Unit→3 (Ion-exchange chromatography)

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Introduction:→ (Defination of Ion-exchange chromatography).

Ion-exchange chromatography is a technique used in seperating and purifying molecules, particularly proteins based on their charge. The stationary phase in this method consists of anion-exchange resign, which is either positively or negatively charged. When a mixture is passed through the column containing the resign based on their charge and the strength of the interaction.

Key components of Ion-exchange chromatography:→

(I) **Ion-exchange Resigns:→**

These are typically beads with functional groups that carry either positive or negative charges.

- Cation exchange:→ uses negatively charged resigns to attract positively charged ions.
- Anion exchange:→ uses positively charged resigns to attract negatively charged ions.

(II) **Buffer solutions:→**

Used to control the PH and ionic strength helping to regulate the binding and elution (release) of the molecules.

(III) **Gradient Elution:→**

Molecules bound to the resign are eluted by gradually changing the ionic strength OR PH of the buffer, allowing the molecules to be seperated based on how strongly they interact with the resign.

Steps in Ion exchange chromatography :→

(i) Sample Application :→
The sample is loaded into the column and molecules bind based on their charge.

(ii) Washing :→
Weakly bound molecules are washed away using a low ionic strength buffer.

(iii) Elution :→
Bond molecules are selectively eluted by increasing the ionic strength or changing the pH.

(iv) Analysis :→
The eluted fractions are collected and analyzed for the target molecules.

Applications :→

- Protein purification :→ used to seperate proteins with slight difference in charge.
- Water softening :→ Removing unwanted ions like calcium and magnesium.
- Pharmaceuticals :→ Purifying drugs or seperating active ingredients.

Classification of Ion-exchange chromotography :→

Ion exchange chromatography is classified based on the type of ion exchanger used the charge of the molecules being seporated.
The two main types of ion exchange chromatography are cation exchange chromatography and anion exchange chromatography. These are further categorized based on the specific interactions and applications :→

① Cation Exchange chromatography (CEX)

• Mechanism :→ In cation exchange chromatography, the stationary phase is negatively charged, and it binds to positively charged molecules.

• Applications :→

(i) Purification of positively charged proteins or peptides.

(ii) Seperation of metal ions, like Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}).

Subtypes :→ (i) Strong cation Exchangers.
(ii) Weak cation Exchanges.

(ii) Anion-exchange chromatography (AEX) :→

• Mechanism :→ In anion exchange chromatography, the stationary phase is positively charged, allowing it to bind to negatively charged molecules (anions.)

• Applications :→

(i) purification of negatively charged proteins, nucleic acids, and some polysaccharides.

(ii) Seperation of anion such as - chloride, sulphate and phosphate.

Subtypes :→ (i) strong Anion Exchangers.
(ii) weak anion exchangers.

(iii) Mixed - Bed Ion Exchange chromatography :→

• ~~Mechanism :→~~ Mechanism :→ Mixed bed ion exchange chromatography is a form of ion exchange chromatography where both cation and anion exchangers are combined in a single column. Applications :→ (i) Water purification :→ one of the most common applications of mixed-bed ion exchange is the production of deionized water. (ii) Nuclear Industry :→ It is used to remove radioactive ions from nuclear waste water, playing a critical role in reducing environmental contamination.

(i) Layered Bed Ion Exchange chrom.
(ii) Homogeneous " " " "
(iii) Regenerable Mixed Bed Ion Exchange Chromat.
(iv) Non- Regenerable " " " " "
(v) Parallel " " " " ".

(IV) Polyelectrolyte complexation :-> Mechanism :-> Polyelectrolyte complexation refers to the process in which oppositely charged polyelectrolytes interact and form complexes through electrostatic interactions. Applications :-> (i) Drug delivery Systems (ii) Tissue Engineering (iii) Water Treatment.

Subtypes :-> (i) Homopolymer complexation.
(ii) Hetropolymer complexation.

(V) Affinity - Based Ion Exchange Chromatography :->

Mechanism :-> Affinity - based Ion exchange chromatography combines principles from both affinity chromatography and ion exchange chromatography to selectively purify biomolecules.

Applications :-> (i) Protein Purification (ii) Antibody Isolation (iii) Enzyme characterization (iv) Metabolite Seperation.

Sub-types :-> (i) Affinity chromatography (ii) Ion-Exchange chromatography (iii) Mixed-Mode chromatography (iv) Immuno-affinity chromatography (v).

Ion Exchange Resigns :->

Ion-Exchange resigns are a key components in ion exchange chromatography, responsible for the actual seperation of ions or charged molecules. These resins are typically small, porous beads made from organic polymers that contain charged functional groups.

Types of Ion Exchange Resins:→

(I) Cation Exchange Resins:→

These resins contain negatively charged functional groups that attract and bind positively charged ions (cations). The functional groups vary based on the stregth of ion exchange and PH range.

- Strong Cation Exchange Resins:→
  - Functional Group:→ sulfonic acid group (SO_3^-)
  - PH Range:→ stable over a wide PH range (typically 0-14)
  - Applications:→ Seperation of strongly basic ions, removal of melat ions in water treatment.
  - Example:→ Dowex 50W.
- Weak Cation Exchange Resins:→

There are two primary types of Ion exchange Resigns:→

(i) Cation-Exchange Resins:→

These resins exchange positive ions (cations) such as calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^+). They are often used in water softening, where calcium and magnesium ions in water are replaced with sodium ions, preventing scale buildup.

(ii) Anions Exchange Resigns:→

These Resins exchange negative ions (anions) like chloride (Cl^-), sulphate (SO_4^{2-}) or nitrate (NO_3^-). They are used in applications like removing anions from water or purifying certain solutions.

These resins are often used in water treatment, purification process for pharmaceuticals and chemicals, an ion seperation technique in laboratories.

Structure of Ion-Exchange Resin :->

Most ion exchange resins are made from cross-linked polymers polystyrene, which is further modified to add functional group. The cross-linking determines the pore size of the resin, which affects the flow of ions and molecules through it.

→ **Polystyrene - Divinylbenzene copolymer :->**
The most common base material for ion exchange resigns. It provides a porous structure that allows ions to diffuse in and out of the beads.

Properties of Ion-exchange Resigns :->

(i) Capacity :-> Refers to the number of ion exchange sites available per unit ~~length~~ of resign. It is measured in miliequivalents per gram (meq/g). Higher capacity means the resin can bind more ions.

(ii) Selectivity :->
Resigns have varying affinities for different ions. The selectivity depends on factors like :- the charge density and size of ions.

(iii) Swelling :->
When exposed to aqueous solutions ~~like~~, ion exchange resins tend to swell as they take in water, which increases the ion exchange capacity, but can affect flow properties.

Mechanism of Ion exchange process :-> PYQ

The Ion exchange process is a method used to separate and purify ions based on their charge. It involves the exchange of ions between a solution and a solid resign.

Types of Ion exchange :->

1. cation exchange :-> Exchange of positively charged ions. (cations)
2. Anion exchange :-> Exchange of negatively charged ions. (anions)

Mechanism of Ion Exchange :-> ~~chromatography~~ PYQ

(i) **Resign Structure** :-> Ion exchange resigns are typically made of polymer beads that contain functional groups capable of binding ions.

(a) **Cation Exchange Resigns** :-> contains acidic functional groups (eg:- sulphonic acid) that releases H^+ ions.

(b) **Anion Exchange Resigns** :-> contains basic functional groups. (eg:- Quaternary ammonium) that releases OH^- ions.

(ii) **Equilibrium** :-> When the resin is immersed in a solution, an equilibrium is established between the ions in the solution and those on the ~~Resion~~ resin. For eg :->

(a) **In cation Exchange** :-> Na^+ ions in the solution can replace H^+ ions on the resin.

(b) **In anion Exchange** :-> Cl^- ions can replace OH^- ions on the resign.

(iii) **Ion - Exchange Reaction** :->

$$\text{Resign} - Na + H^- \rightleftharpoons \text{Resign} - H + Na^+$$

$$\text{Resign} - Cl + OH^- \rightleftharpoons \text{Resign} - OH + Cl^-.$$

(iv) Selectivity :->
The selectivity of the resins depends on factors such as size, charge, and concentration of the ions. For eg :-> Smaller ions with higher charge density are typically exchanged preferentially.

(v) Regeneration :->
After the resign becomes saturated with the exchange ions, it can be ~~regenerated~~ regenerated by flushing it with a solution containing a higher concentration of specific ions (usually the original ions) to restore its ion-exchange capacity.

Application :->
(i) Water purification (removal of hardness)
(ii) Seperation and purification of metal ions.
(iii) Pharmaceutical and biochemical applications.

Advantages and Disadvantages :->

Advantages: High selectivity, effectiveness at low concentrations, and the ability to regenerate the resign.

Disadvantages: Can be sensitive to the presence of competing ions and requires careful control of conditions.

* Factors affecting ion Exchange chromatography :->

(i) Ion concentration :->
High concentrations of ions in the solution can enhance the driving force for ion exchange, affecting the rate and extent of exchange.

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(II) Temperature :→

Increased temperature typically increases the rate of ion exchange by enhancing the movement of ions, though it may also affect resin stability.

(III) P_H :→

The P_H of the solution can influence the charge of the functional groups on the resin and the speciation of ions in the solution, affecting selectivity and capacity.

(IV) Ionic strength :→

Higher ionic strength can compete with target ions for binding sites on the resin, potentially reducing the efficiency of exchange.

(V) Flow Rate :→

The rate at which the solution flows through the resin affects contact time and thus the efficiency of ion exchange. Too fast a flow rate may not allow sufficient interaction.

(VI) Resign Regeneration :→

The ability to regenerate the resin affects the long-term effectiveness and efficiency of the ion exchange process.

(VII) Competing Ions :→

The presence of other ions in the solution can compete with the target ions for binding sites, impacting the selectivity and capacity.

* Methodology :→

Methodology refers to the systematic, theoretical ana of the methods applied in a particular field of study a research. It encompasses the principles, process a techniques used to collect data, analyze information and draw conclusions.

It involves the rationale behind choosing speci techniques and approaches, ensuring that the process is coherent and aligned with the goals of the study or proje

Importance of Methodology :→

(I) Provides a clear framework for conducting resear

(II) Ensures reproducibility and transparency.

(III) Guides researchers in selecting appropriate methods and techniques.

(IV) Facilitates critical evaluation of the research.

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Application of Methodology :→

The application of methodology varies across differen fields and research types, guiding how research is conducted and results are analyzed.

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Some common application of Methodology are :→

① Scientific Research :→

Example :→ In biology, a researcher might use a quantitative methodology to investigate the effects of a drug on cell growth. This involves designing controlled experiments, selecting appropriate controls, and using statistical analysis to interpret the data.

(II) Social Science :→

Example :→ In sociology, qualitative methodologies suchas interviews or focus groups may be employed to explore community behaviors or attitudes. This approach involves coding and analyzing textual data for themes and patterns.

(III) Business and Market Research :→

Example :→ A market researcher might use mixed methods to evaluate customer satisfaction. Surveys (quantitative) can provide numerical data, while in-depth interviews (qualitative) offer insights into customer experiences.

(IV) Education :→

Example :→ Educational researchers may apply action research methodology to assess the effectiveness of a new teaching strategy. This involves iterative cycles of planning, implementing, observing and reflecting on the outcomes.

(V) Public health :→

Example :→ Epidemiologists may use cohort studies to investigate the impact of lifestyle factors on health outcomes. This involves tracking a group over time and analyzing data for correlations.

(VI) Environmental Studies :→

Example :→ Researchers might employ a case study
methodology to examine the impact of pollution on
local ecosystem. This involves detailed observation,
data collection, and analysis of specific instances.

Example of cation Exchange chromatography :→

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- Purification of hemoglobin :→

In this process, hemoglobin, which carries a positive charge at a specific PH, binds to a cation exchange Resin. other proteins and impurities are washed away and haemoglobin can be eluted by gradually increasing the salt concentration in the buffer.

Example of anion Exchanger chromatography :→

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- Purification of antibodies :→

In this process antibodies which typically carry a net negative charge at a certain PH, bind to +ly charged groups on an anion exchange resin. unbound proteins are washed away and the antibodies can be eluted by increasing the salt ~~conc~~ concentration or altering the PH.

Example of mixed-bed ion exchange chromatography :

- Purification of water for pharmaceutical applicatio

In this process both cation and anion exchange resi are combined in a single column, allowing for the simultaneous removal of both +ly and -vly charged impurities.

Example of polyelectrolyte complexation :→

- formation of complexes between chitosan and alginate :→

This interaction occurs in aqueous solutions where the oppositely charged polyelectrolytes ~~form~~ form a stable complex, which can be utilized in drug delivery systems, wound dressings etc.

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Example of Affinity based ion exchange chromatography :→

- Purification of proteins using a resin that has a specific ligand attached, such as protein A or G, which binds to the Fc region of antibodies.

Q. (PYQ) Arrange the following ions in order of capacity to undergo exchange reaction :

Na^+ , Ca^{2+} , Al^{3+} .

The order of capacity to undergo exchange reactions for the ions are :→ $Al^{3+} > Ca^{2+} > Na^+$

This ranking is based on the charge and size of the ions. Higher charged ions generally have a greater capacity for exchange due to their stronger interactions with the exchange sites.

Advantages of Methodology :→

(I) Structure approach :→
Provides a clear, systematic framework for achieving objectives, enhancing consistency and reliability.

(II) Reproducibility :→
Detailed documentation allows for the methodology to be replicated by others, validating results.

(III) Optimization :→
Enables fine-tuning of conditions and parameters to improve efficiency and effectiveness.

(IV) Problem - Solving :→
Structured methodologies help ~~hel~~ in identifying and addressing potential issues systematically.

Disadvantages of Methodology :→

(I) Rigidity :→ A strict methodology may limit creativity and flexibility in addressing unique problems or adapting to new information.

(II) Time-consuming :→ Developing and following a detailed methodology can be time-intensive and resource-heavy.

(III) Overhead costs :→ Requires investment in training, equipment and materials which can be costly.

(IV) Complexity :→

May involve complex processes that are difficult to understand or implement for non-experts.

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Q. How is demineralization of water is done using ion exchange chromatography.

→ Demineralization of water using ion exchange chromatography is a process that removes dissolved mineral ions such as calcium, magnesium, sodium and chloride making the water suitable for various applications including industrial processes and laboratory use.

The process done in ion exchange chromatography given below :→

(i) Equipment setup :→ The process typically involves two column :- cation Exchange column and Anion exchange column.

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(II) Cation exchange :->

Cation exchange resin is typically in a sodium form before the process begins. The water passes through the cation exchange column, where cations like Ca^{2+}, Mg^{2+}, Na^{+} in water are exchanged for sodium ions (Na^{+}) from the resin.

(III) Anion Exchange :->

The anion exchange Resins is often in hydroxide form (OH^{-}). The water then passes through the anion exchange column, where anions like Cl^{-}, SO_4^{2-}, CO_3^{2-} in the water are exchanged for hydroxide ions from the resin.

(IV) Demineralized water production :->

After passing through both columns, the water is ~~now~~ substantially demineralized containing mostly sodium ions and hydroxide ion which react to form water. The output is demineralized water which is often very low in conductivity.

(V) Regeneration of Resins :->

The ion exchange resins become saturated with the exchanged ions and need to be regenerated. A strong sodium chloride (NaCl) solution is passed through the cation resin displacing the bound cations and restoring the sodium form.

Again, A strong sodium hydroxide (NaOH) solution is used to regenerate the anion resin, displacing the bound anions and restoring the hydroxide form.

Advantages of Exchange Demineralization:→

(i) High efficiency:→ Effectively removes a wide range of ions from water.

(ii) Customization:→ Different resins can be used for specific ion removal.

(iii) Scalability:→ Suitable for both small-scale and large scale applications.

Disadvantages of Exchange Demineralization:→

(I) Cost:→ The process can be expensive due to the need for chemicals and resin replacement.

(II) Regeneration:→ Ion exchange resins require regular regeneration, which involves additional chemicals and downtime.

(III) Waste disposal:→ The regeneration process generates waste that must be properly managed and disposed of.

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* Principle of Demineralization:→

Ion exchange chromatography operates on the principle of exchanging undesirable ions in water with ions that are retained on the resin. Two main types of ion exchange resins are:-

Cation exchange Resins:→ These resins contain -ly charged functional groups that attract and holds +ly charged ions.

Anion exchange Resins:→ (opposite).

Q What are Zeolites ?

Ans:-> Zeolites are naturally occurring or synthetic crystalline aluminosilicates with a porous structure, composed mainly of Silica (SiO_2) and alumina (Al_2O_3). They feature a three-dimensional framework that contains interconnected channels and cavities. Zeolites are widely used in applications such as ion exchange, gas separation, water purification etc.

Principle of cation exchange chromatography :->

Cation exchange chromatography utilizes a resin that carries negatively charged functional groups. This allows +ly charged ions (cations) in the sample to bind to the impurities.

Principle of Anion Exchange chromatography :->

Principle :->

Anion exchange chromatography employs a resin with positively charged functional groups, allowing it to attract and bind negatively charged ions (anions) from the sample.

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*Characteristics of cation Exchange chromatography :-

(1) Binding site :->
Contains -vly charged groups that attact and bind +vly charged ions (cations).

(II) PH Sensitivity :->
The binding of cations canbe influenced by the PH of mobile phase, affecting protein charge.

(III) Selectivity :->
cation exchangers can selectively seperate cations based on their charge and size.

(IV) Elution :->
Typically achieved by increasing ionic strength or changing the PH to release bound cations.

(V) Applications :->
Commonly used for purifying proteins, peptides and other biomolecules with a net +ve charge.

*Characteristics of anion exchange chromatography :->

(I) Binding site :->
Contains +ly charged groups that attract and bind -vly chargedions (anions).

(II) PH Sensitivity :->
The binding of anions can also be influenced by the PH, affecting the charge of the target molecules.

(III) Selectivity :->
Anion exchangers seperate anions based on their charge density and size.

(IV) Elution :->
Typically achieved by increasing the salt concentration or adjusting the PH to disrupt interactions with bound anions.

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(v) <u>Applications</u> :→
used for purifying proteins, nucleic acids and other biomolecules with a net -ve charge.

<u>Characteristics of Mixed Bed Ion exchange Chromatography</u> :→

(I) <u>High purity</u> :→ Effectively removes both cations and anions, resulting in highly purified products.

(II) <u>Compact design</u> :→ Requires less space compared to separate cation and anion exchange columns.

(III) <u>~~Continuous~~ complexity in Maintenance</u> :→
Requires careful monitoring and maintenance to manage both resin types effectively.

(IV) <u>Continuous operation</u> :→
can be operated in a continuous flow mode, enhancing efficiency in large-scale applications.

(V) <u>Cost</u> :→ Higher initial costs due to the need for dual resin types and more complex setups.

<u>Characteristics of Affinity-based ion exchange chromatography</u> :→

(I) <u>Targeted Binding</u> :→ Utilizes specific interactions between a ligand and its target molecule, enhancing selectivity.

(II) <u>Dual Mechanism</u> :→
combines ionic interaction with affinity binding, allowing for more efficient separation of biomolecules.

(III) High purity :-> capable of achieving high purity levels by selectively isolating target molecules while minimizing impurities.

(IV) Mild conditions :-> often preserves the biological activity of sensitive biomolecules due to gentle elution conditions.

(V) Versatility :-> Applicable to a wide range of biomolecules, including proteins, peptides and nucleic acids.

* Characteristics of polyelectrolyte complexation chromatography :->

(I) ~~charge-based separation~~ :-> charge Neutralization: Oppositely charged polyelectrolytes neutralize each other, forming stable complexes.

(II) Bio compatibility :-> Often suitable for biomedical applications due to ~~other~~ their non-toxic nature.

(III) Reversible interactions :-> Many complexes can dissociate and reform, allowing for dynamic behaviour.

(IV) Phase Seperation :-> Can lead to distinct phases useful in coatings and films.

(V) Versatility :-> Applicable in pharmaceuticals, food science, and wastewater treatment.

Advantages of Ion Exchange chromatography :→

(I) High selectivity :→ Ability to separate a wide range of charged biomolecules based on their ionic properties.

(II) High Resolution :→ Provides sharp peaks and good resolution for complex mixtures.

(III) Scalability :→ Suitable for both analytical and preparative applications, easily scaled up for large quantities.

(IV) Versatility :→ Applicable to proteins, nucleic acids, and small molecules, making it useful across various fields.

(V) Mild conditions :→ often operates under conditions that preserve the biological activity of sensitive biomolecules.

Disadvantages of Ion exchange chromatography :→

(I) Limited Range :→ Effectiveness can be PH dependent, weak ion exchangers may have limited utility.

(II) Buffer compatibility :→ Requires careful selection of buffers to avoid interference with separation process.

(III) Time – consuming :→ Gradient elution can increase overall processing time.

(IV) column Fouling :→ High salt concentrations or particulates can lead to clogging and reduced column life.

(V) Regeneration needs :→ Requires regular regeneration of the resin, which can be complex and time-consuming.

Principle of Ion exchange chromotography :->

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The principle of Ion exchange chromatography is that charged molecules in a sample bind to oppositely charged sites in a stationary phase. The seperation is based on the ionic interactions between the solute and the stationary phase.

How it works :->

the stationary phase is made up of inert organic matrix that has been derivatized with ionizable groups. the charged groups on the matrix interact with the charged groups on the molecules in the sample.

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Q. Describe the process of seperation of lanthanides from ion exchange chromatography :->

Ans :-> Seperation of lanthanides using ion exchange chromatography involves several key steps :->

(i) Preparation of the column :->

A ~~col~~ chromatography column is packed with an ion exchange resin, typically a cation exchange resin, which has functional groups that can bind +ly charged ions.

(ii) Sample loading :->

The lanthanide mixture is dissolved in a suitable buffer solution and loaded onto the column. The lanthanides being cations, will bind to the resin based on their charge and ionic Radius.

(iii) Elution :-> A gradient elution is often employed. An increasing concentration of a competing cation in the mobile phase is introduced.

(iv) Seperation :-> Different lanthanides will elute at different times due to their varying affinities for the resin which was influenced by their ionic radii and charges.

(v) Detection :-> As the lanthanides elute they can be detected using techniques like UV-vis spectroscopy or mass spectrometry, allowing for identification and qualification.

(vi) Fraction collection :-> The eluted fractions can be collected separately for further analysis or use.

Q. Describe the process of seperation of actinides from ion-exchange chromatography.

Ans: The separation of actinides using ion exchange chromatography involves several key steps :->

(i) Column preparation :-> A cation exchange resin is packed into a chromatography column. The resin is chosen for its ability to interact with actinide cations.

(ii) Sample loading :-> The actinide solution is loaded onto the column. The cations in the sample will interact with the resin based on their charge and ionic radius.

(iii) Elution :-> A gradient elution is applied, often using a mobile phase containing a competing cation. The concentration of competing cation is gradually increased, facilitating the release of bound actinides.

(iv) Seperation :→ Different actinides will elute at different times due to variations in their affinity for the resin. Factors like ionic radius, charge and oxidations state play a significant role in their seperation.

(v) Detection :→ As the actinides elute, they can be detected using techniques such as gamma spectroscopy or mass spectrometry, which allow for identification and quantification.

(vi) Fraction collection :→ The fractions containing the separated actinides are collected for further analysis or processing.