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

<table>
<thead>
<tr>
<th>C (mM)</th>
<th>$A_{400}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.01</td>
<td>0.18</td>
</tr>
<tr>
<td>0.02</td>
<td>0.37</td>
</tr>
<tr>
<td>0.03</td>
<td>0.55</td>
</tr>
<tr>
<td>0.04</td>
<td>0.72</td>
</tr>
<tr>
<td>0.05</td>
<td>0.91</td>
</tr>
</tbody>
</table>

- Measure the absorbance for each cuvette
- Plot the data on a graph of $A$ vs $C$

$$A = \varepsilon Cd = (\varepsilon d)C; \text{ in a graph of } A \text{ vs } C, \text{ the slope is } \varepsilon d.$$

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

$d = 1$ cm

so

$$\varepsilon = \frac{18.2(mM)^{-1}}{1cm} = 18.2mm^{-1}cm^{-1}$$
However.... $\varepsilon$ is usually written with the units M$^{-1}$cm$^{-1}$.
How do we get $\varepsilon$ 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

$\varepsilon = 18200 M^{-1} / 1 \text{ cm} = 18200 M^{-1}.\text{cm}^{-1}$

2
Another method is to say

1000 mM = 1 M,
so
1000 mM.M$^{-1}$ = 1

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

$\varepsilon = 18.2$ mM$^{-1}$.cm$^{-1} \times 1000$ mM.M$^{-1}$

then the mM$^{-1}$ cancels with the mM

$\varepsilon = 18.2$ mM$^{-1}$.cm$^{-1} \times 1000$ mM.M$^{-1}$

and you are left with

$\varepsilon = 18200$ M$^{-1}$cm$^{-1}$