Ion Exchange Chromatography

Ion exchange chromatography is used to remove ions of one type from a mixture and replace them by ions of another type.

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. 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. 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 (Fig. 1).

Exchange-able matrix counter ions may include protons (H^+), hydroxide groups (OH-), single charged mono atomic ions (Na^+, K^+, Cl^-), double charged mono atomic ions (Ca^{2+}, Mg^{2+}), and polyatomic inorganic ions (SO_4^{2-}, PO_4^{3-}) as well as organic bases (NR_2H^+) and acids (COO^-). Cations are separated on cation-exchange resin column and anions on an anion exchange resin column. 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 (Fig. 1).

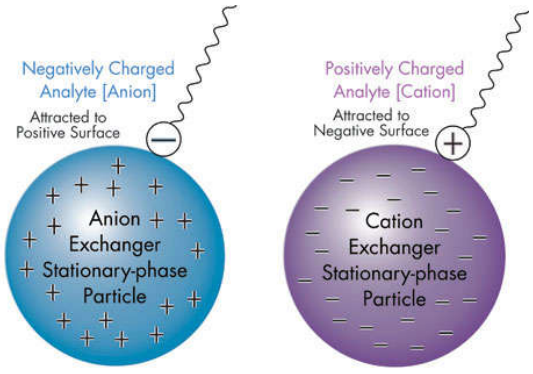


Fig 1. Types of ion exchangers

The column is packed with porous beads of a resin that will exchange either cations or anions. There is one type of ion on the surface of the resin and these are released when other ions are bound in their place – e.g. a basic anion exchange resin might remove nitrate (V) ions (NO_3^-) from a solution and replace them with hydroxide ions (OH^-).

Many of the resins used are based on phenylethene (styrene) polymers with crosslinking via 1,4-bis-ethenylbenzene (divinylbenzene, Fig. 2).

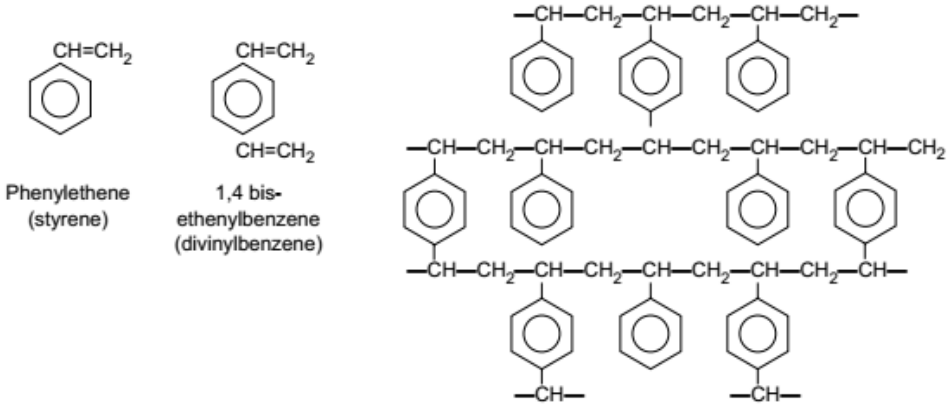


Fig 2. Copolymer of cross-linked styrene–divinylbenzene

If the ion is a quaternary ammonium group the resin is strongly basic (eg –CH $2N(CH_3)_3^+$ OH^-) then the resin will selectively remove the ions: I^- > NO_3^- > Br^- > NO_2^- > Cl^- > OH^- (> F^-), thus liberating hydroxide ions while nitrate(V) ions, for example, are removed. The exchange site can be strongly or weakly acidic or basic depending on the group present. Examples of such groups are as;

- Strongly acidic $–SO_3^- H^+$
- Weakly acidic $–R–COO^- H^+$
- Weakly basic $–CH_2NH(CH_3)_2{+}Cl^-$
- Strongly basic $–CH_2N(CH_3)_3^+ OH^-$

The degree of cross-linking of the copolymer can have a significant effect on the behavior of the resin, and can change selectivity. The range of cross-linking in commercial resins is approximately 4–12 % of the monomer units and the

characteristics of resins having low and high degrees of cross-linking can be compared (Table 1).

Table 1 : Characteristics of resins having low and high degrees of cross-linking

Low degree of cross-linking	**High degree of cross-linking**
Less rigid	More rigid
More porous	Less porous
Rapid exchange of ions	Slower exchange of ions
Greater swelling in water	Little swelling in water
Lower exchange capacity	Higher exchange capacity
Lower selectivity	Greater selectivity

The selectivity for certain ions can change with the degree of cross-linking because a highly cross-linked resin has smaller pores than one with little crosslinking, and bulky ions cannot get into the smaller pores, so they are not exchanged.

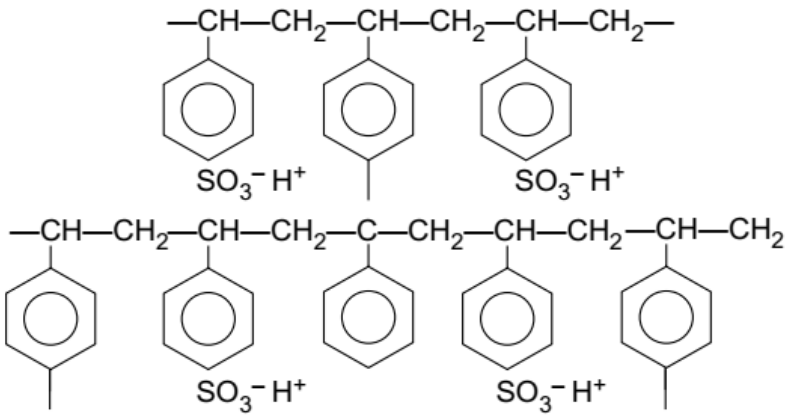


Fig 3. The presence of ionic groups bonded to the polymer of phenylethene and 4-ethenyl phenylethene provides the ability to exchange ions.

Ion exchange equilibrium

If two ions are competing for the binding sites of a resin an equilibrium will be established. For example, a cation–exchange resin used to remove ammonium ions and replace them with sodium ions would have the equilibrium:

$$\text{Resin–Na}^+ + \text{NH}_4{}^+ \rightleftharpoons \text{Resin–NH}_4 + + \text{Na}^+$$

This equilibrium can be described by an equilibrium constant (K) which is called the selectivity coefficient:

$$\text{Selectivity coefficient} = \frac{[\text{Resin}^-\text{NH}_4^+][\text{Na}^+]}{[\text{Resin}^-\text{Na}^+][\text{NH}_4^+]}$$

The equilibrium position can be changed in either direction by changing the concentration of ions in the aqueous phase. A column might remove relatively small amounts of ammonium ions almost quantitatively from a solution, but they can be liberated from the column equally efficiently by passing an excess of a concentrated solution of sodium ions through the column. The removal occurs despite the fact that ammonium ions are preferentially bound to the resin, and this principle is useful in regenerating the column. Once a column's exchange capacity is approached, it is regenerated simply by passing a concentrated solution of sodium ions through it. This shifts the equilibrium back to the left hand side.

Under most circumstances absorption and release of ions is effectively quantitative, so it is possible to remove selected ions and determine their concentrations by titration – e.g. if calcium ions are removed from a sample of hard water and are replaced by hydrogen ions from the acidic form of a resin, then the concentration of hydrogen ions can be determined by titrating the eluate with a standard alkaline solution.

Macromolecules can also be exchanged by using resins with larger pore sizes. The resins are generally derived from proteins or polysaccharides such as cellulose, and the exchange sites are bound to the OH groups of the saccharide units. The sites can be either weakly basic or weakly acidic, with polar covalent groups (e.g. amines); or they can be strongly acidic or strongly basic, with ionic groups (e.g. carboxylic or sulphonic acids).

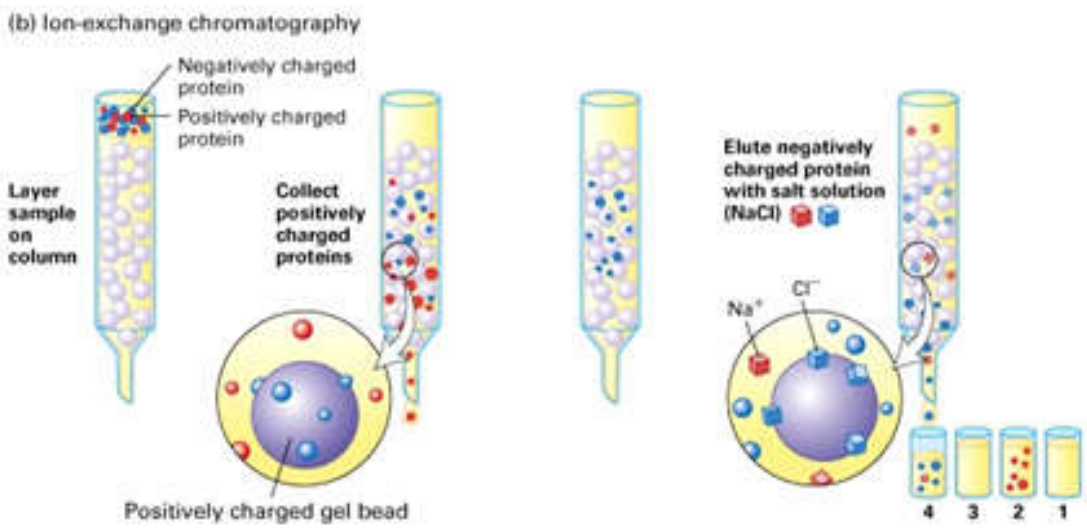


Fig 4. Mechanism of Ion exchange resins

Applications of Ion Exchange Chromatography

1. Solubility of Substances:

The solubility product of some partially soluble substances can be determined by using exchange methods. For example, a saturated calcium sulphate solution can be passed down a cation exchange column that replaces the calcium ions with hydrogen ions:

$$Ca^{2+}_{(aq)} + 2(Resin^{-}H^{+}) \rightarrow Ca^{2+}(Resin^{-})_2 + 2H^{+}_{(aq)}$$

The hydrogen ions liberated can then be determined by titration using a standard sodium hydroxide solution (phenolphthalein as indicator). Once $[Ca^{2+}]$ has been calculated from $[H^{+}]$, the solubility product of the salt can be calculated.

By passing a water sample through two columns, one a cationic exchanger and the other an anionic exchanger, it is possible to remove any ionic impurities. Tap water, for example, can be passed through one column where the metal ions present, such as Mg^{2+} and $Ca^{2+,}$ are replaced by H^{+}; then the anions present, such as F^{-}, Cl^{-} and NO^{3-}, are replaced by OH^{-} in the second column. The product is deionised water.

2. Water Softening:

A more sophisticated application of ion exchange resins is the water softener installed in many modern automatic dishwashers. The cleaning efficiency of dishwashers using detergents increases if the water is heated. This causes problems if the water supply has temporary hardness because when it is heated the hydrogen carbonate ions decompose to form carbonate ions and these are deposited on the heating element, reducing its efficiency. Further problems arise if the ions causing hardness are not removed, because inside the dishwasher they can deposit a film on the items that are meant to be cleaned. To overcome these problems calcium and magnesium ions are removed from the water entering the dishwasher by a cationic exchange resin. They are replaced by sodium ions. ***For example;***

$$(Na^{+})_2\ Resin^{2-} + Ca^{2+}_{(aq)} \rightleftharpoons Ca^{2+}\ Resin^{2-} + 2Na^{+}_{(aq)}$$

The exchange capacity of the resin is regenerated at the start of the next washing cycle by passing saturated sodium chloride (salt) solution through the resin. The high concentration of sodium ions favours the back reaction in the above equilibrium. The unwanted calcium and magnesium ions and the excess sodium chloride are pumped out of the dishwasher during the next drain cycle. It is important that no sodium chloride remains because it is corrosive.

The sodium chloride solution is formed by pumping fresh water through the salt chamber that the user periodically refills. The type of salt put into the dishwasher is important – table salt contains magnesium chloride to keep it free-flowing and the magnesium ions can both cause hardness and interfere with resin regeneration. The small crystal size of table salt also means that it can easily be washed into the resin chamber and cause a blockage. Granular or dendritic salt crystals are recommended to eliminate this risk (minute amounts of potassium hexacyanoferrate(II), $K_4[Fe(CN)_6]$, are added to salt solution to encourage the growth of dendritic crystals).

To minimize unnecessary consumption of salt some dishwashers can be set to take account of the relative hardness of the supply in the area where the dishwasher

is used. Hotpoint dishwashers, for example, pump different volumes of salt solution through the resin according to the hardness of the water.

3. In HPLC Columns

Ion exchange resins can be used in HPLC columns, and a variety of such resins exist. It is possible to monitor the solutes leaving the HPLC column by measuring the conductivity of the solution, and extremely small ion concentrations can be determined by using this method. This is routinely done to monitor water purity in environmental analysis. An anionic exchange resin is used to separate ions. The sample to be analysed is added to an aqueous mobile phase containing sodium carbonate and sodium hydrogen carbonate, which is passed through an anionic exchange resin in an HPLC column. The ions to be detected exchange with carbonate ions on the resin surface. The carbonate ion concentration in the mobile phase is high enough to elute them off again, but at different rates. The anions in the sample are recognized by their retention times on the column, and their concentrations are determined from the conductivity of the eluate.

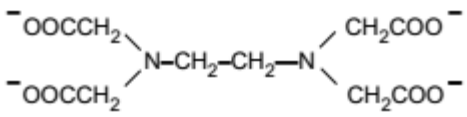
Anions typically elute from the column in the order F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, SO_4^{2-}. Concentrations in the order of 10–3 g dm^{-3} (ppm/μg cm^{-3}) are routinely measured. Measuring concentrations down to 10-6 g dm^{-3} is required in the nuclear power industry, where the leak of radioactive contaminants must be detected (and stopped) immediately.

4. Complexometric Analysis

Many EDTA (ethylenediaminetetraacetate) complexes absorb ultraviolet light, so transition metal concentrations can be determined by making complexes with EDTA. Alternatively, EDTA can be estimated by adding transition metal ions to its solution. This is done in the food industry, where canned shellfish have added EDTA.

Crustacean blood is based on a Cu-haem complex (and not an Fe-Haem complex as in humans). On cooking a blue/black coloring appears where the blood was and this coloring spreads into the flesh making it look unattractive. If EDTA is added it competes for the copper ions and complexes them so that discoloration does not occur. To conform

to legislation the total EDTA concentration must be within certain limits. Excess copper ions are added to ensure that all the EDTA has complexed as $[Cu(EDTA)]^{2-}$, and an ion exchange HPLC column is used to separate all the anions present according to their charge. The eluate containing all the EDTA (identified by its retention time on the column) is then passed through a cell where its ultraviolet absorption at 280 nm is measured; and from this measurement the EDTA concentration can be calculated.



EDTA