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Unit-II

Spectrophotometry

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KEY CONCEPTS

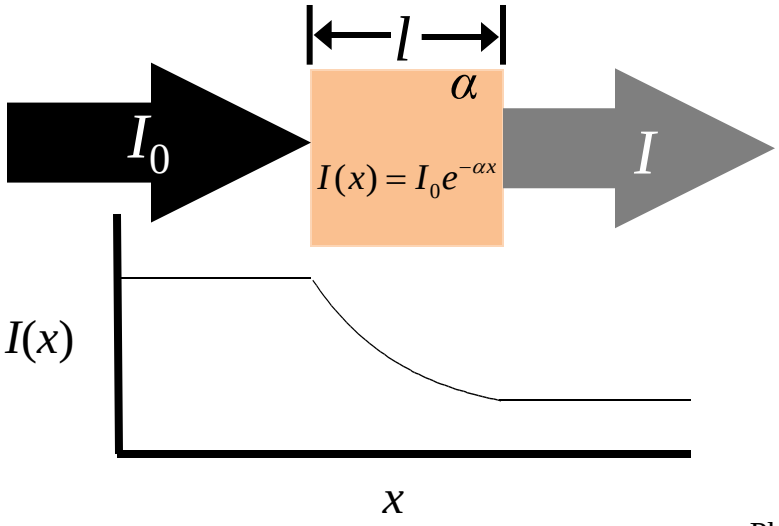
- Lambert's Law of Absorption
- Beer's Law
- Beer-Lambert Law
- Photometric Measurements
- Spectrophotometer

Lambert's Law of Absorption

Lambert described how intensity changes with distance in an absorbing medium.

- In a uniform absorbing medium, the intensity of a beam of light (I_0) decreases exponentially with the linear decay constant α *(path length)*.
- The distance traveled through the medium is called the *path length*.
- The linear decay constant α is a characteristic of the medium. It has units of reciprocal length.

Johann Heinrich Lambert
(1728-1777)



$$\mathrm{d}I = -\alpha I \,\mathrm{d}x \qquad \frac{\mathrm{d}I}{\mathrm{d}x} = -\alpha I$$

$$I = I_0 e^{-\alpha x} \qquad \frac{I}{I_0} = e^{-\alpha x}$$

Photo: http://www-history.mcs.st-andrews.ac.uk/history/PictDisplay/Lambert.html

Beers Law (1825-1863)

- Beer found that Lambert's linear decay constant *(k)* for a solution is linearly related to its concentration *(c)* with a constant absorptivity *(ε)*, a characteristic of the absorbing substance.

$$\boxed{k = \varepsilon c}$$

Units: k cm^{-1}; c M (moles/liter); ε $M^{-1}cm^{-1}$

A colored absorber has an absorptivity that is dependent on wavelength of the light ε (λ). The absorptivity is the fundamental property of a substance.

Photometric measurements

- Photometry (Colorimetry) **measures** the *intensity* of light and characterize its change by an object or substance.
- Expressed as *percent transmittance* or *absorbance*.

Transmittance (T)

Frequently when the light beam is studied

$$T = \frac{I}{I_0}$$

Absorbance (A) (Optical Density, O.D.)

Used almost exclusively when the properties of the material is studied

$$A = -\log\left(\frac{I}{I_0}\right) = -\log T$$

by convention, base 10 logs are used

Beer-Lambert's Law

- Lambert's and Beer's Laws are combined to describe the attenuation of light by a solution. It is easy to see how the two standard photometric quantities can be written in terms of this law.

$$I = I_0 10^{-\varepsilon c x}$$

Transmittance

$$T = \frac{I}{I_0}$$

$$T = 10^{-\varepsilon c x}$$

Absorbance

$$A = -\log\left(\frac{I}{I_0}\right) = -\log T$$

$$A = \varepsilon c x$$

Beer-Lambert's Law

- The fraction of the incident light absorbed by a solution at a given wavelength is related to

  a) Thickness of the absorbing layer (path length).

  b) Concentration of the absorbing substance.

$$A = -\log\left(\frac{I}{I_0}\right) = -\log T$$

$$A = \varepsilon c x$$

Beer-Lambert's Law

$$A = -\log\left(\frac{I}{I_0}\right) = -\log T$$

$$A = \varepsilon c x$$

Where,

A = Absorbance

ε = Absorptivity of the substance under standard conditions

c = Concentration of the substance

x = Light path of the solution

%T = Percent transmittance

Beer-Lambert's Law

- Absorbance

$$A = K \text{ x } C = Log_{10} \frac{I_o}{I}$$

Where,

I_o = Amount of light absorbed by the solution expressed as absorbance (or) optical density.

K = Constant

C = Concentration of the substance

Instrumentation

- **Spectrometer:** Instrument that are used to study emission of light as a function of wavelength (*I* vs *λ)*.
  - Simply measures the spectrum of the electromagnetic radiation. (e.g. Emission spectroscopy).

- **Spectrophotometer:** Instrument that are used to study absorption of light as a function of wavelength (I/I_0 vs *λ)*. (e.g. Transmission, Reflection, Scattering, Fluorescence).

- **All spectrophotometers contain a spectrometer.**

Spectrophotometry

- Beer-Lambert's Law:

$$Log_{10} \frac{I_o}{I} = \varepsilon c l$$

Where,

I_o = Intensity of incident light

I = Intensity of transmitted light

ε = molar extinction coefficient

c = concentration of the absorbing substance (mol/L)

l = path length of the light-absorbing sample (cm)

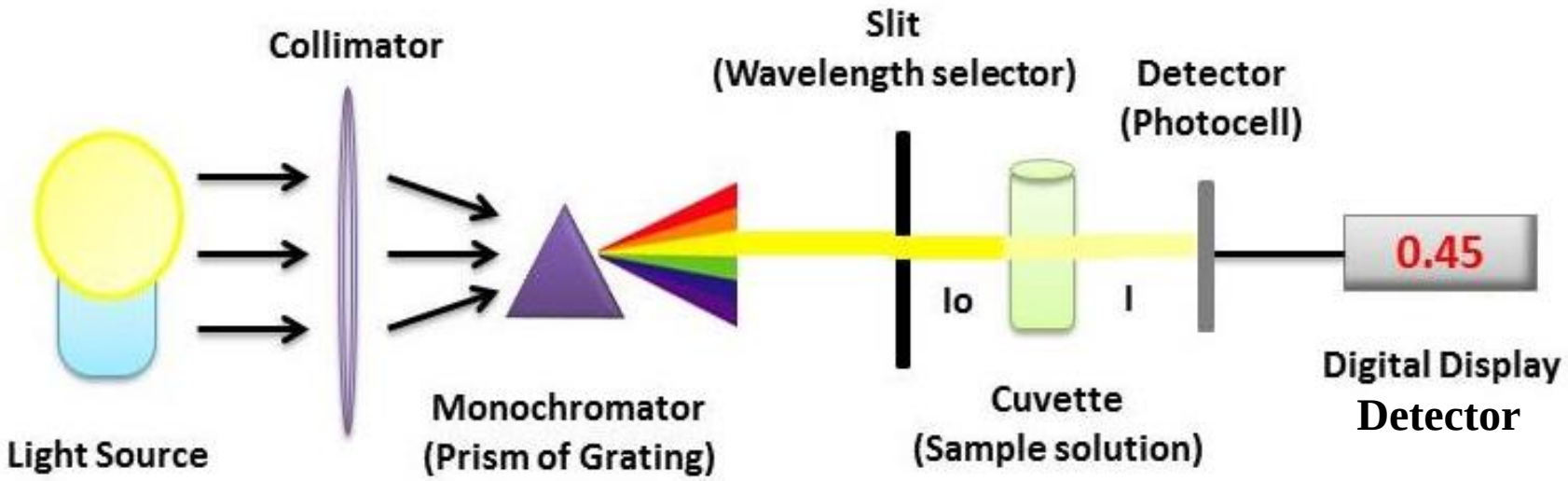
Spectrophotometry-Principle

- Advanced version of a Colorimeter.
- In a colorimeter, filters are used to allow a broad range of wavelengths to pass through, whereas in the spectrophotometer a prism (or) grating is used to split the incident beam into different wavelengths.
- By using filters or monochromators waves of specific wavelengths can be restricted to fall on the test solution.
- The range of the wavelengths of the incident light can be as low as 1 to 2nm.

Spectrophotometer - Instrumentation

- The five essential components include:
  - Radiant energy sources (UV or Visible radiation)
    - UV radiation (<380nm): Hydrogen/Deutrium lamp/Xenon lamp
    - Visible radiation (380-2500nm): Tungsten filament/Halogen lamp
  - Wavelength selectors
    - Optical Filters
    - Monochromators - Resolving element (Prisms or Gratings)
  - Sample Containers (Cuvettes: Glass or Quartz)
  - Photoelectric detectors (Photocell)
  - Amplifiers and Potentiometric recorders (generate electronic signals that are proportional to the transmitted light)

Spectrophotometer - Instrumentation



Basic Instrumentation of a Spectrophotometer

https://www.biochemden.com/wp-content/uploads/2016/05/Spectophotometer-instrumentation-768x330.png

Spectrophotometer - Applications

- ✓ Measurements of Concentration:
  - All chemical analysis and trace analysis.
- ✓ Detection of impurities..
- ✓ Elucidation of structure (or) type of organic compounds, both in pure state and in biological preparations.
- ✓ Enzyme Assays and Kinetics.
- ✓ Chemical Kinetics.
- ✓ Particle size.
- ✓ Detection of functional groups.
- ✓ Molecular weight determination.