Chromatography
(Coloured Writing)

Separation of molecules based on differential properties

Chromatography

- Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.
- The Russian botanist **Mikhail Tswett** coined the term chromatography in 1906
- The first analytical use of chromatography was described by **James and Martin** in 1952, for the use of gas chromatography for the analysis of fatty acid mixtures
- A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties to separate materials
- It is a powerful separation tool that is used in all branches of science and is often the only means of separating components from complex mixtures

Principle

- Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase
- Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into the mobile phase, and leave the system faster
- Separation of a mixture of components into individual component through equilibrium distribution between two phases.
- Each individual component have its own equilibrium between two phases called equilibrium coefficient (Kd)
- Mixture of molecules having different Kd get separated on the given two phases

The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights

So:

- **A partition equilibrium between a stationary liquid and mobile liquid**
- **An adsorption equilibrium between a stationary solid and mobile liquid**
- **An ion exchange equilibrium between ions of similar charges on ion exchange resins (solid) and electrolyte (liquid)**
- **An equilibrium between stationary liquid and mobile gas**
- **An equilibrium between a liquid phase inside and outside of a porous structure**
- **An equilibrium between a macromolecule and a small molecule with biological affinity**

Three components thus form the basis of the chromatography technique.

1. Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface solid support”

2. Mobile phase: This phase is always composed of “liquid” or a “gaseous component”

3. Separated molecules: Sample mixture to be separated

The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on the separation of molecules from each other

- **Stationary phase – Non reactive and non soluble, solid or liquid**

Ex. Solid – Cellulose, Resin, Alumina powder, Silica gel, Activated Charcoal

Liquid – Lloyds solution, Water, Ca, Mg, Na carbonates, Benzoic acid

- **Mobile phase – Usually solvent or mixture of liquids or gas**

Ex. Liquid – Water, Acids, Alkali, Alcohol, Ether, Acetone, Benzene, Toluene, Chloroform, CCl_4

Gas – Volatile gases, compounds stable at gaseous phase at NTP

Types of Chromatography

Substances can be separated on the basis of a variety of methods and the presence of characteristics such as size and shape, total charge, hydrophobic groups present on the surface, and binding capacity with the stationary phase

This leads to different types of chromatography techniques, each with their own instrumentation and working principle

- Paper chromatography (Partition Chromatography): Solubility
- Thin-layer chromatography: Adsorption
- Ion-exchange chromatography: Ionic properties
- Gel-permeation (molecular sieve) chromatography: Molecular shape and size
- Affinity chromatography: Bioaffinity
- Gas chromatography (GS): Gaseous sample
- High-pressure liquid chromatography (HPLC)

Applications of Chromatography

Pharmaceutical sector

- To identify and analyze samples for the presence of trace elements or chemicals
- Separation of compounds based on their molecular weight and element composition
- Detects the unknown compounds and purity of mixture
- In drug development

Chemical industry

- In testing water samples and also checks air quality
- HPLC and GC are very much used for detecting various contaminants such as polychlorinated biphenyl (PCBs) in pesticides and oils
- In various life sciences applications

Food Industry

- In food spoilage and additive detection
- Determining the nutritional quality of food

Forensic Science

- In forensic pathology and crime scene testing like analyzing blood and hair samples of crime place.

Molecular Biology Studies

- Study of metabolomics and proteomics along with nucleic acid research
- HPLC is used in Protein Separation like Insulin Purification, Plasma Fractionation, and Enzyme Purification and also in various departments like Fuel Industry, biotechnology, and biochemical processes