S. S. College, Jehanabad

Department: Zoology

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Subject: Zoology

Topic: Ion Exchange Chromatography (IEC)

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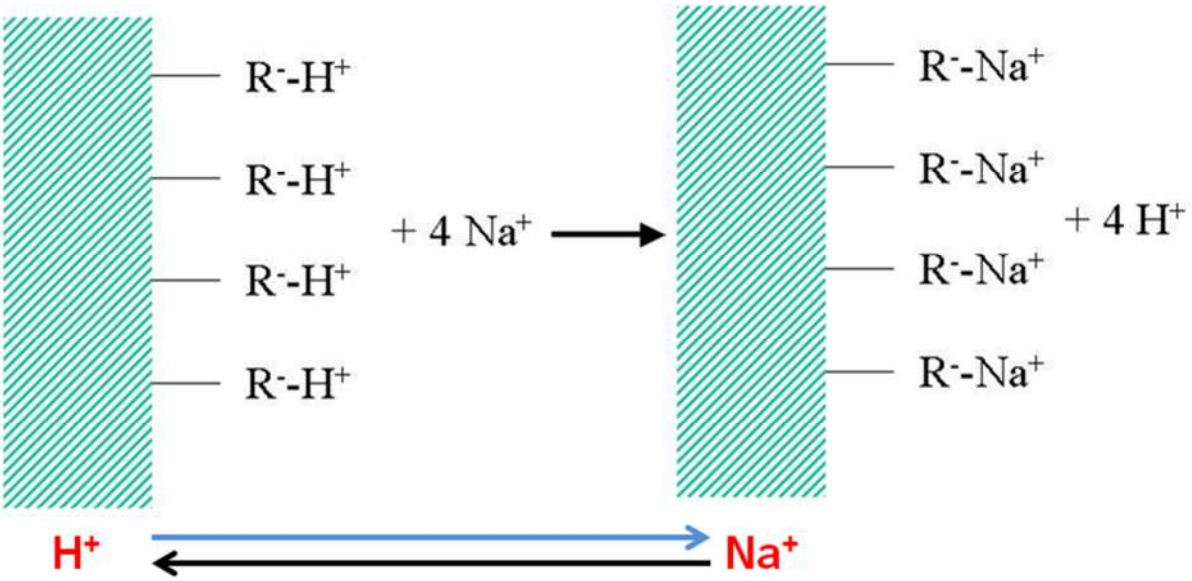
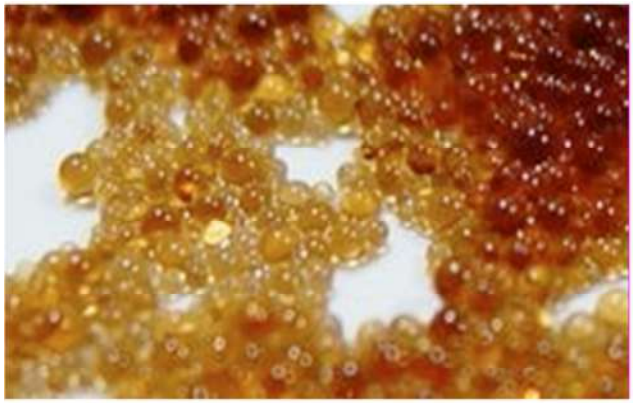
ION EXCHANGE CHROMATOGRAPHY (IXC)

Ion exchange chromatography (IXC) is a type of chromatography technique in which ionic and polar molecules are separated on the basis of ions exchange property between the samples and the stationary phase that is commonly resin. Ion exchange resin exchanges ions according to their relative affinities. Stationary phase contain ionic sites that create bipolar interactions with the analytes present in the sample. If a compound has a high charge density, it will be retained a longer time by the stationary phase. Separation solely depends upon the reversible adsorption of charged solute molecules to immobilized ion exchange groups of opposite charge. *Ion exchange is an adsorption phenomenon where the mechanism of adsorption is electrostatic. Electrostatic forces hold ions to charged functional groups on the surface of the ion exchange resin. The adsorbed ions replace ions that are on the resin surface on a 1:1 charge basis.* Biomolecules generally have charged groups on their surfaces, which change with the buffer pH. Elution can be accomplished by changing the ionic strength or the pH, of which changing the ionic strength by increasing the salt concentration is most common.

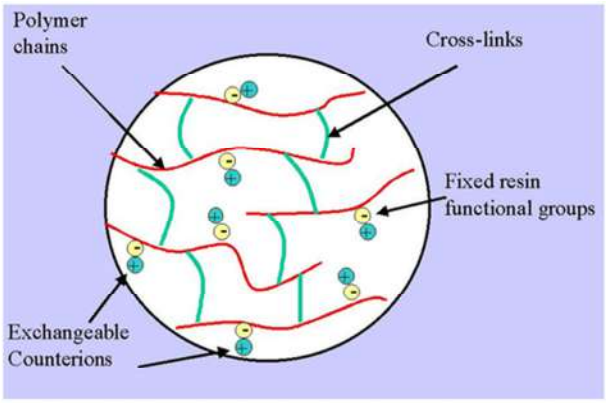


Exchange of ions in stationary phase and analytes in the sample in Ion Exchange Chromatography

Ion exchange is the predominant form of ion chromatography to date. This chromatography is one of the most important adsorption techniques used in the separation of peptides, proteins, nucleic acids and related biopolymers which are charged molecules in different molecular sizes and molecular nature. The separation of biomolecules is dependent on the reversible exchange of ions between the targets ions present in the sample solution to the ions present in ion exchangers such as resins. The first ion exchangers were synthetic resins designed for applications like demineralization, water treatment, and recovery of ions from wastes. The first ion exchangers designed for use with biological substances were the cellulose ion exchangers developed by Peterson and Sober. These polymeric resins are made in 3-D networks by cross-linking hydrocarbon chains and later attaching the ionic functional groups to the structure. The resulting resin is insoluble, inert and relatively rigid.



A typical ion exchange resin

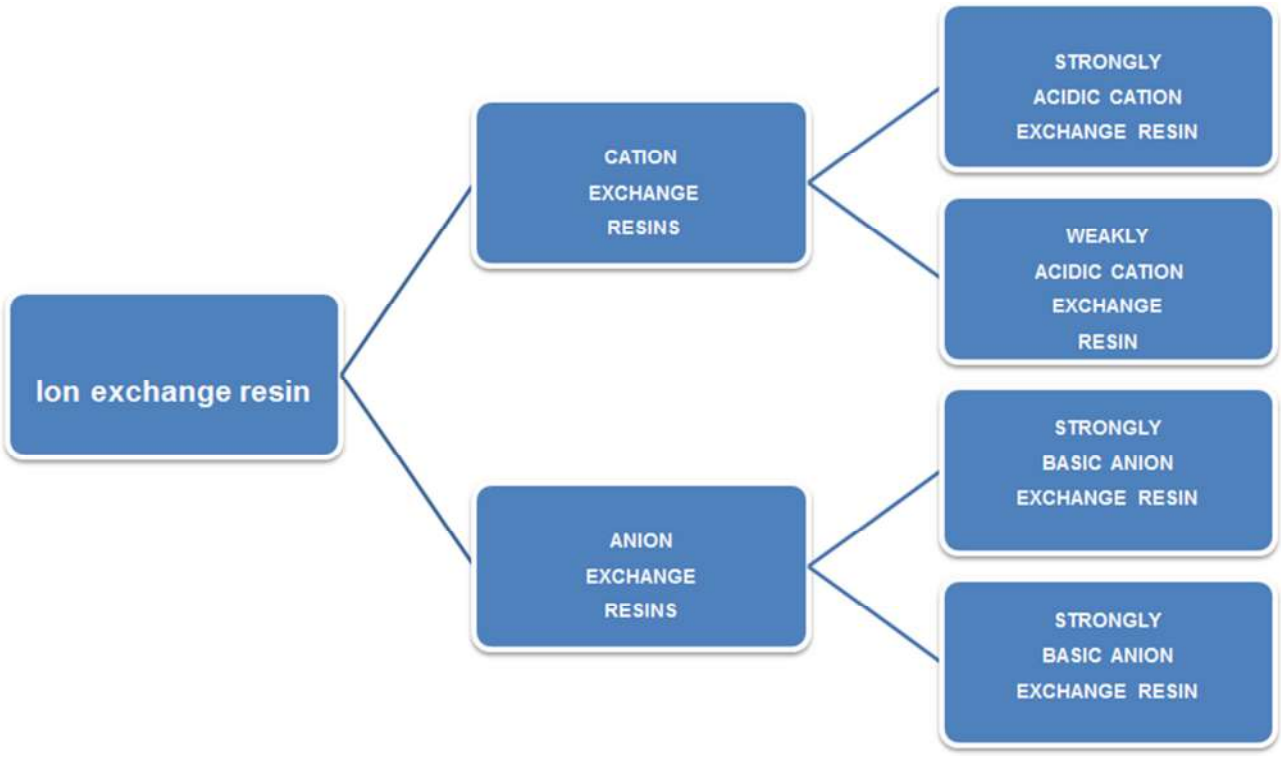


Crosslinking of molecules in an ion exchange resin

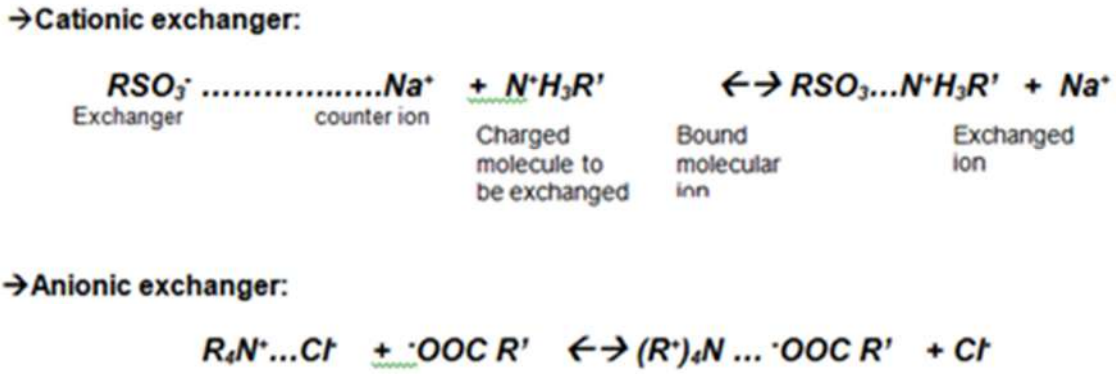
Characteristics of the ion exchange resin: There should be following characteristics of an ion exchange resins;

- It should be insoluble in aqueous medium.
- It should be denser than water.
- It should have loose porous polymeric structure.
- It should have high degree of crosslinking.
- It should be inert in nature.
- It should have large exchangeable sites.

There can be two types of exchangers used for ion exchange in the ion exchange chromatography, namely cationic and anionic exchangers.



- ***Cationic exchangers:*** It possesses negatively charged group, and these will attract positively charged cations. These exchangers are also called "acidic ion exchange" materials, because their negative charges result from the ionization of acidic group e.g. carboxyl group, sulfonic group etc. Here carboxyl group is weakly acidic ion, whereas sulfonic acid group strongly acidic ion.
- ***Anionic exchangers:*** It possesses positively charged groups that will attract negatively charged anions. These are also called "basic ion exchange" materials, e.g. amino group, hydroxyl group, etc. Amino group is weak basic ion, whereas hydroxyl group is strong ion.



Therefore, on the basis of source of resins, it is of two types;

- ***Natural resins:*** In which cation resins include zeolytes, clay, and etc. while anion resins include dolomite.
- ***Artificial:*** It may be of two types; inorganic and organic. Organic resins are the most widely used resins. Organic ion exchange resins are polymeric resin matrix containing exchange sites for exchangeable functional groups. In organic resins, divinyl benzene acts as a cross linking agent that offers adequate strength i.e. mechanical stability.

Resins can also be classified as on the basis of chemical nature as;

- Strong cation exchange resin → SO_3H
- Weak cation exchange resin → COOH, OH, SH, PO_3H_2
- Strong anion exchange resin → N^+R_3, NR_2
- Weak anion exchange resin → NHR, NH_2

The properties and applications of these ion exchange resins are following in the table;

Exchange Type	Ion exchange group	Buffer counter ions	pH range	Commercial samples	Applications
Strong cation	Sulfonic acid (SP)	Na^+, H^+, Li^+	4-13	Capto®S SP Sepharose® SP Sephadex® TSKgel SP_5PW Capto®S	Fractionation of cations, inorganic separions, peptides, aminoacids, B vits

Weak cation	Carboxylic acid	Na^+, H^+, Li^+	6-10	CM Cellulose CM Sepharose® CM Sephadex® CM Sepharose® CL6B TSKgel CM-5PW	Fractionation of cations, biochemical separations, org bases, antibiotics
Strong anion	Quaternary amine (Q)	Cl^-, $HCOO_3^-$, CH_3COO^-, SO_4^{2-}	2-12	Q Sepharose® Capto®Q Dowex®1X2 Amberlite® / Amberjet® QAE Sephadex®	Fractionation of anions Alkaloids, vitamins, fattyacids
Weak anion	Primary amine Secondary amine Tertiary amine (DEAE)	Cl^-, $HCOO_3^-$, CH_3COO^-, SO_4^{2-}	2-9	DEAE-Sepharose® Capto® DEAE DEAE Cellulose	Fractionation of anionic complexes, anions of diff valency vitamins, amino acids

Structurally, there are four types of ion exchange resins; pellicular type with ion exchange film, porous resin coated with exchange beads, macroreticular resin bead, and surface sulfonated and bonded electrostatically with anion exchanger.

- ***Pellicular type with ion exchange film:*** In this type of resins, the particles have a size of 30-40µ with 1-2µ film thickness. They have very low exchange capacity with ion exchange efficiency: 0.01 – 0.1meq/g of exchange resin.
- ***Porous resin coated with exchange beads:*** This type of ion exchange resins is totally porous and highly efficient. It has 5 - 10µ size of beads and has exchange capacity of 0.5-2 meq/g of exchange resin.
- ***Macroreticular resin bead:*** A reticular network of the resin is seen superficially on the resin beads. They are not highly efficient and have very low exchange capacities.
- ***Surface sulfonated and bonded electrostatically with anion exchanger:*** In this type of resins, the particles are sulfonated, and they are bonded electrostatically with anion exchanger resin. They are less efficient and have low exchange capacity. Exchange capacity is 0.02meq/g of exchange resin.

Selection criteria of ion exchange resins for ion exchange chromatography;

- *Type of the ions to be separated:* Cations or anion.
- *Nature of the ions to be separated:* Strong or weak.
- *Efficiency of the resin:* Measured by ion exchange capacity[1].
- *Particle size of the resin:* 50-100 mesh or 100-200.
- *Structural type of the resin:* Porous, pellicular, etc.
- *Amount of cross linking agent present:* It decides swelling of the resin.

Packing of the column: Usually wet packing of the column is done in ion exchange chromatography (IXC). The method of packing of the column is as follows;

[1] Ion exchange capacity is the total ion exchange capacity in terms of the exchangeable functional groups expressed as m.eq/g of the ion exchange resin (m.eq/g = 1000/eq.wt).

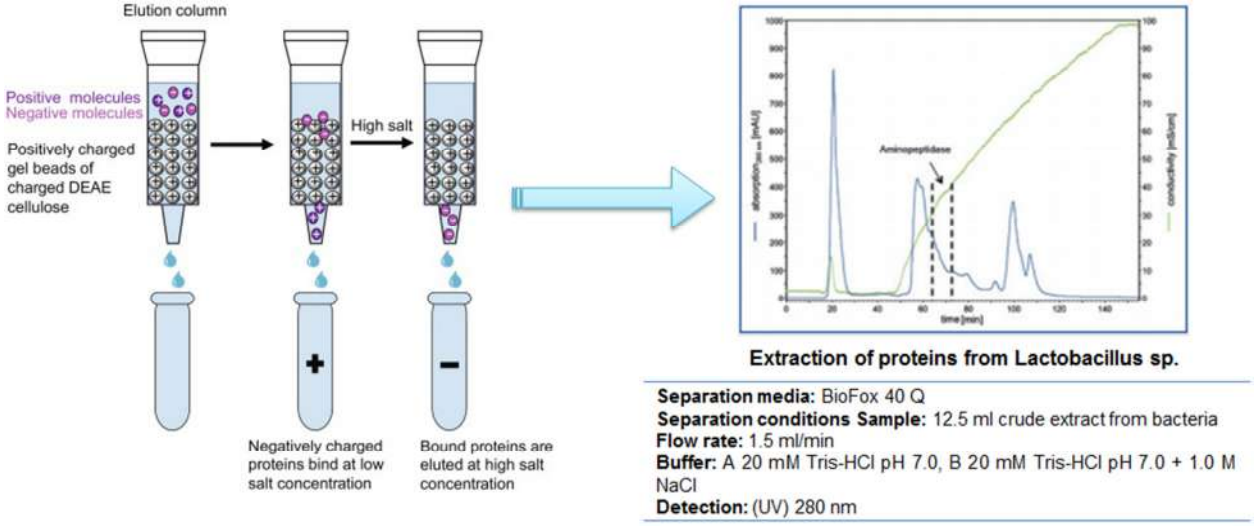
The resin is mixed with the mobile phase & packed in the column uniformly.

The sample to be separated is dissolved in the mobile phase and introduced all at once into the column.

Mobile phase: Organic solvents are less useful and they are not used at all. Only different strengths of acids, alkalis and buffers are used as eluting solvents, e.g. 0.1N HCl, 1N NaOH, phosphate buffer, acetate buffer, borate buffer, phthalate buffer, etc.

Principle

This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte. The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix which can be positively or negatively charged. When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge. In this "ion cloud", ions can be reversibly exchanged without changing the nature and the properties of the matrix. Therefore, on the basis of nature of ions, the ion exchange chromatography (IXC) is further divided into cation exchange and anion exchange chromatography. Anion and cation exchange phases are classified as strong or weak, depending on how much the ionization state of the functional groups vary with pH. A strong ion exchange phase has the same charge density on its surface over a broad pH range, whereas the charge density of a weak ion exchange phase changes with pH, affecting its selectivity, which differs at different pH values.



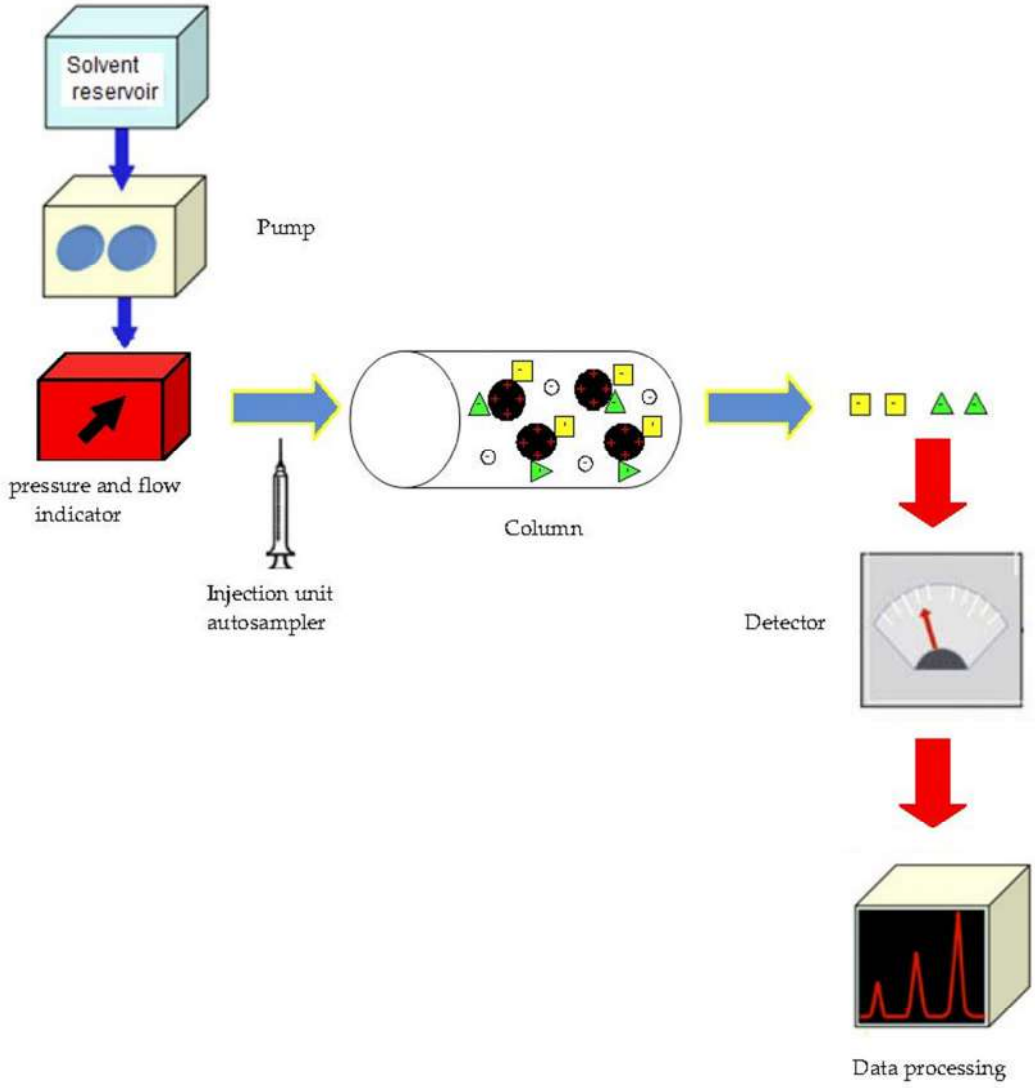
Extraction of proteins from Lactobacillus sp.

Separation media: BioFox 40 Q
Separation conditions Sample: 12.5 ml crude extract from bacteria
Flow rate: 1.5 ml/min
Buffer: A 20 mM Tris-HCl pH 7.0, B 20 mM Tris-HCl pH 7.0 + 1.0 M NaCl
Detection: (UV) 280 nm

Instrumentation of ion exchange chromatography (IXC)

Typical ion exchange chromatography instrumentation includes pump, injector, column, suppressor, detector and recorder or data system.

Pump: The ion exchange chromatography pump is considered to be one of the most important components in the system which has to provide a continuous constant flow of the eluent through the ion exchange chromatography injector, column, and detector.



Injector: Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. Liquid samples may be injected directly and solid samples need only to be dissolved in an appropriate solvent. Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100ml of volume with high reproducibility and under high pressure (up to the 4000psi).

Column: Depending on its ultimate use and area of application, the column material may be stainless steel, titanium, glass or an inert plastic such as PEEK. The column can vary in diameter from about 2mm to 50mm and in length from 30mm to 500mm depending on whether it is to be used for normal analytical purposes, microanalysis, high speed analyses or preparative work. Another column that is is placed anterior to the separating column is called a guard column. This

serves as a protective factor that prolongs the life and usefulness of the separation column. They are dependable columns designed to filter or remove particles that clog the separation column

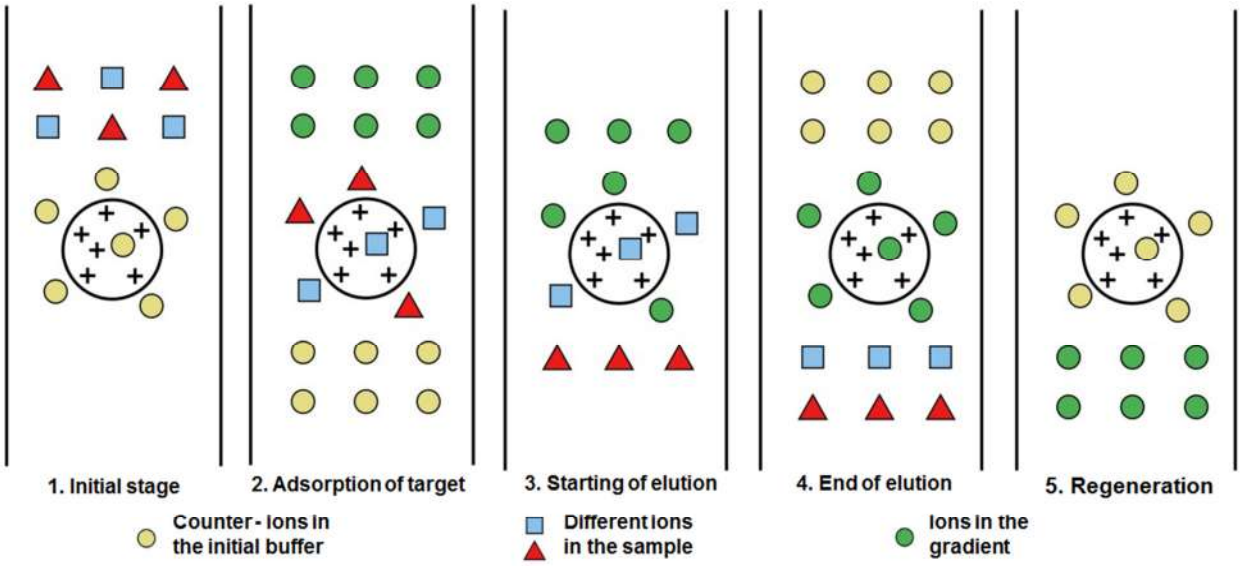
Suppressor: The suppressor reduces the background conductivity of the chemicals used to elute samples from the ion-exchange column which improves the conductivity measurement of the ions being tested. Ion exchange chromatography suppressors are membrane-based devices which are designed to convert the ionic eluent to water as a means of enhancing the sensitivity.

Detectors: Electrical conductivity detector is commonly used in the ion exchange chromatography.

Data system: In routine analysis, where no automation is needed, a pre-programmed computing integrator may be sufficient. For higher control levels, a more intelligent device is necessary, such as a data station or minicomputer.

Procedure of ion exchange chromatography (IXC)

Ion exchange separations are carried out mainly in columns packed with an ion-exchanger. These ionic exchangers are commercially available. They are made up of styrene and divinyl benzene. Examples are DEAE-cellulose, which is an anionic exchanger, and CM-cellulose, which is a cationic exchanger. The choice of the exchanger depends upon the charge of particle to be separated. To separate anions "anionic exchanger" is used and to separate cations "cationic exchanger" is used.



Different stages in the process of ion exchange chromatography

First the column is filled with ion exchanger then the sample is applied followed by the buffer. The tris-buffer, pyridine buffer, acetate buffer, citrate and phosphate buffers are widely used. The particles, which have high affinity for ion exchanger, come down the column along with buffers. In next step using corresponding buffer separates the tightly bound particles. Then these particles are analyzed spectroscopically.

Applications of ion exchange chromatography (IXC)

It has various applications, such as;

- An important use of ion-exchange chromatography is in the routine analysis of **amino acid** mixtures.
- The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
- This is most effective method for water purification. Complete deionization of water (or) a non-electrolyte solution is performed by exchanging solute cations for hydrogen ions and solute anions for hydroxyl ions. This is usually achieved by method is used for softening of drinking water.
- In the analysis of products of hydrolysis of nucleic acid, information is also gained about the structure of these molecules and how it relates to their biological function as carriers of hereditary information.
- Chelating resins are used to collect trace metals from seawater.
- To analyze lunar rocks and rare trace elements on Earth.

Advantages of ion exchange chromatography (IXC)

There are following advantages of ion exchange chrpmatography;

- It is one of the most efficient methods for the separation of charged particles.
- It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.
- Ion exchange is used for both analytical and preparative purposes in the laboratory, the analytical uses being the more common.
- Inorganic ions also can be separated by ion-exchange chromatography.

Limitations of ion exchange chromatography (IXC)

- Only charged molecules can be separated.
- Buffer Requirement

Ion exchange capacity

The number of mill equivalent ion exchange by one gram of dry resin is called as ion exchange capacity. Efficiency of ion exchange process depends upon exchange capacity of resin.

Calculation of cation exchange capacity: One gram of cation exchange resin is soaked in HCl to convert the resin in H^+ form. It is then placed in water. The glass tube like that of burette is packed with the resin. 10 ml of 0.5N solution of Na_2SO_4 is placed on the resin column. The elution is carried out using distilled water. The eluate is collected in conical flask and then titrated with 0.1N NaOH solution using phenolphthalein indicator. Volume of NaOH required for the titration is found out and cation exchange capacity is determined by

using following formula.

$$\textbf{\textit{Cation exchange capacity}} = \frac{\boldsymbol{V} \times \boldsymbol{N}}{\boldsymbol{W}}$$

Where 'V' = volume of Burette reading; 'N' = Normality of NaOH; and 'W' = Weight of dry resin.

Calculation of anion exchange capacity: One gram of anion exchange resin is soaked in HCl to convert the resin in Cl^- form. It is then placed in water. The glass tube like that of burette is packed with the resin. 10 ml of 0.5N solution of Na_2SO_4 is placed on the resin column. The elution is carried out using distilled water. The eluate is collected in conical flask and then titrated with 0.1N $AgNO_3$ solution using potassium chromate indicator. Volume of $AgNO_3$ required for the titration is found out and anion exchange capacity is determined by using following formula;

$$\textbf{\textit{Cation exchange capacity}} = \frac{\boldsymbol{V} \times \boldsymbol{N}}{\boldsymbol{W}}$$

Where 'V' = volume of Burette reading; 'N' = Normality of $AgNO_3$; and 'W' = Weight of dry resin.

Some terminology in IXC:

Elution: The process of removing adsorbed ions is known as elution.

Eluent: The solution used for elution is called as eluent .

Eluate: The solution resulting from the elution is called as eluate.

References

1. Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
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