scattered. Dyes absorb some wavelengths of light and not others, causing them to look coloured. The technique of spectroscopy measures the amount of light absorbed by a substance. As the technique was developed, careful measurements were made of the intensity of incident and transmitted light in absorbing solutions. The following formula, the Beer-Lambert law, was found to hold:



$$A = \varepsilon \, . \, c \, . \, l$$

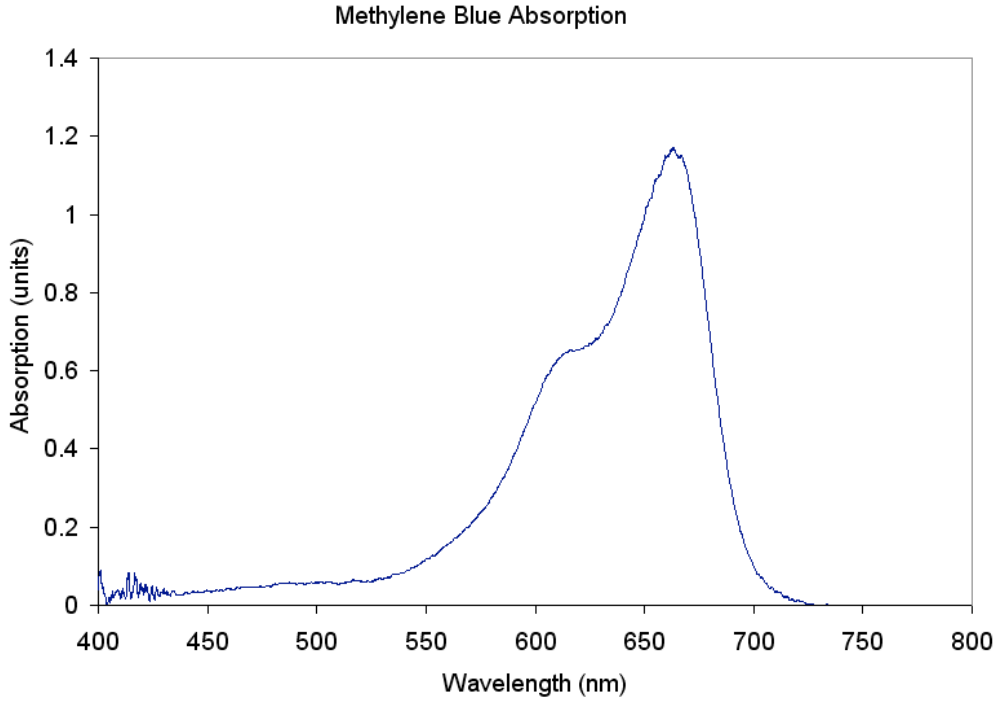
Where A is the absorbance, c is the concentration of the absorbing substance and l is the path length, the distance the light travels through the solution. ε is the molar absorptivity of the sample at a particular wavelength – for a given substance at a particular wavelength, this is a constant. The solution to be tested is held in a standard container called a cuvette, which has a standard path length, usually 1cm.

This means that the absorbance of a solution at a particular wavelength is proportional to the concentration of the absorbing substance in it. We can measure how much dye is left in a coloured solution, like the ink solution, by measuring its absorbance.

Methylene blue dye

The ink we used this morning contains a dye which is used to test the activity of photocatalysts. Another dye used for this is methylene blue or MB.

The absorption spectrum of MB looks like this:



The peak absorption is at a wavelength of 665 nm. By measuring the absorbance of a cuvette of dye at 665 nm, we can work out the concentration of the dye in it.

In this experiment, you are going to show that absorbance is proportional to concentration by making a series of dye solutions of known concentration, and then measuring their absorbance using a spectrophotometer. Using the data you get from this, you will then work out the concentration of an unknown dye sample.

Method

Equipment list:
1 x 100 mL beaker
1 x 250 mL beaker
5 x 100 mL volumetric flasks
Pipettes and pipette pump – 1 x 5 mL, 1 x 10 mL
Pasteur pipettes
Wash bottle of distilled water
1 x flask of unknown solution

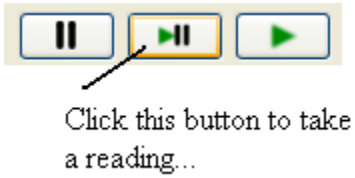
First collect some standard MB solution in a 250 mL beaker.

Next, make up five standard solutions from this.

- Pipette 5 mL of the dye into a 100 mL volumetric flask.
- Fill up to the mark with distilled water and mix well.
- Do the same for four other solutions in four other flasks, with 10, 15, 20 and 25 mL of dye in each.

Now make absorbance measurements of the solutions.

- For each of the five standard solutions, use a Pasteur pipette to transfer some to a clean cuvette - the cuvettes should be nearly full.
- Wipe the clear faces of the cuvettes with a tissue to make sure they are clean and dry – keep track of which cuvette is which.
- Fill one more cuvette with distilled water.
- Take the six cuvettes over to the spectrometer.
- Put a cuvette into the holder, so that the optic fibres are pointing at the clear faces of the cuvette.
- On the laptop,



A new absorbance graph should appear on the left hand side, and a new peak absorbance value at 665 nm should appear in the table on the right.

- Write down the peak absorbance values:

Percentage of dye in solution	Absorbance
0	
5	
10	
15	
20	
25	

Next, draw your calibration curve:

- On graph paper, plot the absorbance value against the percentage of dye solution.

Finding the concentration of an unknown

- Fill a cuvette with some of the unknown solution.
- Take the cuvette to the spectrometer and take a reading:

Absorbance

- Using the calibration graph you made, work out the concentration:

Percentage of dye in unknown solution