

B.PHARMA SEM- 7

UNIT-5

PART-1

INSTRUMENTAL METHODS OF ANALYSIS

**ION EXCHANGE
CHROMATOGRAPHY**



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Analysis

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TOPIC: Ion Exchange chromatography

Ion-Exchange Chromatography

ion + exchange

Cation (+ve) Anion (-ve)

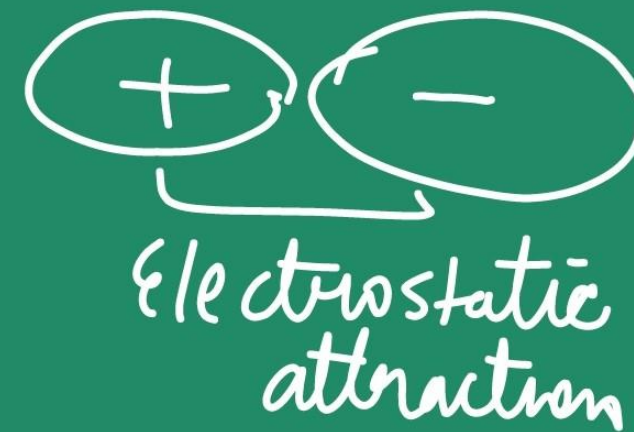
Separation Technique

- Ion-exchange chromatography is a process, which allows the separation of ions.
- This separation is based on their affinity.

This chromatography Technique is used for the separation of large Protein, amino acid, Sugar molecules etc.

Principle :-

ion-exchange chromatography



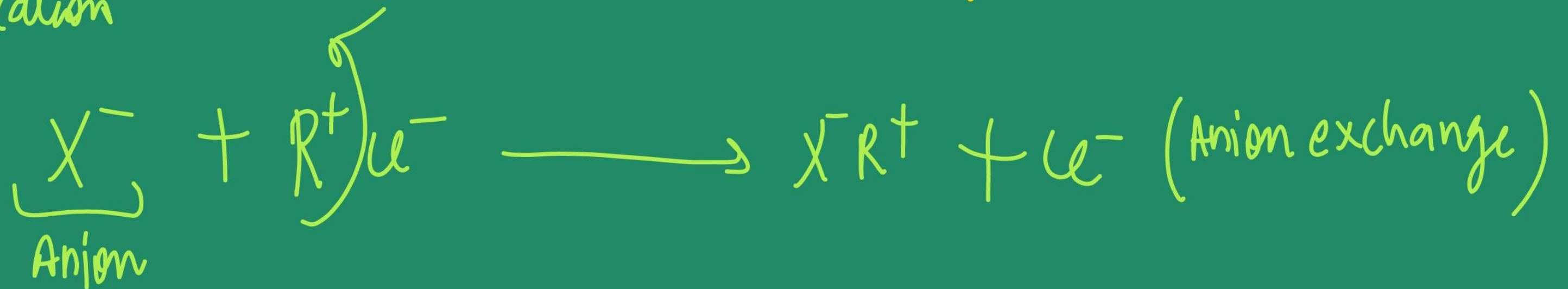
is based on **reversible electrostatic interaction** of ions.
with the separation matrix.

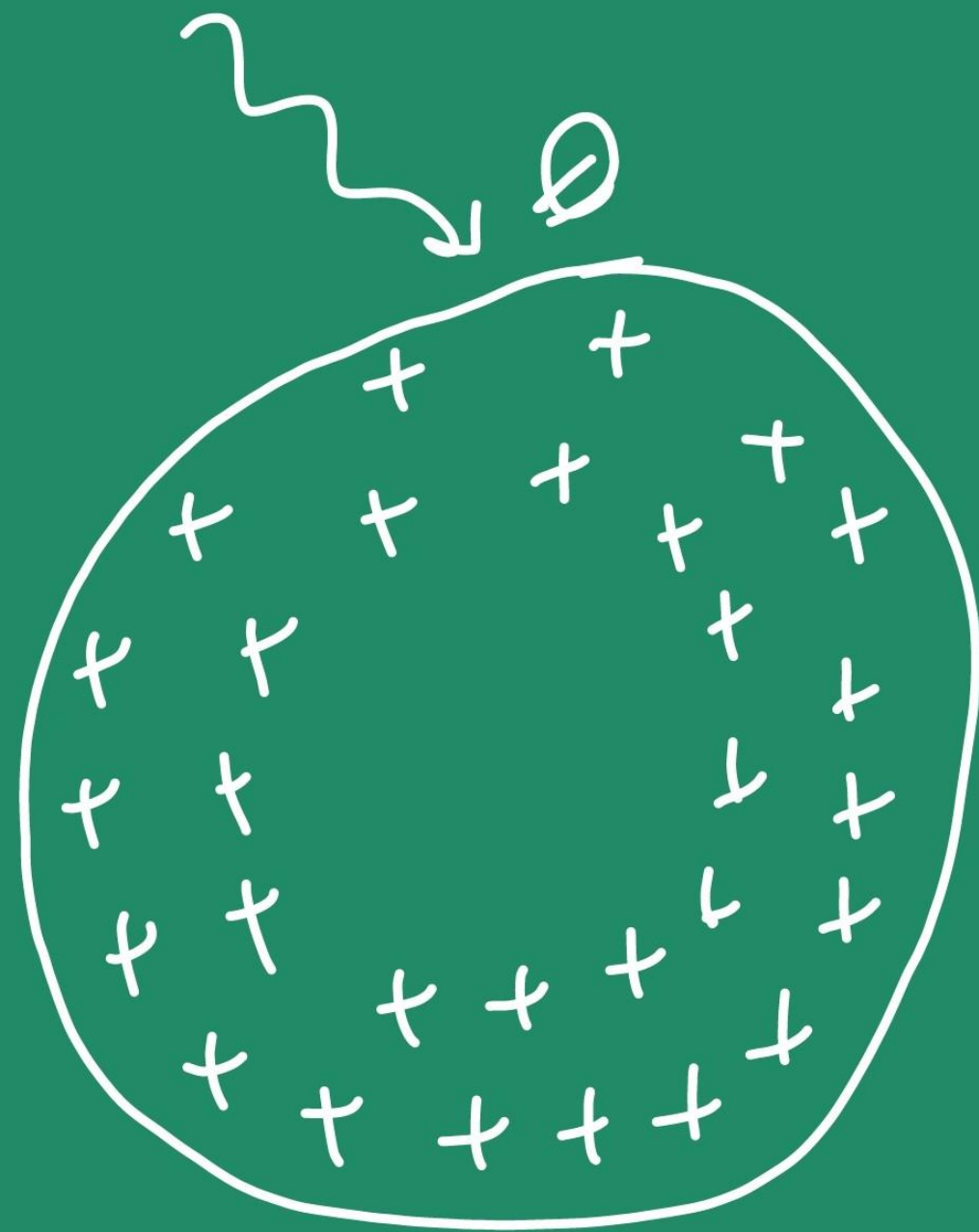
→ Anion exchanger.

→ Cation exchanger.

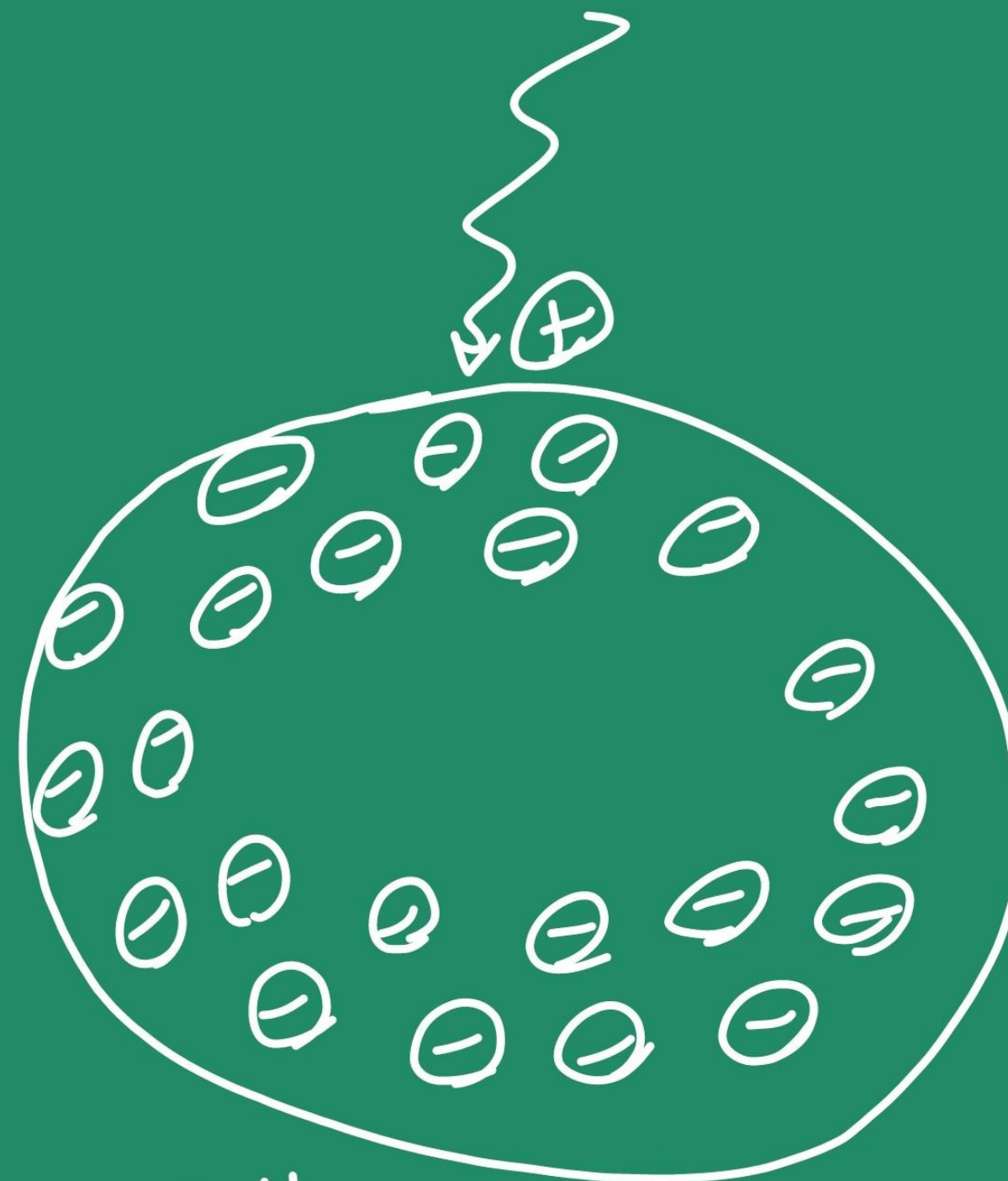
→ Anion exchanger contains bound positive groups

→ Cation exchanger contains bound negative groups.





Anion exchanger
Stationary Phase



Cation exchanger
Stationary Phase.

Classification of Resin

Resins can be classified as follows;

1. Strong cation exchanger Resin!— Sulphonic acid groups attached with Styrene & di-vinyl benzene co-polymer.
2. Weak cation exchange Resin:— Carboxylic acid ($-\text{COOH}$) attached with acrylonitrile & di-vinyl co-polymer.

3. Strong anion exchange resin : Quaternary Ammonium salt attached with Styrene & divinyl benzene co-polymer.

4. Weak Anion exchange Resin : — Poly alkyl amino groups are attached with Benzene co-polymers.

Classification of Resin on the basis of its source

1. Natural Resin:

Cation: Zeolites

Anion: Dolomite

2. Artificial Resin: It is also k/a synthetic resin.

Organic & inorganic resins.

3. Organic Resin: Polymeric resin structure / Matrix.

→ More highly charged molecule, more tightly bound to the resin & travels slower.

Resultant elute very slowly.

→ Moderately charged molecule equilibrate b/w the resin.

→ less charged molecules are bound with resins very weakly and equilibrate more with buffer solution, travels more readily & elute faster.

All these points are based on affinity of ions.

Applications

- For softening & Di-mineralisation of water.
- For the extraction of enzymes & tissues.
- For the separation of Inorganic compounds/ions
- For the separation of sugars, Proteins & amino acids.

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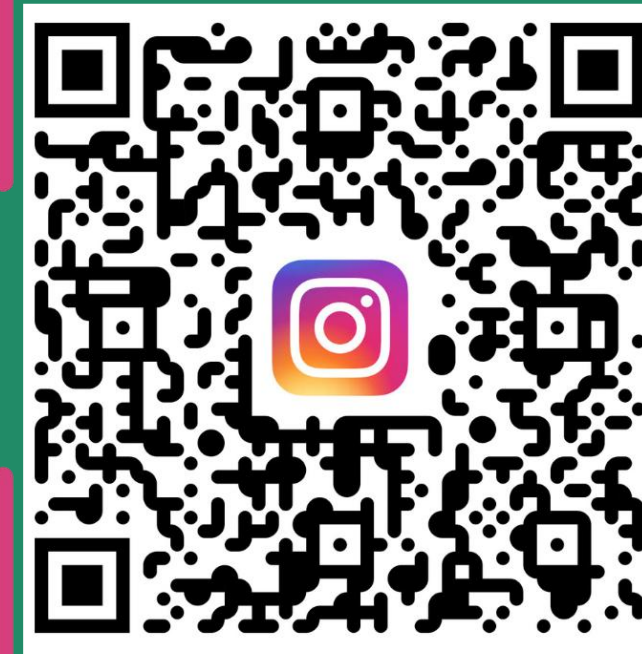
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PART-2

INSTRUMENTAL METHODS OF ANALYSIS

**AFFINITY
CHROMATOGRAPHY**



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Analysis

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TOPIC: *Affinity chromatography*

Affinity Chromatography

Introduction :- Affinity chromatography is highly selective analytical method.



It is based on many types of biological interactions.

→ It is based on 'Lock n Key' interaction between a biomolecule (enzyme, antibody, receptor etc) and a specific ligand (substrate, complementary DNA strand, inhibitor)

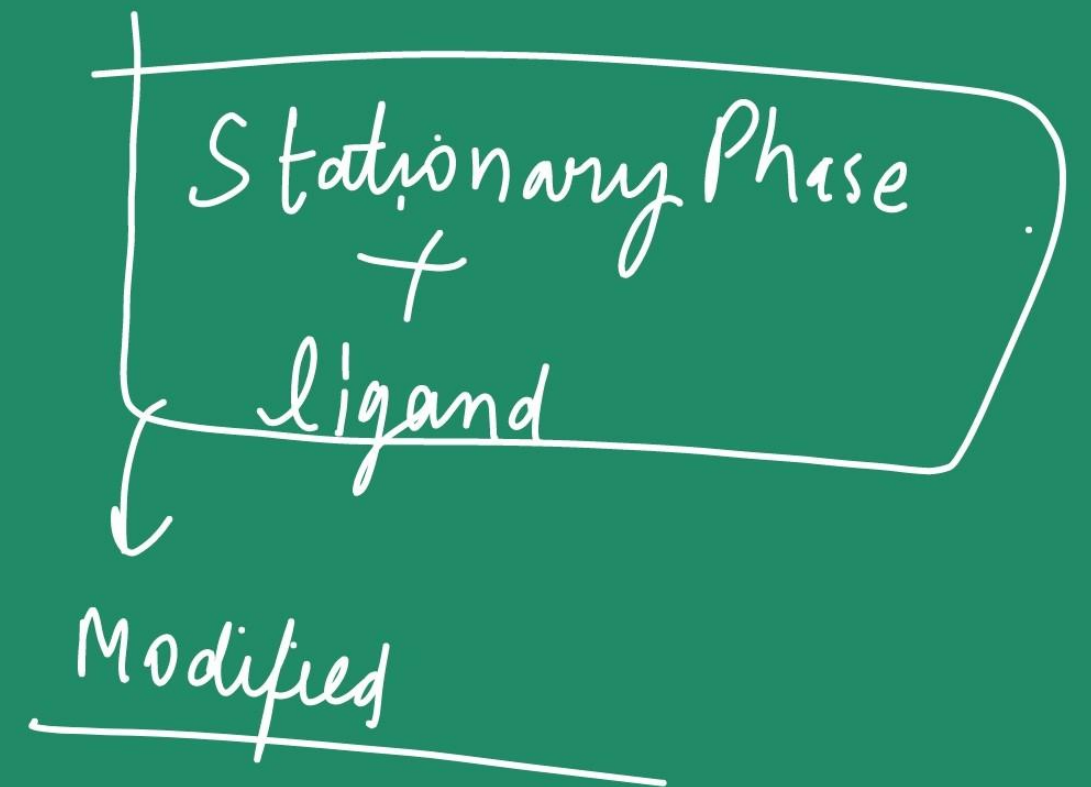
→ It is used for the Purification of Proteins, enzymes, antibodies, hormones, nucleic acid etc.

Principle :-

The stationary Phase is bound with a specific ligand.

→ When the sample mixture is Passes through the column:

- Only molecules with strong affinity to the ligand are retained.
- Non-bonding molecules are Washed out.



→ The bound molecules are then eluted by changing conditions .

(pH, ionic strength etc.)

Steps in Affinity chromatography : This chromatography

Proceed in several steps, which are as follows;

1. immobilization of ligand — In this step, ligands are attached with any

Solid molecule by covalent bond.

eg. Cellulose, Silica etc.

2. Application of Sample :— The crude mixture is applied/loaded in the column.

3. Binding / Adsorption :— In this step, target molecules binds with the specific ligand.

→ All unwanted molecules are washed out.

4. Washing : Buffer washes remove impurities & non-specifically bound molecules elute.

5. Elution : Bound molecules will release by:
increasing ionic strength, optimum pH range, Adding competitive ligand.

6. Regeneration The column is re-generated and re-used.

Types of Affinity chromatography :- It is of many types, which are as follows;

1. Bio-specific Affinity chromatography — Uses natural biological ligands
2. Pseudo-Affinity chromatography :- Uses dye-ligands.
3. Immuno-Affinity Chromatography :- Uses the interaction between Antigen & Antibodies.
4. Metal-chelate Affinity chr. :- Uses different metal ions (Ca^{+2} , Mg^{+2} etc)

Lactin Affinity Chromatography: — Lactins are specifically bound with carbohydrates.

Stationary Phase

Cellulose, Polyacrylamide, silica (solid support Matrix).

Chemical Properties

- ↳ Chemically stable.
- ↳ Inert. (Unreactive).
- ↳ Hydrophilic in nature.
- ↳ Attached to the ligands.

Applications

- Purification of Enzymes, Hormones, anti-bodies and nucleic acids etc.
- Diagnostic kits, biosensors.
- For the removal of contaminants from the mixture.

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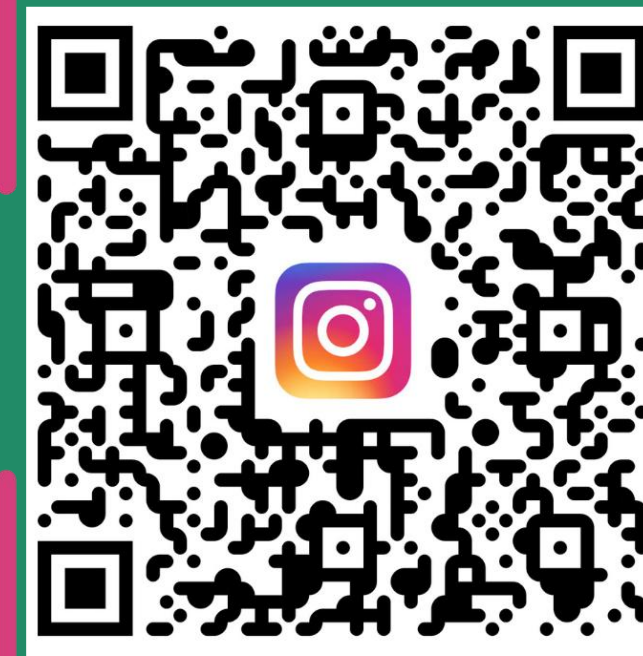
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PART-3

INSTRUMENTAL METHODS OF ANALYSIS

**GEL
CHROMATOGRAPHY**



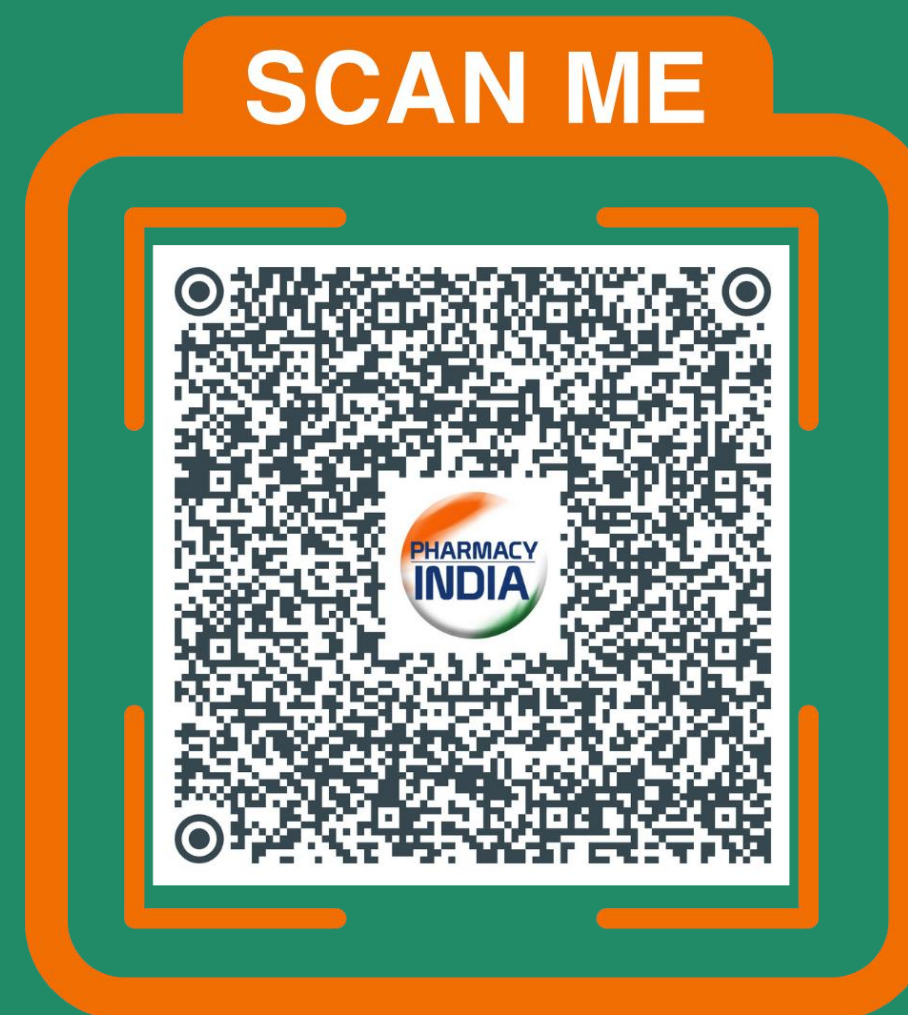
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Analysis

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TOPIC:- Gel Chromatography

Gel Chromatography

This chromatography is also known as 'size exclusion chrom.'

This technique uses Porous Particles to separate molecules of different size.

→ In this Particular Technique molecules are separated on the basis of their size.

→ It is used for the separation of biological molecules & this sep. is based on the molecular weight of different Polymers.

→ This technique is especially used for larger biomolecule or macromolecules.

When any organic solvent is used as a mobile Phase, This is called as Gel filtration chromatography.

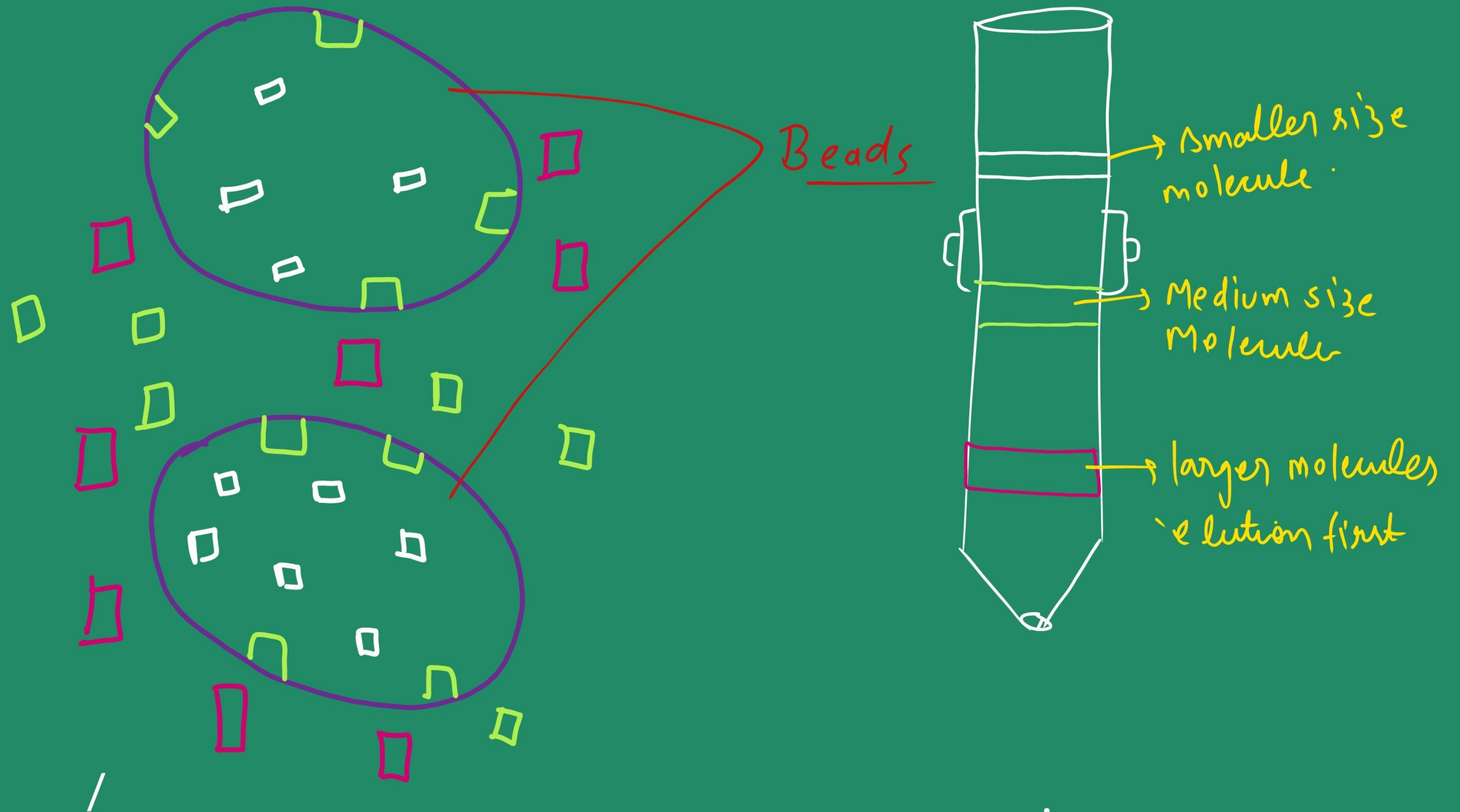
This separation of molecule is called Fractionation.

The size of pores in beads determine the exclusion limit

Principle :— A mixture of molecule is easily dissolved (Mobile Phase)

is applied to chromatographic column which contains a solid support in the form of beads. (Stationary Phase)

- The mass of beads present in column often refers as a column bed.
- The beads act as a trap in which smaller molecules get trapped larger molecule will pass/exclude from the column.
- ∴ larger molecule will elute first, smaller molecules then elute.



Total Volume

$$\left[V_t = V_g + V_i + V_o \right]$$

Where,

V_i = Volume of solvent

V_o = free solvent volume

V_g = volume occupied by packing.

Mobile Phase

Material

Synthetic elastomers

Protein

Solvent

Toluene

Water / Buffer.

Stationary Phase

Soft gel : Sephadex, Polyacrylamide gels.

(Separation of Protein).

Semi-rigid gel : Bio-beads.

(Separation of non-polar polymer).

Highly Rigid gels : Sep. of Polar polymer.

Applications

1. Protein fractionation
2. Molecular weight determination.
3. Purification
4. Separation of Sugar, Proteins, Peptides, nucleic acids, rubbers on the basis of their size.
5. For co-polymerization studies.
6. Various type of RNA & Viruses have been Purified-

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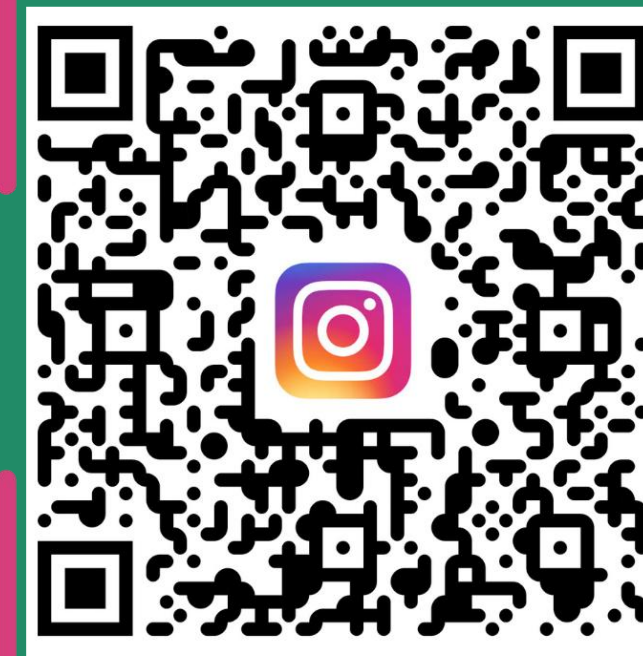
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