# Ion-Exchange Chromatography and Its Applications

Ion-exchange chromatography (IEC) is an important analytical technique for the separation and determination of ionic compounds. This chromatography is one of the most important adsorption techniques used in the separation of peptides, proteins, nucleic acids and related biopolymers which are charged molecules in different molecular sizes and molecular nature [[3](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B3)-[6](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B6)]. The separation is based on the formation of ionic bonds between the charged groups of biomolecules and an ion-exchange gel/support carrying the opposite charge [[7](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B7)]. Biomolecules display different degrees of interaction with charged chromatography media due to their varying charge properties [[8](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B8)].Ion-exchange chromatography has been used for many years to separate various ionic compounds; cations and anions and still continues to be used. The popularity of ion exchange chromatography has been increased in recent years because this technique allows analysis of wide range of molecules in pharmaceutical, biotechnology, environmental, agricultural and other industries [[2](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B2)].

### 1.1. Ion exchange mechanism

Ion-exchange chromatography which is designed specifically for the separation of differently charged or ionizable compounds comprises from mobile and stationary phases similar to other forms of column based liquid chromatography techniques [[9](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B9)-[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)]. Mobil phases consist an aqueous buffer system into which the mixture to be resolved. The stationary phase usually made from inert organic matrix chemically derivative with ionizable functional groups (fixed ions) which carry displaceable oppositely charged ion [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)]. Ions which exist in a state of equilibrium between the mobile phase and stationary phases giving rise to two possible formats, anion and cation exchange are referred to as counter ion ([Figure 1)](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#F1) [[1](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B1),[13](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B13)]. Exchangeable matrix counter ions may include protons (H+), hydroxide groups (OH-), single charged mono atomic ions (Na+, K+, Cl-), double charged mono atomic ions (Ca2+, Mg2+), and polyatomic inorganic ions (SO42-, PO43-) as well as organic bases (NR2H+) and acids (COO-) [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)]. Cations are separated on cation-exchange resin column and anions on an anion exchange resin column [[10](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B10)]. Separation based on the binding of analytes to positively or negatively charged groups which are fixed on a stationary phase and which are in equilibrium with free counter ions in the mobile phase according to differences in their net surface charge.



Ion exchange chromatography involves separation of ionic and polar analytes using chromatographic supports derivatized with ionic functional groups that have charges opposite that of the analyte ions. The analyte ions and similarly charged ions of the eluent compete to bind to the oppositely charged ionic functional group on the surface of the stationary phase. Assuming that the exchanging ions (analytes and ions in the mobile phase) are cations, the competition can be explained using the following equation;

S-X-C+ + M+↔ S-X-M++ C+

In this process the cation M+ of the eluent replaced with the analyte cation C+ bound to the anion X- which is fixed on the surface of the chromatographic support (S).

In anion exchange chromatography, the exchanging ions are anions and the equation is represented as follow;

S-X+A- + B-↔ S-X+B-+ A-

The anion B- of the eluent replaced with the analyte cation A- bound to the positively charged ion X+ on the surface of the stationary phase. The adsorption of the analyte to the stationary phase and desorption by the eluent ions is repeated during their journey in the column, resulting in the separation due to ion-exchange [[2](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B2)].

Molecules vary considerably in their charge properties and will exhibit different degrees of interaction with charged chromatography support according to differences in their overall charge, charge density and surface charge distribution. Net surface charge of all molecules with ionizable groups is highly pH dependent [[13](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B13)]. Therefore pH of the mobile phase should be selected according to the net charge on a protein of interest within a mixture is opposite to that of matrix functional group, that it will displace the functional group counter ion and bind the matrix. On the other hand oppositely charged proteins will not be retained. Adsorbed protein analytes can be eluted by changing the mobile phase pH which effect the net charge of adsorbed protein, so its matrix binding capacity. Moreover increasing the concentration of a similarly charged species within the mobile phase can be resulted in elution of bound proteins. During ion exchange chromatography for example in anion exchange as illustrated in [Figure 2](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#F2), negatively charged protein analytes can be competitively displaced by the addition of negatively charged ions. The affinity of interaction between the salt ions and the functional groups will eventually exceed that the interaction exists between the protein charges and the functional groups, resulting in protein displacement and elution by increasing gradually the salt concentration in the mobile phase [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)].

This technique has been used for the analyses of anions and cations, including metal ions, mono- and oligosaccharides, alditols and other polyhydroxy compounds, aminoglycosides (antibiotics), amino acids and peptides, organic acids, amines, alcohols, phenols, thiols, nucleotides and nucleosides and other polar molecules. It has been successfully applied to the analysis of raw materials, bulk active ingredients, counter ions, impurities, and degradation products, excipients, diluents and at different stages of the production process as well as for the analysis of production equipment cleaning solutions, waste streams, container compatibility and other applications [[2](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B2)]. Wide applicability including high performance and high-throughput application formats, average cost, powerful resolving ability, large sample handling capacity and ease of scale-up as well as automation allow the ion exchange chromatography has become one of the most important and extensively used of all liquid chromatographic technique [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)].

Equilibrium is established for each sample component between the eluent and stationary phases when a sample is introduced into the ion-exchange chromatography. The distribution of component (A) between the two phases is expressed by the distribution coefficient, “DA”.

DA= [A]r/ [A]m

The value of DA is dependent on the size of the population of molecules of component A in the stationary and eluent phases [[1](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B1)]. As the equilibrium is dynamic, there is a continual, rapid interchange of molecules of component A between the two phases.

**Instrumentation:**
General components of an ion-exchange chromatography are presented as below ([Figure 4)](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#F4).

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

In ion-exchange chromatography, adsorption and desorption processes are determined by the properties of the three interacting entities;

* The stationary phase,
* The constituents of the mobile phase
* The solute [[18](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B18)].

### 1.2. Stationary phase

Selection of a suitable ion-exchange matrix probably is the most important in ion exchange protocol and is based on various factors such as; ion exchanger charge/strength, linear flow rate/sample volume and sample properties [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)]. In ion-exchange chromatography, numerous stationary phases are available from different manufacturers, which vary significantly in a number of chemical and physical properties [[6](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B6),[18](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications%22%20%5Cl%20%22B18)]. Stationary phases comprised of two structural elements; the charged groups which are involved in the exchange process and the matrix on which the charged groups are fixed [[18](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B18)]. Ion exchangers are characterized both by the nature of the ionic species comprising the fixed ion and by the nature of the insoluble ion-exchange matrix itself [[1](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B1)].

Ion exchangers are called cation exchangers if they have negatively charged functional groups and possess exchangeable cations. Anion exchangers carry anions because of the positive charge of their fixed groups [[15](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B15)]. The charged groups determine the specifity and strength of protein binding by their polarity and density while the matrix determines the physical and chemical stability and the flow characteristics of the stationary phase and may be responsible for unspecific binding effects [[18](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B18)].

### 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 of the ionic compounds are dissolved better than in others in. Therefore the application of various samples is much easier [[1](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B1),[3](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications%22%20%5Cl%20%22B3)]. Sodium chloride is probably the most widely used and mild eluent for protein separation due to has no important effect on protein structure. However NaCl is not always the best eluent for protein separation. Retention times, peak widths of eluted protein, so chromatographic resolution are affected by the nature of anions and cations used. These effects can be observed more clearly with anion exchangers as compared to cation exchangers [[14](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B14)]. The salt mixture can itself be a buffer or a separate buffer can be added to the eluent if required. The competing ion which has the function of eluting sample components through the column within reasonable time is the essential component of eluting sample. Nature and concentration of the competing ions and pH of the eluent are the most important properties affecting the elution characteristics of solute ions [[1](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B1)].

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. The concentration of competing ion exerts a significant effect by influencing the position of the equilibrium point for ion-exchange equilibrium. The higher concentration of the competing ion in the eluent is more effectively displace solute ions from the stationary phase, therefore solute is eluted more rapidly from the column. Additionally elution of the solute is influenced by the eluent flow-rate and the temperature. Faster flow rates cause to lower elution volumes because the solute ions have less opportunity to interact with the fixed ions. Temperature has relatively less impact, which can be change according to ion exchange material type. Enhancement of the temperature increases the rate of diffusion within the ion-exchange matrix, generally leading to increased interaction with the fixed ions and therefore larger elution volumes. At higher temperatures chromatographic efficiency is usually improved [[1](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B1)].

Isocratic elution or gradient elution can be applied for elution procedure. A single buffer is used throughout the entire separation in isocratic elution. Sample components are loosely adsorbed to the column matrix. As each protein will have different distribution coefficient separation will achieved by its relative speeds of migration over the column. Therefore in order to obtain optimum resolution of sample components, a small sample volume and long exchanger column are necessary. This technique is time consuming and the desired protein invariably elutes in a large volume. However no gradient-forming apparatus is required and the column regeneration is needless. Alteration in the eluent composition is needed to achieve desorption of desired protein completely. To promote desired protein desorption continuous or stepwise variations in the ionic strength and/or pH of the eluent are provided with gradient elution. Continuous gradients generally give better resolution than stepwise gradients [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)].

### Buffer

In ion exchange chromatography, pH value is an important parameter for separation and can be controlled and adjusted carefully by means of buffer substances [[18](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B18)]. In order to prevent variation in matrix and protein net charge, maintenance of a constant mobile phase pH during separation is essential to avoid pH changing which can occur when both protein and exchanger ions are released into the mobile phase [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)]. By means of buffer substances pH value can be controlled and adjusted. 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. Thus a suitable pH range, in which the stability of sample is guaranteed, has to be identified. Keeping of the sample function is related with the preservation of its three dimensional structure as well as with its biological activity [[18](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B18)]. A number of buffers are suitable for ion-exchange chromatography. A number of important factors influences the selection of mobile phase including buffer charge, buffer strength and buffer pH [[11](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B11)]. Properties of good buffers are high buffering capacity at the working pH, high solubility, high purity and low cost.

### Detection

Conductivity detector is the most common and useful detector in ion exchange chromatography. However UV and other detectors can also be useful [[10](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B10)]. Conductivity detection gives excellent sensitivity when the conductance of the eluted solute ion is measured in an eluent of low background conductance. 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 [[1](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B1)].

Although recorders and integrators are used in some older systems, generally in modern ion exchange chromatography results are stored in computer. Retention time and peak areas are the most useful information. Retention times are used to confirm the identity of the unknown peak by comparison with a standard. In order to calculate analyte concentration peak areas are compared with the standards which is in known concentration [[10](https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications#B10)].

## Ion exchange chromatography 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.