

Colorimeter & Spectrophotometer.

Definition :-

Colorimeter or Spectrophotometer is an instrument that utilizes light as a source of radiation and measures changes in optical density or absorbance.

Coloured substance absorb certain wavelengths of light & reflect or transmit rest of it. The light which is reflected or transmitted is the colour of that substance.

The value of wavelengths (λ -max) of some important compounds are given below :-

Compound	λ -max or nm	Compound	λ -max, nm
Protein	210 & 280	NAD (P)H	340
Pyrimidines	245	Carotenoids	440
Pyridines	250	Chlorophyll-a	665
Nucleotides	260	chlorophyll-b	649
Vitamin-A	325	Acetylene	2105-2150.

Principle :->

It has 3 basic principles :-

1. The source of radiation.
2. A unit for dispersing radiation at different wave lengths.
3. A device that detect the amount of radiation at different wavelengths.

For quantitative estimation of a substance can be measured following Beer's Law; because its principle is based on Beer's law. According to this law - absorbance is directly proportional to concentration of a substance.

Mathematical expression of Beer's law is -

$$\log \frac{I_0}{I} = Kc$$

where I_0 = Original intensity of light beam
 I = Intensity of beam passing through a solution.

(2)

C = Concentration of the solution in moles/l

K = Constant.

Spectrophotometer - The spectrophotometer is of many types. Such as -

1. **Visible Range spectrophotometer (VRS)** :- It contains a lamp of 36 watt & 12 volts as a source of energy light. It utilizes all the seven light spectrum from violet to red colours (VIBGYOR) ranging from 400 - 1000 nm wavelengths.

2. **Ultra-violet Visible Spectrophotometer** :- It contains a tungsten bulb or deuterium lamp to emit uv-light. It utilizes 200 - 1000 nm wavelengths of light.

3. **Infra-red Spectrophotometer** :- In this, the tungsten filaments are filled for light source. It uses 1000 - 3000 nm Infra red (I.R.) wavelengths.

A Typical Spectrophotometer :-

A typical spectrophotometer has the following essential components :-

1. Source of light,
2. Monochromator unit.
3. A detector for light transmitted.
4. Meter to read the transmission, and
5. A recorder.

The most common source for working in the visible, near the infra-red and near UV-region is incandescent lamp of tungsten filaments. Hydrogen & ~~de~~ deuterium lamps are also used for UV-region. In many spectrophotometers, the light is rendered parallel by a collimating lens or mirror before passing it to the monochromator unit.

The monochromator unit is normally made up of a rotating prism and a filter with a slit. The prism resolves light into different wavebands and filter allows to pass through only certain wavelengths. The opening of the filter is adjustable and can be made extremely narrow to give

resolution upto about 0.5 nm in some spectrophotometers.

The instrument contains a sample holder between the monochromator unit and the detector. Sample tubes are made up of glass or fused silica with about 1 mm thick wall & 1 cm internal diameter.

The detector is a photosensitive device which has a linear response in the spectral region. It is associated with a photo-electrical device (meter) to read the exact amount of light passing through the sample.

- Uses :-
- (1) Colorimeter or spectrophotometer in the visible range is most useful in determining many inorganic compounds.
 - (2) UV-spectrophotometry is applied to determine mainly some aromatic & heterocyclic organic compounds.
 - (3) Infra red spectrophotometry is used mostly for gases.