

Lecture 6

COLORIMETRY

Colorimetry is the determination of the concentration of a substance by measurement of relative absorption of light or transmitters with respect to a known concentration of the substance. When a beam of radiant energy falls upon a substance, the energy of the beam is partially altered by reflection, refraction, diffraction or absorption and the remaining energy may be transmitted through the substance.

FUNDAMENTAL LAWS OF COLORIMETRY

Spectro-colorimetric and photometric principles are based upon Lambert law and Beers law.

- a). **LAMBERT LAW** - It states that when monochromatic light passes through a transparent medium; the rate of decrease of monochromatic light (radiation of a single frequency) intensity with the thickness of the medium is proportional to the intensity of the light. This is equivalent to stating that the intensity of emitted light decreases exponentially as the thickness of absorbing medium increases automatically; or that any layer of given thickness of medium absorbs the same fraction of the light incident upon it.
- b). **BEER'S LAW** –Thus, according to Beer's law, the intensity of a beam of monochromatic light decreases exponentially as the concentration of the absorbing substance increases arithmetically.

METHODS OF DIRECT MEASUREMENT OF LIGHT INTENSITY

- (I) Klett Summerson Colorimeter
- (II) Bausch and Lomb Spectronic Colorimeter
- (III) Beckman's DU-Ultra Violet Visible Spectrophotometer

FLAME PHOTOMETER

The instrument is used for detection of metal salts such as Na, K, Li, Ca and Ba level in the sample. It is a simple, relatively inexpensive, high sample throughput method used for clinical, biological and environmental analysis. The source of light is from gas flame. The flame vaporizes the liquid sample into a gaseous state. The compound decomposes into simple molecules or atoms which then get excited to emit light at the temperature of the flame. The flame photometer has the following parts:

1. Pressure regulator
2. Atomizer
3. Optical arrangement
4. Burner
5. Photo-sensitive detector
6. Recording and output device

The set-up is provided with a pressure gauge so that the rate of (oxygen) can be regulated.

- 1. Pressure regulator:** this indicates the pressure and flow rate that prevails when the instrument is in operation.
- 2. Atomizer:** Introduces the liquid sample into the flame at a stable and reproducible rate i.e. every drop that falls must be uniform.
- 3. The burner:** the fuel gas should produce a steady flame in the presence of oxygen or air at a constant pressure.
- 4. The optical arrangement:** this consists of light which photosensitive device concave mirror is fixed so that the flame is focused properly.
- 5. Photosensitive Detector:** It is composed of a metal plate of iron on which it is deposited on thin layer of selenium which acts as semi-conductor. Radiation falls on the semi-conductor and measured on a meter after amplification.

CHROMATOGRAPHY

This is a separation process used for the separation of molecular mixture. It is carried out by mechanical manipulation depending on the physical properties;

1. Solubility – ability to dissolve in liquid
2. Adsorption – ability to attach itself to finely divided solid.
3. Volatility – ability to pass into vapour

Every chromatography separation has a “stationary phase” which has a packing within a column and a “mobile phase” which is caused to travel through this column. **CLASSIFICATION OF CHROMATOGRAPHY METHODS**

1. **Partition chromatography:** In, this, the moving phase is the liquid while the stationary phase is a liquid film.
2. **Adsorption chromatography:** the moving phase here is a liquid while the stationary phase is solid.
3. **Gas-liquid chromatography:** the moving phase is gas and the stationary phase is a liquid film.
4. **Gas-solid chromatography:** The moving phase is gas and the stationary phase is a solid surface.

Stationary phase is solid with either acid or base functional group. The acid functional group can be carboxylic or sulphuric, while the base functional group could be amine or quaternary amine hydroxide.

Main chromatography methods are:

- (i) High performance liquid chromatography
- (ii) Thin layer chromatography
- (iii) Paper chromatography
- (iv) Ion-exchange chromatography.

TERMS USED IN CHROMATOGRAPHY

1. **ADSORBENT:** This is a solid material which serves as a stationary phase in adsorption chromatography.
2. **SUPPORT:** This is a support for the liquid film in partition chromatography.
3. **DEVELOPMENT:** this is a process of the flow of moving phase over the adsorbent or support.
4. **ELUTION:** This is the substance on the chromatography which is washed off the adsorbent.