DEPARTMENT OF BIOTECHNOOGY
FACULTY OFENGINEERING
& TECHNOLOGY

Content Outline
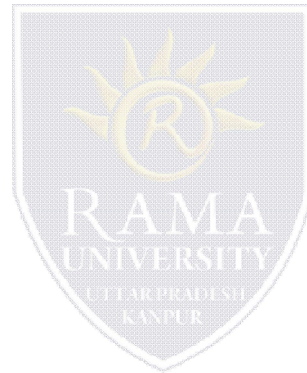
Chromatography Definition

➢Chromatography is a process for separating components of a mixture. Chromatography was first employed in Russia by the Italian-born scientist Mikhail Tsvet in 1900 for the separation of plant pigments such as chlorophyll, carotenes, and xanthophylls. Since these components have different colors (green, orange, and yellow, respectively) they gave the technique its name.

➢**Any chromatography procedure essentially consists of following two components: Mobile phase and stationary phase**

➢Mobile phase: This phase is always composed of "liquid" or a "gaseous component. The mixture to be separated is dissolved in a substance called the **mobile phase. Mobile phase flows over the stationary phase either in gaseous or liquid phase**

➢Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed/ coated on the surface a solid support". The mobile phase carrying mixture is passed over it.

Now question arises how can we separate biomolecules from biochemicals continuously synthesized in biological organisms?

The answer to this question lies in physical and chemical properties of biomolecules. No two biomolecules have same chemical and physical properties and these differences can be utilized to separate them into individual components.

The physical and chemical properties which can be use to separate molecules are

Physical Properties

1. Molecular weight

2. Boiling point (in case both are liquid, as in this case)

3. Freezing point

4. Crystallization

5. Solubility

6. Density

7. Affinity
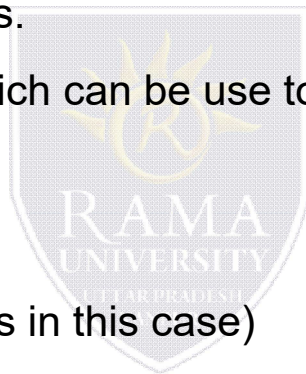
How a physical or chemical property will allow isolating a particular substance?

The basis of all forms of chromatography is the distribution or partition coefficient (Kd), which describes the way in which a compound (the analyte) distributes between two immiscible phases. For two such phases A and B, the value for this coefficient is a constant at a given temperature and is given by the expression:

$$\frac{\text{concentration in phase A}}{\text{concentration in phase B}} = K_d$$

Chemical Properties

1. Functional Group, for example, phenol has –OH where as aniline has NH2.

2. Reactivity towards other reagent to form complex

How these properties are combined in chromatography to effect separation?

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. The mixtures are dissolved in mobile phase and run over stationary phase. Depending upon differences in chemical and physical properties such as adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster.

➤The term effective distribution coefficient is defined as the total amount, as distinct from the concentration, of analyte present in one phase divided by the total amount present in the other phase. It is in fact the distribution coefficient multiplied by the ratio of the volumes of the two phases present.

➤All chromatographic systems consist of the stationary phase, which may be a solid, gel, liquid or a solid/liquid mixture that is immobilised, and the mobile phase, which may be liquid or gaseous, and which is passed over or through the stationary phase after the mixture of analytes to be separated has been applied to the stationary phase. During the chromatographic separation the analytes continuously pass back and forth between the two phases so that differences in their distribution coefficients result in their separation.

Chromatographic Term

Analyte

A substance whose chemical constituents are being identified and measured

Capillary Column

A column whose inner diameter is under 0.5 mm.

Eluate

The mobile phase exiting a column.

**Eluent**

The mobile phase entering a column.

Elution

The passage of the mobile phase through the column to transport solutes.

Flow Rate

The amount of mobile phase that has passed through the column per unit time. The units are millilitres per second (mL/sec) or, more commonly, millilitres per minute (mL/min).

Chromatogram: The plot of elution volume along with the absorbance is known as chromatogram
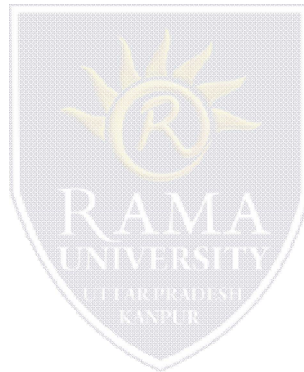
Types of chromatography

- Column chromatography

- Partition chromatography

- Asdorption chromatography

- Ion-exchange chromatography

- Gel-permeation (molecular sieve) chromatography

- Affinity chromatography

- Paper chromatography

- Thin-layer chromatography

- Gas chromatography

- Dye-ligand chromatography

- Hydrophobic interaction chromatography

- Pseudoaffinity chromatography

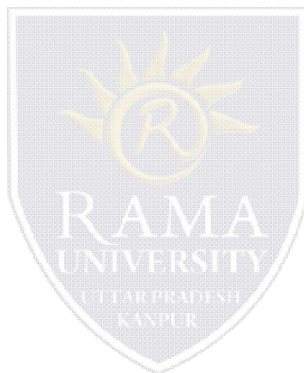- High-pressure liquid chromatography (HPLC)
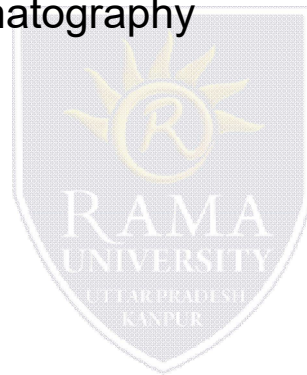
Chromatography is a Chromatography is a

a. process of mixing color

b. process for separating components of a mixture

c. process used in making film

d. process of cinematography

Chromatography is a physical method that is used to separate and analyse _____

a. Simple mixtures

b. Complex mixtures

c. Viscous mixtures

d. Metals

Any chromatography procedure essentially consists of

a. Stationary phase

b. Mobile Phase

c. Both (a) and (b)

d. None of the above

Distribution or partition coefficient (Kd) is given by distribution or partition coefficient (Kd) is given by

a. Concentration in phase 'A'/ Concentration in Phase 'B' Concentration in phase 'A'/ Concentration in Phase 'B'

b. Concentration in phase 'A' + Concentration in Phase 'B' '

c. Concentration in phase 'A' × Concentration in Phase 'B'

d. None of the above

References & Further reading

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3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.

4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.