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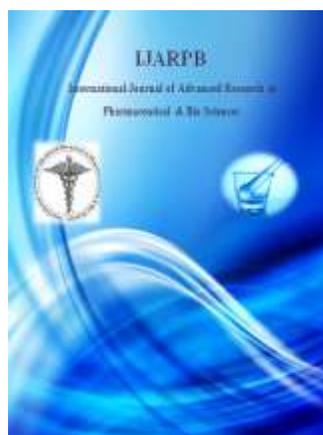
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## An Overview on Ion Exchange Chromatography

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### ABSTRACT

Ion exchange chromatography is a process in which separation of ions and polar molecules occur on the basis of charge present on them. It is the most versatile technique and provides greater degree of selectivity because this stationary phase is called as ion exchanger and is capable of removing reversibly ions from a solution and replacing them with ions of equivalent charge. Capacity of this ion exchanger depends on number of sites available for exchange which in turn depends on ionic dimension and exchange of ion depending on nature of ion exchange resin i.e. nature of functional group, pH of solution and concentration of solution in contact with the resin.

**KEY WORDS:** Ion exchanger, resins, polar molecules, Eluate.

**(Review Article)****INTRODUCTION**

Ion exchange chromatography is a process which allows the separation of ions and polar molecules based on their charge and can be used for any kind of charged molecules like protein, amino acid and small nucleotides.

Solution to be injected is called as sample and individual separated components are called analytes.

This technique is used in water purification, water analysis and quality control. It provides greater degree of selectivity due to large number of combination of mobile and stationary phase which are used. The stationary phase materials are called as ion exchangers, and they are capable of reversible removing ions from a solution while at the same time replacing them with ions of equivalent charge. Among all the techniques ion exchange chromatography is most versatile and is helpful method in separation of ion of similar properties of whose analysis is difficult. It is also useful in determining complicated mixture of very closely related properties and in separation of organic acid, amino acid, peptide and nucleotide<sup>1</sup>.

**Principle**

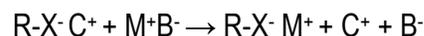
Ion exchange chromatography is based on attraction between oppositely charged particle. Example in amino acid and protein which have ionisable group and carry net positive or negative charge and used in separation of mixture of such compounds. The net charge shown by these compounds depend on the pKa and on pH of the solution according to Henderson Hesselbalch equation<sup>2</sup>.

In Ion exchange chromatography analyte is retained on column on the basis of ionic interaction. The Stationary phase display ionic functional group R-X which interacts with analyte ion of opposite charge.

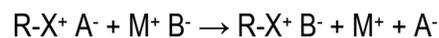
On the basis of this Ion exchange chromatography is divided into

- (a) Cationic Ion exchange chromatography.
- (b) Anionic Ion exchange chromatography.

Ionic compounds consist of cationic species M<sup>+</sup> and anionic species B<sup>-</sup> can be retained on stationary phase. Cationic Ion exchange chromatography retains positive charged cation because stationary phase display negative charged functional group<sup>5</sup>.



Anionic Ion exchange chromatography retains anion using positively charged functional group.

**Ion Exchange Materials**

Various materials possess varying ion exchange capacity. Some important materials of ion exchange phenomenon can be classified as follows-

- A) **Synthetic inorganic ion exchangers-**  
These materials possess a relatively open three dimensional frame work structure with channel and interconnecting cavities e.g. alumino-silicates, TiO<sub>2</sub>, ThO<sub>2</sub>, zirconium oxide, phosphate etc. the hydrous oxide of tri and tetravalent metals are useful as cation exchanger. Besides phosphate, molybdate, tungsten

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etc. of some metal acts as cation exchanger materials.

**B) Natural organic ion exchangers-**

Substance like coal, paper, cotton etc can be converted into cation exchange by reaction of sulphonation or phosphorylation. They act as cation exchanger since they carry sulphonic acid or carboxylic group attached to them. These materials are less uniform in structure and get readily affected by other chemicals.

**C) Synthetic organic ion exchangers-**

The synthetic organic ion exchanger resin are made of cross linked polymer network to which are attached various functional group. The nature of functional group determines whether it is a

(i) Cation exchanger

(ii) Anion exchanger

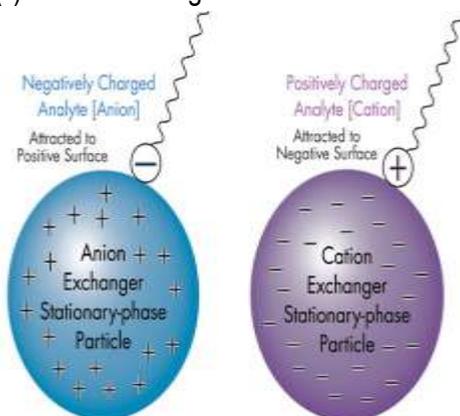


Fig:1 Types of exchanger

In cation exchanger materials the acid group are sulphonic acid, carboxylic acid or phenolic, while in anion exchanger resin the group are basic as amine, quaternary ammonium etc.

On the basis of strength of group, they are further divided into four categories:

- a) Strongly acidic cation exchange resin: sulphonic group.
- b) Weakly acidic cation exchange resin: carboxylic group.
- c) Strongly basic anion exchange resin: quaternary ammonium group.
- d) Weakly basic anion exchange resin: polystyrene, formaldehyde.

For strongly acidic and basic resin exchange capacity is independent of pH, and for weakly acidic and basic resin exchange capacity is dependent on pH of the solution<sup>3</sup>.

**Synthetic Ion Exchange Resins**

These may be regarded as polymers consisting of 3-D hydrocarbon network to which are bonded a large number of electrically charged groups. Originally the term resin was applied to naturally occurring amorphous solid such as amber, shellac, rosin, copal etc. the term resin is now used in case of synthetic polymers which are similar to natural resin in physical properties.

These polymers are cross linked, show thermosetting properties (that is polymers which change irreversibly into hard and rigid materials on heating) due to 3-D network structure. These synthetic polymers are made from small units of chemicals by polymerization either by addition or condensation reactions<sup>1</sup>.

**Requirements of ion exchange resin**

1. It should have a sufficient degree of cross linking for use in chromatography.
2. It should be insoluble in common solvents.
3. It should be sufficiently hydrophilic to permit diffusion of ions through its structure at a constant and finite rate.

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4. It should have desired particle size and shape.
5. It must be chemically stable.
6. It must contain sufficient number of ion exchanger group.
7. It must have the ability of regeneration and reuse.
8. The swollen resin must be denser than water<sup>3</sup>.

**Physical properties of Ion exchange resin**

The ion exchange resin behave as hygroscopic gel, swelling or shrinking reversibly with desorption or absorption of moisture or water.

1. Cross linking: It affects many properties like swelling and strength of ion exchange resin like as it increase swelling decrease.
2. Swelling: In polar solvents swelling occurs while in non polar solvents contraction occurs. The degree of swelling is also affected by the electrolyte concentration.
3. Particle size and porosity: Large surface area and small particle size will increase the rate of ion exchange.
4. Regeneration: The ion exchange resins after use get deactivated and can be regenerated by treatment with aqueous acid followed by washing with water for cation exchanger and with sodium hydroxide for anion exchanger<sup>4</sup>.

**Mechanism of Ion Exchange Process**

The ion exchanger behaves as a porous network which carries a surplus electric charge distributed over the surface and throughout the pores. The surplus charge is compensated by the ions of opposite charge. When the ionization takes place

they are exchanged with the ions of opposite charge. When ionization takes place they are exchanged with the ions which migrate into the solution. In this process chemical bonds are not formed but the exchange occur by the diffusion in two different stages-

- (a) **Film diffusion:** It is diffusion of counter ions through the surface liquid film which surrounds the ion exchanger. The film is extremely thin. It is prominent in dilute solutions and has smaller counter ions.
- (b) **Particle diffusion:** It refers to diffusion of counter ions within the pores of ion exchanger. It is predominant at high concentration and with large ions. This is increased by exchangers with low degree of cross linking, high exchange capacity, small particle size and by increasing temperature.

Non electrolytes and weak electrolytes are usually sorbed by ion exchanger much more strongly than electrolytes. Sorption is enhanced by formation of complexes between the ionic portion of the exchanger and the non electrolyte. Sorption is decreased when the solute molecules become too large to enter the exchanger network. The exchanger acts as sieve or filter. In crystal lattice approach, the exchanger acts as a completely dissociated solid in which each ion is surrounded by a fixed number of ions of oppositely charged. Since the ions on the surface are less influenced by attractive forces, the surface ions are readily influenced for other ions, when exchanger is placed in highly polar solvents like water. The ion selectivity depends on how strongly the surface ion is held by attractive forces within the crystal<sup>4</sup>.

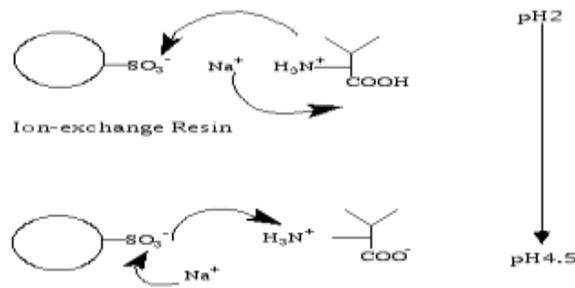
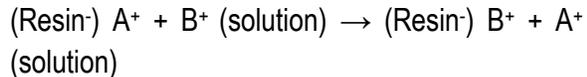
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Fig: 2 Ion exchange process

**Ion Exchange Equilibrium**

Ion exchange resin contains fixed ions on matrix and counter ions balanced opposite replaceable charge. Free replaceable charge is readily exchanged with charge on ion from solution. Therefore in case of cation exchange resin positive charge of resin is replaced by positive charge of solution.



If a solution contains several ion of cation then exchanger shows different affinity for the cation. Exchange of ions is governed by nature of ion exchange resin i.e. strong or weak, nature and number of functional group, pH of the solution and concentration of the solution in contact with the resin<sup>1</sup>.

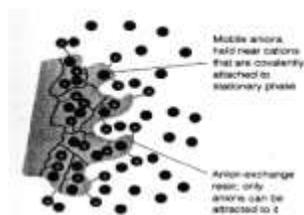


Fig: 3 Ion exchange process

**Ion Exchange Capacity**

The capacity of ion exchanger depends upon the number of sites available for the exchanger which in turn depends on the ionic dimensions. Depending on the form of the resin, cation resins are stable up to  $150^\circ\text{C}$  while anion resins are stable up to  $70^\circ\text{C}$ . The degree of ionization at a given pH and the nature and concentration of ion in solution are also important. When pH increases, the exchange capacity of cation exchanger increases and that of anion exchanger decreases.

The exchange capacity of sulfonated cation exchanger containing only one type of active particles does not depend on pH of the medium because it is a strong electrolyte. Therefore exchange capacity depends on the pH in polyfunctional cation exchangers (like one which contain carboxylic, hydroxyl etc group).

The exchange capacity is expressed in gram equivalent of the ions retained by one cubic meter of the swollen ion exchange resin. In general ion exchange resins are stable towards strong acids at all concentration, strong bases, and almost all organic solvents. The capacity of weakly basic and acidic ion exchanger depends on pH<sup>4</sup>.

**Ion Exchange Techniques**

There are two types of techniques available-

1. **Batch Method** It involves single step equilibrium. The resin and the solution are mixed in a vessel until equilibrium is attained. The solution is filtered off, and further fresh portion of the resin is added to the solution. This process is known as batch operation, but is very little

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important in quantitative analysis. Used for producing deionised and demineralised water and for softening of water.

2. **Column Method** It is better and efficient method. The resin is placed on the top of a glass or wool plug or sintered glass disc in a vertical tube or burette. By passing sufficient concentration of solution through the resin column, a strongly acidic cation exchange resin can be easily converted completely into the desired ionic form<sup>2</sup>.

**Procedure of Ion Exchange Chromatography**

In this charged protein bind to the medium as it is loaded into the column. Then physiological condition are changed in such a way that the bound protein are eluted differentially like on increasing salt concentration or by changing pH mostly common protein are eluted with NaCl using gradient elution and target protein is collected in a purified crystalline form.

The net surface charge of protein vary depending on surrounding pH of the medium if it is high then isoelectric point then protein will bind to anion exchanger and if it is low then it will bind to cation exchanger. Hence ion exchange chromatography can be used to separate several different proteins depending upon surface charge.

Ion exchange chromatography involves two steps:

1. Step first includes binding of a protein to a charged resin.
2. Step two involves elution or displacement of the protein from the charges of the resin.

Points which are to be taken into consideration before performing Ion exchange chromatography are- Preparation of resin, pH of buffer and buffer selection, and elution technique<sup>4</sup>.

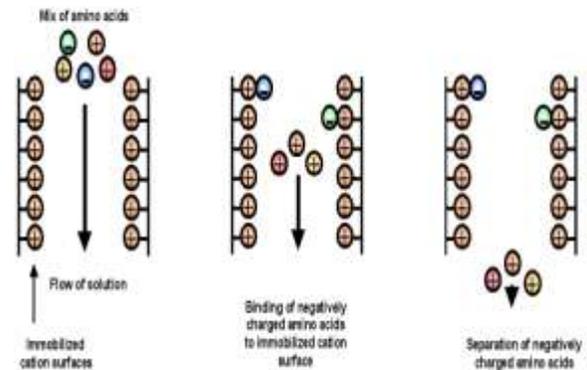


Fig: 4 Anion exchange

**Technique of Ion Exchange Chromatography**

Ion exchange chromatography is performed on column with adsorbents, exchangers and solution which is to be separated is poured on the column then elute from the column is investigated.

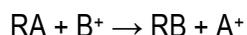
**[A] Column for Ion exchange chromatography**

They are designed in such a way that no disturbance occur in flow of liquid and all operation are carried out in downward direction as the liquid moves down, ion comes in contact with unreacted resin and get completely exchanged with resin. Two points should be kept in mind that no air bubble should come and it is not drained out. Column geometry depends on separation factor and can be improved if length is increased but up to a certain limit, diameter of the column depends on the material which is to be separated. Ratio of 10:1 or 100:1 between height and diameter is maintained generally. Column

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should not be too wide nor should be too narrow because it will produce uneven flow of liquid.

Separation factor: Ion exchange chromatography is based on exchange of ion between solid ion exchanger and ions present in the solution and it obey the law of mass action i.e.



$$[B^-] / [A^-] = K_{A,B} [B^+] / [A^+]$$

Where  $B^-$  and  $A^-$  are concentration of ion in a solid phase and  $B^+$  and  $A^+$  are concentration of ion in liquid phase and  $K$  is ion exchange equilibrium constant<sup>5</sup>.

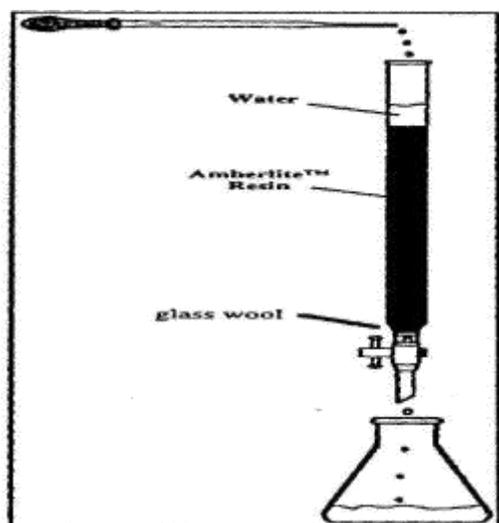


Fig: 5 Column of ion exchange

**[B] Packing of column**

The resin is treated with the solvent and equilibriums are achieved before packing the column. The slurry of the resin is poured in the column. The solvent which is to be used as an eluent should be used for making the slurry. The slurry is added in several portions allowing the resin to settle down. When packing is complete

the eluent is permitted to pass through the column for certain time so to ensure uniform rate of flow over whole cross section area of the column, and level of liquid is so adjusted that it remain below the top of the resin bed. Now the column is ready for experiment and sample solution which is to be separated is introduced or poured on top of the resin in column by using micropipette. It is observed that high rate of exchange is affected with a resin of low cross linking and small particle size. The rate of exchange has also been found to increase with ions of small size, high temperature and concentration of solution<sup>2</sup>.

**[C] Operation of the column**

There are three methods of operation- the basis of all of these operation is that the substance in solution has some affinity for the substrate over which it flows. The affinity is due to ion exchange properties of the column.

1. **Displacement Analysis:** Most strongly absorbed material displaces less strongly absorbed material and each component in turn displaces less readily adsorbed component.
2. **Frontal Analysis:** If solution of mixture of ion having different affinity coefficient is passed through a column of ion exchange resin in sufficient quantity to exceed the exchange capacity of the resin then the least absorbed ion breaks first through the column.
3. **Elution Analysis:** Mixture to be separated is adsorbed in a narrow band at the top of the resin column and is desorbed by passing down the column of

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a solution of another ion which has a lower affinity coefficient than that of the component of the mixture and which is already absorbed on a major portion of the column. The components of the mixture separate to an extent depend upon their distribution coefficient<sup>2</sup>.

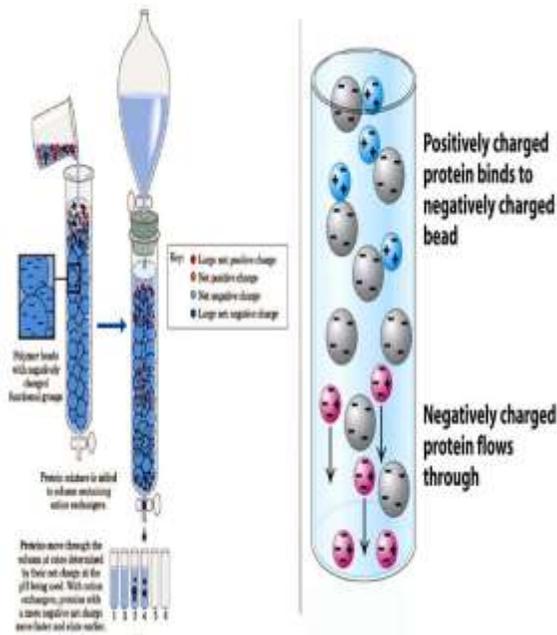


Fig: 6 Ion exchange technique

### Elution

The components of mixture separate and move down the column individually at different rates depending upon the affinity of the ion for the ion exchanger. The ions with least attraction will move most rapidly with the solvent. And as they move downward the distance between them increases. The eluates are collected at different stages. The efficiency of separation increases with increasing length of column and low flow rates.

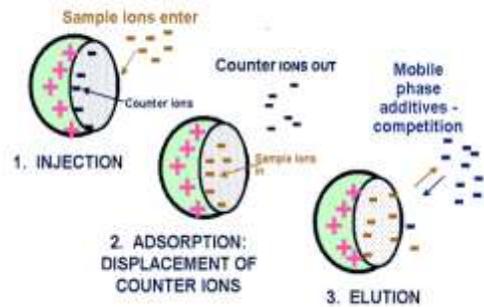


Fig: 7 Ion exchange inside a pore in stationary phase

### Analysis of eluates

After flowing down the column the solution is passed through automatic fraction collector for continuous determination of pH, refractive index etc. these readings are then plotted against elute volume. Other methods are-

Spectrophotometric Methods- Used for direct analysis.

Polarographic Methods- In this diffusion current under constant potential is recorded as function of time and amount of solute present in solution can be calculated from area under the curve.

Conductometric Methods- Electrical conductance of eluate from column is recorded.

Radiochemical Methods- Radioactivity of eluate is recorded by Gieger Muller Counter<sup>1</sup>.

### Applications of Ion Exchange Resins

Ion exchange resins are used for-

1. Demineralization of water.
2. Softening of water.
3. Desalting of water.

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4. Removing carbonates from a solution of sodium hydroxide.
5. Determining total cation content of the sample.
6. Preparation of standard solution.
7. Separation of isotopes.
8. Determination of concentration of traces of ion.
9. Equilibriums and kinetics studies.
10. metallurgy: i.e.
  - For separation of rare earth.
  - For isolation of transuramic elements.
  - For production of uranium and plutonium.
  - For separation of zirconium and hafnium.
9. Analysis natural and industrial water.
10. Separation of complex mixture of biochemical compounds.
11. Radiochemistry.
12. Production of analytical concentrates.
13. Purification and recovery of pharmaceuticals like antibiotic, vitamins, alkaloids and hormones.
14. Medicinal use: Anion exchange chromatography is used in treatment of ulcers. Cation exchange chromatography is used for removing sodium ions and treating hypertension and edema.
15. As diagnostic aid in gastric acidity test.

**Application of Ion Exchange Chromatography<sup>1, 2, 3, 4</sup>**

Ion exchange chromatography is used in-

1. Biological field for separation of hydrolyzed product of nucleic acid.
2. Organic chemistry for separation of acid, amino acid and peptide.
3. Inorganic chemistry for separation of rare earth.
4. Separation of Na and K ions in a mixture.
5. Separation of transition metal.
6. Separation of interfering ions of opposite charge.
7. Separation of amphoteric metal like zinc, aluminum from non amphoteric metals like iron, copper.
8. Separation of metal, alloy and substance possessing related properties.

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