Spectroscopy
Lecture 2

Beer-Lambert Law (Absorption of Light by Molecules)

- Different biomolecules absorb light at different wavelengths and differ in their wavelength maximum that in turn depends on their chemical structure.

- For example, tryptophan absorbs light at 280 nm while nucleic acid (DNA & RNA) absorb at 260 nm.

- The amount of light absorbed and at the characteristic wavelength is exploited for measuring the biomolecules both qualitatively and quantitatively.

  - ❖ The fraction of the incident light absorbed by a solution at a given wavelength is directly related to the thickness of the absorbing layer (path length) ***(Lambert's Law).***

  - ❖ The fraction of the incident light absorbed by a solution at a given wavelength is directly related to the concentration of the absorbing species ***(Beer's Law).***

  - ❖ These two relationships are combined into the ***Beer-Lambert.*** The combined law states that the fraction of the incident light absorbed by a solution at a given wavelength is directly related to both path length ***(Lambert Law)*** and concentration ***(Beer's Law).***

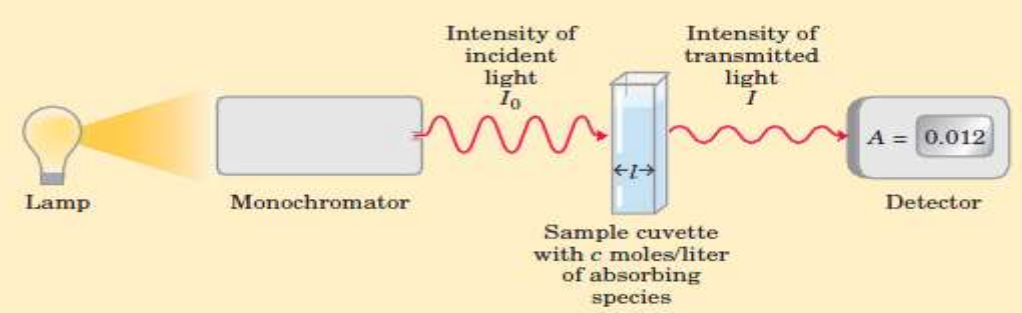
    The equation is as follows:

$$\frac{Log\ Io}{I} = \varepsilon\ c\ l$$

I_o	=	Intensity of the incident light
I	=	Intensity of the transmitted light
ε	=	Molar extinction coefficient
c	=	Concentration of the absorbing species
l	=	Path length of the light absorbing sample

- ✓ The Law assumes that the incident light as parallel and monochromatic.
- ✓ The solvent and solute molecules as randomly oriented.
- ✓ The expression log (I_0/I) is called the **absorbance,** designated *A.*

- ✓ It is important to note that each successive millimeter of path length of absorbing solution in a 1.0 cm cell absorbs not a constant amount but a constant fraction of the light that is incident upon it.
- ✓ However, for the practical purposes, most often same cuvette is used for blank, standards and test solutions, therefore, the path length remains fixed for these estimations.
- ✓ In this way, the absorbance doesn't depend on the path length but depends only on *concentration of the absorbing solute.*
- ✓ The molar extinction coefficient varies with the nature of the absorbing compound, the solvent, and the wavelength, and also with pH if the light-absorbing species is in equilibrium with an ionization state that has different absorbance properties.



5.5.1 Molar Extinction coefficient

log I_o / I	**=**	**ε c *l***
A	**=**	**ε c *l***
ε	**=**	**A / c *l***

- The symbol "ε" is proportionality constant.
- It is an ***absorption coefficient*** or ***absorptivity*** when defined for a particular chromophore at a specific wavelength.
- In old biochemical literature, the term extinction coefficient is still exists.
- For biomolecules, "ε" is often used in the form ***molar extinction coefficient***.
- The ***molar extinction coefficient*** is defined as the absorbance of a 1 M solution of a pure absorbing material in a 1-cm cell (cuvette) under specified conditions of wavelength and solvent.

5.5.2. Units of Molar Extinction Coefficient

log I_o / I	=	ε c l
A	=	ε c l
ε	=	A / c × l
ε	=	A (unit less) **/** M (Molar) × cm (length)
ε	=	1 **/** M × cm
ε	**=**	**M^{-1} cm^{-1}**
Alternatively		
Since, M (Molarity)	=	moles/ liter
	=	moles × liter $^{-1}$
	=	moles × l^{-1}
Substituting the same		
ε	=	M^{-1} cm^{-1}
ε	=	(moles liter $^{-1}$) $^{-1}$ × cm^{-1}
ε	=	$moles^{-1}$ $liter^{+1}$$cm^{-1}$
ε	**=**	**m^{-1} L cm^{-1}**

Determination of MOLAR EXTINCTION COEFFICIENT

STUDY EXERCISE 7.3 Beer-Lambert Law

The absorbance, A, of a 5×10^{-4} M solution of the amino acid tyrosine, at a wavelength of 280 nm, is 0.75. The path length of the cuvette is 1 cm. What is the molar absorption coefficient, ε?

Solution:

$$A = \varepsilon lc = 0.75$$

$$l = 1\,\text{cm}$$

$$c = 5 \times 10^{-4}\,M$$

$$\varepsilon = \frac{0.75}{(1\,\text{cm})(5 \times 10^{-4}\,\text{mole/liter})}$$

$$= 1500\,\frac{\text{liter}}{\text{mole} \times \text{cm}} = 1500\,M^{-1}\,\text{cm}^{-1}$$

Spectroscopy
Lecture 2

Significance /Applications of MOLAR EXTINCTION COEFFICIENT

- The term molar extinction coefficient (ε) is a measure of how strongly a chemical species or substance absorbs light at a particular wavelength.
- It is an intrinsic property of chemical species that is dependent upon their chemical composition and structure.
- The units are $M^{-1}cm^{-1}$.
- The molar extinction coefficient is frequently used in spectroscopy to measure the concentration of a chemical in solution.

You can use the Beer-Lambert Law to calculate a chemical species' ε:

$$A = \varepsilon Lc$$

Where:

- ✓ A is the amount of light absorbed by the sample for a particular wavelength
- ✓ ε is the molar extinction coefficient
- ✓ L is the distance that the light travels through the solution
- ✓ c is the concentration of the absorbing species per unit volume

Rearrange the Beer-Lambert equation in order to solve for the molar extinction coefficient:

$$\varepsilon = A/Lc$$

Use the molar extinction coefficient to determine the brightness of a fluorescent molecule, by using the following equation:

Brightness = Extinction Coefficient (ε) x Fluorescence Quantum Yield (Φ)

What does molar absorptivity depend on?
Remember that the **absorbance** of a solution **will** vary as the concentration or the size of the container varies. **Molar absorptivity** compensates for this by dividing by both the concentration and the length of the solution that the light passes through.

Why do compounds absorb light?
As a general rule, molecules containing conjugated systems of pi electrons absorb light closer to the visible region than saturated molecules or those with isolated double or triple bonds. The longer the conjugated system, the longer the wavelength of the light absorbed.