Absorption of light: Beer-Lambert Law

Up to this point, we have learned how electromagnetic waves are generated, how molecules can scatter light (and how we can determine molecular weight from the amount of scattering, using a Zimm plot) and how helical molecules interact with circularly polarized light.

In this section, we will learn how compounds absorb ultraviolet (UV) or visible light:

\[ P_0 \] is the incident radiant power or intensity
\[ P \] is the radiant intensity that remains
\[ b \] is the path length
Physical principle

Recall the potential energy function that we had for a diatomic molecule.

Figure 8.2
Energy levels for a simple diatomic molecule. The potential energy (heavy curve) is a function of the internuclear distance ($X_{AB}$). Two electronic states are shown (I and II). They differ in their potential energy and in the position of the minimum, the equilibrium internuclear distance. For each electronic state there are different possible levels of vibrational energy (long, thin lines) and rotational energy (short lines, shown only for two vibrational levels). A possible electronic transition is shown by (a) and a vibrational transition in the electronic ground state by (b).
We can describe how the light is absorbed by either the transmittance or the absorbance:

**Transmittance**

\[ T = \frac{I}{I_0} \]

**% Transmittance**

\[ %T = 100 \times T \]

**Absorbance**

\[ A = \log_{10} \frac{I_0}{I} \]

\[ A = \log_{10} \frac{1}{T} \]

\[ A = \log_{10} \frac{100}{%T} \]

\[ A = 2 - \log_{10} %T \]

Both the transmittance and absorbance are related:
Beer-Lambert Law

The Beer-Lambert law relates the absorbance to the concentration:

\[ A = \varepsilon bc \]

where \( A \) is absorbance (no units, since \( A = \log_{10} \frac{P_0}{P} \)), \( \varepsilon \) is the molar absorptivity or extinction coefficient with units of L mol\(^{-1}\) cm\(^{-1}\), \( b \) is the path length of the sample – i.e. the path length of the cuvette in which the sample is contained (in cm) and finally, \( c \) is the concentration of the compound in solution, expressed in mol L\(^{-1}\).

Of course, we can also express transmittance in terms of concentration, but the Beer-Lambert law is more useful because the relationship between absorbance and concentration is linear.
Derivation of the Beer-Lambert law

In order to derive the law, we need to approximate the absorbing molecules in the cuvette as opaque disks, of cross-sectional area, $\sigma$

\[ I_0 = \text{intensity of light entering the sample} \]
\[ I_z = \text{intensity at point } z \text{ in the sample cell} \]
\[ dl = \text{intensity of light absorbed by the slab} \]
\[ I = \text{intensity of the light leaving the sample} \]

\[ \Phi = \frac{\text{molecules}}{\text{cm}^3} \]

http://www.chem.ufl.edu/~itl/3417_s98/spectroscopy/beerslaw.htm
Total opaque area in the slab = N \sigma \, dz \, A

i.e. fraction of opaque areas in the slab times the total area. This fraction of opaque areas is also a measure of the fraction of light absorbed:

\[ \frac{dI}{I_z} = - N \sigma \, dz \]

We can integrate this equation from \( z=0 \) to \( z=b \), to give us

\[ \ln(I) - \ln(I_0) = - N \sigma \, b \]

or

\[ \ln \left( \frac{I_0}{I} \right) = N \sigma \, b. \]

\( N \), the number of molecules per cm\(^3\), can be related to concentration by

\[ \frac{N * (1000 \, \text{cm}^3/\text{L})}{N_A} = c \, (\text{M}) \]

and

\[ 2.303 \times \log(x) = \ln(x) \]
Therefore, we have

$$\log \left( \frac{I_0}{I} \right) = \frac{\sigma N_A c b}{2.303 \times 1000}$$

or

$$A = \varepsilon b c$$

with

$$\varepsilon = \frac{\sigma N_A}{2.303 \times 1000}$$

This shows how the extinction coefficient is related to the cross-sectional areas of absorption. E.g.

Absorption – atoms
- molecules
- 9 kDa protein

<table>
<thead>
<tr>
<th>$\sigma$</th>
<th>\begin{tabular} {c} $10^{-12}$ cm$^2$ \ $10^{-16}$ cm$^2$ \ $10^{-17}$ cm$^2$ \end{tabular}</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon$</td>
<td>\begin{tabular} {c} $3 \times 10^8$ M$^{-1}$cm$^{-1}$ \ $3 \times 10^4$ M$^{-1}$cm$^{-1}$ \ $7 \times 10^3$ M$^{-1}$cm$^{-1}$ \end{tabular}</td>
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</tbody>
</table>
Non-linearity of Beer-Lambert’s law

Beer’s law is linear in most cases, except:

- at high concentrations
- if there is scattering of light due to particulates in the sample
- if the sample fluoresces or phosphoresces
- if the radiation is not monochromatic
- if there is stray light

Taking a closer look at concentration effects – high concentration results in non-linearity because:

- at high concentration, we have strong electrostatic interactions between molecules
- at high concentrations, we may get changes in refractive index
- if we have a system in chemical equilibrium, equilibrium may shift at high concentrations.
Application of absorbance measurements

1 – Estimate the concentration of a protein which has 1 or more Trp residues

if $c$ is too high, dilute!

You can simulate your own sequences at http://www.proteinchemist.com/Multbot.html
set absorbance wavelength = 280 nm
3 – Changes in configuration

![Graph showing changes in DNA configuration](image)

**Figure 8.10**
Hypochromism of polyriboadenylic acid. Note that the form of the polymer spectrum differs little from that of the monomer but that the intensity of absorption is considerably reduced.

![Graph showing DNA melting](image)

**Figure 8.11**
The “melting” of DNA as followed by absorption at 260 nm. Note the sharpness of the transition in cycle I. This is typical of a cooperative process (see Chapter 3). Also note that recovery of the ordered structure is not complete on rapid cooling.