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Spectrophotometer

Introduction:

Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain range of wavelength. Spectrophotometry is a method to measure how much a chemical substance absorbs or transmits light. Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical engineering, clinical applications, industrial applications, etc.).

A spectrophotometer consists of two words as 'spectrometer' for producing light of selected wavelength and 'photometer' for measuring the intensity of light. Thus, a spectrophotometer is an instrument which is used to measure the amount of light (electromagnetic radiation) that a sample absorbs. The instruments are so arranged such that the sample can be placed between the spectrometer beam and photometer thereby measuring an unknown analyte concentration. In other words, it operates by passing a beam of light through a sample and measuring the intensity of the light reaching the detector.

Definition :

A spectrophotometer is an analytical instrument that measures the amount of light that can pass through a solution. It is apparent that less light is

allowed to pass through a highly turbid or coloured solution than through a clear solution.

Fig-A: Labelled Parts Of A Spectrophotometer.

Instrumentation and Mechanism:

A spectrophotometer contains the following:

- A light source.
- A collimator.
- A monochromator (to obtain a single fixed wavelength.
- A sample compartment (which holds a sample tube or cuvette of fixed path length).
- A photomultiplier (to magnify emergent light because its intensity is too diminished to be accurately read).

- And a phototube (to measure this light and display a reading on the meter panel; i.e., adjusts for the magnification relative to the original light source and gives accurate O.D. readings).

The basic structure of Spectrophotometer is illustrated in the figure-B below.

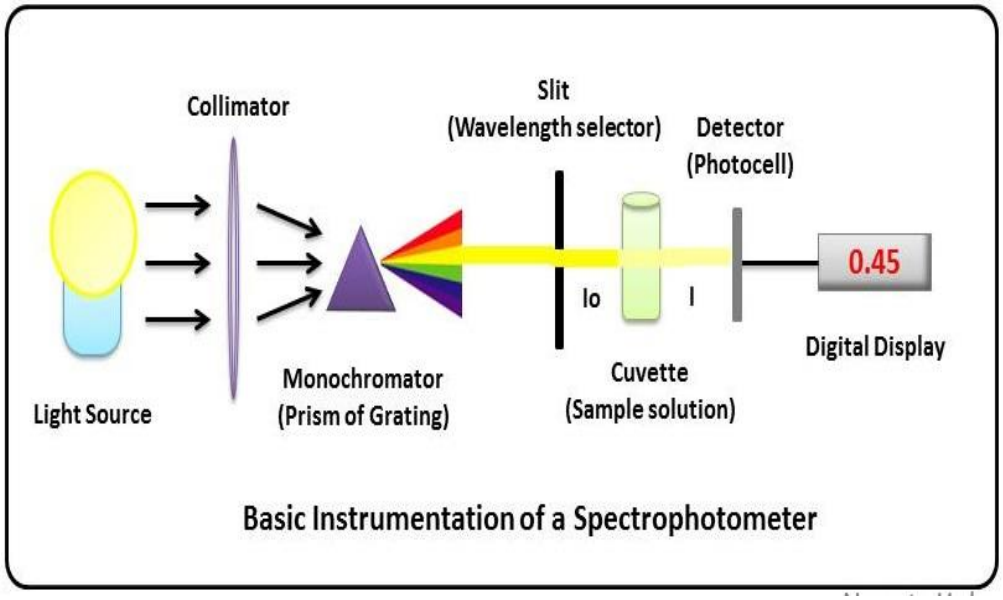

Figure -B

As we have seen above, the working of spectrophotometer is described as two instruments namely, **'spectrometer' and 'photometer'**. Hence the role of each to make things easy to understand.

Spectrometer: It consists of a light source which produces a desired range of wavelength of light. Then there is a collimator which is a lens that will transmit a straight beam of light (photons). This beam of light will then pass through a monochromator which can be a prism or a grating that will split the light into several component wavelengths which will give rise to a spectrum. Then the slit will

transmit only the desired wavelength (as shown in the figure-B, the desired wavelength being transmitted is in the range of yellow).

Photometer: After the desired range of wavelength of light passes through the solution of a sample in cuvette, the detector or photocell detects the amount of photons that is absorbed and then sends a signal to galvanometer or a digital display.

PRINCIPLE OF SPECTROPHOTOMETER

Spectrophotometer is based on the photometric technique which states that When a beam of incident light of intensity I_0 passes through a solution, a part of the incident light is reflected (I_r), a part is absorbed (I_a) and rest of the light is transmitted (I_t)

$$\text{Thus, } I_0 = I_r + I_a + I_t$$

In photometers (spectrophotometer), (I_r) is eliminated because the measurement of (I_0) and It is sufficient to determine the (I_a). For this purpose, the amount of light reflected (I_r) is kept constant by using cells that have identical properties. (I_0) & (I_t) is then measured.

The mathematical relationship between the amount of light absorbed and the concentration of the substance can be shown by the two fundamental laws of photometry on which the Spectrophotometer is based.

The Beer-Lambert Law

Beer's Law: This law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution.

$$\text{Log}_{10}\, I_0/I_t = a_s c$$

where,

a_s = Absorbency index

c = Concentration of Solution

Lambert's Law

⇒ The Lambert's law states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.

$$A = \log_{10} I_0/I_t = a_s b$$

Where,

A = Absorbance of test

a_s = Absorbance of standard

b = length / thickness of the solution

The mathematical representation of the combined form of Beer-Lambert's law is as follows:

$$\text{Log}_{10} I_0 / I_t = a_s bc$$

If *b* is kept constant by applying Cuvette or standard cell then,

$$\text{Log}_{10} I_0/I_t = a_s c$$

The absorbency index a_s is defined as

$$a_s = A/cl$$

Where, c = concentration of the absorbing material (in gm/l).

l = distance travelled by the light in solution (in cm).

In general,

The working principle of the Spectrophotometer is based on Beer-Lambert's law which states that the amount of light absorbed by a colour solution is directly proportional to the concentration of the solution and the length of a light path through the solution.

$$A \propto cl$$

Where,

A = Absorbance / Optical density of solution

c = Concentration of solution

l = Path length

or, $\boxed{A = \epsilon cl}$

ϵ = Absorption coefficient.