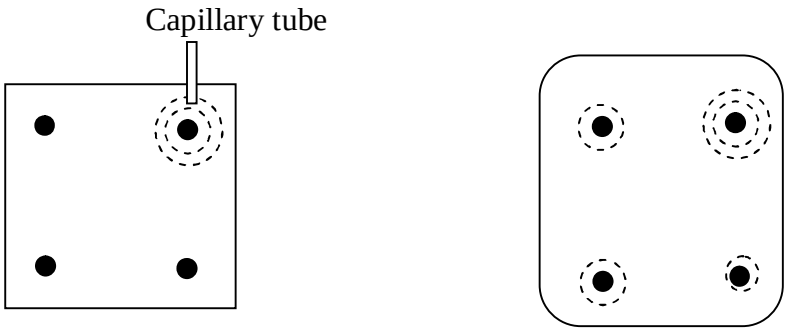
incorporated in small quantity. Other additives to thin layer media include “special fluorescing agent”, which allows spots on the developed chromatogram to be seen under UV light.

The thickness of layers for thin layers for thin layer chromatography is 0.25mm. Thinner layer usually gives a more rapid but less effectives separation.

Choice of medium and solvent

Almost any material can be used for thin layer, and the factors involved in the choice have been discussed earlier. The choice of solvent will depend on the nature of substances being separated and on the material on which it is being separated. A general rule is to match the polarity of the solvent to that of the substances being separated. Sometimes, trial solvents are applied by capillary tube to the center of each spot as shown below.



APPLICATION OF SAMPLE

The method is similar to that of paper chromatography except that the delicacy of many of the layers makes it necessary to take much more care.

Application of Thin – Layer Chromatography

Thin layer does not provide quantitative information of the highest precision and accuracy. The same procedure for quantitative evaluation in PC can also be followed. TLC is very widely used for qualitative purposes. Almost any mixture can be at least partially resolved. Inorganic applications such as separation of metals in alloys, soil and geological samples, and polar organic system, such as mixture of amino acids or sugars in urine are particularly suited for this exercise. TLC is ideally suited for a lot of complex reactions, quality control, purity checks, clinical diagnosis and forensic tests.

ION EXCHANGE CHROMATOGRAPHY

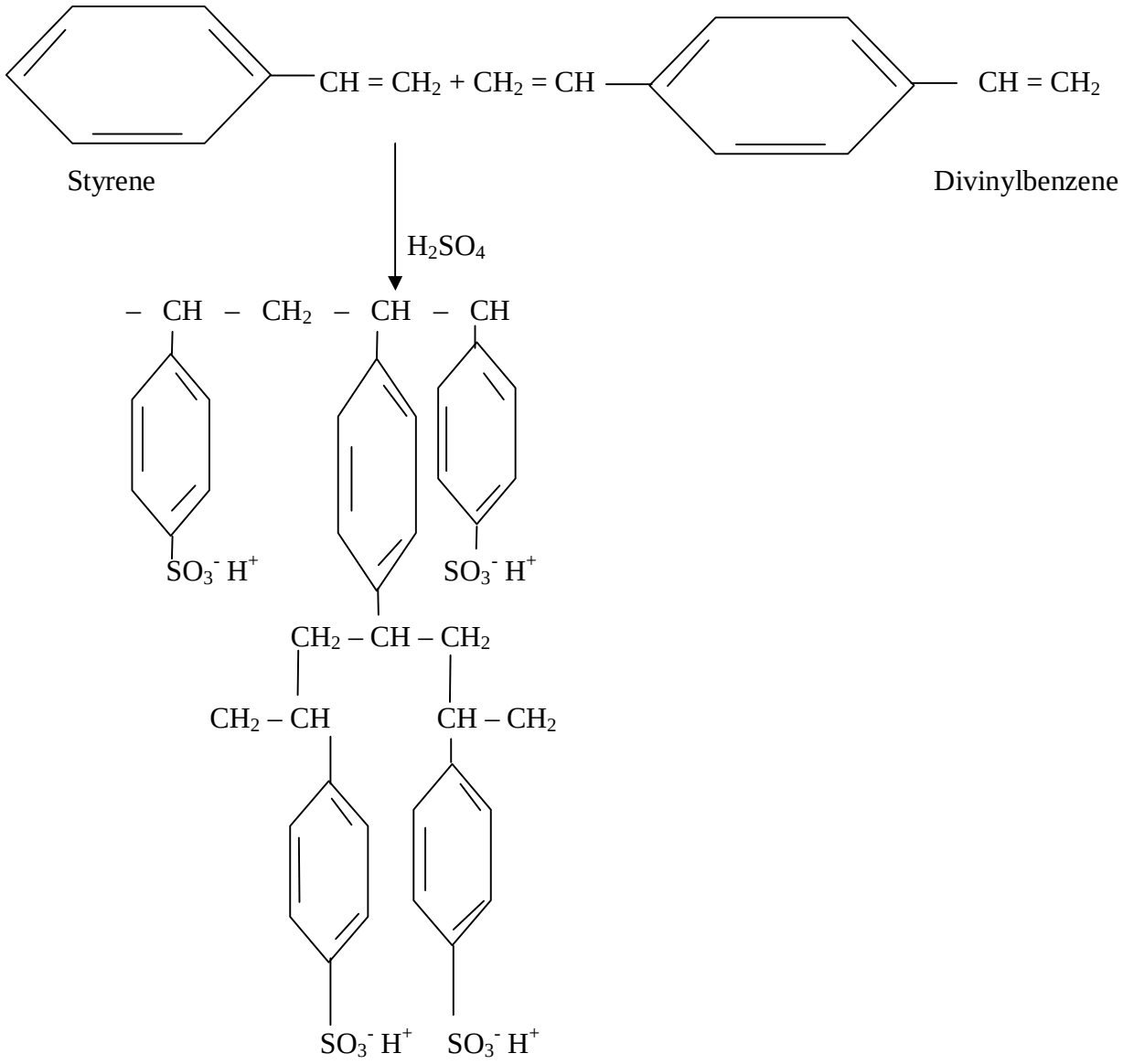
Ion-exchange is generally understood to mean the exchange of ions of like signs between a solution and a solid highly insoluble in contact with it. The solid (ion-exchanger) contains ions of its own and for the exchange to proceed sufficiently; the solid must have an open, permeable molecular structure, so that ions and solvent molecules can move freely in and out. All ion-exchanger sub value in analysis must have the following characteristics:

1. They are almost insoluble in water and in organic solvent.
2. They contain active or counter ions that will exchange reversibly with other ions in surrounding solution without any appreciable physical change occurring in the materials.
3. The ion-exchanger is of complex nature and is in fact polymeric.

The polymer carries an electric charge that is exactly neutralized by the charges on the counter ion. These active ions are cations in CATION EXCHANGER and anions in ANION EXCHANGER. Thus, a cation exchanger consists of a polymeric anion (R^- X^+) with active cation, while an anion exchanger is a polymeric cation (R^+ $X^{-)}$ with active anions.

CATION EXCHANGER

We may define a cation exchange resin as a high molecular weight cross linked polymer containing sulphonic, carboxylic, phenolic etc group, as an integral part of the resin and an equivalent amount of cations e.g. the copolymerization of styrene and divinylbenzene, followed by sulphonation.

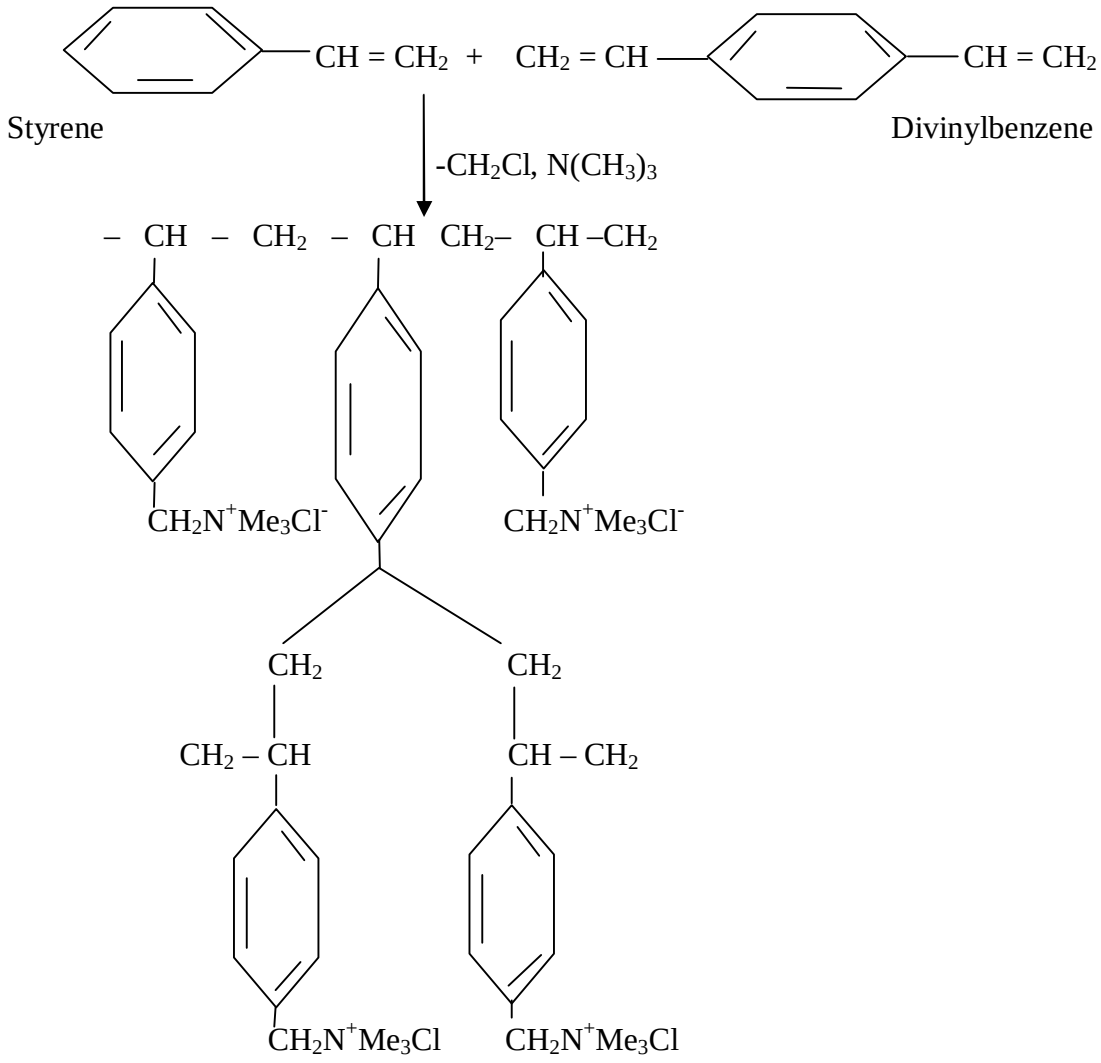


Cation Exchanger

ANION EXCHANGER

Anion exchangers are likewise cross linked high molecular weight polymer. Their basic character is due to the presence of amino, (NH_2), substituted amino or quaternary ammonium group. The resin is a polymer containing the amino, or quaternary ammonium group as integral part of polymer lattice and an equivalent amount of anions such as chlorides, hydroxyl or sulphate ions e.g. copolymerization of

styrene and a little of divinylbenzene followed by chloromethylation (addition of CH_2Cl) and interaction with a base such as trimethylamine.



Anion Exchanger

ACTION OF ION EXCHANGE RESIN

Cation exchange resin can be exchanged for cations in solution.

$(\text{Resin } A^-)B^+ + C^+ \text{ solution} \leftrightarrow (\text{Resin } A^-)C^+ + B^+ \text{ solution}$

If the solution contain several ions (C^+, D^+, E^+), the exchanger may show different affinities for them thus making separation possible.

FACTORS AFFECTING ION EXCHANGE BETWEEN RESIN AND SOLUTION.

Nature of exchanging ions

At low aqueous concentration and at ordinary temperature, the extent of exchange increases with increasing valency of the exchanging ions, i.e. $Na^+ < Ca^{2+} < Al^{3+} < Th^{4+}$.

Under similar condition and constant valency for univalent ions, the extent of exchange increases with decrease in size of the hydrated cation i.e. $Li^+ < H^+ < Na^+ < NH4^+ < K^+ < Rb^+ < Cs^+$, while for divalent ions, the ionic size is an important factor, but incomplete dissociation of salts of bivalent metals also plays a part ($Cd^{2+} < Be^{2+} < Mn^{2+} < Mg^{2+} = Zn^{2+} < Cu^{2+} = Ni^{2+} < Co^{2+} < Ca^{2+} < Sr^{2+} < Pb^{2+} < Ba^{2+}$

With strongly basic anion exchange resins, univalent anions appear to behave similar to univalent cation.

$$F^- < OH^- < HCO_3^- < Cl^- < HSO_3^- < CN^- < Br^- < NO_3^- < I^-$$

In dilute solution, polyvalent anions are generally absorbed preferentially.

When a cation in solution is being exchanged for anion of different valency, the relative affinity of the high valent ion increases in direct proportion to the dilution. Thus, to exchange a higher valent ion on the exchanger for one of lower valency in solution, exchange will be favoured by increasing the concentration, while if the lower valent ion is in the exchanger, and the higher valent ion is in solution, exchange will be favoured by high dilution.

Nature of Ion-Exchange Resin

The absorption of ion will depend upon the nature of the functional group in the resin. It will also depend upon the degree of cross linking. As the degree of cross linking is increased, resin becomes more selective towards ions of different sizes.

ELECTROPHORESIS

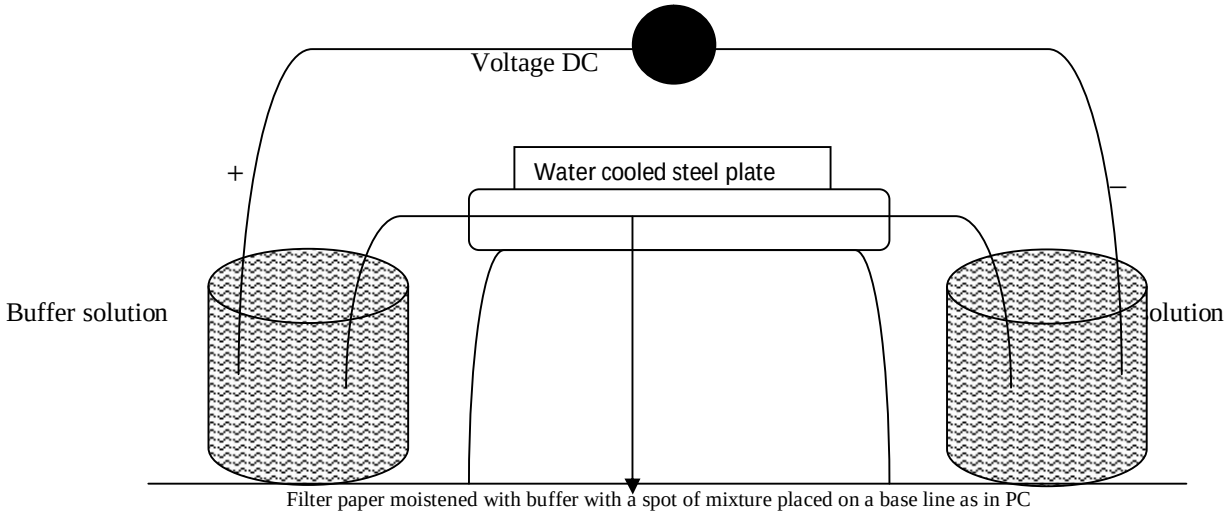
Electrophoresis is a technique which is closely associated with chromatography, and is often used in conjunction with it.

Separations depend upon the difference in the electrical properties of the components in the mixture, so that often substances which would be difficult to separate by chromatographic methods are readily separated by this technique. Electrophoresis is an incomplete form of electrolysis in which the charged particles are stopped somewhere along their path to the electrode.

There are two types:

a. ***Free Electrophoresis,*** in which the separated substances are in solution and are therefore free to diffuse the moment the current, is switched off.
b. ***Zone Electrophoresis,*** in which the separation is carried out on a supporting medium such as starch gel or strips of filter paper.

A pencil line is drawn perpendicular to the length of the filter paper and spots of samples applied to positions mount on the line. The paper is carefully moistened with a buffer solution suitable to effect separation, and the two ends dipped into pots of the same buffer solution as shown below.



Filter paper moistened with buffer with a spot of mixture placed on a base line as in PC

When the current is passed, the supporting medium act as a 'bridge' between the two pots of buffer solution and any substance in the mixture which bears an electrical charge will migrate. In

many cases, molecules which bear no electrical charge can have complex ions with ions present in the buffer solution.

Many sugar molecules can be separated in this way. After a suitable time, the current is switched off and the paper is removed from the apparatus. The rate of migration of substance during electrophoresis depends on several factors, e.g. the voltage applied, the structure of the ion e.t.c.

After allowing the paper to dry, the separated components can be located with a ‘locating reagent’ if they are not naturally coloured. In the same way described for paper chromatography, it is necessary to put on the filter paper a non-moving marker, i.e. a compound which will have no electrophoretic mobility since there is a tendency for substances to move in a direction opposite to that of their electrophoretic movement by electro osmosis.