

Spectroscopy in an Envelope, Step 1: set up and test the colourimeter

Build and test the colourimeter according to the instructions in the packet. Take a voltage reading from a cuvette of clear water and record it here. Make sure the cuvette is oriented correctly (clear side in the path of the light). Disconnect the battery after taking this reading.

Initial blank reading: 73.1 mV

Step 2: Measuring a calibration curve

Prepare a dilution series, or other set of standards, and record your concentration in the first column of this table. Make sure to note your units correctly as they may not be the same for every experiment (M, mM, uM, or g/dm³). Connect the battery of the colourimeter, and measure each standard, recording voltage data in the second column.

Concentration (units)	Voltage reading, mV	Transmittance, %T	Absorbance, Abs
100	7.1	10%	1.01
50	22.1	30%	0.52
25	39.7	54%	0.26
12.5	53.0	73%	0.14
6.25	60.7	83%	0.08
3.125	63.9	88%	0.06

As soon as you have collected all of your voltage data, measure a cuvette of clear water again and note it down below, then disconnect the battery.

Final blank reading: 72.7 mV

Calculate the average of both of the blank readings, and note it below. However, if “Initial” and “Final” are more than about 5% different, you may need to replace the batteries or re-take the data; check with a teacher.

Average blank reading: 72.9 mV

Fill in the third column of the table by calculating %T using Equation 1 with the value in the second column, and your average blank reading above. Values will be between 0 and 100%, closer to 100% for lower concentrations.

$$\%T = \frac{\text{Voltage reading}}{\text{Average blank reading}} \times 100 \quad (\text{Equation 1})$$

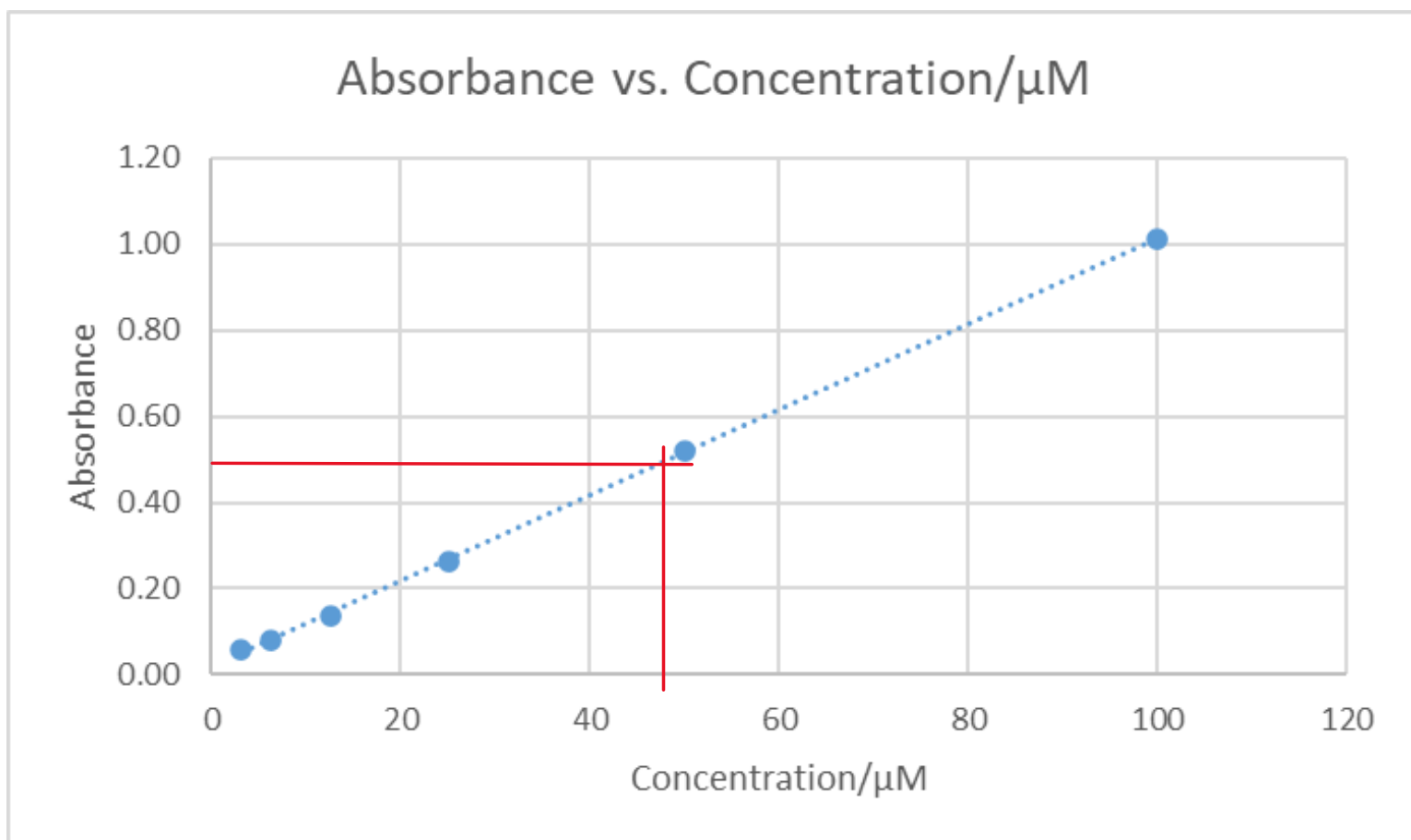
Fill in the fourth column of the table by calculating absorbance (Abs) from %T using equation 2. Abs values will be close to 0 at low concentrations, and trend towards +1 at higher concentrations. Values above 1 may not be reliable, and show that solutions may be too concentrated (check with a teacher before using values above 1).

$$\text{Abs} = 2 - \log (\%T) \quad (\text{Equation 2})$$

Step 3: Plotting and using a calibration curve

Plot a graph of absorbance (Abs) on the Y-axis, and concentration on the X-axis. Absorbance has no units. Ensure you use a linear spacing for concentration, even if your concentrations were non-linear (for example, if they came from a dilution series).

This graph will be your "Calibration Curve", and you should then draw a straight line of best fit.



This line of best fit can be used to determine the concentration for any unknown sample. First, measure the voltage for an unknown sample, and double-check that the blank readings have not changed by quickly measuring a blank (a sample of clear water) again afterwards. Then, calculate the %T and Abs values, using Equations 1 and 2.

New blank reading: 72.9 mV

New unknown reading: 25.4 mV

%T for the unknown (using Equation 1): 34.8 %

Abs for the unknown (using Equation 2): 0.46

Look up this Abs value on your calibration curve, and read off the concentration.

Concentration of the unknown (using calibration curve): 45 μM