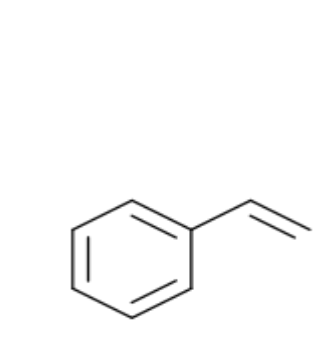
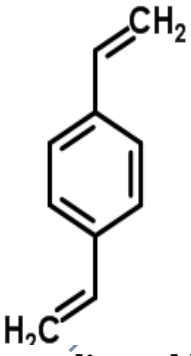
Ion exchange methods

An ion exchanger is a material which consists of resin or matrix carrying certain ion , this material is packed in a column & is used for the same purpose as the column chromatography but here the separation will take place by exchange of the ions between the resin material & the solute . Thus , there is an insoluble phase with fixed ionic sites of one charge , while the oppositely charged species are free to move about & be replaced by other ions of like charge .

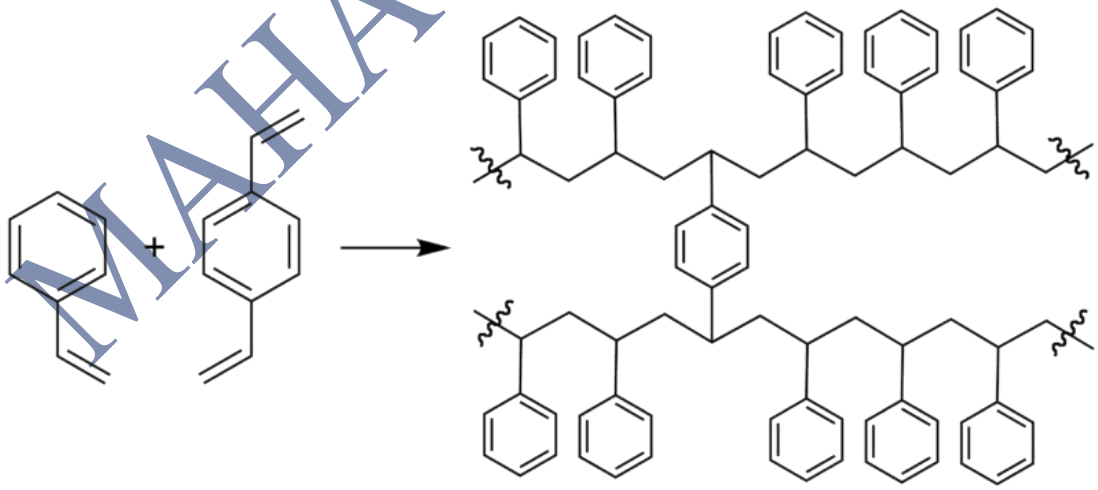
Types of resins : A typical resin is prepared by polymerization of styrene & divinylbenzen



styrene



divenyl benzen



Matrix

There are two major types of ion exchangers:

Cationic exchanger : Acidic functional groups are easily introduced , ex : by sulfonation in which a sulfonic acid group is attached to nearly every aromatic nucleus .Sulfonic acids are strong acids with essentially completely dissociated protons , these protons are not free to leave the resin unless replaced by other positive ions.

Anionic exchanger : If basic functional groups are introduced , then the resin can exchange anions rather than cations . Strong anion exchangers are prepared with a tertiary amine , yielding a strongly basic quaternary ammonium group . Weaker anionic exchangers can be prepared with secondary amines , yielding a weakly basic tertiary amine .

The principle of action is that the insoluble resin has a chemically bound charge group & there is a mobile phase carrying a solute which has a different ion of the same charge . The different ions may be exchanged with other ions of the same charge with out any change in the insoluble resin . If a separation of a mixed solute is needed then the separation consists on binding all the solute to the resin & then the different components of the solute are recovered one at a time .

The net result of an ion exchange reaction can be expressed as a replacement of equivalent quantities of like-charged ions :

$$HR + Na^{+} = NaR + H^{+}$$

$$2HR + Ca^{++} = CaR2 + 2H^{+}$$

$$RCl + OH^{-} = ROH + Cl^{-}$$

Where R or R represents the resin matrix .

This method of ion exchange is used in cases of the formation of salts & also for the quaternary alkaloids which are separated from plant as reinekate salts then they are changed into another salt ex: chlorides by passing them on a chloride ions containing resin .

Gel chromatography (Gel filteration)

The separation of very high molecular weight substances is most readily accomplished by the use of columns packed with gel . Several varieties of gel have recently become available , all of which separate molecules primarily on the bases of their sizes by a " sieving " or " filtering " process . Hence , the names " gel filteration " used by biochemists , & " gel permeation chromatography " used by polymer chemists describe the same general technique .

The gels a very open , three dimentional network formed by cross-linking long polymeric chains . Instead of ion exchange sites , most of these gels have polar groups capable of adsorbing water or other polar solvents . A few are able to adsorb non-polar solvents . In either case , the adsorption causes an opening of the structure, or" swelling " leaving intersticts within the gel . Depending on the extent of cross-linking , there will be a critical size of a molecule that can just penetrate the interior . Larger molecules will pass through the column with no retardation because they cannot enter the gel . Smaller molecules will penetrate the interior to a degree determined by their size i.e these pores are formed from the molecular structure of the gel , when the gel is packed in a column & perculated with a solution they will let the large molecules of the solute to pass quickly down the column with the solvent , the large molecules of a solute pass through the intergranular spaces of the gel because these molecules of a solute do not enter the pores of the gel . Smaller molecules of the solute which are able to enter the pores of the gel will remain in the gel . These molecules will pass more slowly down the column . Each gel has a range of pore size for this reason different size of solute molecules will enter the gel depending whether they fit the pores or not so they will vary in their elution rate so this method is most useful to separate mixtures containing large molecules of various sizes & also to the separation of large molecules from small molecules .

It is mostly used for fractionation of proteins , amino acids , peptides & poly saccharides .

An example of gel used is sephadex which is prepared from polysaccharide dextrans & is used for proteins & other large molecules .

High-performamce liquid chromatography

(High-speed liquid chromatography) HPLC

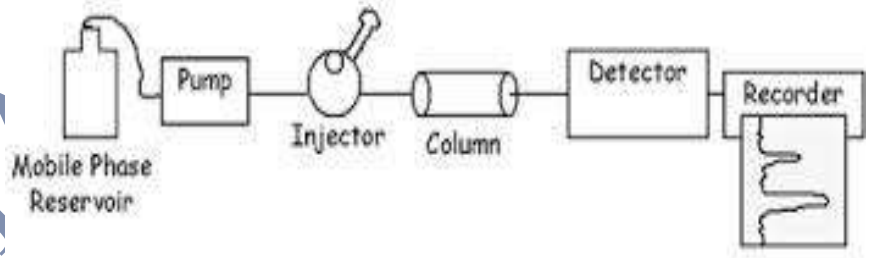
HPLC is a liquid chromatography system which employs relatively narrow columns (about 5mm diameter for analytical work) operating at a temperature up to about 200 C at pressure up to 200 atm . Normal flow rate of eluate are 2-5ml /min but can be up to 10ml/min depending on the diameter of the column & the applied pressure .

The apparatus is suitable for all types of liquid chromatography columns (adsorption , partition , gel filteration , ion exchange , etc) .

Detection of very small quantities of solute in the eluate is possible by continuous monitoring of ultraviolet absorption , mass spectrum , refractive index , fluorescence & electrical conductance etc . For any particular fractionation , some detector systems would be selective for certain groups of compounds & others are universal .

HPLC can give much improved & more rapid separations than can be obtained with the older liquid chromatography methods & it is therefore finding increasing use in numerous areas .

Many stationary phases are available , the most widely used being silica based . in these , which consist of porous particles 5-10um in diameter , the silanol groups Si-OH afford a polar surface which can be exploited in separation using an organic mobile phase as in ordinary adsorption chromatography .



HPLC diagram

Figure 1 A flow diagram of an HPLC system.