WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 8, 1181-1193. Review Article ISSN 2277– 7105

# ION EXCHANGE CHROMATOGRAPHY AND ITS APPLICATIONS

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Article Received on
20 May 2021,

Revised on 10 June 2021,
Accepted on 30 June 2021

DOI: 10.20959/wjpr20218-20946

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## ABSTRACT

Ion exchange chromatography is one of the most powerful modes of liquid chromatography. The principle involves separation of the molecules by using ion exchange resins like cation exchange resin and anion exchange resin. The popularity of ion exchange chromatography has been increased as this separation technique can be applied in various fields like Biotechnology, Pharmaceutical, Environmental, and Agricultural and in various Industries. The topic includes Introduction, advantages, and various factors affecting separation process, Instrumentation and applications of Ion exchange chromatography.

## INTRODUCTION

It mainly involves the separation of the insoluble molecules which is based on their total charge. This method or technique mainly allows the separation of the molecules of similar type which is difficult to separate the molecules by some other techniques, mainly because of the charge caused by the selective or specific molecule that can be alter by the change in pH buffer. Anion exchanger mainly consists of positive groups whereas the cation exchanger consists of negative groups.[1]

### Advantages of ion exchange chromatography

1) It is mainly useful for the detection of organic ions and inorganic salts (Amines)
2) It is useful to separate the mixture of biological origin of inorganic salts and organomettalics.
3) It is useful for complex sample to be resolve, mainly for the multi step separation.
4) It provides a long life to the resin.
5) Maintenance is cheap.
6) It is economically friendly[2]

**Factors affecting ion exchange chromatography**

**Nature and properties of ion exchange resin:** The most important factor is swelling and cross linking which mainly depends on the properties of polystyrene and cross linking agent. When the cross linking agent is more, then it is more rigid and swelling is less, then the separation of ions is difficult because it cannot pass through the pores. When the cross linking agent is less , then it is less rigid and swelling is more, then the separation of ions is not much efficient because the exchange functional groups does not occur due to the wide pore size. So in order to get a effective separation of ions, a supreme quantity of cross linking agent is added.[3]

**Nature of exchange resin**

1. **Valency of ions:–** The ions at less concentration and at normal temperature then the exchange of ions increases as increase in valency.
   Order of valency of ions= Na+< Ca++<Al+3<Th+4
2. **Size of ions:-** For the same charged ions, the exchange of ions increases and then the hydrated ion size is decreases.
   Order of size of ions= Li+<H+<Na+<NH4+<K+<Rb+<Cs+
3. **Polarizability:-** For the greater polarisability of ions, exchange of ions is preferred.
   Order of polarizability of ions= I-< Br-< Cl-<F-
4. **Concentration of solution:-** The polyvalent anions are mostly adsorbed in dilute solution.
5. **Concentration and Charge of ions-** Exchange of ions is favored at high concentration when the resin has high positive charge and the solution has lower positive charge. Exchange of ions is favored at low concentration when the resin has low positivity charge and the solution has high positive charge.

**pH of the mobile phase**

A) Ionic strength

B) Mobile phase modifiers

C) Temperature[4]

**Ion exchange mechanism**

Mobile phase mainly consists of an aqueous buffer system into which the mixture is introduced. Stationary phase is made up of organic matrix which carries oppositely charged ions. Ions which generally exist in the state of equilibrium which is between mobile phase

and stationary phase which leads to Anion, Cation exchange which are called as counter ions (Fig.1).

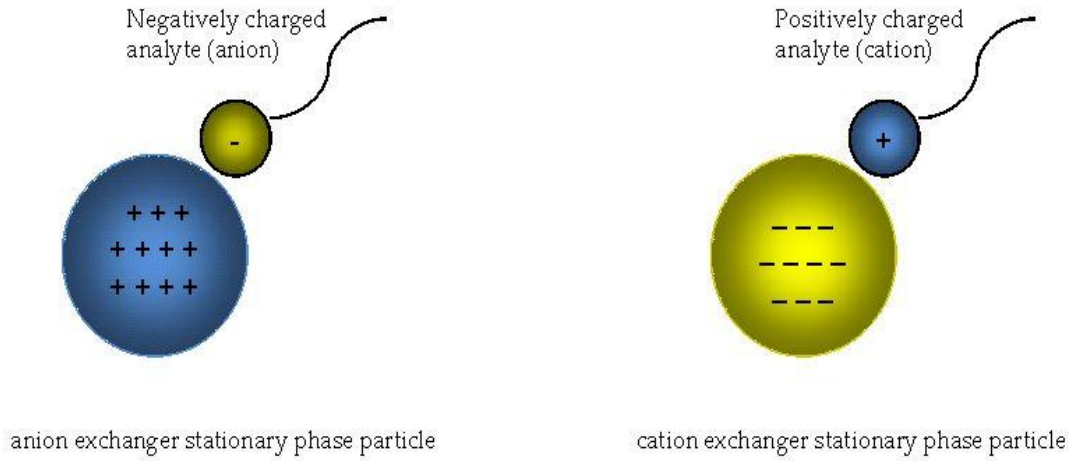


**Fig. 1: Types of ion exchangers.**

These counter ions generally consist of protons, hydroxide groups, single and double charged mono atomic ions and poly atomic ions, inorganic ions as well as organic base and acids. Cations are generally separated on cation exchange resin column and the anions are separated by the anion exchange resin column and the separation is based on the binding of analytes to the positive and negative charge groups. Let us assume that exchanging ions I.e., analytes and ions in the mobile phase are the cations then it is explained as S-X-C+M+. In this procedure the cations i.e. M+ of the mobile phase is replaced which cations I.e., C+bond bound to anions I.e., which is attached on to the surface of the chromatographic support. Molecules differ from other molecules based on the charge properties which will have different degree of interaction with which are charged chromatography surface. The net surface charge of all the molecules with the ionic able groups is of highly pH dependent so the net charge of selected protein. So, protein analyte which can be eluted by charging the mobile phase of the pH which effect the net charge of the adsorbed protein. Increase in the concentration of the same charged specific within the mobile phase which can be resulted during the elution of bond proteins. During the ion exchange chromatography, the negatively charged proteins analytes can be competitively displaced by the addition of the negatively charged ions. These groups are attached to the matrix. The ion exchange process between the ions in the solution takes place mainly on these functional groups.[5]

Ion exchange chromatography is based on the principle of the forces called electrostatic forces of attraction in order to separate a mixture of the charged proteins. These proteins are

of 2 types i.e., positively charged and negatively charged proteins based on the various functional groups of certain charges. The instrument I.e., used in mainly comprise of a chromatography column which is filled stationary phase. When the mobile phase is passed through the column the proteins with identical charge then the polymer charged beads get repelled and it is avoided from the column.[6]

The exchange of ions between the ion exchange resins and the solution depends on 2 principles.

1. The process is reversible, only rare exceptions are known.
2. The exchange reactions take place on the basis of equivalency in accordance with the principle of electro neutrality.

- Ion exchange chromatography is also called as adsorption chromatography which is useful as a popular method based on the following features:

1. High capacity.
2. High resolving power.
3. Mild separation condition.
4. Versatility and widespread applicably.
5. Tendency to concentrate of the sample.
6. Relatively low cost.

**The components of ion exchange chromatography are – (Fig. 2)**

I. A high pressure pump with pressure and flow indicator, to deliver the eluent.
II. An injector for introducing the sample into the eluent stream and onto the column.
III. A column, to separate the sample mixture into the individual components.
IV. An oven, optional.
V. A detector, to measure the analyte peaks as eluent from the column.
VI. A data system for collecting and organizing the chromatograms and data.

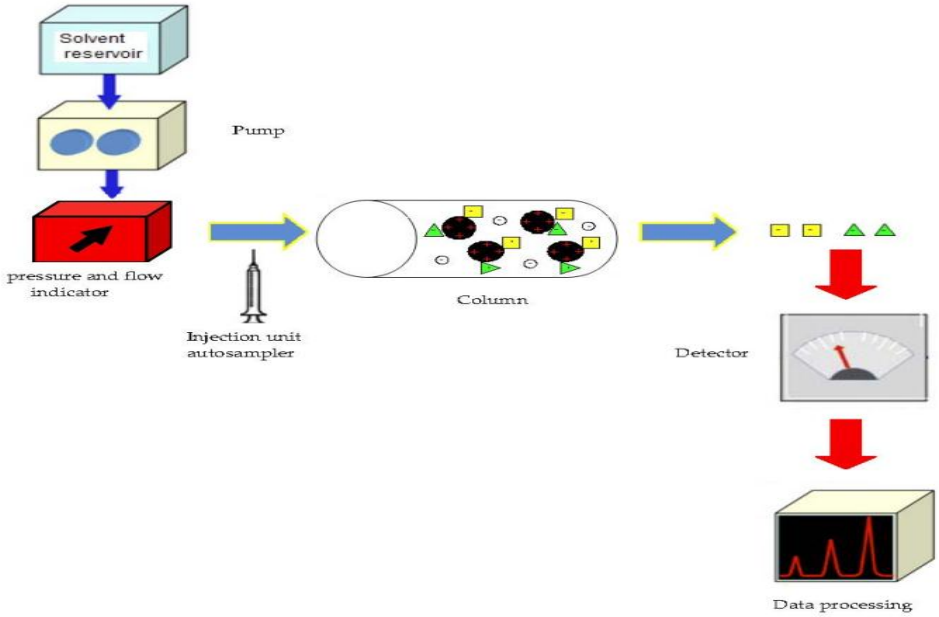


**Fig. 2: Instrumentation of Ion exchange chromatography.**

**Stationary phase**

The elution of a certain ion exchange matrix which is the most crucial in the protocol of Ion exchange chromatography and is mainly depends on certain factors like- ion exchange changers, linear flow rate I.e., sample volumes and the sample properties. In the ion exchange chromatography, there are number of stationary phases which are available from different manufacturers, which mainly differ by chemical and physical properties. The charged groups mainly regulate the specific and strength of the protein binding with the help of polarity and density. The matrix determines the physical and the chemical stabilities and the flow of the stationary phase The most important factors which effect the chromatographic resolution is general structure like fibrous or beaded form, particle size and its variations, pore structures and its dimensions, surface chemistry mainly hydrophobic and swelling characteristics of the matrix. The porosity of the ion exchange beads which are classified into non porous, micro porous, macro porous. (Fig. 3).[7]

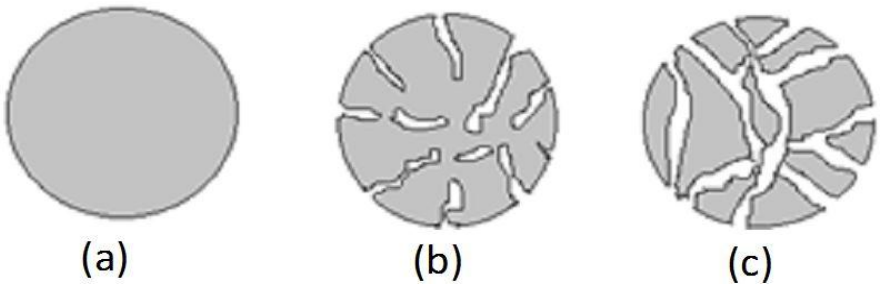


**Fig. 3: a. Non porous b. Micro porous c. Macro porous matrix.**

When it is compared to the beaded matrix fibrous ion exchangers based on the cellulose exhibit the lower chromatographic resolution, where as a high porosity is advantageous, when separating the large molecules. When it is for high resolution separations, the non-porous matrices are preferred and the diffusion effect should be avoided. The binding capacity gets increased by the micro pores as it causes band broadening. There is another disadvantage for the micro pore beads I.e., the protein gets binds with the surface of the beads near to the pores to avoid the protein penetration into the pores is avoided or slows down. These problems can be prevented by using a micro porous particle with the pore dimensions of about 600-800nm. Matrices are mainly obtained with the help of the polymerization of polystyrene with different amounts of the divinyl benzene which are the original matrices for the ion exchange chromatography. These matrices which have hydrophobic surface and the proteins mainly damage due to the strong binding. These ion exchangers are mainly based on the cellulose with the hydrophilic properties for the protein separations.

**Table 1: Few examples of Anion and Cation exchange resins.**

| Exchange Type | Ion exchange group | Buffer counter ions | pH range | Commercial samples |
|---|---|---|---|---|
| Strong cation | Sulfonic acid (SP) | $Na^+$, $H^+$, $Li^+$ | 4-13 | Capto®S |
| | | | | SP Sepharose® |
| | | | | SP Sephadex® |
| | | | | TSKgel SP_5PW |
| Weak cation | Carboxylic acid | $Na^+$, $H^+$, $Li^+$ | 6-10 | CM Cellulose |
| | | | | CM Sepharose® |
| | | | | CM Sephadex® |
| | | | | CM Sepharose® CL6B |
| | | | | TSKgel CM-5PW |
| 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® |
| Weak anion | Primary amine Secondary amine Tertiary amine | $Cl^-$, $HCOO_3^-$, $CH_3COO^-$, $SO_4^{2-}$ | 2-9 | DEAE-Sepharose® |
| | | | | Capto® DEAE |
| | | | | DEAE Cellulose |

**Mobile phase: [Eluent]**

The mobile phase of the ion exchange chromatography generally consists of an aqueous solution of certain salts with organic solvents which are used for dissolution of ionic compounds. The mainly used eluent as mobile phase is sodium chloride for the use of

separation of proteins, as it doesn't cause any important effect on the structure of protein. The chromatographic resolution like retention and peak width of the obtained protein is affected by the anion and cation. In the elution process, the isocratic elution and gradient elution is used. The components of the sample are poorly absorbed to the column. So, to achieve an optimum resolution of sample components, very less content of sample and large exchange column is required.[8]

The pH of the mobile phase is altered due to the adsorption of the proteins when a certain pH is selected which binds with the targeted protein. If the difference in the pH is more then it leads to strong protein binding, sample resolution will decrease. If there is any change in the pH then it leads to the desorption of the targeted protein activity. If the ionic strength is higher, then it leads to the adsorption. If the ionic strength is lower, then it leads to the elution.[9]

Commonly used eluent which mainly used in the Ion exchange chromatography are -

- EDTA; Ethylenediamine tetraacetic acid
- Polyols; glycerol, glucose, saccharose
- Detergents
- Urea
- Lipids
- Organic solvents
- Zwitterion

**Buffer**

In ion exchange chromatography , the important parameters which is used in the separation is pH value and by the help of buffer substances I.e., used in order to control and adjustment of the pH value. H+ concentration and components of buffer effect the binding of protein to the stationary phase, chromatographic resolution, structure and functional integrity of the targeted protein which is to be separated. Many buffers are suitable for ion exchange chromatography. There are so many factors which effect selection of the mobile phase as well as the buffer charge and its strength and buffer pH. A good buffer has certain properties like high buffering capacity at the working pH- High solubility, high purity, low cost. The buffering component which is selected should not react with the ion exchange as it may leads to local pH shifts which occur during the exchange process which impede the elution. Apart

from the interactions of buffer component and stationary phase there are also certain interactions with the mobile phase. To obtain a good buffer, the pka value of the buffer component is to the reversed pH difference should not be more than ±0.5 pH units. The pH value depends on the temperature.[10]

**Selection of buffer conditions**

There are some important factors for the buffer of selected mobile phase

1. Buffer charge
2. Buffer strength
3. Buffer pH

Mostly used buffers are Tris buffers. These are used with the DEAE exchangers. The minimum buffering strength mainly for ion exchange is 10 mm i.e. within the 0.3pH units of pka. A certain buffer pH is selected which permits the selected protein to remain stable. Maintenance of constant pH of mobile phase during the Ion exchange chromatography is important in order to avoid the fluctuations of pH which leads to occur when the protein and the H+ or OH- ions are passed into the mobile phase.

**Theory**

The principle involved in the separation of ions in the reversible exchange of the ions which is present in the solution is added well as in the ion exchange resin. There are two types of ion exchangers

1. Cation exchangers
2. Anion exchangers

**1. Cation exchangers**

These types of exchangers are consists of negatively charged groups. These types of exchangers are mainly attracts positively charged groups. These types of exchangers are also known as acidic ion exchange materials.

**2. Anion exchangers**

These types of exchangers are consists of positively charged groups. These types of exchangers mainly attract negatively charged molecules. These types of exchangers are also known as basic ion exchange materials. The ions i.e. Cation and Anion get separated mainly due to the affinity of the ions towards the matrix. The ions which elute first have less, affinity and the ions which elute later have more affinity towards the matrices.

**Ion exchangers**

Ion exchangers are mainly used in order to separate the metals i.e. uranium from plutonium. Ion exchangers are used to purify the metals. Ion exchangers are divided into 3 types –

I. Resins
II. Gels
III. Inorganic exchangers

**Ion exchange resin**

These ion exchange resins are mainly utilize for the separation of the small molecules. These ion exchange resins are particles of the organic materials made up of amorphous. The benzene group of the ion exchange resin are produce two types of exchange resin i.e. Cation exchange resin and Anion exchange resin by modifying it.

**Classification of ion exchange resin**

1. Based on the chemical nature of the resin it is classified into 4 types

**Strongly acidic cation exchangers:** In this type of exchanger the sulphonic acid groups which are attached to the styrene of co-polymer.

**Weakly acidic cation exchanger:** In this type of exchanger the carboxylic acid groups which are attached to the acrylic of co-polymer

**Strongly basic anion exchanger:** In this type of exchanger the quaternary ammonium groups which are attached to the styrene of co-polymer.

**Weakly basic anion exchanger:** In this type of exchanger the poly alkyl amine groups are attached to the styrene of co-polymer.

2. Based on the source of the resin it is classified into 2 types

Natural =

- Cation - Ex- clay
- Anion - Ex- Delomite

Synthetic =

- Organic resin and inorganic resin. Organic resins are commonly used as ion exchange resin.

**Structural type of ion exchange resin**

**Pellicular type:** The pellicular type of the ion exchange resin with the resin film, the size of the particle of the film is 30-40 micro units with the 1-2 micro units film thickness. To

separate the ions, it uses very low exchange capacity. The efficiency of the pellicular type of the ion exchange resin is 0.01-0.1meq/g of the ion exchange resin.

**Porous resin:** The size of the resin is 5-10 micro units. This type of ion exchange resin is highly efficient. The exchanger capacity of the porous resin is 0.5-2meq/g.

**Macro reticular resin bead:** It is mainly used to identify in the resin beads (superficially). These type of ion exchange resin are not highly efficient as in porous resin. The exchange capacity of the macro reticular resin is very low

**Surface sulfonated and bonded electrostatically:** This type of ion exchange resin is with the Anion exchanger and the particles of this type are sulfonated and are electrostatically bonded. These are less efficient and their capacity is low. The exchange capacity of type of ion exchange resin is 0.02meq/g.

**Physical properties of resins**

**Particle size:** The resins are mainly available in the form of fine powder and the particle size is 500-200 mesh. The resin should allow the mobile phase to flow uniformly and it should contain more exchangeable functional groups.

**Cross linking and swelling:** When the resin is more cross linking agent then the resins is more rigid and swell less. When the swelling of the resin is less, then the ions separation of different sizes is difficult add it cannot pass through the pores which is present. When the resin is less cross linking agent then the resin is less rigid and swells more. When the swelling of the resin is more, then the ions separation will not be efficient. So the cross linking agent used should be is prime quantity is added to the polymeric ion exchange resin for the better separation of the ions.

**Applications of ion exchange chromatography**

1. **Separation of similar ions**

By using cation exchanger resin, a mixture of sodium, hydrogen and potassium are separated from the mixture. By using basic anion exchanger resin, a mixture of chloride, bromide and iodide are separated from the mixture.

2. **Application in water treatment**

- **Softening -** For the softening of hard water a resin is used in the form of sodium is a strong acidic cation exchange resin. The softened water which is mainly used for laundries, textiles and even in domestic water boilers as well as low pressure industrial boilers.

- **Complete demineralization of water-** Cations and anions are removed by 2 steps.

**Step A–** At first, hard water is passed through the acidic cation exchanger like Ca, Mg, and Na which are exchanged by the H+ ions.

**Step B–** The water is then passed through a basic anion exchanger like calcium, Nitrogen dioxide, sulphate ion by OH- ions of the exchanger.

**3. Application in sugar industries**

- **Separation of sugars-** The complex of sugar-borate which us separated on dewax, and the disaccharides are get separated by monosaccharide.
- **Glucose demineralization-** The demineralization is done for glucose syrups in order to increase the purity. Due to the high concentration and high temperature of the glucose syrups, some type of resins are used like strongly acidic resin and widely basic resin of good resistance.

**4. Applications in food industry**

- Beverages
- Treatment of fruit juices
- Citric acid
- Amino acids (Acid amino acids- aspartic, glutamic

Neutral amino acids- Glycine, Valine

Basic amino acids- Lysin, Arginine)

**5. Applications in chemical industries**

- Recovery and removal of metals
- Phenols
- Hydrogen peroxide purification

**6. Applications in pharmaceutical industry**[11]

- Extraction and purification of antibiotics
- Resins used as drugs
- Taste masking

## CONCLUSION

Ion exchange chromatography is a technique that purifies and separates the proteins based on its charge. Ion exchange chromatography is mainly used for the separation of molecules and

analyzing it of different charge like enzymes, amino acids, proteins, peptides, carbohydrates, lectins, nucleic acid, polysaccharides. Ion exchange chromatography is also used for the separation of the organic molecules from the natural sources and purifying it. Ion exchange chromatography is used for more than a half a century for the separation of ionic molecules and isolation of natural products which is useful and popular method in modern drug discovery.

**ACKNOWLEDGMENT**

I am thankful to the Principal, faculty and the management, Vignan Institute of Pharmaceutical technology, Visakhapatnam for helping me in writing this article.

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