IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ION EXCHANGE CHROMATOGRAPHY AND ITS APPLICATIONS: A REVIEW

[1]**N. S. Mali**, [2]S. B. Kanoje, [3]M. S. Charde, [4]R. D. Chakole, [4]A.B.Patil

[1]Research Scholar, [2] Research Scholar,[3] Assistant Professor, [4]Associate professor, [5]Research Scholar

Department of Pharmaceutical Chemistry,

Government College of Pharmacy, Vidyanagar Karad, Dist: Satara,

Pin: 415124, Maharashtra, India,

Abstract:

Ionizable compounds can be separated using ion-exchange chromatography (IEC) based on variations in their charge characteristics. IEC classifies molecules based on differences in their net surface charge. Molecules will interact with charged chromatographic medium to variable degrees due to differences in their total charge, charge density, and surface charge distribution. One of the most adaptable and extensively used liquid chromatography (LC) techniques due to its vast sample-handling capacity, broad applicability (especially to proteins and enzymes), reasonable cost, powerful resolving ability, and simplicity of scale-up and automation. One of the most often used techniques in ion chromatography (IC) for identifying both organic and inorganic ions is the ion exchange chromatography system. In this chapter, we go over the fundamentals of IEC as well as more general standards for deciding on IEC conditions and their use in ion exchange chromatography.

Keywords: Ion-exchange chromatography, liquid chromatography, Anion Exchange, Cation Exchange Matrix etc.

Introduction:

One of the most popular types of column chromatography is ion-exchange chromatography. It is used in research, analysis, and process-scale purification of proteins.[1] The first observations recorded in the literature that refer to ion exchange were made by way and Thompson in 1850.

The researchers discovered that when ammonia ions (NH4+) were leached by ions solutions (Ca2+), the soil had the capacity to remove them and replace them with similar amounts.[2] Ion exchange is ideal for initial capture of proteins because of its high capacity, relatively low cost, and its ability to survive rigorous cleaning regimes. Ion exchange is also ideal for "polishing" of partially purified material on account of the high-resolution at sustainable and the high capacity giving the ability to achieve a high concentration of product.

Together with ion-partition/interaction and ion-exclusion chromatography, ion-exchange chromatography (IEC) is a component of ion chromatography, a significant analytical technique for the separation and detection of ionic substances. Ions present in the eluent, ions present in the analyte, and ionic functional groups fixed to the chromatographic support interact ionically (or electro statically) to separate the analyte. Ion exchange due to competitive ionic binding (attraction) and ion exclusion due to repulsion between similarly charged analyte ions are the two main mechanisms used in ion chromatography and the ions fixed on the chromatographic support. To date, the most common type of ion chromatography has been ion exchange. [3]

The general advancement in fundamental research in the middle (Michael Faraday, notion of ions) and late (Svante Arrhenius, theory of electrolytic solutions) eighteenth century foreshadowed the discovery of ion exchangers. Since both organic and inorganic materials are essentially polyelectrolytes, these concepts were essential for the scientific discovery of ion exchangers. They can therefore be thought of as being made up of two ions with opposite charges. [4]

One of the most adaptable and commonly used liquid chromatography (LC) techniques as a result of its vast sa mple handling capacity, broad applicability (especiallyto proteins and enzymes), reasonable cost, powerful reso lving ability, and simplici-ty of scale-up and automation. [5]

Strategic planning for ion exchange chromatography:

The process of creating an ion-exchange technique involves several fundamental components. The best operating pH and buffer system for the medium and sample, as well as an acceptable ion-exchange media, must first be chosen. In order to determine whether a batch or column chromatography is appropriate given the purity, protein concentration, and physical characteristics of the sample, the sample volume to be used, and the availability of suitable equipment .Next, pilot experiments are conducted to determine conditions for binding and eluting.[6]

To conduct strategic planning of ion chromatography we should get a deep knowledge of various parameters also:

1. Selecting an Ion-Exchange Medium
2. Selecting a Buffer System
3. Selecting Batch Versus Column Purification
4. Capacity of Ion-Exchange Media
5. Chromatography Systems
6. Scale-Up Conditions

Principle of ion exchange chromatography:

On the basis of variations in their net surface charge, IEC divides molecules. Because molecules' overall charge, charge density, and surface charge distribution varied significantly from one another, they will interact with charged chromatographic media to varying degrees. The charged groups within a molecule that contribute to the net surface charge possess different pKa values (acid ionization constant) depending on their structure and chemical microenvironment.[7]

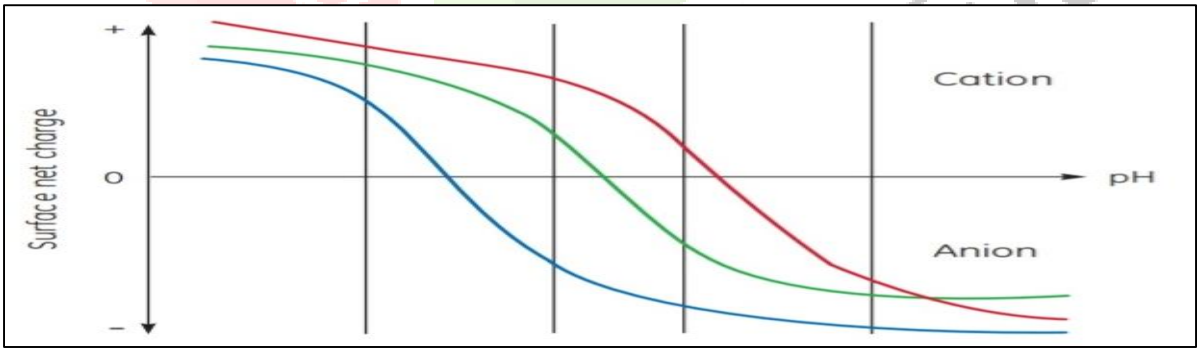

Figure: 1 Theoretical protein titration curves, showing how net surface charge Varies with pH.[6]

The ion exchanger is composed of a base matrix usually in the form of porous beads to provide enough surface area for adsorption. On this base matrix, a charged ligand, either positively or negatively charged, is immobilized. Instead of a tiny charged ligand, a charged polymer has also been grafted onto the matrix to increase capacity. Irrespectively of the molecular size of the charged ligand, we distinguish between cation and anion exchangers. Cation exchangers are negatively charged and anion exchangers are positively charged .The binding of a charged species to an ion exchanger can be described by the mass action law which has been postulated by Boardman and Partridge (1955).[7]

Two principles govern the ion exchange between the solution and the ion exchange resin:
1. There are no known exceptions to the reversibility of the process
2. The exchange reactions take place on the basis of equivalency in accordance with the principle of electro neutrality. It is expected that the amount of milimoles of an ion released from an ion exchange will match the number of milimoles of an identically charged ion that was sorbed by the exchange.

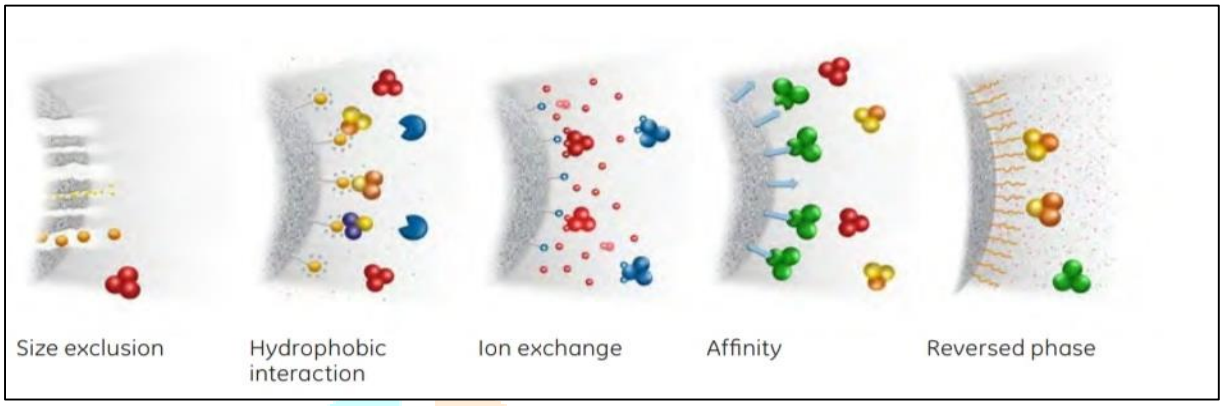

Figure: 2 Separation principles in chromatographic purification [6]

IEC also have two major roles in protein separation. IEC involves two separate events. These are (1) the binding of the protein to the fixed and (2) the elution or displacement of the protein from the fixed charges.[8] Since all compounds with ionizable groups are titrable, the pH has a significant impact on the molecules' net surface charges. Because proteins are composed of a variety of amino acids with weak acidic and basic groups, their net surface charge will gradually alter as the pH of their surroundings varies, making them amphoteric. Each protein has its own unique net charge versus pH relationship which can be visualized as a titration curve. This curve reflects how the overall net charge of the protein changes according to the pH of the surroundings. [9]

Positively charged molecules can be separated using CEC columns, typically packed with 3–10m particles containing negatively charged acidic functional groups. Through ionic contact, these columns bind cationic species like protonated bases. In anion-exchange (AEC) mode, the stationary phase carries positively charged basic Functional groups that are capable of binding anions (e.g. ionized carboxylic acids).In order to manage the retention of sample ions, the mobile phase typically comprises a buffer to maintain a steady pH and variable salt concentrations (counter-ions). The Charge of the counter-ion has the same sign as the sample ions; therefore it can be used to control the retention of protonated bases in CEX or ionized acids in AEX. [10]
Prior modeling of chromatographic behavior and adsorption isotherm characteristics in ion-exchange systems is also possible. It is indeed possible to generate predictive QSPR models of protein SMA parameters and free energy changes in ion-exchange systems using a SVM regression technique. Utilizing two proteins from the test set that were not part of the model training set; the models' prediction power was assessed. Molecular descriptors selected during model generation were examined to gain insights into the important physicochemical factors influencing stereochemistry, equilibrium, stearic effects, and binding affinity in protein ion-exchange systems. [11]

Ion exchange chromatography System:

One of the most popular methods for determining both organic and inorganic ions is ion chromatography (IC). Since the development of this chromatographic technique by Small it has obtained analytical applications in environmental monitoring , food analysis , criminal forensics , analysis in water quality control procedures and pharmaceutical industry .[12]

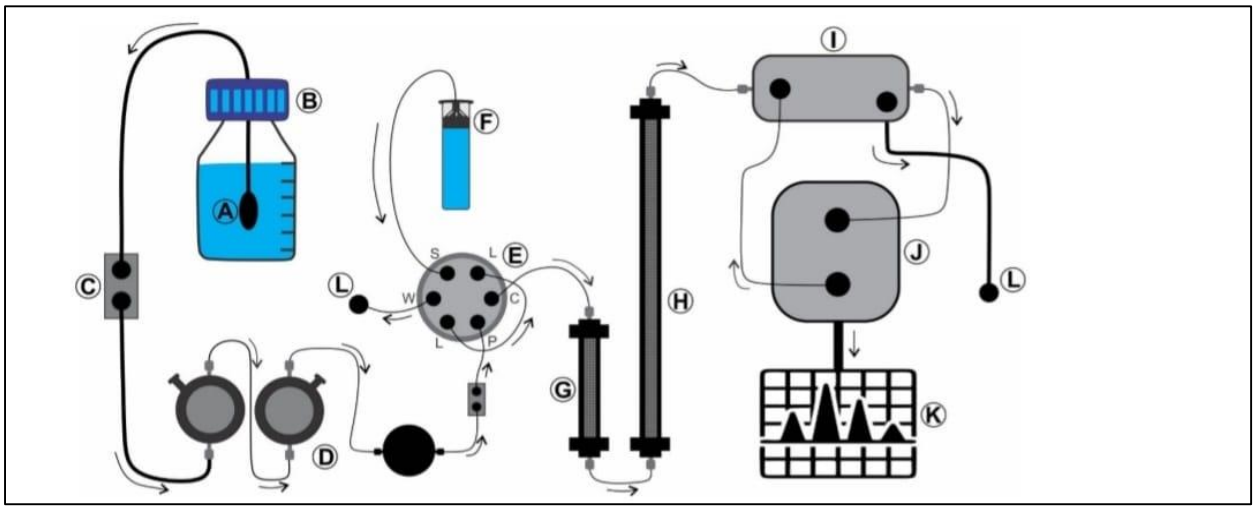

Figure:3 Schematic of an ion chromatography system. A-Filter B-Bottle with eluent; C-Degasser; D-Pump system; E-Injection System; F- Vial Sample; G-Guard column; H-Separation Column; I-Chemical suppressor, J-Detector, K-Chromatogram; L-Waste.[2]

Both selectivity and efficiency in chromatography on ion-exchange packing's are governed by diffusion:

- Diffusion of sample molecules to the ion exchange packing.
- Diffusion past the Donnan layer into the packing Interaction with and diffusion through the packing.[13]

Ion exchange chromatography system comprises various component which helps in the separation of the mixture components. The following list contains the basic elements of an ion-exchange chromatography.

1) A high pressure pump with pressure and flow indicator, to deliver the eluent.
2) An injector for introducing the sample into the eluent stream and onto the column
3) A column, to separate the sample mixture into the individual components
4) An oven, optional
5) A detector, to measure the analyte peaks as eluent from the column
6) A data system for collecting and organizing the chromatograms and data.

The interaction of charged molecules in the mobile phase (buffer+ sample) with opposingly charged groups attached to the stationary phase (column packing matrix) is the basis for ion-exchange chromatography. The charged molecules in a buffer solution come from the buffer components (e.g., salts). The many amino acids in a protein provide the charged groups on a protein. Lysine, arginine, and histidine have a positive charge at physiological pH, whereas aspartic acid and glutamic acid have a negative charge at physiological pH.

Adsorption and desorption procedures in ion-exchange chromatography are governed by the characteristics of the three interacting entities;

- The stationary phase
- The constituents of the mobile phase
- The buffer
- The solute
- Detection

1) Stationary phase

The most crucial step in the ion exchange technique is choosing an appropriate ion-exchange matrix, which is dependent on a number of variables including sample characteristics, linear flow rate, and ion exchanger charge and strength.

If an ion exchanger has negatively charged functional groups and exchangeable cations, it is referred to be a cation exchanger. Anion exchangers carry anions because of the positive charge of their fixed groups.

Inert matrix should be utilized to reduce non-specific interactions with sample components. High physical stability ensures that the packed medium's volume stays constant even in the presence of significant pH or salt concentration variations, enhancing repeatability and preventing the need to repack columns.

2) Mobile phase (Eluent)

In ion exchange chromatography generally eluents which consist of an aqueous solution of a suitable salt or mixtures of salts with a small percentage of an organic solvent are used in which most ionic chemicals dissolve more easily than in other substances. Therefore the application of various samples is much easier. The eluent pH has considerable effects on the functional group which exist on the ion exchange matrix and also on the forms of both eluent and solute ions. The selectivity coefficient existing between the competing ion and a particular solute ion will determine the degree of that which competing ion can displace the solute ion from the stationary phase. As different competing ions will have different selectivity coefficients, it follows that the nature of competing ion will be an important factor in determining whether solute ions will be eluted readily. [7]

3) The buffer

Protein stability and activity must be compatible with buffer pH and ionic strength. The best pH should be close to the point of elution (release) while still allowing the target proteins to bind. Elution becomes more challenging and high salt concentrations may be required if the pH is too low or high.

Maintaining a steady mobile phase pH during separation is crucial to preventing pH changes that may occur when both proteins and exchanger ions are released into the mobile phase. This will help to prevent variations in matrix and protein net charge. Buffering agents allow pH values to be controlled and modified. Concentration of H+ and the buffering component influence the protein binding to the stationary phase, chromatographic resolution and structural as well as functional integrity of the protein to be separated.

4) Detection

The most prevalent and practical detector in ion exchange chromatography is the conductivity detector. Therefore when conductivity detection is used dilute eluents should be preferred and in order for such eluents, to act as effective competing ions, the ion exchange capacity of the column should be low.

Although some older systems used recorders and integrators, most modern ion exchange chromatography results are saved in computers. The most helpful data are retention time and peak areas. Retention times are used to confirm the identity of the unknown peak by comparison with a standard. [8]

Application of ion exchange chromatography:

Ion exchange chromatography can be used to separate and purify a variety of charged or ionizable compounds from both natural and synthetic sources, including proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins, and more. Example of some application ion exchange chromatography as follow:

- **Separation of Actinides**. The actinide series' transplutonium elements were first discovered using the ion exchange chromatographic method. Since some of these elements could only be created in atom quantities, the actinide series also exhibits elution in the opposite order of the atomic number due to actinide contraction. This proved to be the sole method for identifying these elements. One could argue that any other method of separation would not have allowed for their seclusion.
- **Purification of Organic Compounds**. Many natural products extracted in water have been found to be contaminated with ions originally present in water. Ion-exchange procedures can be used to get rid of such ions.
- **Sugar separation**. The approach was created in 1951 by Khym and Zill. Borate complexes are first created from these sugars.
- **Preparation of Pure Reagents**. Carbonate is invariably present in solutions of sodium hydroxide used for volumetric determinations. This gives rise to errors in acid-base titrations. The simplest method for removing carbonate involves running the solutions through a column of a strongly basic anion exchange resin in the hydroxide form. As the carbonate is absorbed, an equivalent concentration of hydroxide is released.
- The preparation of tetra methyl ammonium hydroxide which is not available in the required degree of purity can be undertaken by passing a solution of tetraethyl ammonium chloride through a bed of a cation exchanger

which will be converted to the tetraethyl ammonium form. The bed is then washed with water followed by passing sodium hydroxide. Tetra methyl ammonium hydroxide is created in the effluent and is ready for use.

- Making silicic acid from sodium silicate is another example of commercial significance. During the passage of the silicate through the hydrogen form of a cation exchanger, the sodium ions of the silicate get replaced by hydrogen ions from the hydrogen form of the cation exchanger to yield silicic acid. Compared with the silicic acid formed by treatment of silicate with mineral acids, silicic acid produced by ion exchange is non-flocculent.
- **Lydrometallurgy**. Many metals are recovered and purified commercially by ion exchange. Examples include uranium, thorium, lanthanides, and actinides, gold, silver and platinum. In some cases, the scale of operation is relatively small e.g., lanthanides, But the intrinsic value of these metals are very high. Additionally, a significant use from an environmental standpoint is the isolation and recovery of trace levels of hazardous metals from effluent and waste streams. Some examples include the recovery of chromium from spent metal-plating solutions, and copper and zinc from effluents in rayon and synthetic fiber industry.
- The most common applications for ion exchangers (which remove bicarbonates) include water softening (removal of calcium and magnesium ions), water demineralization (removal of all ions), and dealkalinization.[12]
- Cation exchange resins can be used to remove copper, iron, lead, radium, barium, and other contaminants from water. Anions, also known as anionic exchange units, are capable of removing nitrate, sulphate, and other negatively charged atoms. Researchers are developing resins that will enable more selective nitrate extraction than is currently feasible.
- In the chemical sector, ion exchangers are also used to extract or recover metal ions from effluent. Due to the poor selectivity of the resins, some pollutants (such as arsenic, fluoride, and lithium ions) are difficult to remove using ion exchange.[13]
- **Separation of nitrogen isotopes in ammonia**. The remarkable success achieved in separating adjacent rare earths in high purity suggested that isotopes might also be separated by a similar procedure. It was hoped that the slight differences in the exchange constants of various isotopes of an element might also cause the isotopes to be resolved into distinct zones within a chemically constrained band if the elution step was carried out under proper conditions. [14]
- Membrane ion-exchange chromatography for process-scale antibody purification. The mass manufacture of recombinant monoclonal antibodies necessitates low-cost, high-Throughput purification procedures. For the filtration of low concentrations of contaminants like DNA, host cell protein, and virus, anion-exchange membranes operating in a flow-through mode may be a viable substitute for columns.[15]

- **Conclusion:**

In this chapter, we review the basic principles of Ion exchange chromatography, as well as the broader criteria for selecting IEC conditions and its application. Ion exchange chromatography can be used to separate and purify a variety of charged or ionizable compounds from both natural and synthetic sources, including proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins, and more. Its large sample-handling capacity, broad applicability (particularly to proteins and enzymes), moderate cost, powerful resolving ability, and ease of scale-up and automation have led to it becoming one of the most versatile and widely used of all liquid chromatography (LC) techniques.

Acknowledgments:

The authors are thankful to AICTE New Delhi for providing the financial support during M. Pharm tenure. Also, thankful to the Principle of Government College of Pharmacy, Karad for providing required facilities.

References:

1. Selkirk, Chris. "Ion-exchange chromatography." Protein purification protocols (2004): 125-131.
2. Moura, Alexandre Varão, José Domingos Santos da Silva, and Priscila Gubert. "Ion Chromatography: Principles and Instrumentation." Orbital: The Electronic Journal of Chemistry (2022): 110-115.
3. Acikara, Özlem Bahadir. "Ion-exchange chromatography and its applications." Column chromatography 10 (2013): 55744
4. Kumar, Sanjeev, and Sapna Jain. "History, introduction, and kinetics of ion exchange materials." Journal of chemistry 2013 (2013).
5. Cummins, Philip M., Oonagh Dowling, and Brendan F. O'Connor. "Ion-exchange chromatography: basic principles and application to the partial purification of soluble mammalian prolyl oligopeptidase." Protein Chromatography: Methods and Protocols (2011): 215-228.
6. Williams, Alan, and Verna Frasca. "Ion-exchange chromatography." Current protocols in molecular biology 44.1 (1998): 10-10.
7. Healthcare, G. E. "Ion Exchange Chromatography: Principles and Methods." General Electric Company: GE Healthcare (2016).
8. Jungbauer, Alois, and Rainer Hahn. "Ion-exchange chromatography." Methods in enzymology 463 (2009): 349-371.
9. Rossomando, Edward F. "[24] Ion-exchange chromatography." Methods in enzymology. Vol. 182. Academic Press, 1990. 309-317.
10. Fekete, Szabolcs, et al. "Ion-exchange chromatography for the characterization of biopharmaceuticals." Journal of pharmaceutical and biomedical analysis 113 (2015): 43-55.
11. Ladiwala, Asif, et al. "A priori prediction of adsorption isotherm parameters and chromatographic behavior in ion-exchange systems." Proceedings of the National Academy of Sciences 102.33 (2005): 11710-11715.
12. Vedantu. "Application of ion chromatography".Vedantu improvement promise.
13. Wood, Roy, Larry Cummings, and Tom Jupille. "Recent developments in ion-exchange chromatography." Journal of Chromatographic Science 18.10 (1980): 551-558.
14. Fritz, James S. "Early milestones in the development of ion-exchange chromatography: a personal account." Journal of Chromatography A 1039.1-2 (2004): 3-12.