3.B.3. Calculating the molar absorbance coefficient (ε) from absorbance and concentration data

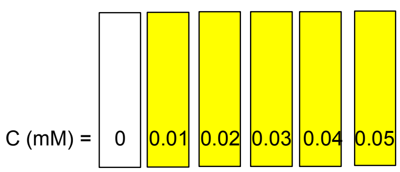
.

Learning Objective.
To calculate a value for ε from experimental data of absorbance and concentration.
.

In this example we are using data for p-nitrophenol which is a yellow-coloured reagent commonly used in diagnostic tests (ELISA's).

p-nitrophenol absorbs well with light of about 400 nm so we measure the absorbance using light of that wavelength in a cuvette of pathlength 1 cm and call the absorbance A_{400}.
.

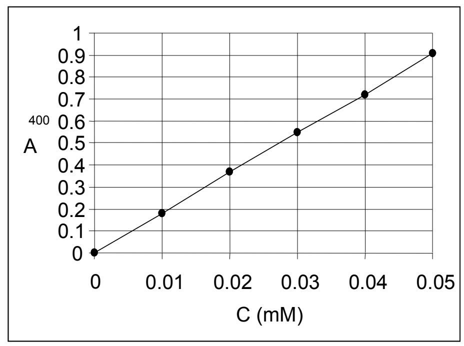
set up some cuvettes containing a range of concentrations of p-nitrophenol from 0 to 0.05 mM



measure the absorbance for each cuvette

C (mM)	A_{400}
0	0
0.01	0.18
0.02	0.37
0.03	0.55
0.04	0.72
0.05	0.91

plot the data on a graph of A vs C



A = εCd = (εd)C; in a graph of A vs C, the slope is εd.

$$slope = \varepsilon.d = \frac{y_2 - y_1}{x_2 - x_1} = \frac{0.91 - 0}{(0.05 - 0)mM} = 18.2(mM)^{-1}$$

d = 1 cm
so

$$\varepsilon = \frac{18.2(mM)^{-1}}{d} = \frac{18.2(mM)^{-1}}{1cm} = 18.2mM^{-1}cm^{-1}$$

Creative Commons Attribution Non-commercial Share Alike
Author: Dr Jenny A Koenig

However.... ε is usually written with the units M^{-1}.cm^{-1}.
How do we get ε in the right units?

Two possible methods:

1
Possibly the easiest way is to start with M rather than mM in the first place.

$$\varepsilon d = \frac{0.91 - 0}{(0.05 - 0) \times 10^{-3} M} = 18.2\left(10^{-3} M\right)^{-1} = 18.2 \times 10^{3} M^{-1} = 18200 M^{-1}$$

then
ε = 18200 M^{-1} / 1 cm = 18200 M^{-1}.cm^{-1}

2
Another method is to say
1000 mM = 1 M,
so 1000 mM.M^{-1} = 1

If ε = 18.2 mM^{-1}.cm^{-1}
then you can multiply both sides by 1 (=1000 mM.M^{-1})
ε = 18.2 mM^{-1}.cm^{-1} x 1000 mM.M^{-1}

then the mM^{-1} cancels with the mM
ε = 18.2 ~~mM^{-1}~~.cm^{-1} x 1000 ~~mM~~.M^{-1}

and you are left with
ε = 18200 M^{-1}cm^{-1}
.

Creative Commons Attribution Non-commercial Share Alike
Author: Dr Jenny A Koenig