Case-study: The Beer-Lambert Law and Spectrophotometry

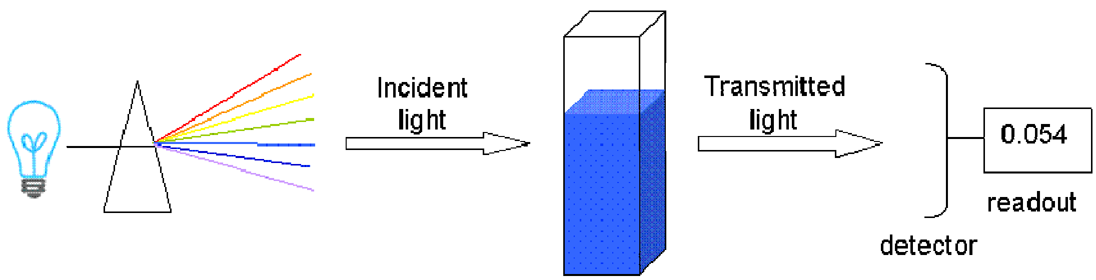
Learning objectives:

* describe the basic principles of spectrophotometry
* state the Beer-Lambert Law
* define ε, the Molar Absorbance Coefficient.

Spectrophotometry.
Many substances dissolve to give coloured solutions

The higher the concentration of solute, the more light is absorbed and the less light is transmitted through the sample.

Spectrophotometry is a simple technique used to measure absorbance of solutions. The sample is placed inside a cuvette which is a rectangular prism-shaped vessel. Light of a particular wavelength is directed to one side of the cuvette and the intensity of light reaching the detector is measured.



The intensity of light hitting the detector after passing through a "blank" solution is measured – this is a solution that is identical to the sample but doesn't contain the solute. This measurement is called "I_0".

Then the intensity of light hitting the detector after passing through the sample is measured. This measurement is "I".

The transmittance (the amount of light reaching the detector) is calculated as $T = \frac{I}{I_0}$

The absorbance is calculated as $A = -\log_{10} T = -\log_{10} \frac{I}{I_0}$

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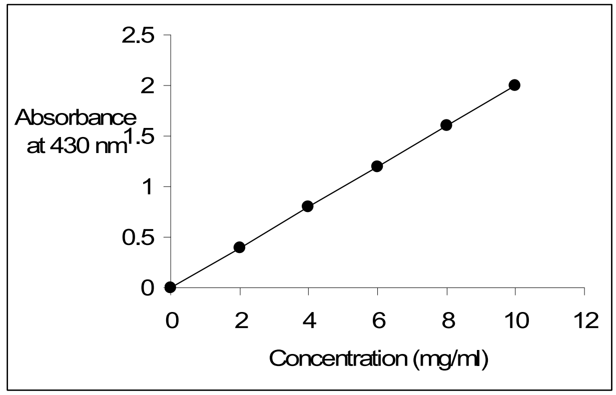
The Beer-Lambert Law

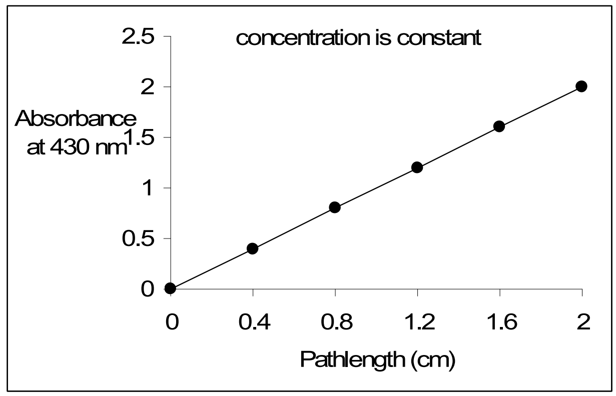
Let the concentration = C,
and absorbance = A.
As C increases, A increases in a proportional manner i.e.

A α C



Also, as the pathlength the light has to travel through (i.e. the width of the cuvette) increases, the absorbance increases proportionally. We are going to use the symbol d for pathlength (sometimes l is used)

A α d

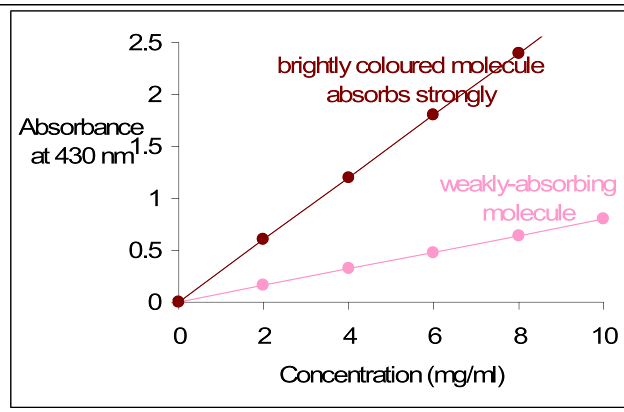


Some molecules are very brightly coloured – i.e. they absorb a lot even at very low concentrations whereas others are not so good at absorbing light.

The molar absorbance coefficient, ε, is a characteristic for each type of molecule.

$A = \varepsilon C d$

This is known as the Beer-Lambert Law



A large value for ε gives a steep slope and reflects strong absorbance.

If you plot A on the y axis and C on the x axis (as in the graph on the right),
then the slope is $\varepsilon d = A/C$

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