4.2.3 Plate Theory

This theory was developed by **A.J. Porter Martin** and **Richard Laurence Millington Synge.** This theory describe the chromatographic system as being in equilibrium.

The partition coefficient K is based on this equation and is defined by following equation -

$$K = \frac{\text{Concentration of solute in stationary phase}}{\text{Concentration of sample in mobile phase}}$$

K is the partition coefficient and can be changed if experimental condition of chromatographic system is changed. For a column of fixed length and flow, the retention time (Rt) and retention volume (Vr) can be measured that is useful to calculate partition coefficient (K).

4.2.4 Basic Procedure

The mixture to be analyzed dissolved in a suitable solvent and passed through a column containing an adsorbing material. The component of the mixture would be held back by the material to different extant, the extant of such retention depends upon the "Partition Coefficient" i.e. the relative affinity of each compound to the solvent and adsorbing material as the solvent continuously flow through the column. The components are separated and appear in the effluent at different times. The components with the least affinity to the stationary phase appear first and one with stronger affinity appear at the last.

4.3 Classification of Chromatography

Chromatography is a technique that involves separation of solutes based on selective interaction between a stationary phase and a liquid (mobile phase).

(i) If the stationary phase is a solid support or a liquid coated solid support and the mobile phase is a liquid, the technique is known as liquid chromatography.

(ii) If the stationary phase is applied as a layer, the technique is referred to as thin layer and paper chromatography.

(iii) When the stationary phase is placed in a column it is said to be liquid column chromatography.

(iv) In gas chromatography the stationary phase is a solid support or a liquid coated solid support and the mobile phase is a gas.

components having closely related Rf values. The Rf value affected by gravity so that the substance is compared with suitable standard compound (Fig. 4.4).

(iii) One Dimensional Method

A complex mixture is generally separated by this method. In this the sample is placed at the corner of the paper instead of their margin. And then put into suitable solvent and allowed to run. The different components separated at different levels according to their size, nature and other parameters. But there are many substances which cannot be separated by this method due to very closely related Rf values. To overcome this problem we can use Two-Dimensional Chromatography.

(vi) Two-Dimensional Chromatography

In this the chromatogram obtained by one dimensional chromatography is dried and rotated at 90° and allowed to run in other solvent system parallel to second edge (i.e. at right angle to the first axis). Then the mixture becomes separated completely into its components.

(v) Radial or Disc Development

In this type of development a circular piece of paper is taken which has a wick cut parallel to the radius, from edge to center. The sample material that is to be examined, spotted to the center of the paper and upper end of the wick. This paper is laid on the edge of a circular disc and wick dipping in the solvent at the bottom of the dish. The liquid ascends through the wick and flows radially through the paper and separates the components present in the sample. It will take more time due to slow movement towards radii. It separates the mixture completely (Fig. 4.5).

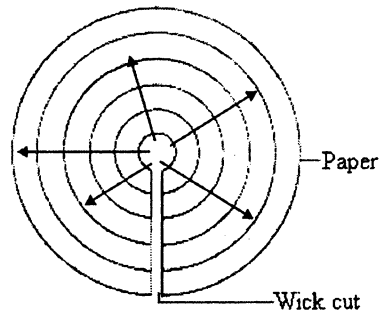

Fig. 4.5. Radial Paper Chromatography having Round Paper and Wick cut in it

4.4.3.5 Drying of Chromatogram

After developing the chromatogram it is removed from the glass jar and allowed to dry in an oven or by hair-drier for few minutes.

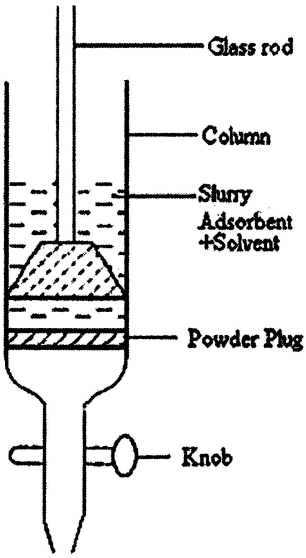

Fig. 4.14. Packing of Column with the help of Glass Rod and Silica Gel

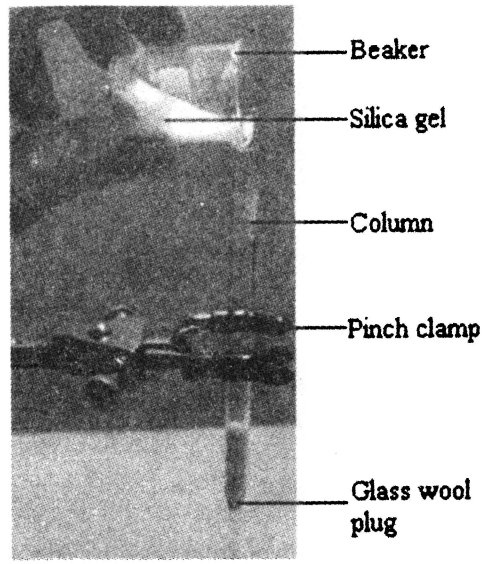

Fig. 4.15. Packing of Column with Silica

4.6.2.3 Procedure of Column Chromatography

The sample to be analyzed is dissolved in a very small amount of solvent to make a concentrated solution. This solution is then added to the top of column with the help of a pipette. The pinch clamp is opened and the solvent is allowed to run down. Then the eluting solvent is added into the column at the top and allow to flow through the medium. The solvent may be changed to more polar during the eluting processes. The flow of solvent continues until the sample mixture is completely separated into its components in the form of band or different zones (Fig. 4.16, 4.17).

After separation the adsorbent with the zone is taken out and each zone is separated with the help of a knife, and each zone may be dissolved in suitable solvents for further identification of the components. Such a set-up of separation is based on the polarity of the substances. The silica and associated solvents forms an extremely polar matrix. Thus more polar substances in the sample will adsorb more strongly to the stationary phase and elute slowlv from the column.

The solute particles retain through stationary phase by the process of adsorption. The adsorbent material may be silica or alumina.

(i) Normal Phase Chromatography

Normal phase chromatography retains analytes based on hydrophilicity. In this the mobile phase is a highly non-polar solvent like hexane or methylene chloride and stationary phase is a polar solvent.

(ii) Reversed Phase Chromatography

In this type of chromatography the non-polar polymer beads can be used as a stationary phase with relatively polar solvents such as water, acetonitrile or methanol as the mobile phase. This type of separation mode is referred to as reversed phase adsorption.

4.7.2.2. Partition

In partition chromatography the separation is based on partition coefficient of sample between the stationary phase and mobile phase.

(i) Liquid - Liquid Partition

In this chromatographic mode the stationary phase is a liquid (i.e. tightly bound water act as stationary phase) and the separation occurs as a result of partitioning between two liquid - liquid phase.

If the stationary phase is polar and the mobile phase is non-polar, the chromatographic mode is known as Normal Phase Partition Chromatography. But in the opposite case it refers to as Reversed Phase Partition Chromatography. It suffers from the disadvantages of that stationary phase always, has some solubility in the mobile phase; thus precaution must be taken.

(ii) Chemically Bonded Partition

To overcome the disadvantage of liquid-liquid partition chromatography the stationary phase is developed with chemically bonded or organo bonded soluble matrix. The most common one is Octadecylsilane (ODS), which is bonded to a silica support via a silylether linkage (Siloxane). Such a packing material is used with a polar eluent like methanol or water - methanol. Thus the stationary phase is stable and chromatographic mode does not suffer any disadvantages. If the bonded phase is polar then non-polar mobile phase is employed, and technique is known as normal phase chromatography.