

B.PHARMA
7TH SEMESTER

UNIT-5

INSTRUMENTAL METHODS OF ANALYSIS

**QUESTION
BANK**

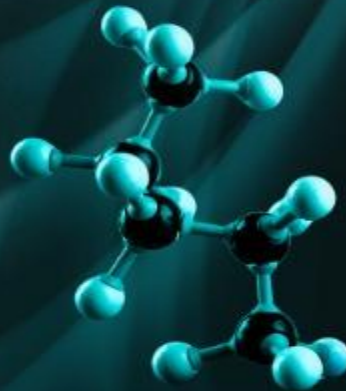
CLICK ON BANNER TO WATCH VIDEO

FREE

PDF



NOTES



INSTRUMENTAL METHODS OF ANALYSIS
B.Pharm | Sem -7
Unit – 5
Question Bank

DOWNLOAD "PHARMACY INDIA" MOBILE APP



SCAN ME



Mobile Phone Par Click karein

Download Pharmacy India Mobile App from Play Store



DAILY UPDATES

जुड़िए PHARMACY INDIA के साथ.....

WHATSAPP & TELEGRAM SE JUDNE KE LIYE ICONS PAR CLICK KARE

SCAN ME



WhatsApp



SCAN ME



Download Pharmacy India Mobile App from Play Store



1

Define ion exchange chromatography. Explain its principle and application.

- Ion exchange chromatography (IEC) is a separation technique used to separate and purify charged molecules (ions) based on their affinity to an ion exchange resin.
- The separation occurs due to reversible electrostatic interactions between charged solutes and oppositely charged functional groups attached to a stationary phase.

Principle:

- The principle of ion exchange chromatography relies on the exchange of ions between the **mobile phase (solution containing analytes)** and the **stationary phase (ion exchange resin)**.
- The **stationary phase consists of a solid matrix (often a polymer like polystyrene)** with charged functional groups attached:
 - **Cation exchange resins have negatively charged groups** (e.g., sulfonate, $-\text{SO}_3^-$), which bind positively charged ions (cations).



1

Define ion exchange chromatography. Explain its principle and application.

- Anion exchange resins have positively charged groups (e.g., quaternary ammonium, $-NR_4^+$), which bind negatively charged ions (anions).
- When a solution containing ions passes through the column packed with ion exchange resin, ions in the solution compete with ions already bound to the resin.
- Ions with greater affinity to the resin will replace the ions already attached, effectively exchanging places.
- The ions are separated based on differences in charge, size, and affinity for the resin.
- The bound ions can later be eluted (washed out) by changing the ionic strength, pH, or using a competing ion in the mobile phase.

Applications:

- **Purification of proteins and peptides:** Separating proteins based on their net charge at a given pH.
- **Separation of amino acids, nucleotides, and sugars:** Especially useful for charged biomolecules.
- **Water treatment:** Removing heavy metals or unwanted ions.
- **Drug analysis:** Separation and purification of ionic drugs and their metabolites.
- **Quality control:** Monitoring ionic impurities in pharmaceutical formulations.
- **Enzyme purification:** Based on differences in charge properties of enzymes.
- **Removal of contaminants:** Such as removing salts, buffer components, or charged impurities from samples.

2

Outline the mechanism involved in Ion exchange chromatography with factors affecting.

Mechanism of Ion Exchange Chromatography:

- **Preparation of Stationary Phase:**

- The stationary phase is an insoluble resin containing fixed charged groups.
- For **cation exchange resin**, the functional groups are negatively charged (e.g., $-\text{SO}_3^-$).
- For **anion exchange resin**, the functional groups are positively charged (e.g., $-\text{NR}_4^+$).

- **Sample Application:**

- The sample containing ions (charged molecules) is passed through the column packed with the ion exchange resin.
- Ions in the sample compete with ions that are initially bound to the resin (usually H^+ , Na^+ for cation exchangers or OH^- , Cl^- for anion exchangers).

2

Outline the mechanism involved in Ion exchange chromatography with factors affecting.

■ Ion Exchange Process:

- Electrostatic attraction causes oppositely charged ions from the sample to bind to the resin.
- The bound ions on the resin are simultaneously released into the mobile phase, thus the name “ion exchange.”

■ Separation:

- Different ions bind to the resin with varying affinities depending on their charge, size, and hydration energy.
- Ions with stronger affinity bind more tightly and elute later; weaker ions elute earlier.

■ Elution:

- After binding, ions are eluted by changing the mobile phase conditions:
- **Increasing ionic strength** (e.g., adding NaCl) competes with bound ions.
- **Changing pH** affects ionization states and charge of analytes.



Factors Affecting Ion Exchange Chromatography

■ pH of the Mobile Phase:

- Affects the charge state of the analyte and resin functional groups.
- Optimal pH ensures the analyte is ionized and able to interact with the resin.

Ionic Strength of the Mobile Phase:

- Higher ionic strength (more competing ions) reduces binding by competing for sites, causing earlier elution.
- Lower ionic strength promotes stronger binding and longer retention.

Charge and Size of the Ions:

- Ions with higher charge (e.g., divalent vs. monovalent) have stronger affinity for the resin.
- Larger ions might have steric hindrance, reducing binding.

Type of Ion Exchange Resin:

- Different resins have different functional groups and capacities.
- Choice of resin affects selectivity and capacity.

Flow Rate of Mobile Phase:

- Slower flow rates improve resolution by allowing sufficient interaction time.

Download Pharmacy India Mobile App from Play Store



3

Differentiate between cation exchange and anion exchange chromatography.

Feature	Cation Exchange Chromatography	Anion Exchange Chromatography
Definition	Separates positively charged ions (cations)	Separates negatively charged ions (anions)
Resin Functional Group	Negatively charged groups (e.g., $-\text{SO}_3^-$, $-\text{COO}^-$)	Positively charged groups (e.g., $-\text{NR}_4^+$, $-\text{NH}_3^+$)
Ions Retained	Cations (e.g., Na^+ , Ca^{2+} , NH_4^+)	Anions (e.g., Cl^- , SO_4^{2-} , NO_3^-)
Elution Method	Increase salt concentration with competing cations (e.g., Na^+) or change pH	Increase salt concentration with competing anions (e.g., Cl^-) or change pH
Application Examples	Purification of basic proteins (positively charged at given pH)	Purification of acidic proteins (negatively charged at given pH)
Charge on Stationary Phase	Negative	Positive
Common Resins	Sulfonated polystyrene (strong acid cation exchanger)	Quaternary ammonium groups (strong base anion exchanger)
Effect of pH	Sample must have positively charged ions ($\text{pH} < \text{pI}$ of protein)	Sample must have negatively charged ions ($\text{pH} > \text{pI}$ of protein)
Typical Use	Removal of metal cations, purification of peptides/proteins with positive charge	Removal of negatively charged impurities, purification of proteins with negative charge



4

What are the applications of ion exchange chromatography in pharmaceutical analysis?

Applications of Ion Exchange Chromatography in Pharmaceutical Analysis:

- **Purification of Drugs and Biomolecules:**

Separation and purification of **proteins, peptides, and antibiotics** based on their charge.
Isolation of active pharmaceutical ingredients (APIs) from complex mixtures.

- **Analysis of Amino Acids:**

Quantitative and qualitative analysis of amino acids in formulations and biological samples.
Useful in **monitoring amino acid content** and impurities.

- **Determination of Drug Impurities:**

Separation and quantification of **ionic impurities**, such as counter ions, residual solvents, and degradation products.

- **Separation of Charged Metabolites:**

Analysis of metabolites in **pharmacokinetic and drug metabolism** studies.

5

What is affinity chromatography? Explain its principle with an example.

- **Affinity chromatography** is a highly selective separation technique based on specific **biological interactions** between a molecule in the mixture (target molecule) and a **ligand immobilized on a stationary phase (resin)**.
- It is used to purify biomolecules such as **enzymes, antibodies, proteins, and nucleic acids**.

Principle:

- Affinity chromatography exploits the **specific and reversible binding** between a **target molecule** and its **ligand** attached to the stationary phase.
- The stationary phase contains a ligand that **specifically binds** to the target molecule based on biochemical affinity (e.g., **antigen-antibody, enzyme-substrate, receptor-ligand interactions**).
- When the sample passes through the column, the target molecule binds to the ligand on the resin, while other components pass through.
- The bound target is then **eluted** by changing conditions (**pH, ionic strength, or by adding a competitive molecule**), disrupting the interaction and releasing the purified molecule.



5

What is affinity chromatography? Explain its principle with an example.

Steps in Affinity Chromatography:

- **Preparation of Column:** Ligand is covalently attached to an inert matrix (like agarose beads).
- **Sample Application:** The mixture containing the target molecule is passed through the column.
- **Binding:** The target molecule binds specifically to the ligand; non-target molecules are washed away.
- **Washing:** Unbound impurities are washed off.
- **Elution:** The target molecule is released by changing buffer conditions or adding a competitor.

5 What is affinity chromatography? Explain its principle with an example.

Example:

➤ Purification of Antibodies using Protein A/G Column:

Ligand: Protein A or Protein G (bacterial proteins) that specifically bind to the Fc region of antibodies.

Target: Antibodies present in a mixture.

Process: Antibody-containing solution passes through a Protein A column; antibodies bind tightly, impurities wash away.

Elution: Antibodies are eluted by lowering the pH or changing salt concentration, disrupting the binding.

6

Differentiate between affinity and ion exchange chromatography.

Feature	Affinity Chromatography	Ion Exchange Chromatography
Basis of Separation	Specific biological interactions (e.g., enzyme–substrate, antigen–antibody)	Electrostatic interactions between charged molecules and charged resin
Stationary Phase	Resin with immobilized specific ligand	Resin with charged functional groups (cation or anion exchangers)
Selectivity	Highly selective for target molecules	Less selective; separates based on charge differences
Target Molecules	Proteins, enzymes, antibodies, nucleic acids	Charged molecules such as ions, amino acids, peptides
Elution Method	Changing buffer conditions (pH, salt) or adding competing ligand	Changing ionic strength (salt concentration) or pH
Application	Purification of specific biomolecules like antibodies, enzymes	Separation and purification based on charge; protein fractionation, removal of ionic impurities
Complexity	More complex due to ligand immobilization	Simpler, relies on charge interactions
Binding Mechanism	Highly specific binding to ligand	Non-specific ionic interactions

Download Pharmacy India Mobile App from Play Store

7

Explain the principle of gel filtration chromatography.

Principle:

- Gel filtration chromatography, also known as **size-exclusion chromatography**, separates molecules based on their **size and shape**.
- The stationary phase consists of porous beads made of polymers like **dextran, agarose, or polyacrylamide**.
- When a mixture passes through the column, **smaller molecules** enter the pores of the beads and take a **longer path**, thus eluting **later**.
- **Larger molecules** cannot enter the pores and **move around the beads**, traveling a **shorter path**, so they elute **earlier**.
- Therefore, molecules are separated primarily by their **hydrodynamic volume** (effective size in solution), with **larger molecules eluting before smaller ones**.

8

What types of stationary phases are used in size exclusion chromatography?

Common Stationary Phases:

Stationary Phase Material	Description & Use
Cross-linked Dextran (Sephadex)	Hydrophilic polysaccharide beads ; widely used for proteins, polysaccharides, and nucleic acids ; available in different cross-linking degrees for size range tuning
Cross-linked Agarose (Sephарose)	Another hydrophilic polysaccharide ; ideal for biological macromolecules ; mechanically stable and used for larger molecules
Polyacrylamide Beads	Synthetic polymer beads ; used for proteins and peptides; can be finely controlled for pore size
Polystyrene-divinylbenzene (PS-DVB) Beads	Hydrophobic synthetic beads ; used for organic solvents and small molecules ; resistant to harsh chemical conditions
Silica-based Beads	Rigid and used mainly in HPLC SEC columns ; suitable for small molecules and peptides; limited pH range

Download Pharmacy India Mobile App from Play Store

9

Name commonly used ion exchange resins and explain their role.

Types of Ion Exchange Resins:

- Ion exchange resins are solid, insoluble polymers with charged functional groups. They are classified into:
 - **Cation Exchange Resins:** Contain negatively charged groups; bind positively charged ions (cations).
 - **Anion Exchange Resins:** Contain positively charged groups; bind negatively charged ions (anions).

Common Cation Exchange Resins:

Resin Name	Functional Group	Role/Use
Sulfonated Polystyrene (Strong Acid Cation Resin)	$-\text{SO}_3^-$ (sulfonate group)	Binds strongly to cations (e.g., Na^+ , Ca^{2+}); used in water softening, protein purification, and drug analysis.
Carboxylic Acid Resins (Weak Acid Cation Resin)	$-\text{COO}^-$ (carboxyl group)	Binds weakly to cations; used for selective separation and purification of proteins, amino acids.

9

Name commonly used ion exchange resins and explain their role.

Common Anion Exchange Resins:

Resin Name	Functional Group	Role/Use
Quaternary Ammonium Resins (Strong Base Anion Resin)	$-\text{NR}_4^+$ (quaternary ammonium)	Binds strongly to anions (e.g., Cl^- , SO_4^{2-}); used for removal of acidic impurities, nucleic acid purification, and drug analysis
Primary, Secondary, Tertiary Amine Resins (Weak Base Anion Resin)	NH_3^+ , $-\text{NH}_2^+$ groups	Binds weakly to anions; useful in selective separations and mild purification conditions.

10

What are the advantages and limitations of Size Exclusion Chromatography (SEC)? Explain with pharmaceutical applications.

Advantage	Explanation
Non-destructive technique	No interaction with the stationary phase — ideal for sensitive biomolecules
High resolution for large molecules	Effectively separates proteins, polysaccharides, and polymers
Simple separation mechanism	Based purely on molecular size , no need for chemical modifications
Preserves biological activity	Mild conditions prevent denaturation of proteins or enzymes
Fast and reproducible	Separation occurs quickly and results are consistent

10

What are the advantages and limitations of Size Exclusion Chromatography (SEC)? Explain with pharmaceutical applications.

Limitation	Explanation
Limited separation range	Can only separate molecules within a specific molecular weight range
Low capacity	Not suitable for processing large sample volumes
Resolution decreases for small differences in size	Similar-sized molecules may co-elute (not separate well)
Requires calibration	Molecular weight must be estimated by comparing with known standards
Not suitable for ionic interactions	Cannot separate compounds based on charge or polarity

FOR MORE CLASSES & VIDEOS

जुड़िए PHARMACY INDIA
के साथ.....

INSTAGRAM & YOUTUBE SE JUDNE KE LIYE QR SCAN KARE

SCAN ME



SCAN ME



Download Pharmacy India Mobile App from Play Store

**GET
FREE**

NOTES- *B.PHARMA*

TYPE → B.PHARMA

SEND-

6397576299