3. Some Analytical Techniques

3.1 Introduction

There has been a systematic development in the techniques used for analysis of chemical substances. In this chapter we are going to look into basic analytical techniques, namely, purification and separation techniques. Chemical substances occur in nature in impure stage. Also, when synthesized in the laboratory they are obtained in crude and impure form. Before investigating their composition and properties it is essential to obtain them in the pure form. Methods of purification and separation of compounds depend on the difference in their physical properties.

3.2 Purification of solids

A solid substance may contain two types of impurities, those (i) which are soluble in the same solvent as the main substance and (ii) which are not soluble in the same solvent as the substance. The second type of impurity can be separated easily using a suitable solvent to dissolve the main compound when the impurities remain undissolved and can be separated by a simple process called **filtration**. This process is similar to separating tea leaves from a decoction of tea, or sand from mixture of sand and water. Filtration is carried out with the help of a filter paper cone placed in a funnel as shown in the Fig. 3.1. A circular piece of filter paper is folded to form a cone and fitted in the funnel. The funnel is fixed on a stand and a beaker kept below it. The paper is made moist, the solution to be filtered is poured on the filter paper. The insoluble part remaining on the filter paper is called **residue** and the liquid collected in the beaker is called **filtrate**.

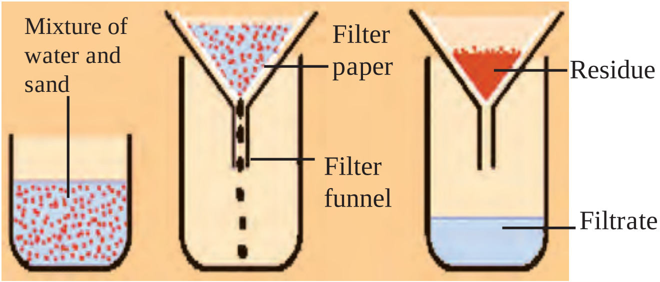


Fig. 3.1 : Separation of mixture of water and sand by simple filtration

Filtration under suction : When filtration is carried out using a vacuum pump it is called filtration under suction. It is a faster and more efficient technique than simple filtration.

The assembly for filtration under suction consists of a thick wall conical flask with a side arm. The flask is connected to a safety bottle by rubber tube through the side arm. The safety bottle is used to prevent sucking of the filtrate into suction pump. A special porcelain funnel called Buchner funnel is fitted on the conical flask with the help of a rubber cork as shown in Fig. 3.2.

The Buchner funnel has a porous circular bottom. A circular filter paper of correct size is placed on the circular porous bottom of the Buchner funnel and the funnel is placed on the flask. It is moistened with a few drops of water or solvent. Suction is created by starting the pump and filtration is carried out. Crystals are collected on the filter paper and filtrate in the flask.

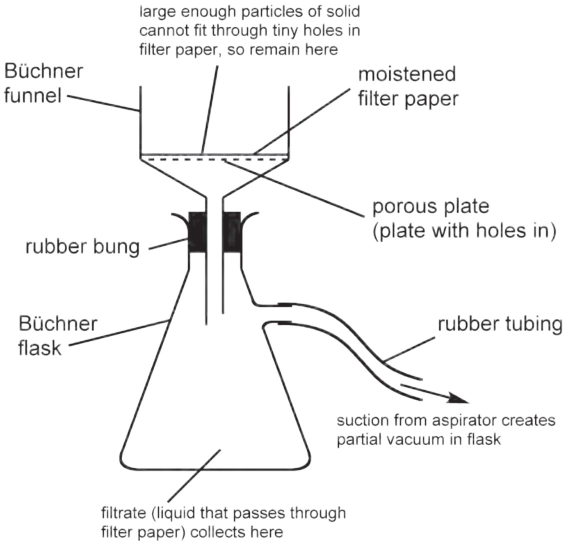


Fig. 3.2 : Setup for filtration under suction

3.2.1 Crystallization : When a crude solid is made of mainly one substance and has some impurities, it is purified by the process of crystallization. It is done in four steps:

(i) Prepartion of saturated solution : A saturated solution is a solution which cannot dissolve addtional quantity of solute. A saturated solution of the crude solid is prepared by boiling it in a small but sufficient quantity of a suitable solvent. On doing so the main solute forms an almost saturated solution, but the solution is not saturated with respect to the soluble impurities, as they are in small proportion.

(ii) Hot filtration : The above solution is quickly filtered while hot. Filtration under suction allows rapaid filtration. Undissolved impurities get removed in this process as residue.

(iii) Cooling of the filtrate : The hot filtrate is allowed to cool. Solubility of a substance decreases with lowering of temperature. As a result, the filtrate becomes supersaturated with respect to the main dissolved solute. The excess quantity of the dissolved solute comes out of the solution in the form of crystals. The dissolved impurities, however, do not supersaturate the solution, as their quantity is small. These continue to stay in the solution in dissolved state even on cooling. The separated crystals are, therefore, free from soluble impurities as well.

(iv) Filtration : The crytals of the pure substance are separated by filtration. The filtrate obtained is called **mother liquor**. The crystals so formed are free from soluble as well as insoluble impurities.

Choice of the solvent : The solvent to be used for crystallization must have following properties :

1. The compound to be crystallized should be least or sparingly soluble in the solvent at room temperature but highly soluble at high temperature.
2. Solvent should not react chemically with the compound to be purified.
3. Solvent should be volatile so that it can be removed easily.

Water, ethyl alcohol, methyl alcohol, acetone, ether or their combinations are generally used as solvent for crystallization. The choice is done by trial and error method.

Activity : Crystallization of common salt from impure sample.

Many times the common salt obtained from the market may contain some siliceous matter and other impurities. These can be removed and larger crystals of pure NaCl can be obtained.

Apparatus : Beaker, glass rod, funnel, filter paper and stand, evaporating dish, etc.

Materials : Impure market salt or a mixture of salt and fine sand, etc.

Procedure : Arrange apparatus as shown in Fig. 3.3. Take some water in a beaker. Add salt to the beaker and stir it with a glass rod. Add more salt and stir till no more salt dissolves. Heat the solution. Filter the hot solution, insoluble impurities will remain on the filter paper. Collect the filtrate in an evaporating dish and allow to cool. Crystals of pure salt NaCl will separate leaving soluble impurities in the mother liquor. Filter the solution and collect the crystals on the filter paper and dry them.

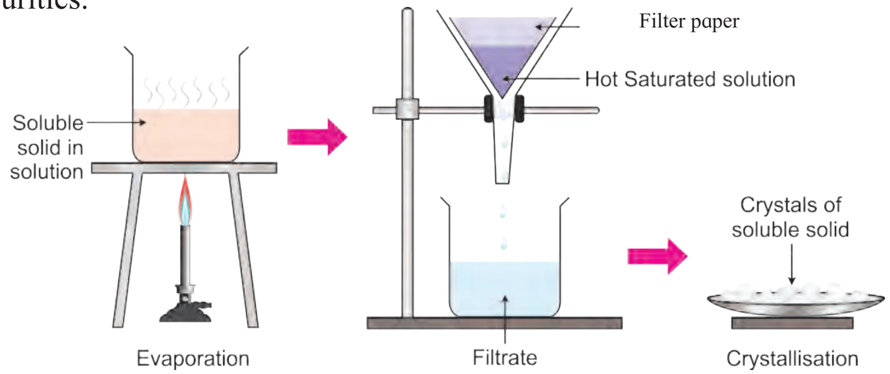


Fig. 3.3 : Process of crystallization

Impure copper sulphate can be purified by crystallization using water as solvent. Similarly, Benzoic acid can also be purified by crystallization using water as solvent.

3.2.2 Fractional crystallization :

Two or more substances in a mixture can be separated by fractional crystallisation process. Fractional crystallisation is a process wherein two or more soluble substances having widely different solubilities in the same solvent at room temperature are separated by crystallization.

Mixture of two solutes A and B are dissolved in a suitable hot solvent to prepare a saturated solution. The saturated solution is filtered to remove dust particles and then allowed to cool. As the solution cools, the solute which is less soluble crystallizes out first. The crystals are filtered, washed with solvent and dried. The mother liquor is concentrated by evaporating the solvent. The second solute crystallizes from the mother liquor. These crystals are filtered to obtain the separated and purified second component.

3.3 : Distillation : Distillation is an important method used to separate. **(i)** Volatile liquids from non-volatile impurities **(ii)** Liquids having sufficient difference in their boiling point.

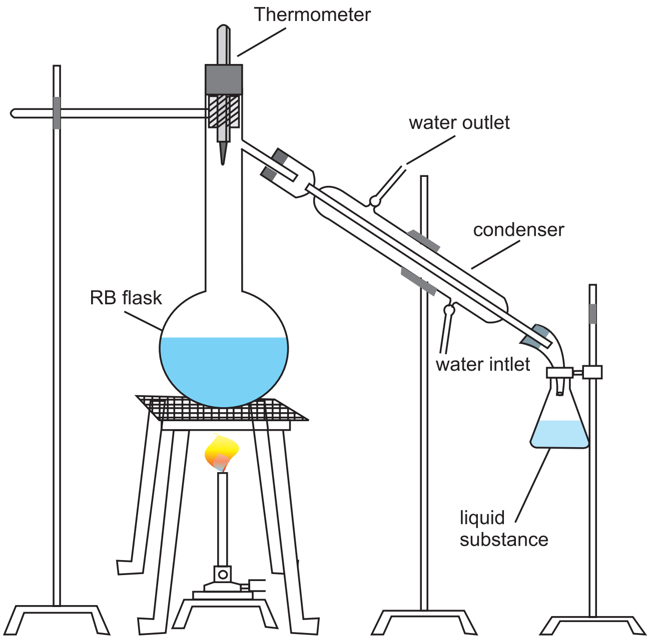


Fig. 3.4 : Simple distillation

3.3.1 Simple distillation :

Liquids which boil without decomposition at atmospheric pressure are purified by the process of **simple distillation**. In this process the liquid is first converted into its vapour by boiling and then the vapour is condensed back into liquid by cooling, and the pure liquid trickles down in the reciever.

The apparatus used for simple distillation is shown in Fig. 3.4. It consists of round bottom flask fitted with a cork having a thermometer. The flask has a side arm through which it is connected to a condenser. The condenser has a jacket with two outlets through which water is circulated. The liquid to be distilled is taken in the round bottom flask fixed by clamp. The flask is placed in a water bath or oil bath or sometimes wire gauze is kept on a stand as shown in Fig 3.4.

The condenser is connected to receiver to collect the purified liquid. Care is taken that the bulb of the thermometer is just below the side arm of the round bottom flask.

The flask is heated. As the boiling point of the liquid is reached it starts boiling and the vapors rise to the neck of the flask and pass through the side arm into the cooler parts of the condenser, which is kept cool by circulating water through its jacket. The vapours condense and the liquid is collected in the receiver.

Activity : To separate the components of a liquid mixture containing acetone (b. p. 56^{0}C) and water (b. p. 100 ^{0}C)

Apparatus : Distillation flask with condenser, two receivers, thermometer, etc.

Chemicals : mixture of acetone and water.

Principle : Acetone and water are two miscible liquids having a wide difference in their boiling points. Acetone boils at 56 ^{0}C while boiling point of water is 100 ^{0}C. When the mixture of acetone and water is heated and temperature of the mixture reaches 56 ^{0}C acetone would distil off. When all acetone distils out and when the temperature rises to 100 ^{0}C water would to distil out.

Procedure : Take the mixture of water and acetone in the distillation flask and arrange the apparatus. Heat the flask on a water bath carefully. At 56 ^{0}C acetone will distil out, collect it in reciever number 1. After all acetone distilled, change the receiver. Discard a few ml of the liquid. As the temperature reaches 100 ^{0}C water will begin to distil. Collect this in reciever number 2.

3.3.2 Fractional Distillation :

If in a mixture the difference in boiling points of two liquids is not appreciable, they cannot be separated from each other using the simple distillation assembly.

To separate such liquids, the process called fractional distillation is employed in which a special assembly is used (see Fig. 2.5(a)). In this assembly the distillation flask is fitted with a fractionating column (Fig. 3.5 (b)). Hence, the vapours first pass through the fractionating column. Vapours of more volatile liquid with lower boiling point rise up more than the vapours of liquid having higher boiling point.

Suppose we have a mixture of two liquid (A) and (B) having boiling points 363 K and 373 K respectively. A is more volatile and B is less volatile. As the mixture is heated, vapors of (A) along with a little of (B) rise up and come in contact with the large surface of the fractionating column. Vapors of (B) condense rapidly into the distillation flask. While passing through the fractionating column there is an exchange between the ascending vapors and descending liquid. The vapors of B are scrubbed off by the descending liquid, this makes the vapors richer in (A). This process is repeated each time the vapors and liquid come in contact with the surface in the fractionating column. Rising vapors become richer in (A) and escape through the fractionating column and reach the condenser while the liquid in the distillation flask is richer in B. The separated components are further purified by repeating the process. Mixtures of acetone (b.p. 329 K) and methyl alcohol (b.p. 337.7 K); acetone and benzene (353 K) can be separated by fractional distillation.

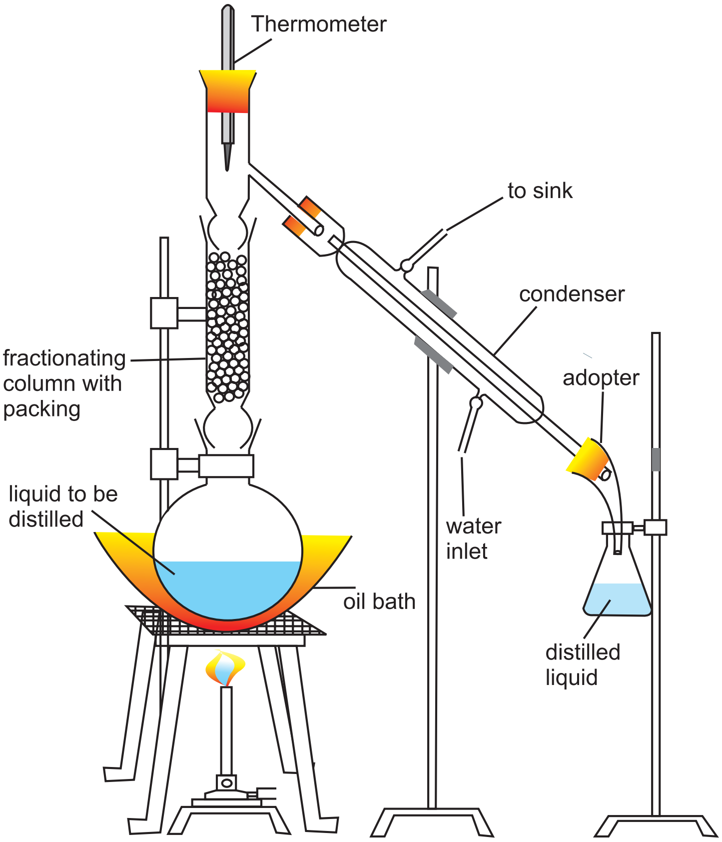
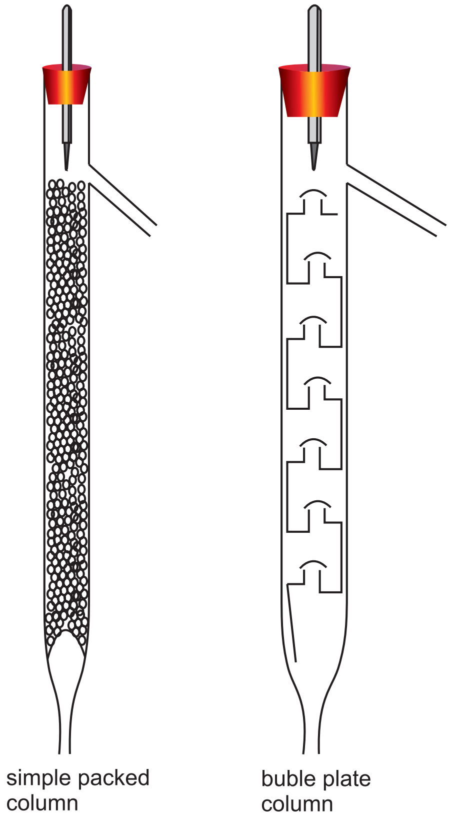


Fig. 3.5 (a) :Fractional distillation


simple packed column

buble plate column

Fig 3.5 (b) : Fractionating Columns

This process is used in petroleum industry to separate different fractions of crude oil.

3.3.3 Distillation under Reduced Pressure :

Liquids having very high boiling point or those which decompose on heating are purified by carrying out distillation under reduced pressure. In this method the liquid is made to boil at a temperature which is below its normal boiling point by reducing the pressure on the surface of the liquid. Pressure is reduced using a water pump or vacuum pump. In soap industry glycerol is separated from soap by using this technique.

3.4 Solvent Extraction : When an organic substance is present in an aqueous solution, it can be extracted from that solution by shaking it with an organic solvent in which the substance is more soluble. The organic liquid should be immiscible with water and be able to form two layers. In this process the solute distributes itself between two immiscible liquids. From the aqueous phase the solute gets extracted in the organic phase. Extraction of compound takes place based on the difference in solubility of compound in two liquids (See Fig. 3.6). On shaking for a few times with small volumes of organic phase, most of the solute gets extracted into the organic phase. The organic solvent is, then, removed by distillation and the solute is collected.

The solvent extraction process is important as it helps clean separations in a short time span.

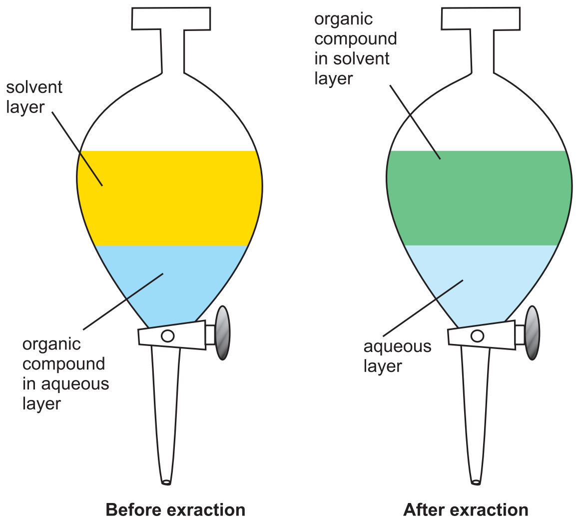


Fig. 3.6 : Solvent Extraction

If the solute is less soluble in organic phase then a technique called **continuous extraction** is used where the same amount of organic solvent is used repeatedly for extraction. This technique involves continuous distillation of the solvent within the same assembly. Thus use of large quantity of organic solvent is avoided.

Internet my friend

Get more information about continuous extraction/soxhlet extraction from YouTube.Royal Scociety of chemistry Soxhlet extraction.

3.5 Chromatographic techniques :

Chromatography is a technique used to separate components of a mixture, and also purify compounds. The name of the technique comes from the Greek word Chroma meaning Colour.

In 1903, Tswett discovered this technique for separating the coloured components found in plants. The principle of separation of substances in this technique is similar to solvent extraction i. e. distribution of the solutes in two phases. In chromatography we use two phases for separation. **(a)** Stationary phase and **(b)** Mobile phase. This technique is based on the difference in rates at which components in the mixture move through the stationary phase under the influence of the mobile phase. First the mixture of components is loaded at one end of the stationary phase and then the mobile phase, which is a pure solvent or a mixture of solvents, is allowed to move over the stationary phase. Depending on the relative affinity of the components toward the stationary phase and mobile phase they remain on the surface of the stationary phase or move along with the mobile phase, and gradually get separated.

The stationary phase can be a solid or a liquid. Depending on the stationary phase, chromatography is classified into Adsorption Chromatography and Partition Chromatography.

3.5.1 Adsorption Chromatography :

This type of Chromatography is based on the principle of Differential Adsorption. Different solutes are adsorbed to different extent on the stationary phase. Adsorption Chromatography is of the following two types.

i. Column Chromatography : This type involves the separation of components over a column of stationary phase. The stationary phase material can be Alumina, Silica gel. A slurry of the stationary phase material is filled in a long glass tube provided with a stopcock at the bottom and a glass wool plug at the lower end. The mixture to be separated is dissolved in a small amount of appropriate solvent and is then loaded on top of the adsorbent column. A suitable mobile phase which could be a single solvent or a mixture of solvents is then poured over the adsorbent column. The mixture along with the mobile phase slowly moves down the column. The solutes get adsorbed on the stationary phase and depending on the degree to which they are adsorbed, the solutes get separated from each other. The most strongly adsorbed component is retained on the column and others move down the column to various distances forming bands as seen in Fig. 3.7. The component which is less strongly adsorbed is desorbed first and leaves the column first, while the strongly adsorbed component is eluted later. The solutions of these components are collected separately. On evaporating the solvent the solutes can be recovered.

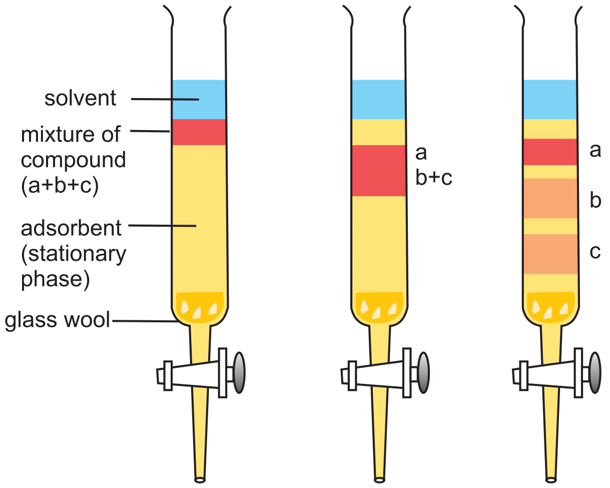


FIG 3.7 Column Chromatography. Different stages of separation.

ii. Thin Layer Chromatography : A thin layer (0.2 mm thick)of adsorbent silica gel or alumina spread over a glass plate acts as the stationary phase. The plate is called the TLC plate or chromplate. The mixture of solutes is applied on the Chromplate as a small spot about 2 cm from one end of the plate as shown in Fig. 3.8.

The plate is then placed in a closed jar containing the mobile phase such that the spot is well above the mobile phase. As the mobile phase rises up the components of the mixture move along with it. They move upto different distances depending upon their degree of adsorption and thus get separated. If the components are colored they appear as separated colored spots on the plate. If the components are not colored but have property of fluorescence they can be visualised under UV light, or the plate can be kept in a chamber containing a few iodine crystals. The Iodine vapors are adsorbed by the components and

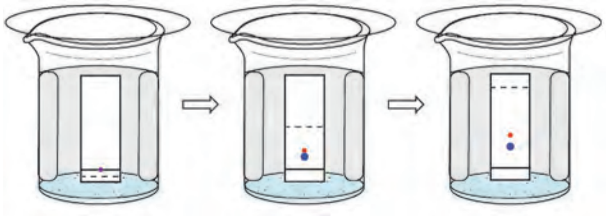
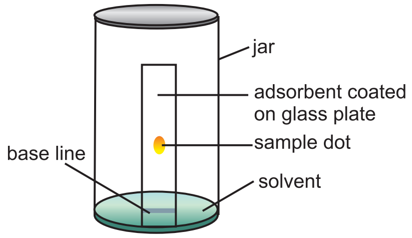


Fig. 3.8 (a)Stages in Thin Layer Chromatography



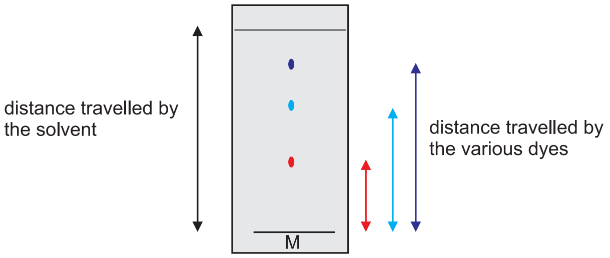


Fig. 3.8 (b) Developed chromatogram.

the spots appear brown. Amino acids are visualised by spraying the plate with a solution of ninhydrin. This is known as spraying agent.

3.5.2 Partition Chromatography : In this type of chromatography the stationary phase and mobile phase both are liquids. Separation of components takes place by continuous differential partitioning of the components between the stationary and mobile phases. For example, Paper Chromatography : In this technique a special quality paper, Whatmann paper number 1, is used. The water trapped in the fibres of the paper acts as the stationary phase. The solution of mixture is spotted on the strip of the Chromatography paper at the about 2 cm from one end of the paper using a glass capillary. The paper is then suspended in a chamber containing the mobile phase taking care that the spot does not dip in the mobile phase (Fig. 3.9).

The mobile phase rises up by capillary action and flows over the spot. Partitioning of the components takes place between stationary phase (water) and the mobile phase.

Different solutes are retained differently on the paper depending on their selective partitioning between the two phases. This developed paper strip is the chromatogram. Similar to TLC (Thin layer chromatography) the colored components are visible as colored spots and the colourless components are observed under UV light or using a spraying agent.

Retention factor (R_f) : Migration of the solute relative to the solvent front gives an idea about the relative retention of the solutes on the stationary phase. This is termed as the R_f of the solute.

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

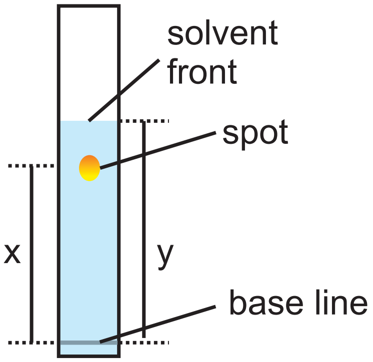


Fig. 3.10 : Retention factor

Internet my friend

Column chromatography
https ://youtube/KqQITxvFDLB8

Fig. 3.9 : Stages in Paper chromatography

Exercises

1. Choose the correct option

A. Which of the following methods can be used to seperate two compounds with different solubilities in the same solvent?
   a. Fractional crystallization
   b. Crystallization
   c. Distillation
   d. Solvent extraction

B. Which of the following techniques is used for seperation of glycerol from soap in soap industry ?
   a. Distillation under reduced pressure
   b. Fractional distillation
   c. Filtration
   d. Crystallization

C. Which technique is widely used in industry to seperate components of mixture and also to purify them ?
   a. Steam distillation
   b. Chromatography
   c. Solvent extraction
   d. Filtration

D. A mixture of acetone and benzene can be seperated by the following method :
   a. Simple distillation
   b. Fractional distillation
   c. Distillation under reduced pressure
   d. Sublimation

E. Colourless components on chromatogram can not be observed by the following :
   a. Using UV light
   b. Using iodine chamber
   c. Using the spraying reagent
   d. Using infrared light

2. Answer the following

A. Which of the following techniques is used for purification of solid organic compounds?
   a. Crystallisation b. Distillation

B. What do you understand by the terms
   a. residue b. filtrate.

C. Why is a condenser used in distillation process?

D. Why is paper moistened before filtration?

E. What is the stationary phase in Paper Chromatography?

F. What will happen if the upper outlet of the condenser is connected to the tap instead of the lower outlet?

G. Give names of two materials used as stationary phase in chromatography.

H. Which properties of solvents are useful for solvent extraction?

I. Why should spotting of mixture be done above the level of mobile phase ?

J. Define : a. Stationary phase b. Saturated solution

K. What is the difference between simple distillation and fractional distillation?

L. Define a. Solvent extraction b. Distillation.

M. List the properties of solvents which make them suitable for crystallization.

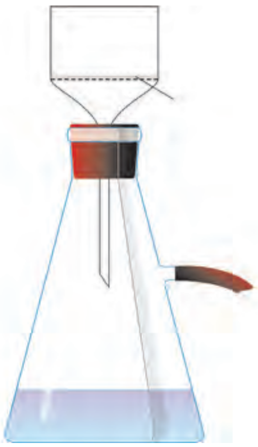
N. Name the different types of Chromatography and explain the principles underlying them.

O. Why do we see bands separating in column chromatography?

P. How do you visualize colourless compounds after separation in TLC and Paper Chromatography?

Q. Compare TLC and Paper Chromatography techniques.

3. Label the diagram and explain the process in your words.



Activity :

Use any one analytical technique in laboratory and discuss it in groups.