

SPECTROPHOTOMETRY

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KEY CONCEPTS

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

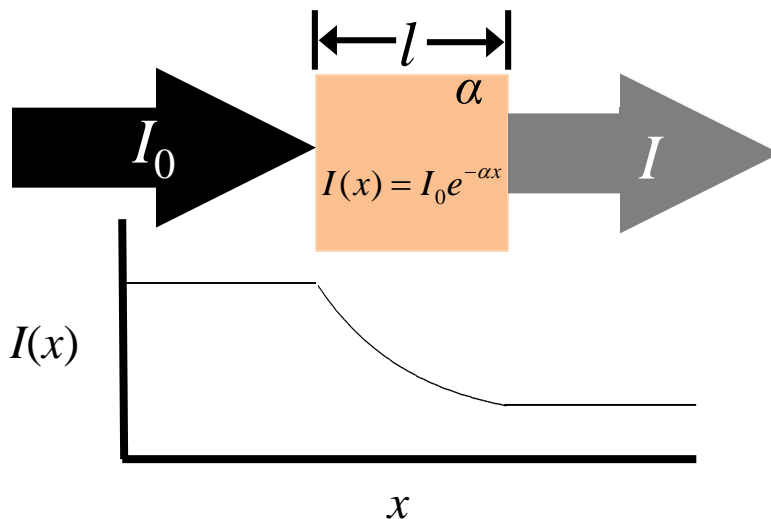
LAMBERT'S LAW OF ABSORPTION

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)



$$dI = -\alpha I dx$$

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

$$\frac{dI}{dx} = -\alpha I$$

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

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.

$$k = \varepsilon c$$

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

A colored absorber has an absorptivity that is dependent on wavelength of the light $\varepsilon(\lambda)$. 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.

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

Transmittance

$$T = \frac{I}{I_0}$$
$$T = 10^{-\epsilon c x}$$

Absorbance

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

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.

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

$$A = \epsilon c x$$

BEER-LAMBERT'S LAW

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

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

$$A = K \times C = \text{Log}_{10} \frac{I_o}{I}$$

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:

$$\text{Log}_{10} \frac{I_o}{I} = \epsilon cl$$

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)

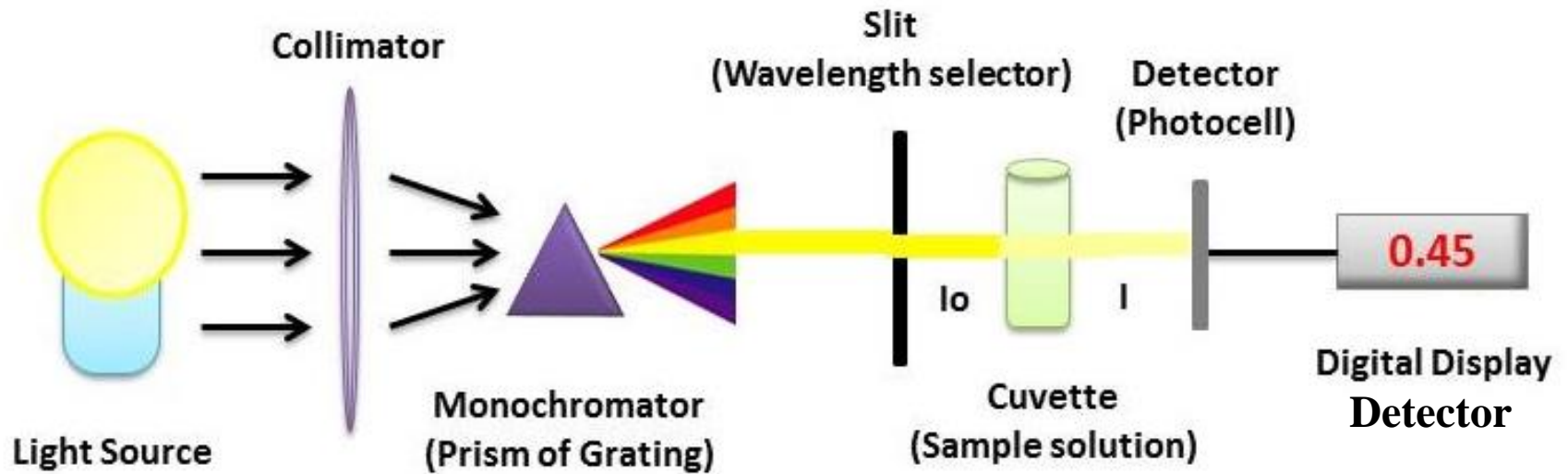
SPECTROPHOTOMETRY-PRINCIPLE

- 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/Deuterium 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

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.