Chromatography

Chromatography is a modem and sensitive technique used for rapid and efficient analysis and (or) separation of components of a mixture and purification of compounds. The basic principle of chromatographic technique is based on the differential migration of the individual components of a mixture through a stationary phase under the influence of moving phase. The stationary phase may be a porous solid (such as silica, alumina, etc.) packed in a column or supported by filter paper or a glass strip. The moving (or mobile) phase may be some solvent or a gas and is referred as an eluent.

Types of Chromatography

There are various types of chromatographic techniques which differ from one another on the basis of difference in the moving phase and the stationary phase.

1. Column Chromatography

(a) Adsorption chromatography. In this type, the mixture is dissolved in some suitable solvent such as alcohol, ether, benzene etc. and the resulting solution is poured down a vertical column filled with the adsorbing material such as alumina, chalk, charcoal, silica gel, etc. The process of addition of the mixture to the column is called loading. Depending upon the

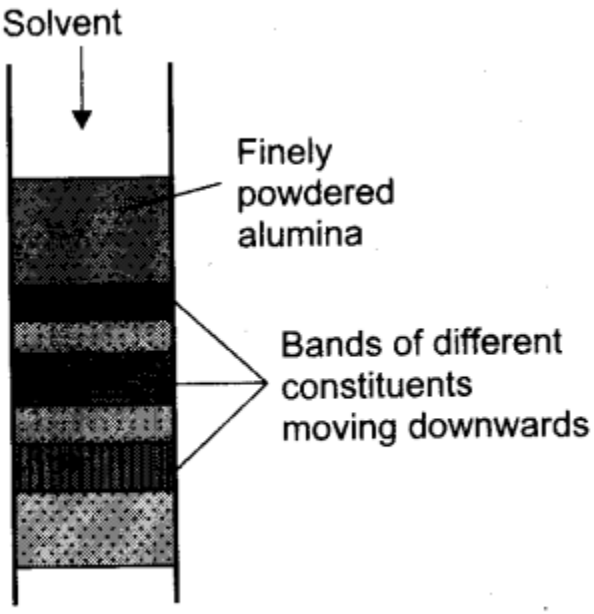

Fig. Column chromatography.

rate at which different components of the mixture are adsorbed, different zones or bands are formed down the column; substance which is adsorbed most, remains at the top of the column. Later, the constituents are washed down and collected separately, with a suitable solvent. This process is called ‘elution’.

(b) Partition chromatography. This is based on the principle that' if to a mixture of two immiscible liquids A and B, a substance which is soluble in both A and B is added, then it distributes itself in such a way that the ratio of its concentrations in two liquids A and B is constant at a particular temperature. The technique used here is the same as for the adsorption type. Separation of different constituents takes place because each constituent distributes itself to different degrees between the solvent which flows down the column and the stationary liquid.

2. Thin Layer Chromatography (TLC)

In this technique the adsorbent (alumina or silica gel) is pasted on a thin strip of glass and is dried. The substance under investigation is dissolved in some suitable solvent A drop of this solution is put on one end of the glass plate and it is kept vertically in a vessel containing a solvent. Due to capillary action the solvent rises up carrying along with it the constituents which are adsorbed on the plate at different distances depending upon the extents of adsorption. The weakly adsorbed components rise to greater heights.

3. Gas Chromatography

This is relatively new technique used to analyse mixtures of gases, liquids and volatile solids. A small quantity of the mixture is introduced into a stream of a gas which acts as moving phase. The stream of gas along with the substance to be analysed passes through a column and different constituents come out one by one at different intervals and are recorded automatically on a chromatogram.

4. Paper Chromatography

It is mainly a type of partition chromatography in which a special adsorbent paper is used instead of a column. Moisture adsorbed by this acts as a stationary phase and the solvent as a moving phase. The mixture to be separated or analysed is put at one end of the paper strip as a small spot. The paper is placed in a container, with a suitable solvent, vertically in such a way that the lower end (where the mixture spot is put) dips in the solvent and the spot remains slightly above the solvent level (Fig). The solvent rises up the paper due to capillary

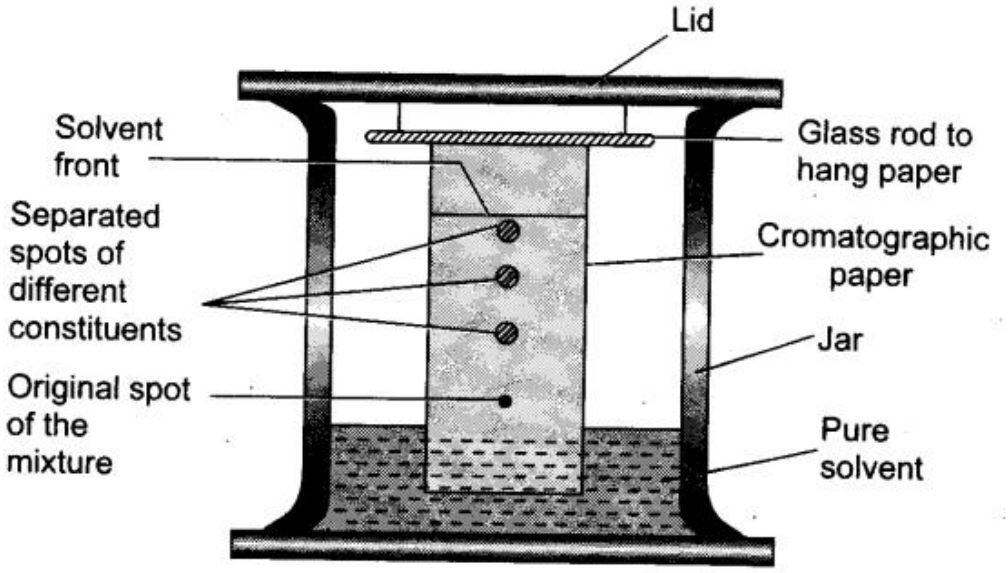

Fig. Paper chromatography.

action and the components of the mixture rise up at different rates and thus get separated from one another as shown in Fig.
This type of paper chromatography in which the solvent rises up is called Ascending paper chromatography. Alternatively, the solvent may be taken on the top in a container and be allowed to come down in which case it is termed as 'Descending paper chromatography.

R_f Values

It represents retention factor or ratio of fronts. It may be defined as the ratio of the distance moved up or travelled by the component from the origin or point of application to the distance moved up by the solvent from the same point.

$$R_f = \frac{\text{Distance travelled by the solute from the original line}}{\text{Distance travelled by the solvent from the original line}}$$

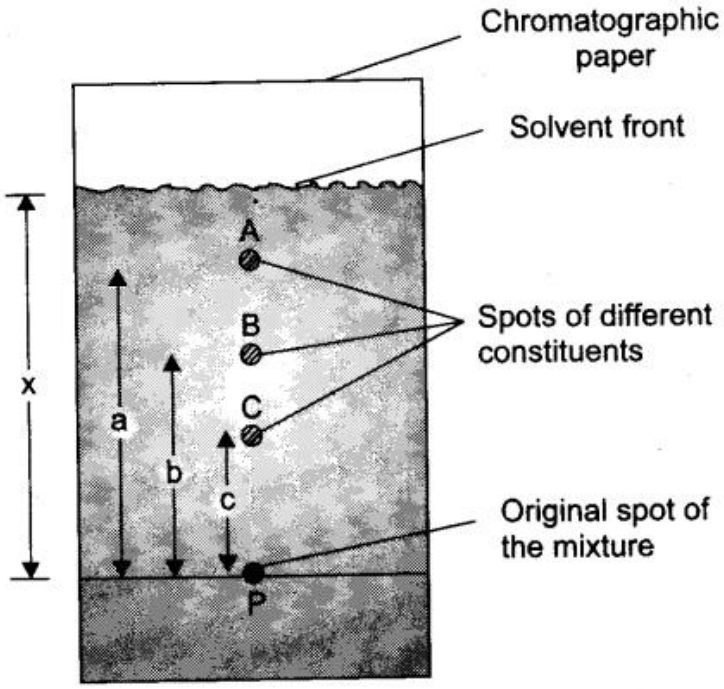

Fig. Calculation of R_f values.

For example, the R_f values of substances A, B and C will be as given under :

$$R_f \text{ value for A} = \frac{a}{X}$$

$$R_f \text{ value for B} = \frac{b}{X}$$

$$R_f \text{ value for C} = \frac{c}{X}$$

Different substance possess different R_f values. R_f depends upon a number of factors:

1. Nature of the substance.
2. Nature of the solvent.
3. Temperature.
4. Presence of impurities.
5. Quality of the filter paper used.