CHROMATOGRAPHIC METHODS OF SEPARATION

ANKITA OJHA
DEPARTMENT OF CHEMISTRY
MAHARAJA COLLEGE, ARRAH

WAYS TO SEPARATE COMPOUNDS

Chromatography:

- **Paper Chromatography**
- **Thin Layer Chromatography**
- **Column Chromatography**
- **Gas Chromatography**
- **High Pressure Liquid Chromatography (HPLC)**

<u>Characterization</u>

UV-Vis spectra, IR spectra, NMR and Elemental Anlaysis

Chromatographic techniques mainly involves the separation of mixtures due to differences in the distribution coefficient (equilibrium distribution) of sample components between two different phases.

One of these phases is a mobile phase and the other is a stationary phase.

DISTRIBUTION COEFFICIENT (EQUILIBRIUM DISTRIBUTION)

It is the ratio of Concentration of any component of in stationary phase and mobile phase.

Distribution coefficient =

$$\frac{Concentration\ of\ component\ A\ in\ stationary\ phase}{Concentration\ of\ component\ A\ in\ mobile\ phase}$$

Chromatography is an effective method of separation because different components within a mixture are attracted to adsorbent surface (such as silica gel in Thin layer chromatography) of the stationary phase with varying degrees depending on each components polarity and its unique structural characteristics, and also its interaction with the mobile phase.

Analysis and separation techniques

- **Paper chromatography**
- **Thin layer chromatography**
- **Gas chromatography (GC)**
- **High Pressure Liquid Chromatography (HPLC)**
- **Thin layer chromatography (Microgram Quantity)**
- **Column chromatography (Gram Quantity)**
- **High Pressure Liquid Chromatography, HPLC(milligram quantity)**

- **Gas chromatography (milligram quantity)**

Plate Theory

- **Plate theory describes a chromatography system to be in equilibrium between the stationary and mobile phases.**
- **The column is assumed to be divided into a number of imaginary theoretical plates.**
- **As the number of plates in a column increases or the height equivalent theoretical plates increases, and hence the separation of components.**
- **It provides an equation which describes the elution curve or the chromatogram of a solute it can also be used to find the volume and the column efficiency.**
- **H= L/N where, L = Column Length and N= number of theoretical plates.**

Rate Theory

- **Rate theory describes the migration of molecules in a column.**
- **This included band shape, broadening of the band and the diffusion of a solute.**
- **This theory actually follows the Van Deemter equation, which is the most appropriate for prediction of dispersion in liquid chromatography columns.**

- **This theory takes into account about the various pathways that a sample must travel through a column. Van Deemter equation helps to find the optimum velocity and a minimum plate height needed for separation.** $H = A + \frac{B}{u} + C.u$ **where A = Eddy-Diffusion, B = Longitudinal Diffusion, C = mass transfer, u = linear velocity**

Types of Chromatography

1. Normal Phase Chromatography:

- **The components in a mixture will elute at different rates depending on each one's polarity relative to the next component. When the column to be used for the separation is more polar than the mobile phase, the experiment is said to be a normal phase method.**
- **In this the stationary phase is polar and more polar solutes are separated adhering more to the stationary adsorbent phase. On passing the solvent or gradient of solvents through the column, less polar components will be eluted faster than more polar components.**
- **The components are collected separately, assuming adequate separation was achieved, in order of increasing polarity.**