BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Instrumentation and Application of Ion Exchange Chromatography

Namita G. Bhandarkar*, Rani T. Bhagat, Tulsidas P. Nimbekar
Shri Laxmanrao Mankar Institute of Pharmacy Amgaon

ABSTRACT

Chromatography is a crucial technique in various industries, particularly in pharmaceuticals, where it is used to separate, purify, and analyse compounds. Ion exchange chromatography (IEC) is a type of chromatography that separates ions based on their electrostatic interactions with a stationary phase. The technique is widely used in biotechnology, pharmaceutical processing, and water treatment. IEC is essential for the purification of charged proteins, nucleic acids, and other biomolecules. The separation process involves the interaction between ions and a charged stationary phase, allowing for the selective retention and elution of ions. The technique has various applications, including the purification of proteins, peptides, enzymes, nucleotides, and DNA. IEC is also used in the food industry to adjust the pH of liquid media and extract important components. The instrumentation of IEC includes columns, sample injection systems, mobile phase reservoirs, pumps, detectors, and data systems. Overall, IEC is a powerful tool for the separation, purification, and analysis of ions and biomolecules, and its applications continue to grow in various industries.

Keywords: Ion exchange; Resin; Anion exchange; Cation exchange.

*Corresponding Author Email: namitabhandarkar15@gmail.com
Received 01 November 2025, Accepted 15 December 2025

Please cite this article as: Bhandarkar NG *et al*., Instrumentation and Application of Ion Exchange Chromatography. British Journal of Medical and Health Research 2025.

INTRODUCTION

A Forms of column chromatography to separate, identify, and quantify the compounds. Developed in 1970s. The widely used analytical separation technique. 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. 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 (NH_4^+) were leached by ions solutions (Ca2+), the soil had the capacity to remove them and replace them with similar amounts. 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 ironically (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 [1]

Chromatography:

Chromatography is a technique which separates components in a mixture due to the differing time taken for each component to travel through a stationary phase and carried through it by a mobile phase. It is technique used for separation, purification, identification and extraction of compound. It can consist of two phases' stationary phase and mobile phase. Stationary phase is constant phase or column packaging material. Mobile phase is movable phase. The basic principle of chromatography is based on adsorption partition chromatography.

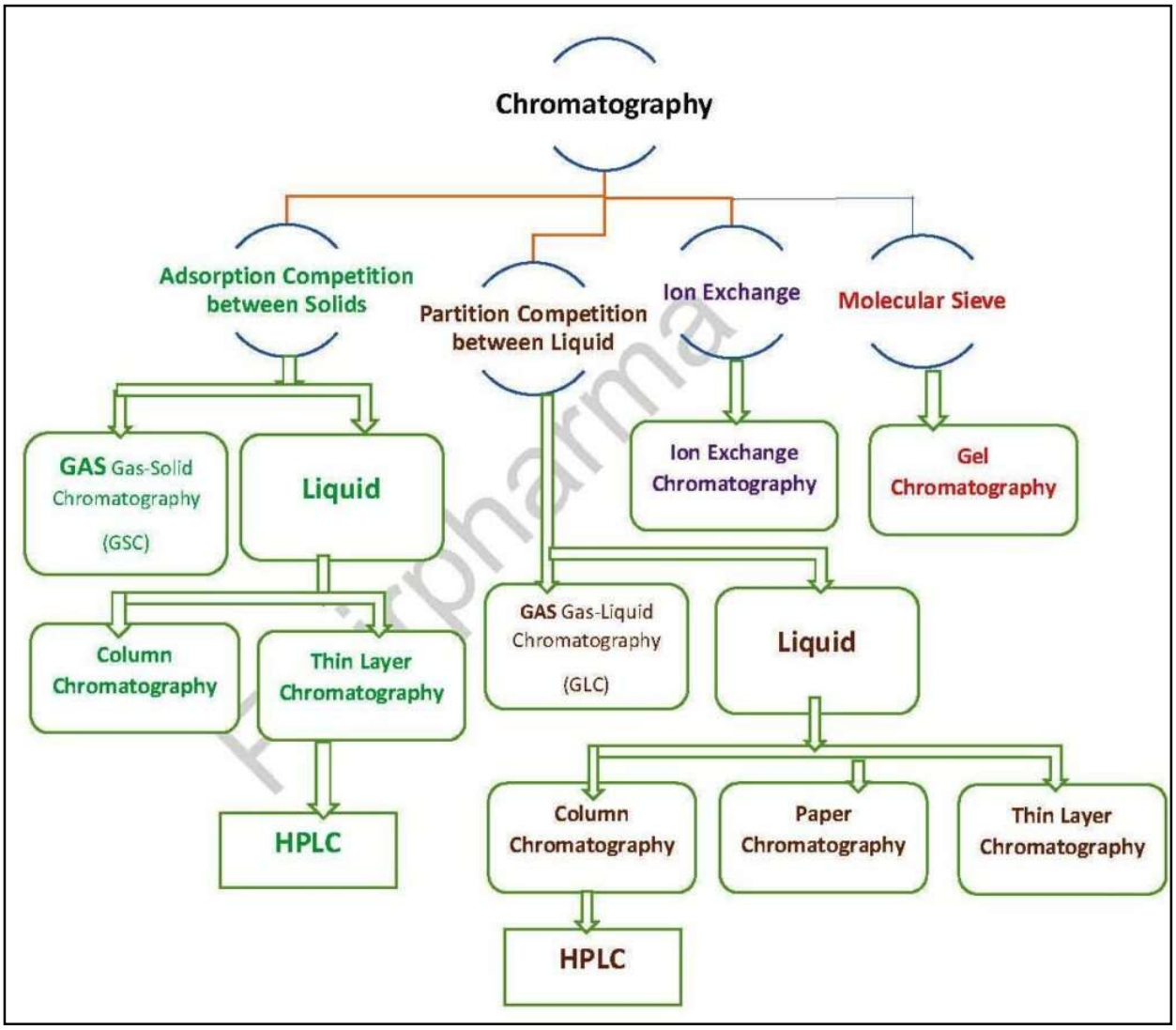
Adsorption chromatography:

The affinity to molecules towards stationary phase is known as adsorption chromatography.

Partition chromatography

The molecule can move in two phases of liquid is known as partition chromatography. It is important for qualitative and quantitative analysis.[2]

Types Of Chromatography



Ions Exchange Chromatography

Ion exchange chromatography is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchange. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. Cation or Anions can be separated using this method. Ion exchange chromatography is a part of ion chromatography which is an important analytical technique for the separation and determination of ionic compounds, together with ion partition or interaction and ion-exclusion chromatography. Ion chromatography separation is based on ionic or electrostatic interactions between ionic and polar analytes, ions present in the eluent and ionic functional groups fixed to the chromatographic support. Two distinct mechanisms as follows; ion exchange due to competitive ionic binding (attraction) and ion exclusion due to repulsion between similarly charged analyte ions and the ions fixed on the chromatographic support, play a role in the separation in ion chromatography. Ion exchange chromatography can be defined as a reversible process in which ions of same sign are exchanged between solid and

liquid, a highly insoluble body in contact with it. The soil is known as Cation anion exchange chromatography.

Ion exchange chromatography, which is also known as adsorption chromatography, is a useful and popular method due to its High Capacity

- High resolving power
- Mild separation conditions
- Versatility and wide speared applicability

Tendency to concentrate the sample relatively low cost.[3]

Technical History: -

The name chromatography is derived from the Greek "Chroma + graphein", which means writing of color. At the beginning of the 20th century, Michael S. Tswett developed a technique to extract a variety of plant compounds in glass columns filled with calcium carbonate and passed through the

solvent petroleum ether, thus verifying the separation of the pigments. The first observations recorded in the literature that refer to ion exchange were made by Way and Thompson in 1850.

The researchers found that the soil had the ability to remove ammonia ions (NH_4^+) when leached by ion solutions (Ca^{2+}), replacing them by equivalent amounts It was not until the end of World War I that ion exchange was used for analytical purposes, when Folin and Bell obtained the separation and quantification of ammonia in urine using an artificial ion exchanger, synthetic aluminum silicate. In 1935, Adams and Holmes made synthetic ion exchange resins. The basis of their synthesis was the condensation polymerization of poly substituted benzene compounds or formaldehyde. In 1940, exchange resins based on the copolymerization of styrene and divinylbenzene were developed for use as a water treatment medium. However, the first report of this method occurred only in 1944 by Russell, Svartout, Hume and Kettle, first published in the open literature in 1947. In 1975, Small, Stevens and Bauman performed the separation and detected the samples using a conductivity meter and incorporating a suppressor column between the separator and a conductivity detector to increase sensitivity by reducing background. In the 1980s, Gjerde and colleagues used the ion chromatography system without the suppression device with very low conductivity eluents. Both suppressed and unsuppressed ion chromatography modes can be applied to analyze diverse samples. However, the application of suppressed ion chromatography is more commonly used.[4]

Principle:

The principle of ion exchange chromatography is based on the principle on the reversible exchange of ions between a mobile phase (the liquid solution containing the sample) and a stationary phase (the solid support with charged groups).

Ion exchange chromatography operates on the electrostatic interactions between charged molecules and a solid stationary phase containing fixed charge groups. The stationary phase can be made up of a resin with either positively charged group (cation exchange) or negatively charged group (anionic exchange). The sample containing mixture of charged molecules is loaded into the column, molecules interact with the charged group in stationary phase. Depending on their charge molecules will either be attracted to or repelled by the stationary phase, leading to separation.[5]

Classification

The ion exchange separation is mainly carried out in column packed with an ion exchanger. There are two types of ion exchanger namely,

1. Cationic exchangers
2. Anionic exchangers
3. Zwitterionic exchanger

Cationic exchangers:

It possesses negatively charged groups and these will attract positively charged groups. These exchangers are also called acidic ion exchange materials since their negative charges result from the proteolysis of acidic groups.

Anionic exchangers:

It possesses positively charged groups and these will attract negatively charged molecules. This exchanger is termed as basic ion exchange materials since their positive charges generally result from the association of protons with basic groups.

Zwitterionic Exchanger

Zwitterionic ion exchangers are those stationary phases used in the multi-selective retention mechanism. These exchangers have both positive and negative charges, so they carry a net zero charge. Zwitterionic stationary phases accumulate equal amounts of oppositely charged groups, fixed close to the surface or within the volume of the stationary phase. Ideally, the zwitterionic phase can be considered as a phase containing an equivalent amount of strongly acidic and strongly basic groups.[6]

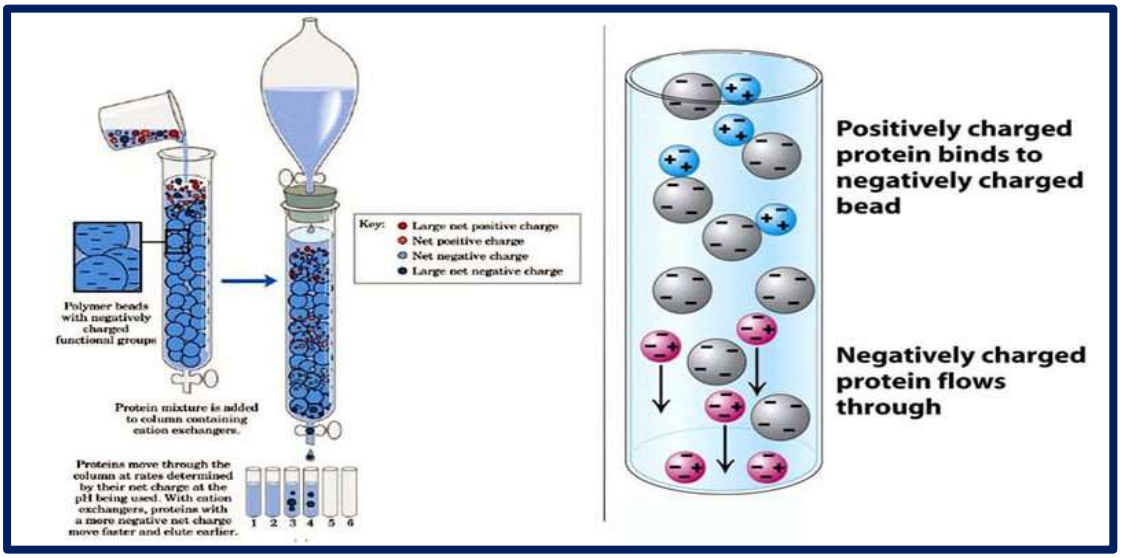
ION EXCHANGE MECHANISM

Ion exchange mechanism are distinct into five types

1. Diffusion of the ion to the exchanger surface. This occurs very quickly in homogeneous solutions.
2. Diffusion of the ion through the matrix to the exchanger site. This is dependent upon the degree of cross linkage of the exchanger and the concentration of the solution.
3. Exchange of ions at the exchange site occurs. This occurs instantaneously in an equilibrium process.
4. Diffusion of the exchanged ion through the exchanger to the surface.
5. Selective desorption by the eluent and diffusion of the molecule into the external solution takes places.[7]

Resin - SO3H + Na+----------> Resins - SO3Na + H+

Resin - N(CH3)3OH + Cl- ----------> Resin - N(CH3)3Cl + OH



ION EXCHANGERS

Ion exchange processes are used to separate and purify metals. Including, separating uranium from plutonium and other actinides.

There are three classes of ion exchangers;

1. Resins
2. Gels
3. Inorganic exchangers

Resins

Resins are amorphous particles of organic materials, which are composed of polystyrene and divinely benzene.

- Polystyrene contains sites for exchangeable functional groups.
- Divinely benzene acts as cross-linking agents and offers adequate strength., mechanical stability. Ion exchange resins are used for separation of small molecules.[8]

CLASSIFICATION OF ION EXCHANGE RESINS

According to chemical nature

- Strong cation exchange resin – sulphonic acid
- Weak cation exchange resin – Carboxy methyl compound
- Strong anion exchange resin – Quaternary ammonium compound
- Weak anion exchange resin – Diethyl amino ethyl compound

According to the source

1. Natural

- Cation – Zeolytes, clay
- Anion – Dolomite

2. Synthetic

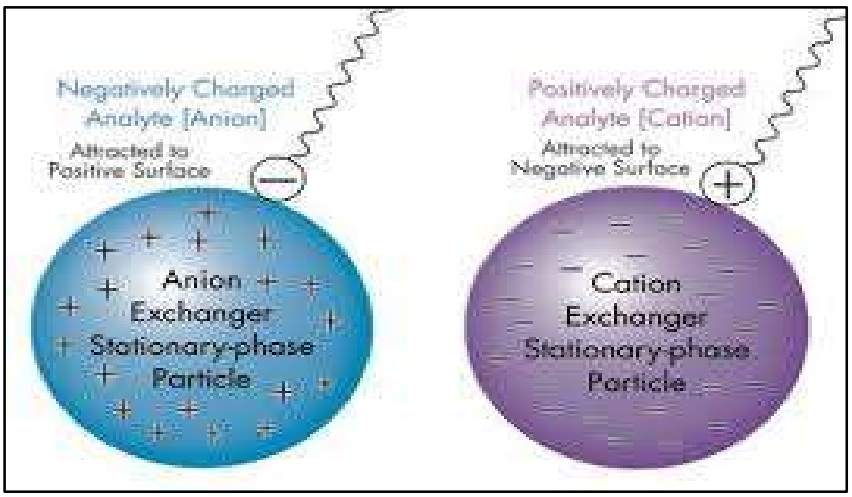
- Inorganic and organic resins Organic resins are polymeric resin matrix.

The resin composed of Polystyrene and Divinyl benzene.

According to the structure

a. Pellicular type with ion exchange film

Particle size of 30-40 micron with 1-2- micron film thickness



- Very low exchange capacity
- Ion exchange efficiency is 0.01-0.1meq/g of ion exchange resin

b. porous resin coated with exchanger bead

- Particle size of 5-10 micrometer
- Porous and highly efficient
- Ion exchange efficiency is 0.5-2meq/g of ion exchange resin

c. Macroreticular resin bead

- Highly efficient
- Very low Not exchange capacity

d. Surface sulfonated and bonded electrostatically with anion exchanger

- Low efficient
- Low exchange capacity

GELS

- Ion exchange gels are used for the separation of large molecules like proteins, nucleic acids.
- Cellouse and dextran ion exchangers which are polymers of sugar glucose possess large pore sizes and lower charge densities.
- They are much softer than polystyrene resins, dextron and its relatives are called as gels.

INORGANIC EXCHANGERS

- The combinations of hydrous oxide of highly charged ions, with one oxide more acidic than the other have been found to have ion exchanging properties.
- The amorphous precipitates have higher exchange capacities than the crystalline compounds, because of the greater surface area of the former type of compounds.
- Inorganic exchangers are employed when separations involving harsh chemical conditions such as high temperature, high radiation level, strong basic solution or powerful oxidizing agent.
- E.g., Titanium arsenate has been used to absorb alkaloids.
- Hydrous antimony peroxide has been used to study exchange equilibrium of Ka and Rb ions with hydrogen and other ions.[9]

Properties Of Ion Exchange Resin

- It must be chemically stable
- They are almost insoluble in water, benzene, ether etc.
- It must contain sufficient no of ion exchange groups.
- It should have a sufficient degree of cross linking.
- The swollen resin must be denser than water.

Practical Requirements

1. Column material and dimension
2. Type of ion exchange resin and physical characteristics

a. Type of ions

b. Nature of ions

c. Efficiency of resin

d. Particle size

e. Structural type IAJPS 2023,

3. Packing of the column

4. Mobile phase

5. Development of the chromatogram

6. Analysis of the elite

7. Regeneration of the ion exchange resin

1**. Column material and dimensions:**

Columns used in the laboratories are made up of glass. In industries are made up of either high quality stainless steel or polymers which are resistant to strong acids and alkalis. The column dimensions are also important and a length: diameter ratio of 20:1 to 100:1 for higher efficiency can be used.

2.Type of ion exchange resin:

- Type of ions – Cations or Anion
- Nature of ions – Strong or Weak

Efficiency of the resin – It is measured by ion exchange capacity Ion exchange capacity: It is the total ion exchange capacity in terms of the exchangeable functional groups expressed as milli equivalents per gram of the exchange resin. m.eq/g = 1000/eq. wt. Particle size of the resin - 50- 100 mesh or 100-200 mesh is used. Structural type of the resin - porous, pellicular Amount of cross-linking agent present -Which decides swelling of the resin [10]

3.Packing in of the column: -

- Wet packing method is used. ¬ Resin + mobile phase
- Packing in the column uniformly

4. 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.: 0.1 N HCl,1N NaOH, Phosphate buffer, acetate buffer, borate buffer, phthalate buffer

5. Development of the chromatogram and elution

After introduction of the sample, development of the chromatogram is done by using different mobile phases. As mentioned earlier, organic solvents are less useful and only acids, alkalis and buffers of different pH are used.

There are two elution techniques:

- Isocratic elution
- Gradient elution

Isocratic elution

Same solvent composition is used. i.e., same strength of acid or alkali or buffer.

Gradient elution

In gradient elution technique, initially less acidic or basic character is used followed by increasing the acidity or basicity of the mobile phase [11]

6**. Analysis of the elute**: -

Several methods of analysis can be used which depends upon the nature and quantity of the sample.

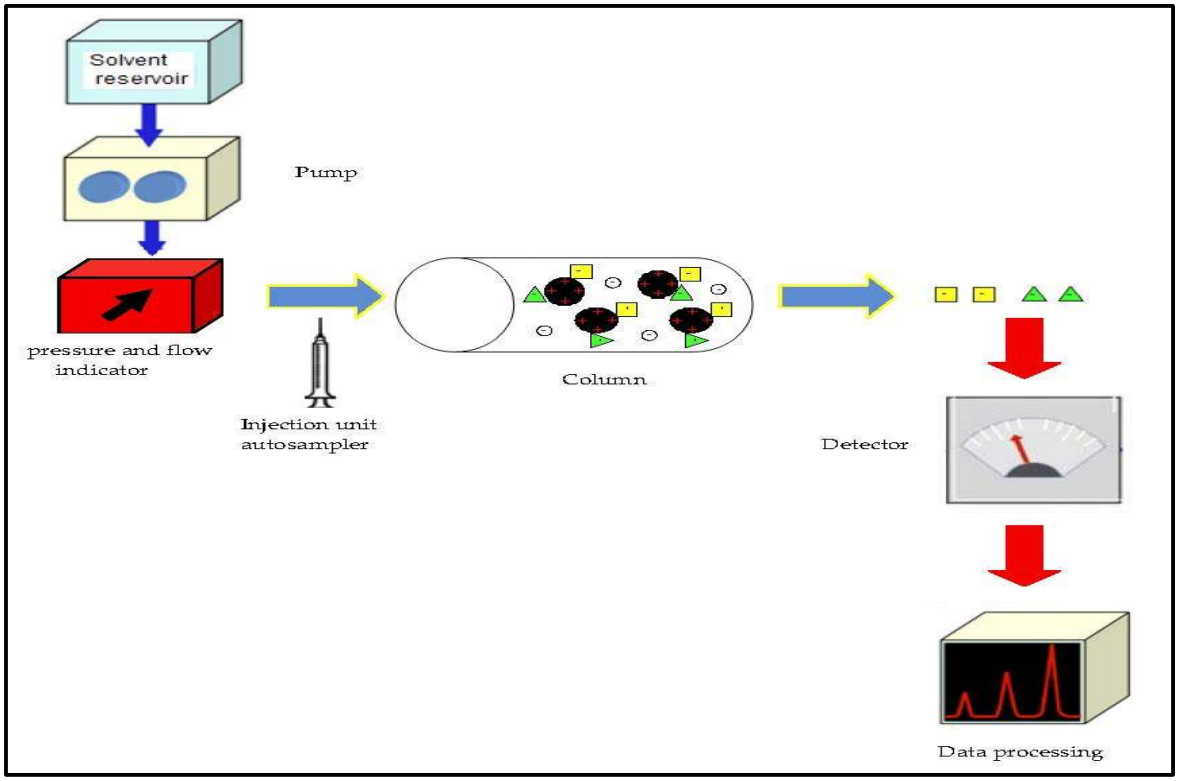
1. Spectrophotometric method
2. Polarographic method
3. Conductometric method
4. Amperometry method
5. Flame photometric method
6. Radiochemical method

Geiger Muller Counter - Ionization Chamber method After analyzing, similar fractions are mixed in order to get pure ion or compound of each type.

7. Regeneration of the ion exchange resin: -

Regeneration makes the used ion exchange resin to be as efficient as a virgin resin. Regeneration refers to the replacement of the exchangeable cations or anions present in the origin resin. Hence regeneration of the Cation exchange resin is done by the charging the column with strong acid like hydrochloric acid. Regeneration of anion exchange resin is done by using strong alkali like sodium hydroxide or potassium hydroxide.[12]

Ion Exchange Chromatography Instrumentation



Components of ion exchange chromatography

- Mobile phase
- Degasser
- Pump system
- Injector
- Column or Stationary phase
- Ionic exchanger
- Suppressors
- Detector

1. **Mobile phase**

   The eluent is the mobile phase that has a fixed or variable concentration during the chromatographic run using an eluent generator cartridge, which contains the appropriate concentrated electrolyte solution for the eluent being generated. The sample is initially dissolved in this eluent, which is then led to the stationary phase. This allows for the separation of different components or elements that are contained in a sample solution. Depending on the analysis of anions or cations, different eluent solutions are used. The most common eluent for anion analysis is a dilute buffer solution containing sodium bicarbonate and sodium carbonate. Alternatively, sodium or potassium hydroxide may be used as the eluent. In cation analysis, the eluent is normally a solution of dilute acid such as sulfuric, acetic, citric, sulfonic and carboxylic acids.

2. **Degasser**

   The quality of the eluent significantly affects chromatographer performance and wear is a way to ensure high quality of the eluent. The degasifier is a high-pressure gas removal device that removes electrolysis gases created during the generation of eluents, preventing the formation of bubbles caused by the gases outlet in the eluent ratio valves, pumps "heads" and detector cell, which may interfere with the chromatographic process.

3. **Pump systems**

   In general, alternative double piston pumps are used to reduce noise in the chromatogram base line, as it provides the constant and pulses free flowing phase needed for sensitive detectors such as conductivity, UV/VIS and amperometry. Therefore, an electronic circuit in combination with pulse shock absorbers is used to reduce the maximum residual pulse. Some pumps operate only in isocratic mode or with isocratic capacities and gradients. While isocratic is the standard choice for

routine cationic analysis, gradient elution allows the separation and analysis of a considerably larger range of ions.

4. **Injector**

   The sample is injected into the system through a valve injector. The injection valve typically contains six ports in a two-position change format. One position is used to load the sample in the injection circuit (Fig) and, after that, there is a change of the injection valve, this being the second position, where the sample is transported by the mobile phase to the guard column and, in then to the separation column.

5. **Column or Stationary**

   Phase Separation columns or chromatographic columns, also known as stationary phase, are supports packed in cylindrical tubes of different diameters and lengths, which have inlet and outlet at their ends. The internal contents of these columns are generally porous and solid substrate particles with positively charged or negatively charged ionic functional groups on their surface. An overview of stationary phases used for ion chromatography was described by Weiss and Jensen illustrated in figure.[13]

6. **Ionic exchangers**

   Functional groups have three different types of ion exchangers. In cation exchange chromatography, negatively charged ligands bond to positively charged molecules (Fig. 4A), whereas the opposite occurs for anion exchange chromatography, whereas Zwitterionic ligands have both charges in a single column.[14]

7. **Suppressors**

   After sample separation and before detection there is a suppressor cell. The main function of the electrochemical suppressor system as part of the detection unit is to reduce the background signal of the mobile phases, increasing the detectability of sample ions. Thus, the high background conductivity of the electrolytes in the eluent is reduced through a selective exchange membrane, lowering the baseline and enabling detection at low concentrations.

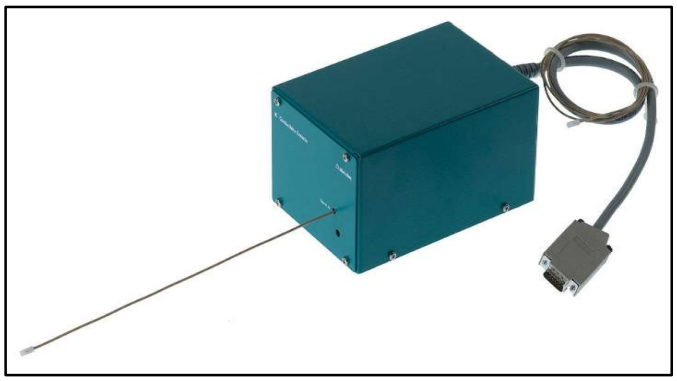
8. **Detectors**

   Passing through the suppressor, the analyte is conducted to the detector of the apparatus. There are several types of detectors, they can be conductivity, amperometry, potentiometric, spectrophotometric or fluorescence.

Conductivity Detector

A conductivity detector in ion exchange chromatography measures the change in electrical conductivity of the eluent as ions pass through it after being separated by the stationary

phase. This detector is ideal for ion chromatography because it is sensitive to ions and produces a signal proportional to their concentration, allowing for the detection and quantification of a wide range of inorganic and organic ions. To improve sensitivity, an ion suppressor is often used to lower the background conductivity of the eluent.[15]

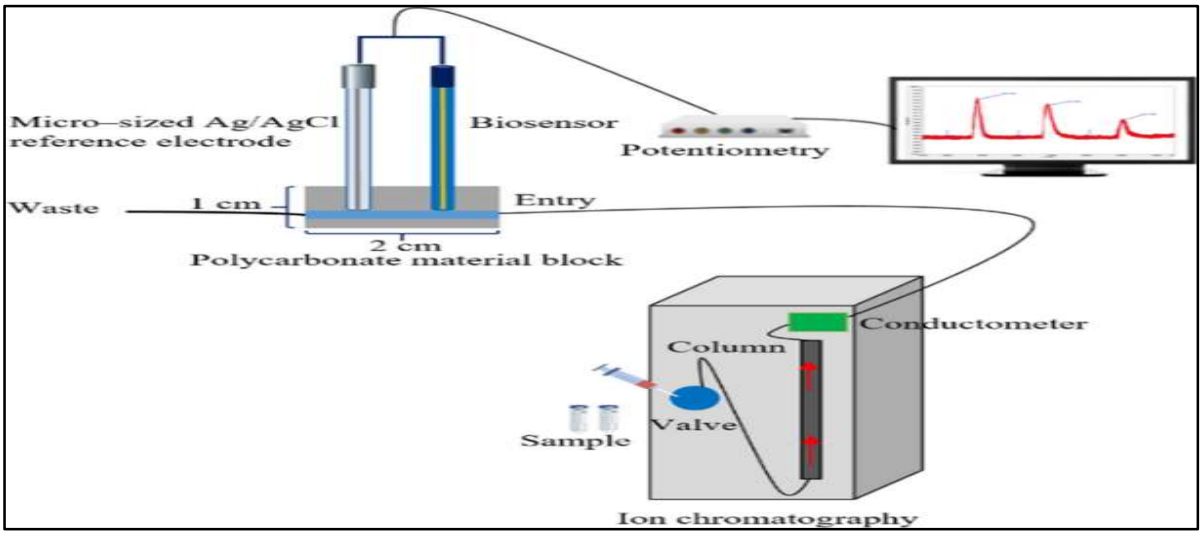


Amperometry detector

Amperometry detection in ion chromatography (IC) is an electrochemical technique that measures the current produced when analytes are oxidized or reduced at a working electrode as they elute from the column. This method is highly sensitive and is used to detect electroactive compounds after separation by IC, with common techniques including constant potential, pulse amperometry detector (PAD) Integrated pulse amperometry detector (IPAD). It is particularly useful for analyzing carbohydrates, amino acids, and certain ions like cyanide.[16]

Potentiometric Detector

A potentiometric detector in ion chromatography (IC) measures ion concentrations by detecting changes in the potential difference between an indicator and a reference electrode. It's used in microfluidic device and is attractive for its simplicity, sensitivity, and ability to analyze samples without interference from dissolved oxygen. Common types include metallic copper electrodes for direct detection of some ions, and ion selective electrode (ISEs) with polymeric membranes for detect in specific ion like ammonia.



ION EXCHANGE TECHNIQUES

There are two methods,

1. Batch method

2. Column method

1. Batch method

- It involves single step equilibrium processes
- Resin + solutions are mixed in a vessel
- Filter the solution
- Only a single portion of the exchange capacity of the resin is utilized
- It is used for softening of water and production of deionized water

2. Column method

- It involves in separation of components of a mixture by selecting different coefficient of resin.
- The difference in selectivity coefficient leads to different migration rates on ion exchange column.
- Chromatography process is classified according to physically state of mobile and stationary phases.[17]

Advantages

1. It is one of the most efficient methods for the separation of charged particles.
2. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.
3. Ion exchange is used for both analytical and preparative purposes in the laboratory, the analytical uses being the more common.
4. Inorganic ions also can be separated by ion-exchange chromatography [18]

Disadvantages

Only charged molecules can be separated.

1. Buffer Requirement.
2. Column efficiency is less.
3. It is difficult to achieve control over selectivity and resolution.
4. Stability and reproducibility of the columns become questionable after repeated use.
5. Nature of exchanging ions is not known.
6. The organic matter Fe3+ occurring in some water which can foul the resin.[19]

APPLICATIONS

Ion exchange chromatography can be applied for the separation and purification of many charged or ionizable molecules such as proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins and etc. from natural sources or synthetic origin. Some of its applications are as follows;

Separation of similar ions

The ion exchange chromatography is used for separation of similar ions as different ions undergo exchange reactions to different extent.

E.g.: A mixture of OH+, Na+ and K+ can be separated by using cation exchange resin. Similarly, Cl-, Br-, I can be separated by passing through basic anion exchange.

Softening of hard water

Hardness of water is due to the presences of Ca2+, Mg2+ and other divalent ions may be removed by passing the hard water through the cation exchanger

$2Na_n R^- + nCa^{2+} = Ca_nR^{2-} + 2Na^+$

charged with Na+ ions. Then the following exchange reaction takes place:

$Ca_n R^- + 2_nNa^+ = nCa^{2-} + 2Na_nR$

The Ca+, Mg+ ions from water are retained on the column while Na+ ions pass into the solution. These Na+ ions are harmless for washing purpose. After using the ion exchange for a time, it becomes in active. Percolating through it a concentrated solution of NaCl. when the following reverse reaction takes place can revive its activity.[20]

Complete demineralization of water

This requires complete removal of ions i.e., both cations and anions. For this, water is passed through an acidic cation exchanger then metallic cations are exchanged with H+ ions. The water obtained is then passed through a basic anion exchanger then the anions present in the water are exchanged by OH- of the exchanger. The H+ and OH- ions which pass into solution combine to form unionized water. E.g.: Cation exchanger- Sulphonic acid resin is commonly used. Anion exchanger- Strong basic resin is used.

Purification of organic compounds

Many natural products extracted in water have been found to contain ions originally present in water. Those ions can be removed by using ion exchange process

Separation of sugars

This method is developed by Khym and Zill in 1951. Sugars are first converted into borate complex and the separation of borate complexes and the separation of borate complexes have been achieved quantitatively on columns by using ion exchange chromatography. In this, disaccharide can be separated from monosaccharides and the individual compounds of hexose, pentose from the mixture can be resolved.

Separation of amino acids

Ion exchange methods can be used to separate the complex mixture of 18 amino acids obtained by the acid hydrolysis of proteins. The mixture of amino acids is first introduced on a very short column at pH 2 and eluted with 0.35N sodium citrate buffers at pH 5.25. Acidic and neutral amino acids at first leave the column unseparated and after that other amino acids are separated. Similarly, a mixture of vitamins like vitamin B1, B2, B6, Niacin, Folic acid, B12 etc. can be separated using ion exchange technique.

Purification and recovery of pharmaceuticals

The process is used for purification and recovery of antibiotics, vitamins, alkaloids, hormones and other chemicals of pharmaceutical importance during their manufacturing process.

Medicinal importance

Anionic resins are introduced in the treatment of ulcer while cation exchangers have been used to remove Na+ from body during the treatment of hypertension and edema. The resins are also used as a diagnostic aid in gastric acidity tests. The resins have been successfully used with other medicinal agents to achieve delayed action dosages.

Biochemical separations

Used for biochemical separations like some drugs or metabolites from blood, urine or other biological fluids.

Ion exchange column in HPLC

For separation of compounds of mixed nature like acidic and basic substances, ion exchange is used in HPLC.

Concentration of ionic solutions

A cation or anion from a bulk of solution can be adsorbed onto ion exchange resins, after adsorption, it can be eluted by using small volume of eluent.

Separation of lanthanides

Solution having mixture of lanthanides is passed through a column packed with particles of a suitable ion exchange resin. Cations present in solution undergo exchange with hydrogen with hydrogen ions.

Separation of actinides

The IEC technique has played a unique role in the discovery on the trans plutonium elements in the actinide series. The power of the method can be judged from the order of elution of lanthanides and actinide ions in the +3-oxidation state from a cation exchange resin column with an aqueous solution of ammonium hydroxyl is butyrate. In the actinide series also, the elution occurs in the reverse order of the atomic number due to actinide contraction and this proved that IEC is only way for identifying these elements.

Removal of interfering radicals

The estimation of Ca+ or Br+ ions is carried out by the oxalate or sulphate method in which phosphate ion is found to interfere. Therefore, its removal becomes necessary which is achieved by passing a solution of Ca+ or Ba+ ions through suitable ion exchanger in the column. The process has to be repeated so that the phosphate ions are completely removed. Now, the calcium and Ba+ ions held by resin will be removed by using suitable eluent. Finally, these ions are estimated by the usual methods.[21]

Other applications

- For the measurement of various active ingredients in medicinal formulations.
- For the measurement of drugs and their metabolites in serum and urine, for residue analysis in food raw materials.
- For the measurement of additives such as vitamins and preservatives in food and beverages.[22]

CONCLUSION:

Ion exchange chromatography is a technique often used in protein purification, water analysis and quality control. It can be used for large proteins, small nucleotides and amino acids. The principle of ion exchange chromatography was that, charged molecules binded electrostatically to oppositely charged groups that have been bound covalently on the matrix. This method is widely applicable to the analysis of a large number of molecules with high capacity. The technique is easily transferred to the manufacturing scales with low cost. High levels of purification of the desired molecule can be achieved by ion exchange step.

REFERENCES:

1. Mohammed JS; A brief review on Ion Exchange Chromatography; Pharma Tutor; 2017; 2nd edition; 5(2); page no; 30-38.

2. "Instrumental methods of Chemical Analysis", 5th edition, Galen W. Ewing, McGraw Hill Book Company 1988.Page no.678-698.
3. Instrumental methods of chemical analysis by B.K. Sharma,8 th edition;2000; Page no;123-150.
4. Instrumental analysis of chemical analysis by Gurdeep. R. Chatwal, Sham.K. Anand,2015; 3 rd. edition; Pg.no; 2.662-2.672.
5. Fritz J.J. Early milestones in the development of ion exchange chromatography: a personal account. Journal of chromatography A 2004; 4 th edition; page no. 10393-12.
6. Haddad P.R, Jackson P.E. Journal of Chromatography Library- Volume 46 Ion Chromatography. Amsterdam: Elsevier Science;1990, 4th edition; page no.78.9-79.9.
7. Wilson. K, Walker, J. (2018). Principles and Techniques of Biochemistry and molecular biology 8th edition. Cambridge University Press: New York; page no. 1.23-1.26.
8. Haffner. T.R. Pharmaceutical chemistry, Theory and application,2017; Volume 1, Pg no: 347-365.
9. Davis B.M. Practical pharmaceutical chemistry" 4 th edition, Part 2, by Beckette and stenlake, CBS publishers and Distributors,2014, pg.no;157-174.
10. Paterson. R,1970. An Introduction to Ion Exchange. London: Heyden and Son Ltd. 5 th edition. Page no. 345-367.
11. Willard Merritt Dean settle; Instrumental methods of analysis; 7th edition;2006; page.no. 633-639.
12. Kenneth A. Connors; A Text book of pharmaceutical Analysis; 3rd edition, 1998; page no. 398-408.
13. Amersham Pharmacia biotech; Ion Exchange Chromatography: Principles and Methods 2016; 6 th edition; page no.283-300.
14. Parimoo.P.; Pharmaceutical analysis;1999;12th edition; Page. No. 284-286.
15. Robert. D. Braun; Introduction to Instrumental Analysis;2001; Page. No. 850-859.
16. Dr. Katsura A. V, Dr K. R. Mahadik, Dr. S.G. Wadodkar, Dr.H.N. More; Pharmaceutical Analysis; Vol-II; Page. No. 39-47.
17. Mendham J., R.C Denny, J. D. Barnes, M. Thomas, B. Siva Sankar; Text Book of Quantitative Chemical Analysis;6th edition;(1998) page. No.213-222.
18. Day.R. A, Underwood; Quantitative Analysis 2016; 6th edition; page. No. 535-543.
19. Mianbin Wu, Xuewan Wang, Zhengyu Zhang and Rutao Wang; Isolation and

Purification of bioactive proteins from Bovine colostrum.2015; 8 th edition page no. 23-27.

19. Ahamed T, N for BK, Verhaert PDEM, van Dedem GWK, van der Wielen LAM, Eppink MHM, et al. Ph -gradient ion exchange chromatography.2012;12th edition; Page no. 181- 188.
20. Harris, Daniel. C. Quantitative Chemical Analysis. 7th edition. Freeman and Company, New York.2005; page no. 456-487.
21. Anees A. Siddiqui; Pharmaceutical Analysis;2012; 6th edition; Volume-1; page.no 13- 18.