LAMBERT-BEER'S LAW

Lambert's Law

"When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light". Mathematically, the Lambert's law may be stated as follow:

$$-\frac{dI}{dt} \alpha I$$

Or

$$-\frac{dI}{dt} = kI \quad \text{.....(i)}$$

Where, I denotes the intensity of incident light of wavelength λ, t denotes the thickness of the medium and k denotes the proportionality factor. On integrating equation (i) and putting $I=I_0$ when $t=0$,

We get

$$\ln \frac{I_o}{I} = kt$$

$$I_t = I_o e^{-kt} \quad \text{..... (ii)}$$

Where I_o denotes the intensity of the incident light, I_t denotes the intensity of the transmitted light and k is a constant which depends upon the wavelength and absorbing medium used.

Beer's Law

Lambert's law shows that there exists a logarithmic relationship between the transmittance and the length of the optical path through the sample. Beer observed that a similar relationship holds between transmittance and the concentration of a solution, i.e., the intensity of a beam of monochromatic light decreases exponentially with the increases in concentration of the absorbing substance arithmetically.

Thus, equation (ii) becomes as:

$$I_t = I_o e^{-k'c} \quad \text{..... (iii)}$$

Where k' is constant and c is the concentration of the absorbing substance.

$$I_t = I_o 10^{-acl}$$

$$\text{Log } I_o/I_t = acl \text{ (absorbance)} \quad \text{..... (iv)}$$

Equation (iv) is termed as mathematical statement of Beer-Lambert law. This is also the fundamental equation of colorimetry and spectrophotometry.

In equation (iv), the value of 'a' depends upon the unit of concentration. If 'c' is expressed in mole dm^{-3} and 'l' in centimeters, then 'a' is replaced by the symbol ε and is termed as the molar absorption coefficient or molar absorptivity.

It is important to remark here that there exists a relationship between the absorbance A, the transmittance T and the molar absorption coefficient ε *i.e.*,

$$A = \varepsilon cl$$
$$= \log I_o/I_t = \log 1/T$$

Absorption of light by molecules

The Lambert-Beer Law

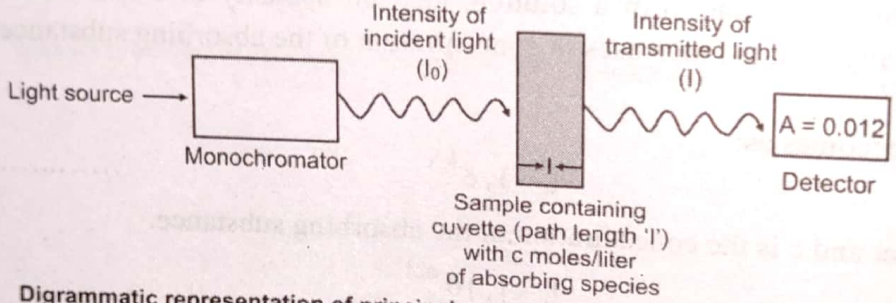
A wide range of biomolecules absorb light at characteristic wavelengths, just as tryptophan absorbs light at 280 nm. Measurement of light absorption by a spectrophotometer is used to detect and identify molecules and to measure their concentration in solution. The fraction of the incident light absorbed by a solution at a given wavelength is related to the thickness of the absorbing layer (path length) and the concentration of the absorbing species. These two relationships are combined into the Lambert-Beer law,

$$\log \frac{I_0}{I} = \varepsilon cl$$

where I_0 is the intensity of the incident light, I is the intensity of the transmitted light, ε is the molar extinction coefficient (in units of liters per mole-centimeter), c is the concentration of the absorbing species (in moles per liter), and l is the path length of the light absorbing sample (in centimeters).

The Lambert-Beer law assumes that the incident light is parallel and monochromatic (of a single wavelength) and that the solvent and solute molecules are 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, with an absorbing layer of fixed path length, *the absorbance,* A, *is directly proportional to the 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.



Digrammatic representation of principal components of a spectrophotometer

Example 26: Molar extinction coefficient of malondialdehyde at 532 nm is 0.155 M^{-1} cm^{-1}. The concentration of malondialdehyde in a solution which has absorbance of 0.31 in a 1cm cuvette will be:

a) 0.5M
b) 1.0M
c) 1.5M
d) 2.0M

Solution

$$\log \frac{I_0}{I} = \varepsilon cl$$

$$A = \varepsilon cl$$

or $$0.31 = 0.155 \times c \times 1$$

$$c = 2$$