

Purification of Organic Compounds

A pure substance contains only one kind of molecules while an impure substance is a mixture of different kind of molecules. Thus to purify an impure substance is to separate the desired molecules from the mixture and therefore, the purification is a separation. The differences in the properties of different molecules are the basis of various separations. Products of organic reactions are seldom pure; they may be contaminated with either the starting material or side-products. To obtain the satisfactory physical constants, the substance must be pure. And to carry out the purification, various techniques have been employed depending on the physical state of the compound, which are discussed in this chapter.

2.1 CRYSTALLIZATION

Crystallization is a simple, effective and very important technique to separate and purify solids. It is based on the fact that all organic compounds are more soluble in hot than in cold solvents, so that solid gets dissolved on heating and is obtained back on cooling.

2.1.1 Steps Involved in Crystallization

The following steps are involved in the purification by crystallization:

- (a) Selection of a solvent
- (b) Dissolution of the sample
- (c) Decolourisation of the solution
- (d) Hot filtration
- (e) Cooling for crystallization
- (f) Cold filtration
- (g) Washing the crystals
- (h) Drying the crystals
- (i) Checking the purity

Table 2.1 Common solvents for Crystallisation

S.No	Solvent	b.p. (°C)	Remarks
1.	Diethylether*	35	Inflammable
2.	Petroleum ether	Boiling fractions 40-60 60-80 80-100 100-120	Inflammable
3.	Dichloromethane**	41	Non-inflammable & toxic
4.	Acetone	56	Inflammable
5.	Chloroform**	61	Non-inflammable & toxic
6.	Methanol**	64.5	Non-inflammable & toxic
7.	Carbon tetrachloride**	77	Non-inflammable & toxic
8.	Ethanol	78	Inflammable
9.	Ethyl acetate	78	Inflammable
10.	Benzene***	80	Inflammable, highly Toxic and Carcinogenic
11.	Cyclohexane	81	Inflammable
12.	Acetic acid	118	Not very inflammable and pungent vapours

* Its use should be avoided wherever possible because of its high inflammability and also its tendency to creep up the walls of the containing vessel and hence results in the deposition of solid material by complete evaporation rather than the crystallization.

** Its vapours are toxic and therefore inhalation should be avoided and the recrystallization should be done in fuming cupboard.

*** Toluene is much less toxic than benzene and so wherever possible it is used preferentially.

Use of carbon disulphide, b.p. 46°C, should be avoided as it has a very low flash point and its vapours form explosive mixture with air.

continued in 0.5 ml portion each time, if the substance is not dissolved completely. The mixture is heated after each addition. If even after adding 3 ml of solvent, the compound does not dissolve on heating, the solvent is unsuitable due to the low solubility of the compound in it. If an almost clear solution is obtained, the tube is cooled by immersing it in cold water and if the solution is not clear, it is filtered and then the tube is allowed to cool. If crystallization does not start rapidly, it may be due to the lack of nuclei for crystal growth and therefore the scratching of the tube below the surface of the solution is done with the help of a glass rod. Crystals often form rapidly after scratching as fine scratches on the walls form the sites for crystal growth. If crystals do not separate even after scratching another

coloured impurities get dissolved in the boiling solvent and adsorbed by the crystals, giving an impure product. Such type of impurities are preferentially adsorbed by the activated charcoal so can be removed by boiling the coloured solution with a small amount of activated charcoal for 5-10 minutes followed by hot filtration. Now the filtrate is usually almost colourless and therefore pure crystals are obtained.

Not only the coloured impurities can be removed by the activated charcoal but also the resinous or finely divided matter can also be removed from the solution. Sometimes the solution is not clear due to the presence of resinous or very finely divided insoluble impurities, which cannot be removed by simple filtration. These impurities can also be removed by heating the solution with activated charcoal. The activated charcoal takes away these from the solution and makes it clear.

Only a small amount of activated charcoal must be used (1-2% by weight of the sample) otherwise some of the compound which is to be purified may also get adsorbed. The same procedure could be repeated with some more fresh charcoal (1-2%), if this amount is not sufficient enough. It should also be noted that the activated charcoal should be added only after the solution is cooled otherwise the solution would boil over when it is added. After this addition, the solution can be reheated to the boiling point.

Animal charcoal contains large proportion of calcium salts and should not be used with acidic solutions and also the impurities present in charcoal itself may get dissolved in hot solvent and separate out with the crystals of the compound. These problems can be sorted out by boiling animal charcoal under reflux with dilute hydrochloric acid (1:1) for 3 hours. Then the mixture is diluted with hot distilled water and filtered through a Buchner funnel. It is washed repeatedly with hot distilled water till all the acid has been removed, drained and finally dried by heating in an evaporating basin in an electric oven.

(d) Hot filtration

Now the desired compound is in the solution in the hot solvent along with insoluble impurities and decolourising carbon, if used. They can be separated by gravity filtration. Vacuum filtration cannot be used as the reduced pressure in the suction flask will boil the filtrate and the solid will get deposited on the walls of the flask.

In hot filtration, main problem is that undue cooling takes place, which results in the crystallization of some of the solid during the filtration. This can be reduced to a minimum by using heated funnel with no stem. A stemless funnel avoids the problem of crystallization and hence clogging in the stem. Therefore to avoid the undesired crystallization, the filtration is carried out through a fluted filter paper* supported in a heated, stemless funnel and pouring only a little of solution at a time and keeping the remainder at the boiling point. If the solid separates out on filter paper during filtration, it must be scraped back into the flask, redissolved and then filtered.

If very large amount of hot solutions are to be filtered, the funnel should be warmed externally during the filtration (hot water funnels). The hot water funnels are shown in Figure 1.8.

The filtrate should be reheated to dissolve any crystals, which are formed during filtration, to give a clear solution.

(e) Cooling for crystallization

After making the filtrate clear by dissolving any crystals formed during

*Preparation of a fluted filter paper.

The filter paper is first divided into eight equal sectors by making fourfolds in the paper. The folding is continued, the edge 2,1 is then folded on to 2,6 and 2,3 to 2,5 to produce new folds 2,7 and 2,8 respectively. Further 2,3 to 2,6 and 2,1 to 2,5 give new folds 2,9 and 2,10 respectively. Finally a fold is made on each of the eight segments e.g. between 2,3 and 2,9/2,9 and 2,6 and so on in a direction opposite to the first series of folds to give a fan arrangement which on opening gives a fluted filter paper. The above sequence is shown in the Figure 2.1

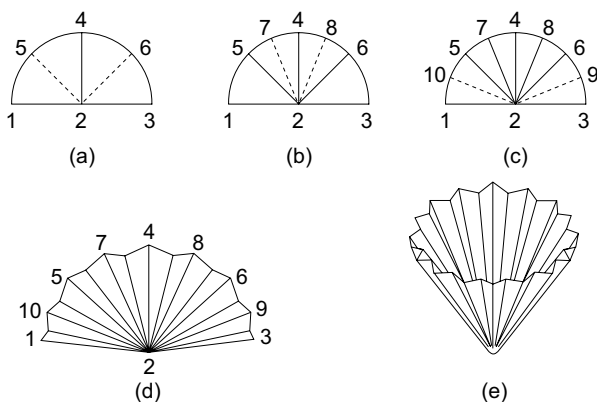


Fig. 2.1 Preparation of a fluted filter paper

These processes (swirling and pouring) are repeated till whole of the solid material has been transferred into the funnel. The suction is continued till most of the liquid is filtered.

The above procedure (filtering the bulk of mother liquor followed by the entire compound) is quicker than bringing the solid material into suspension and then filter it. And also, a gentle suction is more effective and rapid filtration than powerful suction as the finer particles of the solid may drag into the pores of the filter paper in the latter case and hence results in the slower filtration.

(g) Washing the crystals

Crystals are now washed with some fresh cold solvent to remove soluble impurities from the surface of the crystals, otherwise crystals will be contaminated with these impurities when solvent get evaporated.

If the crystals are relatively soluble in the solvent, then a minimum amount of cold solvent must be used. While if they are not very soluble in solvent, washing can be done by large amount of solvent (no need to cool it). If the crystals are not in the form of solid cake, washing can be done by releasing the vacuum and then pouring of the wash liquid over the crystals and again applying the vacuum, while if the solid cake is formed, the solvent is added to the crystals in the funnel and the solid is carefully broken down and suspended by the help of a spatula. Care should be taken during the suspension that the entire solid product gets suspended without the tear or dislodge of the filter paper. Alternatively, the solid cake can be transferred to a breaker and then washing is done by breaking the cake in the solvent and the product is again collected by suction filtration.

When a non-volatile solvent e.g. acetic acid is used for recrystallization, the washing of the crystals is done with a more volatile solvent, in which crystals are not soluble to wash off non-volatile solvent so as to speed up the drying process.

(h) Drying the crystals

The crystals obtained by suction filtration can simply be dried by spreading it on the thick pad of filter paper with the help of a spatula. Another similar pad is placed on the top and then the solid is pressed. These sheet pads are changed by the fresh pads occasionally as the mother liquor wets them.

- (iv) By addition of solid carbon dioxide. On addition of a few lumps of it, cold spots are produced in the solution which facilitate the crystallization.
- (v) In some cases, when all the above methods fail, the crystallization is carried out by keeping the solution in a refrigerator or freezer for long periods of time.

2.1.3 Semimicro Microrecrystallizations

Sometimes very small quantity of a compound may be needed to recrystallize e.g. the compounds obtained by natural products, small-scale expensive preparations or by-products of reactions.

(a) Semimicro recrystallization

In this recrystallization the apparatuses used are shown in Figure 1.10 (b-c). The compound to be recrystallized is dissolved in semi-micro test tubes or centrifuge tubes. Heating should preferably be done in a water or oil bath to regulate the heat. While for inflammable solvents, solution is prepared under reflux conditions by using semimicro glass apparatus. The solutions are allowed to cool and separated crystals are filtered using a small Hirsch funnel as shown in Figure 1.10 (b).

Filtration nail may also be used. It is a thin glass rod flattened at one end, which is then fitted into a small glass funnel as shown in Figure 1.10 (c). The nail head is covered with a circle of filter paper of appropriate size. The size of the funnel and filtration nail may be varied depending upon the amount of crystals to be filtered.

(b) Micro-scale recrystallization

When micro quantity of the compound is to be recrystallized, usual semi-micro techniques of recrystallization give great loss of the crystals.

An easy and cheap way to recrystallize the compound on micro scale is as follows:

A test tube filled with only about 1/8th by the solution should be chosen and the sample is added to it. Solvent is added and the test tube is warmed in a water bath or on a sand bath. As the amount of compound is very small, heating should be done for a brief period only. Relatively large size of test tube helps in condensation of solvent vapours by providing large surface area.

The solution is now filtered into another test tube, smaller than the previous one, by using a Pasteur pipette with a small piece of cotton just at the point where the pipette starts to narrow. Keep it on the tip of the second test tube as in Figure 2.2. Second Pasteur pipette is used to transfer the hot solution from the test tube to the filter pipette. The solution passes down through the pipette to the second test tube, which can be warmed carefully in water bath or sand bath to keep the solution warm. After filtration, the second test tube is kept aside for cooling.

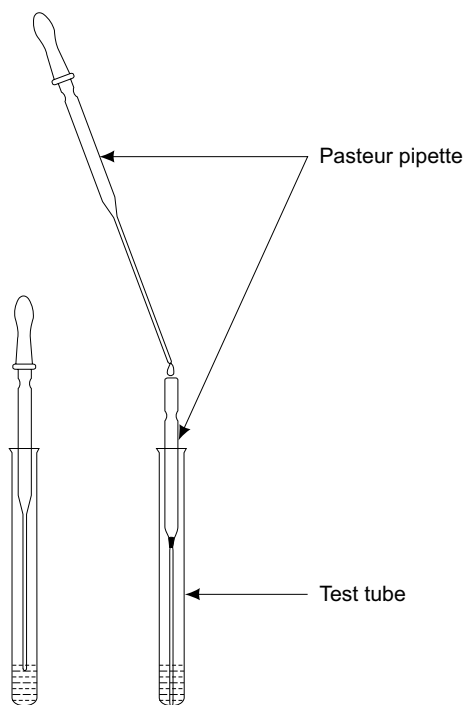


Fig. 2.2 Filtration in a micro-scale recrystallization

When crystallization is completed, the solvent can be removed by using another Pasteur pipette. It withdraws solvent by pushing its tip in the bottom of the test tube. Washing can be done by using cold solvent.

The solvent can also be removed by centrifugation. After centrifugation, crystals are settled down and the solvent can easily be decanted. Washed with the solvent and removed it by centrifugation followed by decantation.

The crystals are dried by connecting it to the vacuum as shown in the Figure 2.3.

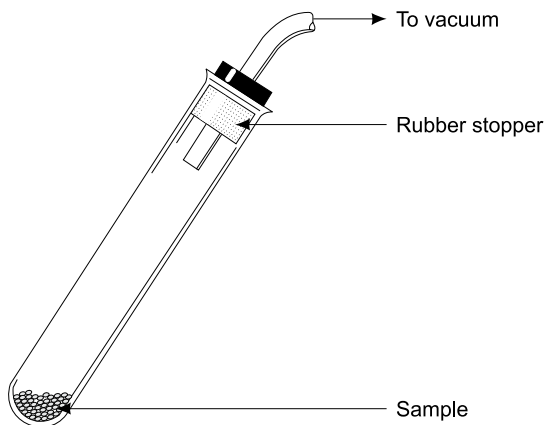


Fig. 2.3 Drying crystals in a test tube

Dried crystals are collected over a piece of filter paper by inverting the test tube and tapping it with a glass rod.

2.1.4 Exercises in Recrystallization

A few examples are given to understand the recrystallization technique.

(a) **Benzoic Acid from distilled water (a non-inflammable solvent)**

Some other compounds which can be recrystallized from water are acetanilide, salicylic acid etc.

Weigh 1 g of crude Benzoic acid into a 100 ml conical flask. Add about 20 ml of hot distilled water. Heat the mixture on sand bath or electric hot plate. Add more of hot water in small portions with continuous stirring and heating until the entire solid has dissolved. If the solution is colourless, filter the hot solution through a fluted filter paper supported on a stemless funnel. During filtration, keep the remaining solution hot with the help of a hot plate or filter the solution by using a hot water funnel (Figure 1.8). If the solution is coloured, allow it to cool slightly and then add about 0.1 g of decolourising carbon and boil the mixture for few minutes to remove the coloured impurities and then carry out the filtration as described earlier.

Collect the filtrate in a 100 ml conical flask, heat it again to dissolve unwanted crystals of the benzoic acid and then cover it through watch glass and allow to cool to room temperature of its own. After an hour when complete crystals separate out, filter them with suction using a small Buchner funnel. Wash the crystals twice with 5 ml portions of cold water to remove the mother liquor adhering to it. Press the crystals in the funnel with spatula to remove the mother liquor as much as possible. Remove the funnel from the filtration set and invert it on a pad of 3-4 filter papers resting upon another pad of newspaper and let them dry in air. For rapid drying, the crystals may be kept in oven on a watch glass. Note the yield and m.pt. of the recrystallized sample. The crystallization may be repeated if the recrystallized sample is not pure i.e. if compound melts over a range. Melting point of pure benzoic acid is 121°C .

(b) Naphthalene from alcohol (an inflammable solvent)

Some other compounds which can be recrystallized with an inflammable solvent are:

- (a) m-dinitrobenzene from alcohol
- (b) tribromoaniline from alcohol

Weigh 1 g of commercial naphthalene into a 50 ml round bottomed flask. Add 5 ml of rectified spirit and 2-3 pieces of pumice stone in it to avoid bumping. Fit a reflux condenser (Figure 1.25). Heat the mixture in a water bath or electric hot plate to boil the solvent. Add 2-3 ml of the solvent, boil carefully after each addition until whole naphthalene apart from insoluble impurities, has dissolved. If it is coloured, use 0.1 g of animal charcoal as described in the above procedure. Filter the hot solution through a fluted filter paper. Care should be taken that no flames must be there in the vicinity. Collect the filtrate in conical flask, heat it and cover it by a watch glass. When the complete crystals are separate out, filter them through a small Buchner funnel. Wash the crystals twice with 5 ml portions of cold rectified spirit. Press the crystals through spatula and dry them as described in the above procedure. Record the yield and melting point of the recrystallized compound. The melting point of pure naphthalene is 80°C .

(c) Sulphanilic acid from water

Crude sulphanilic acid is usually almost black in colour. It is recrystallized from distilled water and decolourized by using animal charcoal.

Weigh 1 g of crude sulphanilic acid in a conical flask and add 20 ml of distilled water. Heat to dissolve almost whole of the compound by using sand bath or hot plate. Cool it slightly and add 0.1 g of animal charcoal to the solution and continue boiling for few minutes. If the filtered solution is coloured, boil it with a further 0.1 g of animal charcoal. Filter it as in the above procedures and collect crystals of sulphanilic acid. Record* the yield.

(d) *Para-aminoazobenzene from solvent pair*

Aminoazobenzene ($C_6H_5-N=N-C_6H_4-NH_2$) is highly soluble in methylated spirit and insoluble in water. So for recrystallization this solvent pair is used. Dissolve the crude sample in boiling methylated spirit using water bath. Remove the conical flask from water bath and then add water drop by drop until the solution becomes just cloudy. Put the solution on a water bath until it becomes clear and then remove it from water bath immediately. Filter if necessary; allow it to cool to room temperature slowly. Filter the crystals and note the yield and melting point. The melting point of pure para-aminoazobenzene is $126^\circ C$.

2.2 FRACTIONAL CRYSTALLIZATION

Fractional crystallization is employed to separate a mixture of two or more substances in their pure states, provided they differ in solubilities in a particular solvent. The solvent to be used can be found out by trial and if there is no appreciable difference in their solubilities, the process of separation is difficult.

In this method, the mixture is treated with a minimum quantity of the hot suitable solvent to dissolve almost all of the more soluble part leaving the less soluble behind. The solution also contains a small amount of less soluble part. Now, the solution is filtered while hot and the more soluble substance present in the solution, is obtained on cooling. The solid, hence obtained, consists of the major portion of more soluble substance and a very small portion of less soluble substance. So, the solid is dissolved once again in the minimum amount of the solvent and recovered by crystallization. This whole procedure of making solution and crystallization is repeated a number of times until the pure component (more soluble) is obtained. Purity of the substance is checked by melting point determination.

* Melting point cannot be recorded as it decomposes on heating and therefore, it has no melting point.

The residue obtained after the first filtration consists of mainly the less soluble part along with a very small amount of the more soluble part. This (less soluble part) can also be obtained in the pure form by working up in the similar manner, the purity of which is also checked by melting point determination.

2.2.1 Exercise on Fractional Crystallization

(a) Separation of benzoic and cinnamic acid

The two acids differ in their solubilities in warm water (40°C). Cinnamic acid is insoluble in water at this temperature and benzoic acid is soluble. And, therefore, these two acids can be separated by fractional crystallization using warm water (40°C) as a solvent. A mixture of 2 g each of the above acids is placed in a 100 ml beaker and 50 ml of warm water (40°C) is added to it. The mixture is stirred for 5 minutes on a hot water bath. The temperature of the mixture is to be maintained at 40°C. The contents of the mixture are allowed to settle down and the supernatant liquid is filtered while hot using a hot water funnel. The filtrate is collected in another beaker. The residue left in the first beaker, is treated similarly with warm water six times and then the final residue is recrystallized from boiling water. The product cinnamic acid hence obtained, is checked for purity by melting point determination (i.e. 133°C). All the filtrates are combined in 400 ml beaker and is concentrated down to 50 ml. On cooling, benzoic acid is crystallized out, which is further purified by recrystallization from hot water. The purity of benzoic acid is also checked by melting point determination (i.e., 121°C).

2.3 DISTILLATION

If a sample of an organic liquid contains impurities, it may be purified by distillation. This method may also be used to determine the boiling point (4.3.1). If impurities are non-volatile, on simple distillation, they are left behind in the distillation flask and when the impurities are volatile, fractional distillation method is used to purify the given liquid.

In this section, simple distillation is discussed. The method is used when the pure liquid and impurities have widely different boiling points (b. pt.) (The b.pt. difference between the two must be at least 50°C). Typical apparatus used for simple distillation is shown in Figure 2.4.

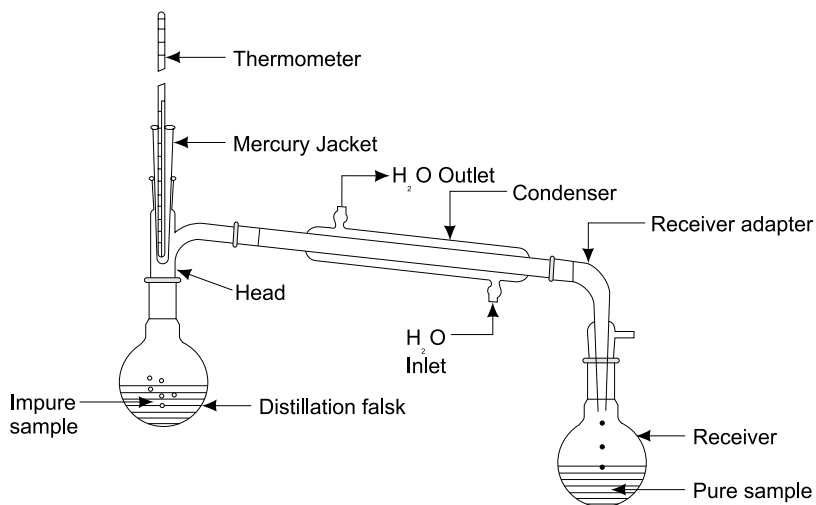


Fig. 2.4 Apparatus used for simple distillation

A distillation flask of suitable size is fitted to a water condenser, to which an adapter is fitted so as to collect the distilled liquid into a receiver. Thermometer is fitted in the neck of the distillation flask by means of a well-bored cork. The bulb of the thermometer should be in centre and just below the level of the side tube otherwise the accurate boiling point will not be obtained.

The following points must be taken into account, while carrying out the distillation process:

- (1) The size of the distillation flask should be such that it will be one half or at the most two-thirds filled by the liquid. If the flask used is too large, then superheating and sometimes, decomposition may occur.
- (2) Few pieces of unglazed porous porcelain or carborundum must be added in the distillation flask to provide a surface on which bubbles of the vapours can be formed and hence results in the gentle boiling. In the absence of these boiling stones, the liquid often reaches a temperature above its b.pt. and leads to the violent eruptions—'bumping'.
- (3) The complete distillation apparatus should be tightly fitted as it may be used for inflammable liquids.

- (4) All the clamps used should be lined with cork as otherwise glass apparatus may have cracks on applying excessive pressure on the clamp.
- (5) In water condenser, the inlet of water should be from the lower end and outlet of water should be above the jacket so that the condenser is full of water.
- (6) A water condenser is replaced by an air condenser [a glass tube with no jacket or emptying the water from condenser jacket] if the boiling point of liquid is above 150°C.
- (7) For low boiling inflammable liquids, heating should be done by a water bath while for liquids having b.pt. more than 80°C, sand bath or direct heating on a wire-gauze can be used. Electrically heated mantles can also be used for all the liquids.

The liquid is poured in the distillation flask with the help of a funnel having stem which extends below the side arm, pumice stones* are added to it and a thermometer is placed in the neck of the flask. The flask is heated by a suitable means, described above. The temperature will first rise rapidly until it is near boiling point of the liquid, then it will rise slowly and then will become constant. This constant temperature is the boiling point of the liquid. So heating can be fast until boiling commences, then the flame must be decreased so that the one or two drops of the liquid are collected quickly. The distillation should not be conducted too slowly as otherwise due to the lack of a constant supply of vapour on the thermometer bulb will result in an irregular boiling point while it should not be too fast so that it causes superheating by heating directly a part of the vapour as well as liquid. If the liquid is pure, most of it will be collected at a constant temperature, i.e. boiling point of the liquid, while if two components are to be separated, the first component will pass over at a constant temperature, then before second component starts coming, an intermediate stage will come in which there is rise in the temperature, it is collected in another receiver and finally the second component will come over again at a constant temperature which is collected in the third receiver. It must be noted that never overheat the flask to distil the last drop of the remaining liquid – a small residue must be left in the flask.

For small quantity liquids, pear-shaped flask may be used. The apparatus is shown in Figure 2.5. A 10 or 25 ml pear-shaped flask having a

* Should never be added to the hot liquid.

long side arm is fitted with a condenser, which in turn is fitted with a receiver. The flask is heated in an air bath and distillate is collected in an appropriate size receiver.

If instead of attaining a constant temperature, there is a steady rise in temperature, simple distillation cannot be used to purify the sample. And for the purification, fractional distillation must be used.

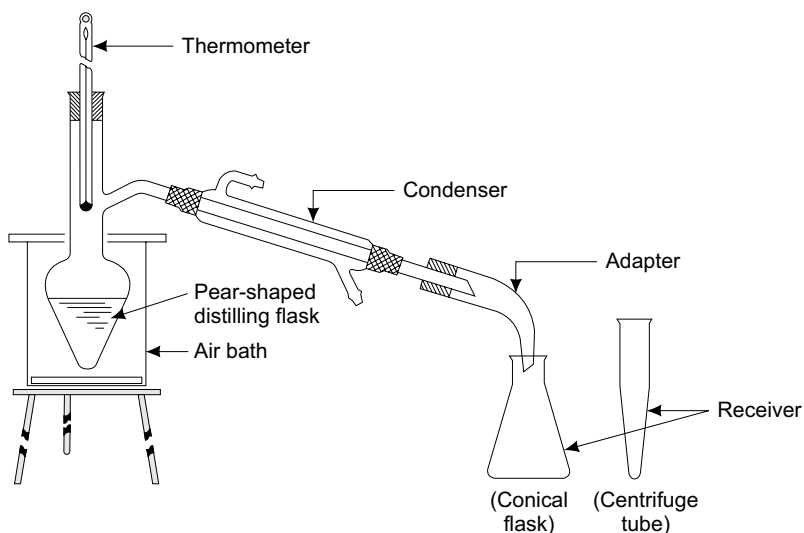


Fig. 2.5 Apparatus for micro-scale distillation

2.4 FRACTIONAL DISTILLATION

This method is used to separate the liquids whose boiling points difference is less than 50°C . For this type of distillation, the fractionating column is vertically inserted between the flask containing the liquid and condenser.

A fractionating column consists of a long vertical tube through which first the vapour goes up and then is partially condensed which comes down to the flask. This condensed liquid (having higher boiling point component) when flows down through the column gets in contact with the ascending vapours and results in the interchange of heat.

To reach the equilibrium within liquid-vapour system, the vapours get enriched with the more volatile component at the expense of the liquid. And therefore, ascending vapours become richer and more volatile i.e. the lowest boiling component while the descending condensate becomes

richer in the highest boiling component. Efficiency of a fractionating column is determined by the extent of separation. Fractionating columns, which may be used in the laboratory, are shown in Figure 2.6 (a-d).

Figure 2.6(a) shows the Vigreux column having moderate efficiency and the most widely used column. It is made by a glass tube with a series of downward slope indentations such that points of each pair of indentations must almost touch each other and form a spiral of glass inside the tube so that the vapour cannot pass directly through the whole length of the column without meeting an indentation.

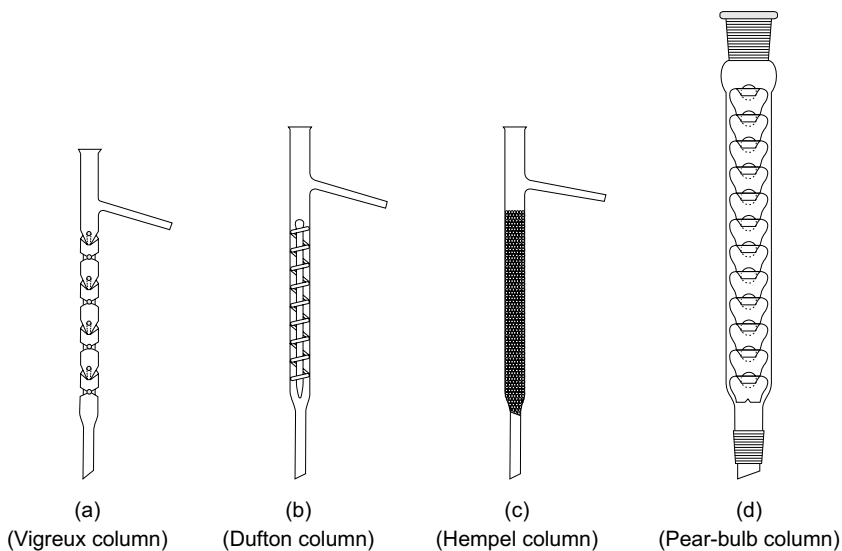


Fig. 2.6 (a-d) Types of fractionating column

While Figure 2.6 (b) shows all-glass Dufton column having efficiency greater than Vigreux column. It is suitable for general use. A glass spiral is fitted tightly in a plain tube around a central tube by grinding the edges of the spiral into the tube. This column has an advantage that a low volume of liquid is retained in the column.

Figure 2.6 (c) shows Hempel column whose efficiency is also greater than the Vigreux column. It is a simple glass tube packed with a suitable packing e.g. $3/16'' \times 3/16''$ glass or porcelain rings. It has very high efficiency.

Figure 2.6 (d) shows pear-bulb column, the increase in cooling surface is provided by these pear-shaped bulbs and the efficiency of this column can be increased by increasing the number of bulbs in the column.

The apparatus employed for the fractional distillation is shown in the Figure 2.7. The impure liquid is placed in a round bottomed flask of suitable size, one-third to one-half, pumice stones are added and then the column is fitted absolutely vertical in the position followed by fixing of a water condenser to the side arm and the distillate is collected in the receiver. The thermometer is placed in the position such that its bulb should be just below the level of the side arm. The mixture is heated with a low flame. Care should be taken that the flame should be devoid of draughts so that uniform heat is supplied (use of an air bath, Figure 1.43 gives better results). Initially heating of the liquid should be slow so as to avoid the choking of the column [on fast heating, extra condensation takes place while the column is warming up and so column may be choked with the liquid]. When once the distillation has started, the flame should be adjusted that about one drop of the liquid passes in 2-3 seconds so that an efficient fractionation can be obtained. First, the low boiling point

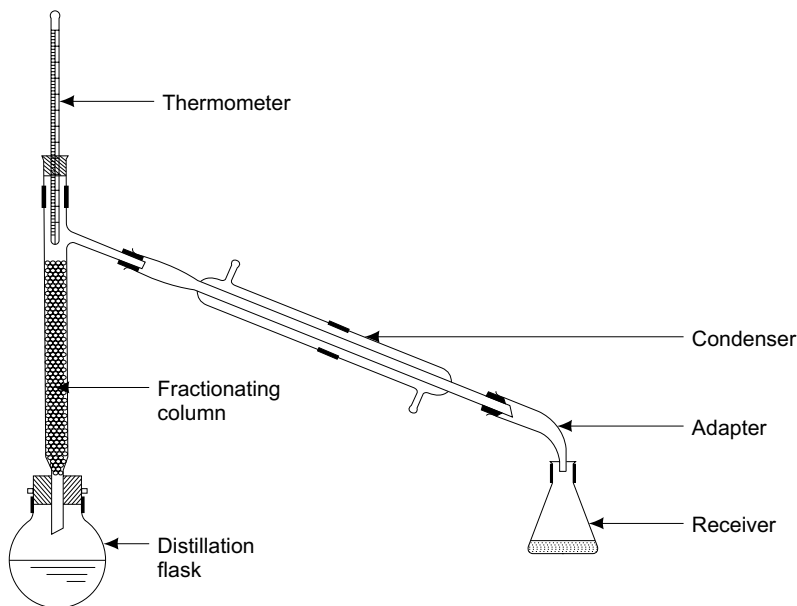


Fig. 2.7 Apparatus for fractional distillation

component will be passed over so when it has passed, distillation should cease. Then heating is slowly increased and a second fraction starts distilling over with a sharp rise in the boiling point. A relative large intermediate fraction may be obtained, if the distillation set-up is inefficient. The distillation should be conducted slowly so as to obtain pure fractions, otherwise the fractionation has to be repeated.

If the boiling point of any of the component exceeds 100°C lagging of the column is necessary, as it will avoid the excessive cooling. Lagging is done by wrapping asbestos cloth or cotton wool around it. Excessive cooling may also be avoided by surrounding the column with a vacuum jacket or an electrically heated jacket.

For small-scale work, the apparatus generally used is shown in Figure 2.8. It consists of a round bottomed flask with a long fractionating sidearm packed with $3/16" \times 3/16"$ glass or porcelain rings.

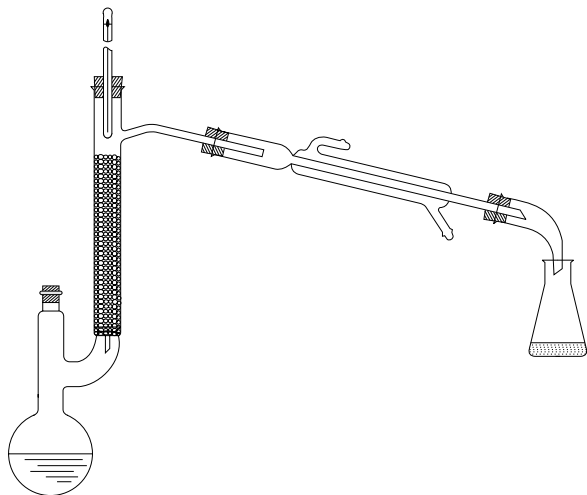


Fig. 2.8 Apparatus for micro-scale fractional distillation

A special receiver may be used to collect the fractions of different boiling ranges of a mixture without interrupting the distillation process, which is shown in Figure 2.9.

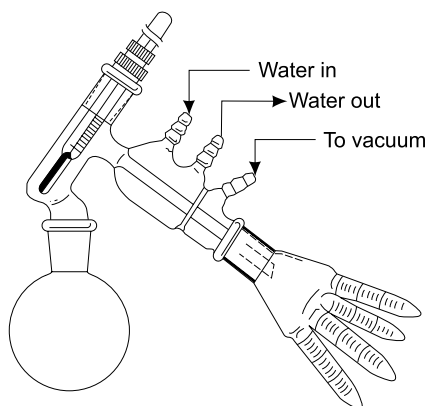


Fig. 2.9 Micro-scale distillation apparatus with a rotating fractional collector

2.4.1 Exercise in Fractional Distillation

(a) *Fractional distillation of a mixture of benzene and toluene*

The boiling point of benzene is 80°C while that of toluene is 110°C and as the b.pt. difference is 30°C (less than 50°C) and so the mixture can be separated by fractional distillation. It is carried out by using the apparatus shown in Figure 2.7, and here the lagging of the column is not required. For about 40 ml of a mixture containing equal volumes of benzene and toluene, about one and a half hour is needed if distillation is carried out slowly. The flame should have no draughts during the process. Fractions of boiling points (i) $80\text{-}85^{\circ}\text{C}$, (ii) $85\text{-}107^{\circ}\text{C}$ and (iii) $107\text{-}111^{\circ}\text{C}$ are collected in different receivers. The approximate volumes of each fraction are 19, 2 and 17 ml respectively.

2.5 DISTILLATION IN VACUUM

Some organic compounds decompose before their b.pt. are reached and so they cannot be distilled under atmospheric pressure. In such cases, the compound is distilled under reduced pressure so the boiling point is reduced and hence can be distilled without decomposition e.g. aniline has a boiling point 184°C at 760 mm Hg while it falls to 151°C at 300 mm Hg and to 77°C at 155 mm Hg.

Generally, as the external pressure is reduced by a factor of two, the boiling point of a compound will decrease by about $20\text{-}30^{\circ}\text{C}$. A nomograph (shown in Fig. 2.10) is useful for estimating the boiling point as a function of pressure. It estimates not only the expected reduced pressure boiling points from the normal boiling points but also the normal boiling points

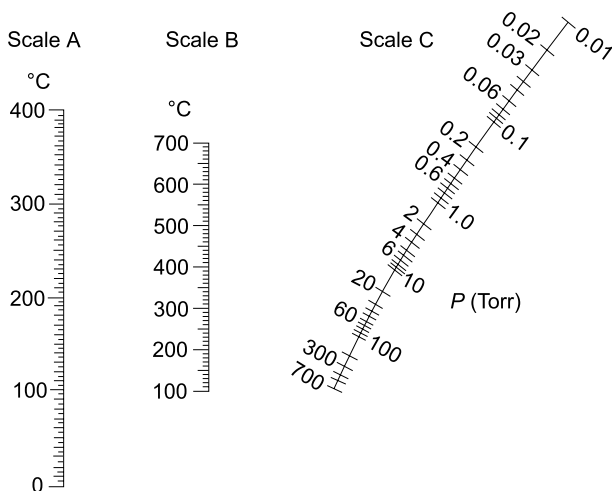


Fig. 2.10 A Nomograph for estimating the boiling point as a function of pressure

from observed reduced pressure boiling points. It is applicable for non-associated liquids only. For associated liquids, the variation of boiling point with pressure is 10-20% less than that for non-associated liquids.

The nomograph relates the normal boiling point of a substance (Scale B) to boiling points at reduced pressures (Scales A and C). A line joining points on two scales will intersect the third scale at some point. So, if the values of A and C are known (boiling point A at reduced pressure C), B (normal boiling point) can be estimated and if the values of B are known, boiling point A at a reduced pressure C can be estimated.

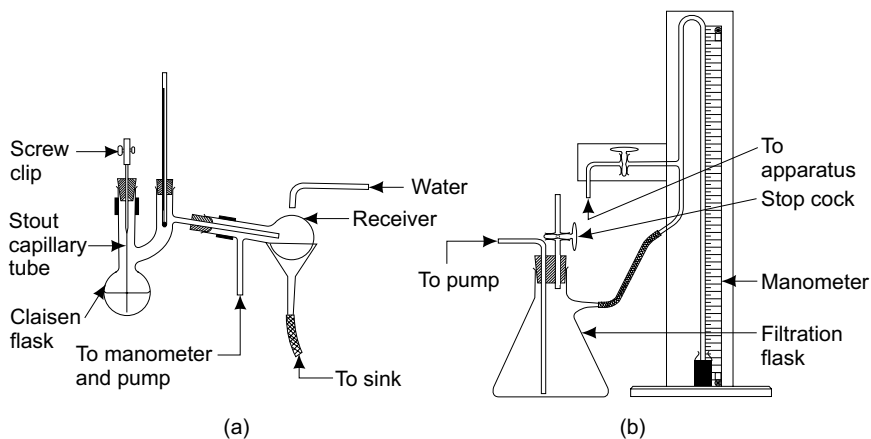
Table 2.2 shows the approximate boiling points of some of the substances at reduced pressure.

Apparatus employed for such type of distillation is shown in the Figure 2.11 (a-b).

A specially designed flask, known as Claisen flask is used [As a liquid can bump violently when superheated even under normal distillation process so the possibility of bumping is greatly increased when distillation is carried out under reduced pressure, so to reduce the risk of superheating and hence danger of bumping, Claisen flask is used]. Claisen flask has two necks. The right-hand neck carries a thermometer with the bulb just below the level of the side arm, while the left hand one is carrying a stout capillary tube, whose upper end is closed by a short piece of pressure tubing and a screw clip to regulate the amount of air passing through the capillary. The capillary used here should not be prepared by ordinary glass "quill" tube, as

Table 2.2 Boiling points ($^{\circ}\text{C}$) at reduced pressures

S.No.	Name of the Compound	Boiling point in $^{\circ}\text{C}$ at reduced pressure in mmHg [$b_{p_{\text{red pressure}}}$ (mmHg)]
1.	Water	$b_{p_5}1^{\circ}/b_{p_{20}}22^{\circ}/b_{p_{25}}26^{\circ}/b_{p_{760}}100^{\circ}$
2.	DMF	$b_{p_{10}}40^{\circ}/b_{p_{39}}76^{\circ}/b_{p_{760}}153^{\circ}$
3.	DMSO	$b_{p_{0.37}}20^{\circ}/b_{p_{5.11}}56.6^{\circ}/b_{p_{17}}83^{\circ}/b_{p_{760}}189^{\circ}$
4.	Chlorobenzene	$b_{p_5}10^{\circ}/b_{p_{25}}39^{\circ}/b_{p_{50}}54^{\circ}/b_{p_{760}}132^{\circ}$
5.	Formamide	$b_{p_{10}}109.5^{\circ}/b_{p_{60}}147^{\circ}/b_{p_{200}}175.5^{\circ}/b_{p_{760}}210.5^{\circ}$
6.	Glycerol	$b_{p_{10}}167^{\circ}/b_{p_{20}}182^{\circ}/b_{p_{30}}192^{\circ}/b_{p_{760}}290^{\circ}$
7.	Quinoline	$b_{p_{17}}114^{\circ}/b_{p_{40}}136.7^{\circ}/b_{p_{100}}163.2^{\circ}/b_{p_{760}}237.7^{\circ}$
8.	Ethyl salicylate	$b_{p_{10}}105^{\circ}/b_{p_{25}}124^{\circ}/b_{p_{50}}139^{\circ}/b_{p_{760}}234^{\circ}$
9.	Diglyme (Dimethyl ether of diethylene glycol)	$b_{p_3}20^{\circ}/b_{p_{35}}75^{\circ}/b_{p_{200}}116^{\circ}/b_{p_{760}}162^{\circ}$

**Fig. 2.11 (a-b)** Apparatus for vacuum distillation

by this method, capillary obtained will be very fragile and probably may snap during the course of the distillation. It should always be prepared by drawing out a piece of thick-walled capillary tube of 3-5 mm external bore and then the length of the fine drawn-out capillary is adjusted so that it reaches to within 1-2 mm of the bottom of the flask. Then, the side arm of the Claisen flask is fitted to an ordinary distillation flask, acting as a receiver, in such a way that it is well into the bulb of the receiver for the complete condensation of the vapours. Well-fitting and well-bored rubber stoppers and heavy-walled rubber tubing should be used throughout the apparatus. Cooling of the receiver is essential for complete condensation of

the vapours and so for liquids having boiling points below 140-150°C, the bulb of the receiver is cooled with a stream of water and the waste water is collected by funnel below, as shown in Figure 2.11 (a) and then carried to the sink with the help of the rubber tubing while for higher-boiling liquids, cooling is usually carried out by dipping the bulb of the receiver in cold water.

The distilling flask (receiver) is then fitted to filter flask, acting as a reservoir to equalize the pressure, by a pressure tubing, which is then connected to a manometer and a water pump as shown in the Figure 2.11 (b). The glass tube, which connects the filter flask to the pump, should touch the bottom of the flask so that any water, which may flow back due to the unequal pressure of water, may be sucked back as the water pressure retains. The larger flow back of water may be checked by opening the stop-cock (connected to the filtration flask) until the original water pressure is restored.

The reduction of pressure is carried out by using a water pump or oil pump. In water pump, the vapour pressure of water at that temperature is the lowest pressure that can be achieved e.g. in winter, the temperature of water is 6-8°C and the vapour pressure of water at this temperature is 6-8 mm, while in summer, when the temperature of water may be 22°C, the vapour pressure of water is 22 mm and therefore, in a water pump, the pressure varies from 7 to 22 mm. For lower pressures, oil pump should be used.

To start the distillation, first the liquid is placed in a Claisen flask (it should not be more than half-filled) and few pumice stones are added to it, the complete apparatus for distillation is fitted as shown in Figure 2.11 (a-b). Reading of barometric pressure is noted down. Now, the flask is heated by an air bath (Figure 1.43) or by a water or an oil bath, by immersing two-thirds of the flask into the bath. The water is supplied to the condenser and then water pump is turned on to attain its maximum capacity, keeping screw clip almost fully closed.* A fine steady stream of air bubbles is passed through the solution by adjusting the screw clip. This will minimise the bumping as the introduction of air prevents the delay in the appearance of the vapour phase and thus superheating. And the volume of air passed in the form of bubbles is so small that there is almost negligible

*If the flask contains traces of volatile solvents, warm the flask slightly and pass a comparatively large volume of air through liquid, so that the volatile solvents get removed down the water pump, otherwise the pressure obtained will have value above the capacity of the pump which can lead to either non-functioning of the pump or leakage in the apparatus. After the removal of all traces of volatile solvent, the screw clip is either almost completely closed or adjusted.

effect of partial pressure on the boiling point when the mercury level in the manometer is constant, the reading is noted. To obtain the value of pressure in the system, the above value is subtracted from atmospheric pressure. The apparatus set-up is considered to be satisfactory if the pressure obtained does not differ by more than 10 mm from the expected value [the temperature of the tap water], while if the pressure is unsatisfactory, the apparatus must be checked for leaks that all glass joints are firmly in position and proper fitting of all the pressure tubings over glass tubings. After achieving the satisfactory pressure, heating of flask is carried out by using a water bath, oil-bath or air bath. When water-bath or air-bath is used, the temperature of the bath should be 20-25°C above the boiling point of the liquid at that pressure, while if an air-bath is used, the temperature is raised slowly until the liquid starts to distil and heating is maintained so that the liquid distils at the rate of 1-2 drops per second. During the distillation, reading of temperature and pressure should be recorded by the thermometer and the manometer respectively. During the distillation, the boiling point of the pure compound should not be raised more than one to two degree, even if the temperature of bath is increased in the end to take off the last drop of the liquid. Sometimes, the liquid distil out first is having the boiling point lower than expected and so in such cases, the heating is continued until the expected temperature is reached and then the liquid is collected in another receiver. To remove the receiver, first the flame is removed and the Claisen flask is allowed to cool slightly by lowering the bath or raising the flask, then vacuum is released gradually by opening the stopcock of the filter flask and screw clip of the filtration flask, the latter will help in preventing the flow of liquid in the capillary.

Now receiver is replaced, again all joints are tightly joined and the whole procedure mentioned above is repeated. When the pressure is having constant value, the heating is started again.

The boiling point at a given pressure may be estimated approximately for the working pressure of a water pump (10-25 mm) by assuming that one mm difference in pressure corresponds to one-degree difference in boiling point. And the more accurate method is by using the expression:

$$\Delta t = 0.00012 (760 - P) (T + 273)$$

where, Δt = correction in the °C to be applied to the observed boiling point T and barometric pressure is P .

For water, alcohols, acids and other associated liquids, the expression used is:

$$\Delta t = 0.00010 (760 - P) (T + 273)$$

2.6 FRACTIONAL DISTILLATION IN VACUUM

If more than one fraction distils, the whole process of simple vacuum distillation has to be stopped after collecting each fraction, as described above and this is the main disadvantage of the simple vacuum distillation. This can be overcome by using a 'Pig' shown in the Figure 2.12 in place of a receiver flask. So various fractions can be collected without disturbing the distillation.

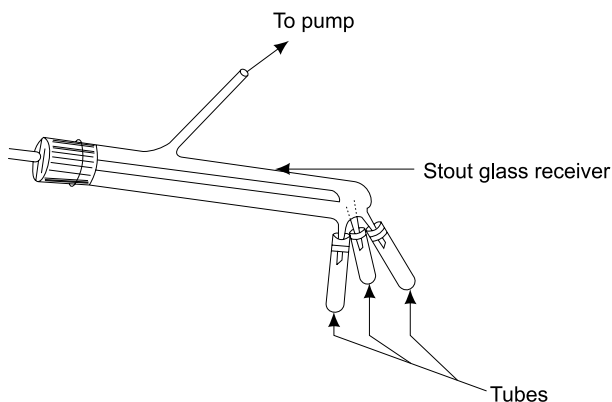


Fig. 2.12 Pig

The 'Pig' contains a glass receiver, which is fitted in place of a receiver flask. It has an outlet tube at the top for the connection to the manometer and pump, and three outlet tubes to which receivers are fitted. So when distillation starts first fraction is collected in one tube and when the second fraction starts coming, then the tube is rotated slightly to bring another outlet in the lowest position so as to collect second fraction in it, similarly third fraction can be collected in third outlet tube. However, this method is unsuitable as if a good vacuum is obtained in the apparatus, rotation of the tube is difficult as then it will be firmly attached while if the rotation is easy around the cork, it means that leakage is there at this point and so varying pressure is recorded by the manometer. The ground glass fitting shown in Figure 2.13 can overcome the leakage problem.

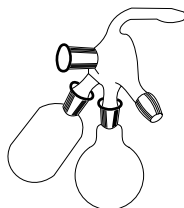


Fig. 2.13 Fractional collector

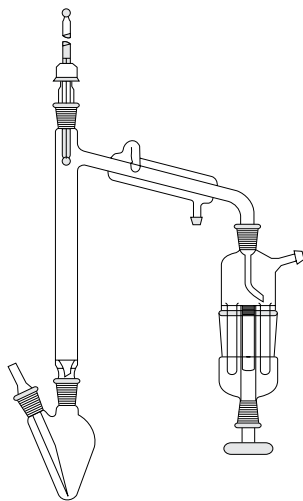


Fig. 2.15 Apparatus for micro scale vacuum distillation

through them]. So the compounds can be purified by passing a current of steam into the mixture of compound and water. The former will distil out with steam and can be easily separated from the distillate (water & compound) as it is insoluble in water. Since steam distillation takes place at a temperature below the boiling point of water,* and so the main advantage is that a compound which decomposes at or near its boiling point can be purified by this method.

So this method is useful for the purification of the organic compound:

- (a) which decomposes at or near its boiling point.
- (b) by separating the non-volatile by-products in many reactions.
- (c) from aqueous mixtures containing dissolved inorganic salts
- (d) from those compounds which are not steam-volatile e.g. separation of *o*-nitro phenol from *p*-nitro phenol (discussed in exercises on steam distillation).
- (e) from steam-volatile by-products e.g. biphenyl and excess of unreacted starting material from the non-volatile triphenylcarbinol (See Sec. 2.7.1).

*As the boiling point, in case of a mixture of two immiscible liquids will be the temperature at which sum of the vapour pressures of these liquids is equal to that of the atmospheric pressure, and its value is lower than the boiling point of the more volatile component and hence the compound distils below its boiling point.

The isolation of the pure organic compound from the steam-distillate depends on the physical state and its solubility in water. The methods of isolation employed are:

- (a) If the compound is water insoluble liquid, it will form a separate layer so can be separated by separating funnel.
- (b) If compound is water insoluble solid, it will crystallize out in the aqueous distillate, so can be separated by filtration.
- (c) If water-soluble organic compound, then the compound can be isolated by solvent extraction [See Sec. 2.15].

To minimize the probability of the splashing of material over into the condenser, the distillation head shown in Figure 2.17 (a) may be used while the probability of leakage problems may also be reduced by using the all-glass head as shown in Figure 2.17(b).

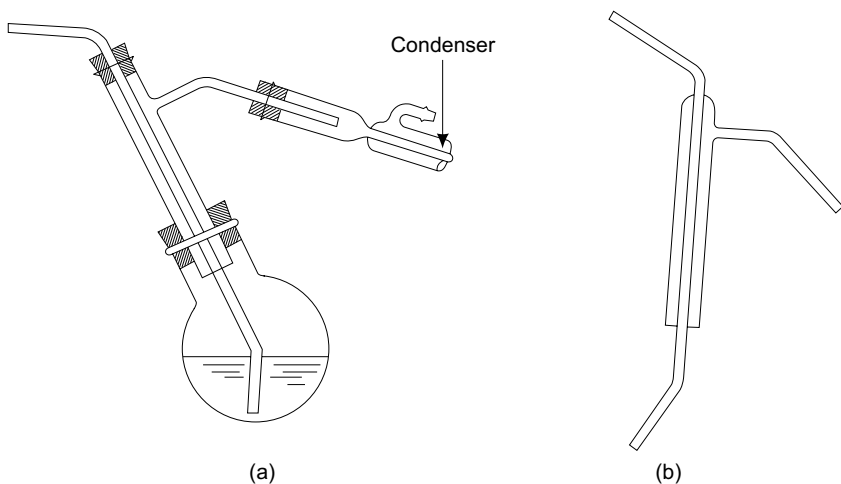


Fig. 2.17 (a-b) Types of distillation head

When the steam distillation is carried out for small quantities, the apparatus, which may be used, is shown in the Figure 2.18. The substance to be distilled is placed in the inner tube, which is dipped in water of the outer flask.

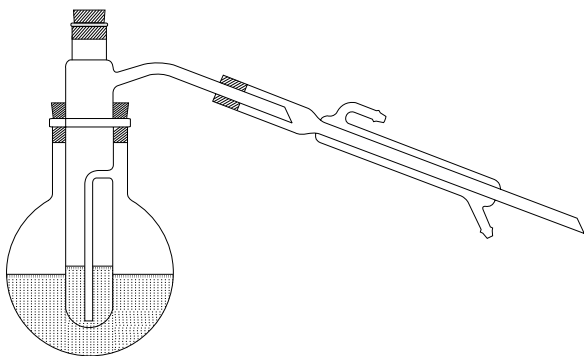
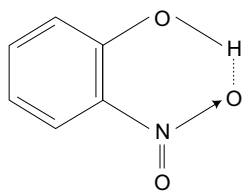


Fig. 2.18 Apparatus for micro scale steam distillation

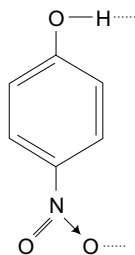
2.7.1 Exercises on Steam Distillation

(a) Separation of *o*- and *p*-nitro phenol

These two can be separated by steam distillation as the ortho compound contains an intermolecular hydrogen bonding, between oxygen of nitro group and hydrogen of OH group in the ring, while in case of para intermolecular hydrogen bonding is present. This results in high volatility of *o*-isomer in comparison to *p*-isomer.



(Intramolecular H-bonding)



(Intermolecular H-bonding)

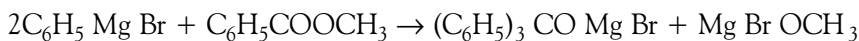
These nitro phenols can be prepared by nitration of phenol. So the mixture obtained by the nitration is steam distilled as discussed above until no more *o*-nitro phenol passes over to the receiver flask. If it gets condensed in the condenser, then the flow of water in the condenser is turned off for a few moments. Distillate is cooled in the ice water for complete solidification of *o*-nitro phenol. It is filtered at the pump and drained thoroughly. The yield of the dry *o*-nitro phenol is 7g, if the

nitration is carried out with 20 g of phenol. The m.pt. of pure o-nitro phenol is 46°C.

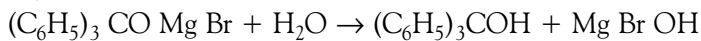
The p-nitro phenol is obtained by cooling the residue of the flask in ice for 20 minutes. The crude p-nitro phenol is filtered and its purification is carried out by boiling it with 200 ml of 2% HCl along with 1 g of decolourized charcoal for 5-10 minutes. It is filtered through a hot water funnel and filtrate is allowed to cool overnight. The pure p-nitro phenol crystals are filtered and dried. The yield of p-nitro phenol (m.pt. 112°C) is 4 g.

(b) Purification of triphenyl carbinol from the unreacted starting material and the by-products

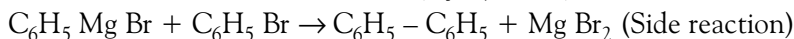
Triphenyl carbinol, a tertiary alcohol can be prepared by a Grignard reaction, shown below:



(Phenyl
Magnesium
Bromide) (Methyl benzoate)



(Triphenyl Carbinol)



(biphenyl)

The residue obtained during this grignard reaction contains triphenyl carbinol, unreacted starting materials and biphenyl, as side product, out of which the former is non-volatile and the latter two are volatile, so can be separated by steam distillation.

To the residue [starting with 6 ml of bromobenzene] 30 ml of water is added and the mixture is placed for steam distillation as discussed above. Steam distillation is carried out until no more oil [unreacted starting material and biphenyl] passes over to receiver. On cooling the residue of the flask, solid separates out, which is filtered at the pump and dried. The triphenyl carbinol so obtained is colourless but crude and so it is crystallized from methylated spirit or benzene. The yield of pure triphenyl carbinol (m.pt. = 162°C) is 4.2 g.

2.8 DISTILLATION WITH SUPERHEATED STEAM

The superheated steam can increase the proportion of higher boiling component in the steam distillate by increasing the vapour pressure of the component in comparison to that of water. The advantage of the use of superheated steam is that there is no need of supplementary heat to the substance as less condensation takes place. The higher temperature of the superheated steam will be obtained by preventing the condensation of steam by surrounding the flask by a liquid bath having the temperature same as that of superheated steam, and this will increase the proportion of higher boiling component in the steam distillate.

The apparatus employed for the distillation in superheated steam is shown in the Figure 2.19.

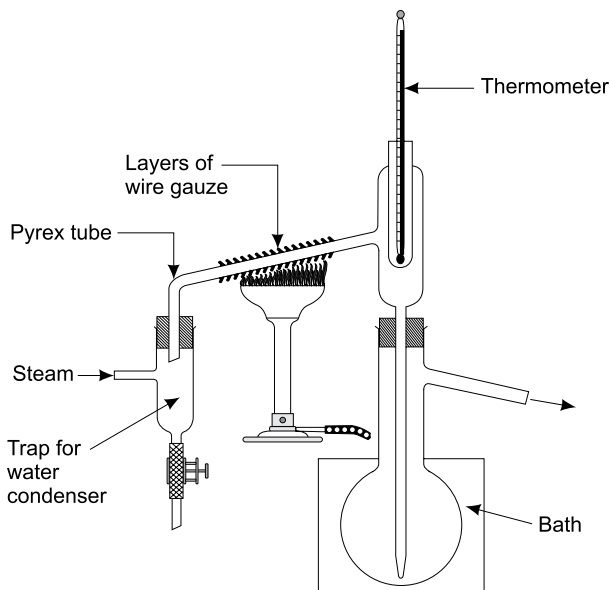


Fig. 2.19 Apparatus for distillation in superheated steam

The flask containing the liquid to be distilled is heated in an oil bath having the temperature approximately same as that of the superheated steam. The Pyrex tube, having diameter (10 mm) is wrapped with a few layers of wire gauze, which is supported by a clamp to prevent sagging of the tube at high temperature. This Pyrex tube is heated with a wing-topped

burner. Since the superheater is close to the steam inlet tube, and thus the cooling of the steam enters in the flask, is reduced to a minimum.

2.9 MOLECULAR DISTILLATION

It is also called as High Vacuum distillation or short path distillation. This method is used to distil the compounds having very high boiling points, and which get decomposed at such a high temperature. So to avoid decomposition, they are distilled at pressures in the region of 10^{-5} mm Hg. The main advantage of this method is that the boiling point of the compound is highly reduced [in some cases, by 200-300°C], and therefore can distill the compounds which decompose at higher temperatures and very sensitive to heat. The distillation of such a high boiling compounds cannot be carried out by using apparatus used for distillation under reduced pressure. To carry out the distillation of above compounds, the main feature of the distillation unit is the short direct path between a heated liquid surface and the cooled condensing area. Since the mean free path of large organic molecules is shorter and therefore the condenser must be quite close to the evaporating surface. A molecular still is a still in which the distance between the evaporating surface and the cold condensing surface is less than the mean free path of the molecules. The temperature of the condenser is maintained comparatively low so that the escaping molecules proceed in a straight path to the condenser.

For small quantities (10-50 mg) of highly viscous high boiling liquids, the vacuum sublimation apparatus (Figure 2.27(a-b)) can be used. The apparatus provides the least hindrance to the flow of vapour from the evaporating surface to the condensing surface. As a liquid sample may contain the dissolved gases or solvents, and so great care must be taken in applying the vacuum. And so first these solvents and gases must be removed. Initially the cold finger is replaced by a stopper. The vacuum is reduced very gradually and the temperature is increased very carefully to avoid excessive frothing and splashing. So initially the vacuum is attained by a water pump and the temperature is raised slowly by immersing the distillation unit in a water bath at a controlled temperature. So the solvent is removed by keeping the froth minimum. When there is no effervescence, the water pump is replaced by an oil pump and the vacuum is reapplied slowly, the heating is continued. When no further solvent is being removed, the stopper is now replaced by the cold finger. The molecular distillation is now started by connecting the apparatus to the high vacuum

source i.e. suitable vapour diffusion pump. The complete assembly of the apparatus required for the distillation is shown in Figure 2.20.

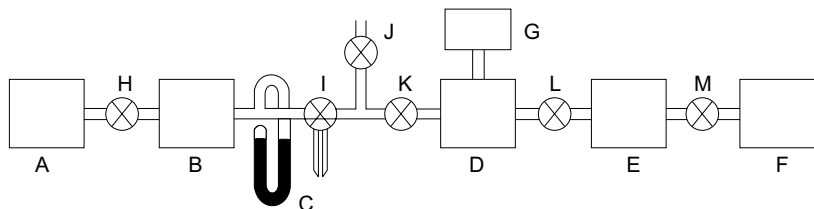


Fig. 2.20 Apparatus assembly for molecular distillation

In the above figure, A is the distillation unit, B is the vapour traps, C is an uncalibrated mercury manometer, D is the vapour diffusion pump, E is a phosphorous acid moisture trap, F is an oil immersion rotatory backing pump and G is the McLeod Gauge. All these components are connected with wide-bore glass tubing having the minimum number of bends and fitted with joints of the O-ring seal type, or with glass joints sealed with Apiezon Wax W, as required. The H-M are the vacuum taps which site at the points indicating that the assembly can be isolated at these points to facilitate the vacuum control and measurement.

The distillation unit A is attached to the ground glass joint which is fitted to tap H, all the taps are in the closed position. The Dewar flasks, which surround the vapour traps, are filled with suitable coolant e.g. nitrogen water in the cold finger of A and vapour diffusion pump is turned on. The backing pump is also switched and all the taps M, L, K and H are opened in the sequence to attain the pressure with this pump. The pressure in the assembled system is shown by the auxillary manometer C. This can also be used to check the leakages in the assembly by closing the tap M and noting the fluctuations in the mercury level. After reconnecting the backing pump by opening of the tap M, the heat is supplied to the vapour diffusion pump and the pressure is noted down by McLeod gauge. When the system attains the minimum pressure, the distillation unit A is heated slowly in an oil bath until the misting of the cold finger is observed. The temperature of oil bath is noted and is maintained at this point. The reading on McLeod Gauge is noted occasionally during the distillation process. When distillation is completed, the heating of the vapour diffusion pump is stopped and the unit A is de-attached by turning off the tap H. The temperature of the fluid of diffusion pump and the unit A is

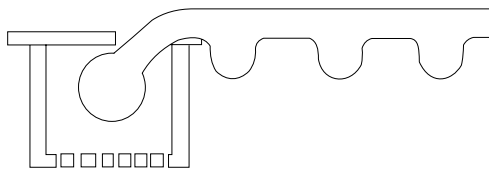


Fig. 2.22 Distillation unit for micro scale apparatus

and clamp in such a way that the receiver tube is sloping downward and the bulb is immersed in an oil or air bath. When the vacuum is attained to a constant value, the heating of the system is started. Liquid distils into the first indentation. The waste of the distilled material is avoided by the further indentations along the tube.

2.10 DISTILLATION OF SOLIDS UNDER REDUCED PRESSURE

The apparatus (Figure 2.14) used for the distillation of liquids under reduced pressure may also be used for solids having comparatively low melting points. In this case, water-cooling of the receiver is not required. While if the solid solidifies in the side arm of the Claisen flask, then the distillation is carried out rapidly and the side arm of the Claisen Flask and the neck of the receiver is warmed with a luminous flame occasionally to overcome this problem.

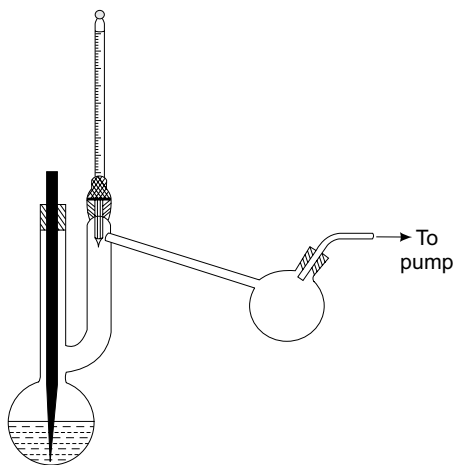


Fig. 2.23 All glass apparatus for distillation of solids under reduced pressure

The all-glass apparatus, shown in the Figure 2.23 is a more satisfactory apparatus and can be used for compounds having melting point as high as 200°C .

This method is mostly used to remove tar, the coloured and non-volatile solid impurities without loss of material. Also, this method of purification of solids is more effective and economical than several crystallizations from a solvent. The purity of the solid can be determined by melting point determination. For purification of solids by this method, the apparatus used is shown in the Figure 2.24. The solid and apparatus must be dry. The flask is heated with a large free flame in rotatory motion round the walls of the flask to avoid bumping which is more frequent in case of heating from the bottom. At the start, if there is frothing, the upper walls and neck of the flask are heating with the flame. After the completion of the distillation, air is allowed to enter the apparatus, and the contents of the receiver are collected by melting it and removed with a bulb pipette. If the distillate is left in the receiver, the side arm may be cut and a new receiver is sealed to the side arm.

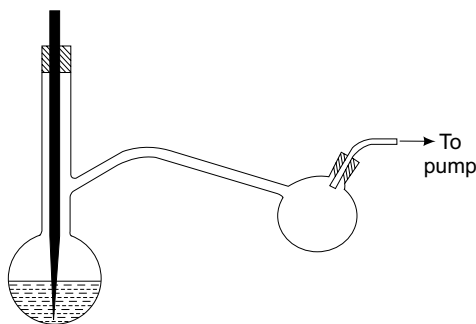


Fig. 2.24 Another type of apparatus for distillation of solids under reduced pressure

2.11 SUBLIMATION

Some of the organic compounds can be purified by the sublimation under normal pressure e.g. naphthalene, anthracene, benzoic acid etc. This method is used for those organic compounds only which directly get converted from vapour to the solid state without passing through the intermediate i.e. liquid state. Such compounds have a high vapour pressure at a temperature below melting point so that the rate of vaporisation from

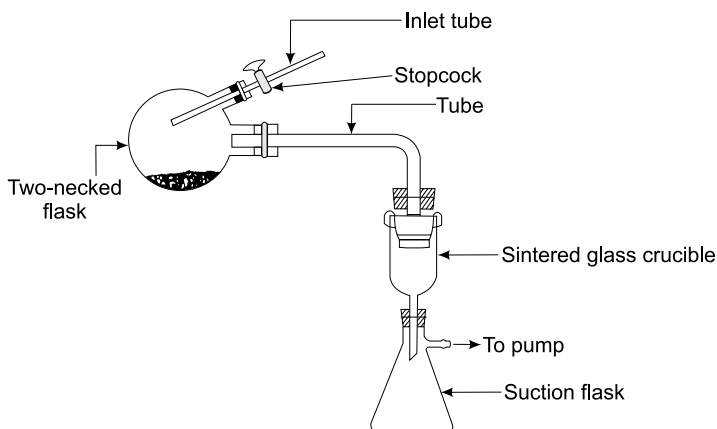


Fig. 2.26 Apparatus for sublimation in an inert atmosphere

2.11.1 Exercise on Sublimation

(a) *Purification of anthraquinone by sublimation*

Place crude, dry anthraquinone in a china dish, cover it with perforated filter paper and place a cold dry glass funnel, slightly smaller in diameter than the dish, over the filter paper. Heat the dish such that the anthraquinone is vapourised steadily and the funnel does not become more than lukewarm. After a few minutes, the vapours come into the funnel through the holes of the paper and get condense on the upper surface of the filter paper and on the walls of the funnel in long yellow needles. Stop the heating when almost all the anthraquinone is vapourised. Collect the pure anthraquinone. Melting point of anthraquinone is 277°C .

2.12 SUBLIMATION UNDER VACUUM

The yield of the sublimate can be improved to large extent if the sublimation is carried out under reduced pressure and sublimation of the substances having low vapour pressures at their melting points, can also be carried out under these conditions. The one more advantage of this method is to reduce the possibility of thermal degradation, as sublimation is carried out at low temperature under these conditions.

The apparatus employed for the vacuum sublimation is shown in Figure 2.27 (a-b). In figure 2.27 (a), the cold finger is inserted in a larger tube with the help of a rubber stopper and is carrying a disc slightly smaller

The stationary phase, i.e. solid can be packed in the column [column chromatography], or can be coated in the form of thin layer on rectangular glass sheet [TLC].

2.13.1.1 Column chromatography

In this chromatography, solid phase is packed in a cylindrical column, the mixture to be separated is added at the top of the column and the solvent, eluent, is allowed to flow down through the column. A certain fraction of each component of the mixture will be adsorbed by the adsorbent and the remaining will be in the solution. And any molecule will spend part of its time on adsorbent and the rest of the time flowing down with the solvent. The substance having strong adsorption towards the adsorbent will have greater fraction of its molecules adsorbed at any time and therefore any molecule of the substance will spend more time on adsorbent and less time in moving down. While, the substance having weak adsorption will have a smaller fraction of its molecules adsorbed and therefore, will spend less time on adsorbent and more time in moving. Hence, the more strongly adsorbed substance will move down slowly while the less strongly adsorbed substance will move down faster and so comes out in early fractions and the former will come out in the later fractions.

So as the solution of the mixture is added to the column, it penetrates into the column and the components are adsorbed. The fresh solvent is added at the top, when the solution has just completely entered the column. The solvent moves down and re-dissolves the solutes in amounts, which can be calculated by adsorption Law [ratio of amount of solute adsorbed per unit surface area to the concentration of the solute in solution is constant]. So solvent carries solute down the column where it is re-adsorbed, amount of which can also be determined by adsorption law. So as more and more solvent passes through the column, the adsorption and de-adsorption occurs continuously and the solutes gradually move down the column and appear in the form of bands. For solutes having sufficiently different adsorption coefficient, the separation is better. And as discussed earlier, the strongly adsorbed substance will form band above the weakly adsorbed substance. When separation of the band is sufficient enough, the solutes are forced out of the column elution at the lower end and are collected separately. Sometimes, due to the much of time required for the elution, this method to obtain the component from the column is not preferred. In such cases, the surplus solvent of the column is drained off, so that the adsorbent comes out as one piece and the various bands of

the column are cut off by laying it down on a sheet of glass and are separately extracted [by adding solvent into it, followed by filtration so that the adsorbent can be removed. Adsorbent is washed 2-3 times with the solvent followed by filtration so as to remove the component completely from the adsorbent. Now the filtrate is distil off to obtain the component as residue].

If the components are coloured, they can be visualized as a series of colour bands. Some compounds are strongly fluorescent under ultraviolet light, so the positions of the bands can be detected by their fluorescence in the U.V. light. But if the components are colourless and non-fluorescent, then the eluent must be collected in successive fractions and the presence of the component is determined by some analytical method. The most common method used for analysis is to distil the eluent. If the residue is a solid, it can be identified by TLC [see Sec 2.13.1.2], melting point, infrared spectrum etc.

2.13.1.1.1 Column

Column is a long narrow glass tube with stopcock. The length and the diameter of the column may vary depending upon the nature of components of a mixture, e.g. if the difference in adsorption coefficient is less, long column is required for the better separation. The apparatus used in the column chromatography is shown in the Figure 2.28. Sometimes

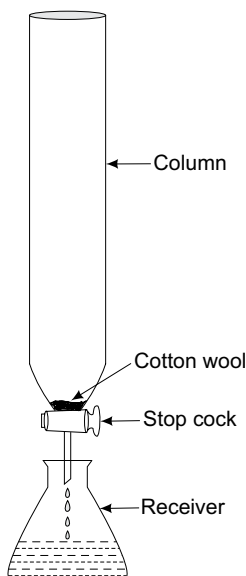


Fig. 2.28 Apparatus for column chromatography

column may be fitted with ground glass joints at its ends so as to allow the attachment of a separatory funnel as solvent reservoir and a Buchner flask via an adaptor with a tap to collect the eluate fractions [Figure 2.29].

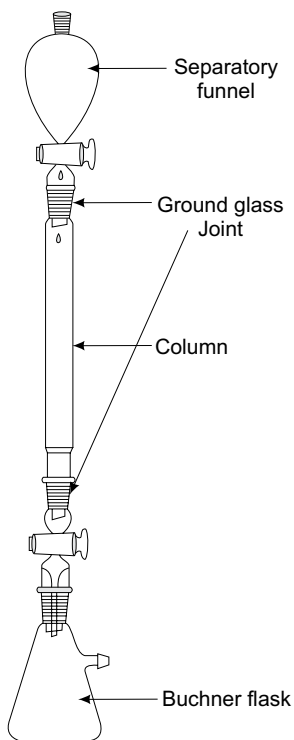


Fig. 2.29 Another type of apparatus for column chromatography

2.13.1.1.2 Adsorbent [Column Packing Material]


Several factors affect the efficiency of a chromatographic separation, adsorbent is one of them. Adsorbent should have following properties :

- (a) It should have high and selective adsorption power.
- (b) It should have high surface area.
- (c) It must be chemically inert.
- (d) It must be readily available.

For the separation of any mixture, some adsorbents may have too strong adsorption power that all the components gets adsorbed near the top while some of them will be too weakly adsorbing that all the

Table 2.3 Eluting solvents for chromatography taking alumina as adsorbent

(1)	Petroleum ether (Hexane; pentane)	
(2)	Cyclohexane	
(3)	Carbon tetrachloride	
(4)	Trichloroethylene	
(5)	Toluene	
(6)	Benzene	
(7)	Dichloromethane	
(8)	Chloroform	
(9)	Ether	
(10)	Ethylacetate	
(11)	Acetone	
(12)	Propanol	
(13)	Ethanol	
(14)	Methanol	
(15)	Water	
(16)	Pyridine	
(17)	Organic acids	



 Decreasing
eluting
power

highly adsorbing, it can be replaced by a more completely hydrated alumina or any weaker adsorbent, which if it is too weakly adsorbing, then trial should be done by a less hydrated alumina.

If the substances in the mixture differ greatly in their polarities, they are separated very easily. And so the order in which the components (adsorbate) are eluted from a column depends upon their relative polarities e.g. a hydrocarbon and an alcohol can be separated much easily as alcohol being more polar is adsorbed more strongly on the adsorbent than the hydrocarbon and so hydrocarbon can be eluted with a relatively non-polar solvent and an alcohol can then be eluted with a more polar solvent. Table 2.4 shows the order of ease of elution of the adsorbate.

Table 2.4 The order of ease of elution of the adsorbate

(1)	Saturated hydrocarbons; alkyl halides	
(2)	Unsaturated hydrocarbons, alkenyl halides	
(3)	Aromatic hydrocarbons; aryl halides	
(4)	Polyhalogenated hydrocarbon	
(5)	Ethers	
(6)	Esters	
(7)	Aldehydes and ketones	
(8)	Amines, alcohols and thiols	
(9)	Phenols, carboxylic acid	


 Decreasing
ease of elution

2.13.1.1.4 Packing of the Column [Loading of Column]

For the best separation, the column must be packed uniformly without holes, air bubbles, cracks or channels. If the distribution of the adsorbent is uneven, it may lead to the formation of cracks and channels, which may result in the distortion of the adsorption band shapes.

The ratio of amount of adsorbent to the weight of sample to be separated should normally be 50:1. Also, the ratio of the height of the adsorbent in column to its diameter should normally vary between 3:1 to 10:1. More is the ratio, the flow of the solvent may be too low. So for large amount of samples, large-diameter column must be used.

To load the column first a small plug of cotton wool is inserted in the lower end of the column. The adsorbent is mixed with the liquid to form a thin slurry, which is then poured through a funnel into a clean dry column held vertically. The column is tapped gently with a wooden rod so as to settle the adsorbent uniformly. Solvent is removed by the stopcock fitted at the bottom of the column and more slurry is added until the complete required uniform bed of the adsorbent is obtained. Fresh solvent is then flow down the column, until complete setting of the adsorbent is obtained. A disk of filter paper, having diameter just a little smaller than the diameter of the column is dropped on the adsorbent so as to cover the top of the column to protect the surface from disturbance when the sample or the eluting solvent is poured onto it. During the whole process, column loading as well as the separation of bands, the level of the solvent should not fall below the level of adsorbent otherwise the cracking of the bed of adsorbent takes place due to the drying of the column and hence result in the diminished ability to separate the components because of the flow of the solution through these cracks.

A concentrated solution* of the mixture is added to the column and the column is allowed to drain just to the surface of adsorbent. The sample is rinsed down from the walls of the column with a small quantity of solvent, which is again allowed to drain just to the surface of the adsorbent. [Figure 2.30 shows the loaded column]. Now the empty space of the column above the adsorbent is filled with the solvent and a dropping funnel filled with the solvent is attached. The development of

*The concentrated solution of the sample to be separated is added so that it gets adsorbed in a minimum layer of adsorbent. And, narrower is the sample bands, overlap is less and hence better is the separation.

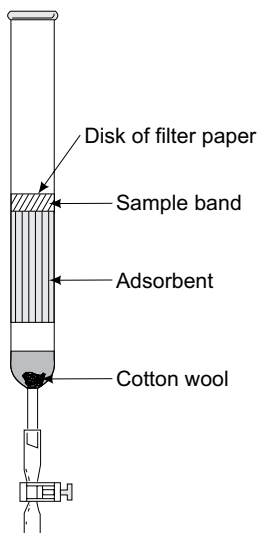


Fig. 2.30 Loaded column

chromatogram, collection, analysis of fractions and recovery of the separated components are as described earlier.

2.13.1.1.5 *Advantages and Disadvantages of Column Chromatography*

This technique is especially used for purifying small amounts of the compounds. And, the separation by this method is far better than the distillation or recrystallization. But, it is a highly empirical method, and several trials are required to establish the acceptable conditions. Also, it cannot be used to purify the sample in large quantities as even reasonable size columns have a small capacity.

2.13.1.1.6 *Exercises on Column Chromatography*

(A) *Purification of Anthracene*

The apparatus employed is shown in Figure 2.29. The column used is 40 cm long and 1.5 to 2 cm in diameter. A 250 ml suction flask is fitted at the bottom of the column and a 50 ml dropping funnel is fitted at the top of the column.

Wash the column with chromic acid mixture followed by distilled water and dry it. Insert a plug of cotton wool at the end of the column. Clamp the column in vertical position. Here alumina is used as an

adsorbent. Introduce 3-4 cm column length of alumina and press it by dropping a glass pestle, [which is made from (60 cm × 7-8 mm) solid glass rod and flattened at one end to 10-12 mm] on to it from a height of 5-10 cm several times.

Repeat the process using 3-4 cm portions of alumina each time to have a column of 20 cm adsorbent bed. Insert another small cotton wool plug on the top of the alumina column. Wet the column by adding 25 ml of n-hexane from dropping funnel. Precaution must be taken to cover the column with the solvent during the whole experiment to prevent the formation of holes, cracks, air bubbles or channels. Prepare the solution of technical anthracene by dissolving 50 mg of it in 50 ml of n-hexane and add the solution from the top of the column. n-Hexane is used as an eluting solvent. Develop the chromatogram with n-hexane and examine the progress of the separation with an ultraviolet lamp. After some time, three bands will be obtained:-

- (a) a narrow, deep-blue fluorescent band near the top due to carbazole (impurity in anthracene)
- (b) a non-fluorescent yellow band in the middle due to naphthacene (another impurity in anthracene)
- (c) a broad blue violet fluorescent band at the bottom due to anthracene.

Continue the progress of the separation with n-hexane. Change the receiver when fluorescent band (anthracene) begins to come off the column. Discard the elutant collected before the anthracene begins to come off. At this point, elute the anthracene with a 1:1 n-hexane – A.R. benzene mixture. Stop the elution when the middle band (naphthacene) reaches the cotton-wool plug. To obtain the anthracene, evaporate the solvent from the eluate under reduced pressure. Pure anthracene of visibly fluorescent in daylight is obtained. Yield = 30 mg and m.pt. = 215°C.

Clean and dry the column. Discard the alumina.

(B) Separation of *o*- and *p*-nitro aniline mixture

Wash the column thoroughly with chromic acid followed by distilled water. Dry it and insert a small plug of cotton wool in the lower end of column and clamp it vertically.

Make a slurry by mixing 100 gm of active alumina with dry benzene and pour it down into the column. Fit a disk of filter paper over the top of the column. And now fit a dropping funnel at the top of the column and a

by means of a cement for glass. In the central depression, the plate to be coated is placed which is held by two uncoated plates one on either side. The required thickness (0.25 mm) of the layer can be adjusted by the spreader.¹

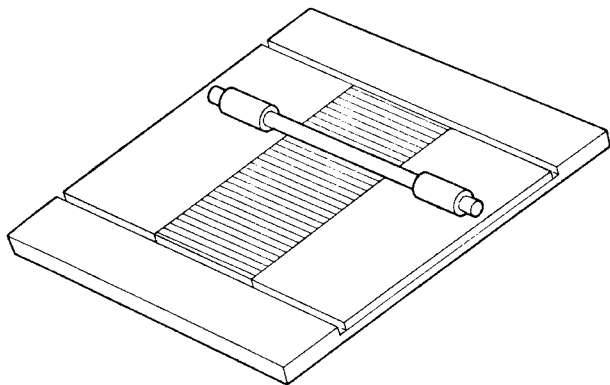


Fig. 2.31 Apparatus for coating a glass sheet

To coat one plate of 20×5 cm, 2 g of dry adsorbent is sufficient enough. 2 g of silica gel² is mixed with 4 ml of distilled water in a small conical flask and is shaken vigorously for exactly 90 seconds. Slurry can also be prepared by grinding the silica gel G and distilled water in a glass mortar to form a smooth paste. The slurry is poured on the upper end of the central glass plate [Figure 2.31] and is spread evenly over the plate with the help of the spreader. This whole process must be completed in 15 seconds. It is now allowed to stand for 5 minutes to set the layer and then the plate is removed and dried at 110°C for 15 minutes. It is then cooled and kept aside in the atmosphere for half an hour.

Adsorbent coated (with or without fluorescent indicators) TLC plates can also be obtained commercially. As they are prepared carefully under standardised conditions, to get reproducible results, they are preferred to the hand-coated plates due to the elimination of the variation in the factors which can affect R_f values e.g. particle size prior to activation of the plates, thickness of the adsorbent etc. [See sec. 2.13.1.2.5].

¹Spreader is a glass rod 14 cm long and 7.5 mm in diameter with equal lengths (12.5cm) of 2.5 cm. cello-tape

²Silica gel G has a particle size of $5\text{--}25\ \mu$ and contains 13% CaSO_4 .

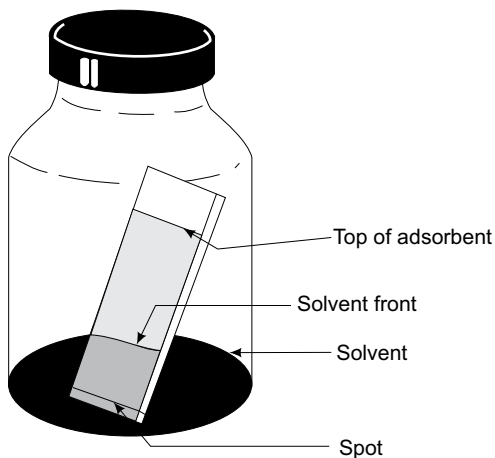


Fig. 2.32 Development of a TLC plate in a corked bottle

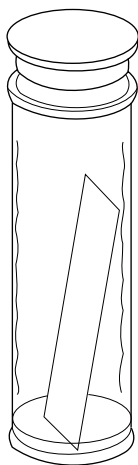


Fig. 2.33 Development of a TLC plate in a glass jar

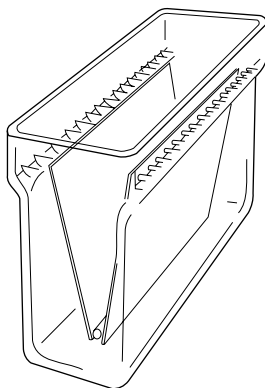


Fig. 2.34 Development of a TLC plate in a rectangular glass tank

so that the lower edge of the adsorbent layer is dipped in the solvent but the spots are far above the level. The jar is closed again and the solvent is allowed to rise on the adsorbent layer by the capillary action. The components of the mixture ascend at different rates depending upon their affinities for the adsorbent. When solvent rises up to 10-12 cm from the

starting line, the plate is removed and immediately the position of the solvent front is marked on the adsorbent layer. The time required for the completion of the development of chromatogram varies as it depends on the nature of the adsorbent and the composition of the solvent.

The plate is now dried in a temperature-controlled oven.

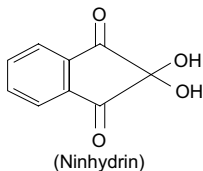
2.13.1.2.4 Visualization of Spots

If the components are coloured, they can be visualized directly. U.V. fluorescent compounds can be visualized by keeping the plate under an ultraviolet lamp. Non-fluorescent compounds can be visualized due to their fluorescent quenching effect, when they are chromatographed on the adsorbent incorporated with a fluorescent indicator.

A detecting agent used generally for the most of the organic compound is iodine vapour. The dried plate is kept in a closed dry tank containing iodine crystals. Iodine vapours make spots brown. The positions of these spots are marked immediately as the plate has been removed from the iodine jar as iodine vapour evaporates on standing in air so the brown spots disappear.

One more general detecting agent is conc. H_2SO_4 or a solution of 4 ml conc. H_2SO_4 in 100 ml methanol. These detecting agents can be used only when the inorganic adsorbents are used and the components are organic materials. They are sprayed on the dried plate and then the plate is kept for heating in an oven to about 200°C until all the organic materials are turned into dark charred spots.

The detection of colourless compounds can also be carried out by using chemical methods with the help of the suitable spraying agent. These spraying agents are highly selective and may be extremely sensitive, e.g. the ninhydrin reagent* is used for the detection of amino acids, indicators are used for the detection of acids and bases. The advantage of these spraying



*Ninhydrin is a 2-hydrate of indane-1,2,3-trione. It reacts with α -amino acids to yield highly coloured products due to the presence of extended conjugation. It produces a long-lasting purple colouration on skin and therefore its contact with the skin should be avoided.

agent is that they can be applied after viewing the plate under ultraviolet light, exposing to the iodine vapour and then allowing the evaporation of iodine. So this extended treatment gives a clearer picture of the composition of the components of a mixture.

The apparatus used for the spray of these agents is called automizer and is shown in the Figure 2.35

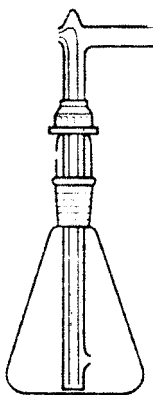


Fig. 2.35 Automizer

2.13.1.2.5 Values R_f

$$R_f = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by solvent front}}$$

Under the same experimental condition, for any substance, R_f value is a constant and is characteristic of that substance.

e.g. In the given chromatogram (Fig. 2.36),

R_f value for A, B & C is

$$R_{fA} = a/d, R_{fB} = b/d, R_{fC} = c/d$$

R_f value depends on various factors. These are:

- (i) The size of the adsorbent,
- (ii) Solvent composition,
- (iii) Degree of saturation of the jar atmosphere with solvent vapour,
- (iv) Activation and storage conditions of the plates,
- (v) Thickness of the adsorbent layer,
- (vi) Atmosphere temperature etc.

sufficient thickness so as to self-support and must also be flexible. The supporting material may be a solvent-resistant polyester sheet or an aluminium foil, former is used usually.

The sheets are coated with the silica gel incorporated with a small amount of a polyvinyl alcohol binder. It results in a highly porous coating and the solvent penetrates it quickly. These sheets can be activated, if required and can be cut down to the required size with the help of scissors and used as a thin layer glass plate. Advantages of these sheets are that there is the less chance of damage of adsorbent layer and also they can be stored in a notebook etc. after use.

These sheets are also available with the fluorescent indicator.

2.13.1.2.8 *Advantages of TLC*

It can be used to identify the substance as well as separate them [already discussed in sec. 2.13.1.2]. Paper chromatography [See sec. 2.13.1.3] is used only for the separations on cellulose as other adsorbents cannot form sheets. This limitation is overcome by TLC as large number of adsorbents can be used as they easily form thin layer supported on glass sheets. In TLC, sample spots remain more compact than paper chromatography and therefore very small amount of substances are required for the separation and identification. Larger quantities of the mixture can be separated by using thicker adsorbent layer on large size plates.

Not only these, but TLC can also be used to monitor the progress of the reaction and to assess the success of purification process. In general, the reaction is monitored by the periodic removal of small portions of reaction mixtures and load them directly on TLC plate after their work up.

2.13.1.2.9 *Exercises on Thin-Layer Chromatography*

(a) *Separation of α -amino acids*

Separation of DL- α -alanine, L-Leucine and L-lysine hydrochloride.

Dissolve 5 mg of each of the above amino acids separately in 0.33 ml of distilled water (with the help of 1 ml graduated pipette). For Leucine, warm to effect the solution, mix one drop of each amino acid solution to have a mixture of these three amino acids. Dilute the rest of the each solution separately to 1 ml to have solutions of the respective amino acids. Apply spots of each solution on to the TLC plate as already described and allow them to dry in the air.

Prepare the developing solvent by mixing n-propanol (70 ml) with conc. aqueous ammonia (30 ml, specific gravity = 0.88). Line the inside of

the jar with the filter paper and allow it to get wet with the developing solvent. Close the jar and allow it to stand for about 10 minutes so that the jar becomes saturated with the solvent vapour. Insert the plate into the jar and add the sufficient developing solvent into it with the help of a pipette, so that the lower edge of the adsorbent layer is dipped into solvent and the spots must be far above the solvent level. Close the jar again and allow the solvent to ascend on to the chromatplate. Remove it when solvent rises up to 10-12 cm and dry it in oven at 100°C for 10 minutes. Violet spots are developed. Mark these spots and evaluate the R_f values from the centre of these spots as discussed in sec. 2.13.1.2.5 [Literature R_f values for alanine, Lysine and Leucine are 0.26, 0.11 and 0.48 respectively].

(b) Separation of 2,4-dinitrophenylhydrazones

Prepare the solutions of 2,4-DNP derivatives of acetone, butan-1-one and hexan-3-one by dissolving 10 mg of each in 0.5 ml of ethyl acetate. Apply the spot of each solution on a flexible silica gel sheet (20 × 5 cm sheets with fluorescent indicator) as already described. Apply the spot of mixture by loading each of the solution one at a time and allow the spot to dry completely after each addition.

Prepare the developing solvent by mixing toluene to light petroleum in 3:1 ratio. Line the inside of the jar with filter paper (b.pt. = 40-60°C) and when the jar becomes saturated with solvent (after 10 minutes), insert the chromatplate into it. Carefully pour more of the developing solvent so that the lower edge is immersed into the solvent but spots are far away from the solvent level. Allow the solvent to ascend on to the sheet. Remove the chromatogram and air-dry it. Calculate directly the R_f values of each component.

(c) Separation of leaf pigments

Prepare the sample solution by crushing the green leaves in a mortar along with few milliliters of ethanol or acetone and twice this volume of petroleum ether or hexane. Filter the mixture. Transfer the filtrate into a separating funnel and wash it with small portions of water thrice. Dry it over anhydrous sodium sulphate.

Here, the developing solvent used is Hexane: acetone, 7:3 (by volume). The developed chromatogram gives the R_f values of carotenes, chlorophyll *a* & *b* and the Xanthophylls.

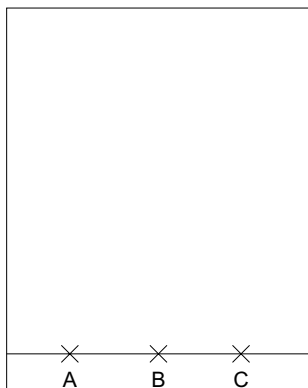


Fig. 2.37 A chromatogram

also be used for each solution. The paper strip is dried in the air. If the solution is dilute, large amount can be applied by making several small applications, and allowing the previous spot to dry before putting the next one.

2.13.2.2 Chromatogram development

After drying the paper, it is placed in a suitable container where it is moistened with moving phase by ascending technique (i.e. moving phase is moving upwards by capillary action) or descending technique (moving downwards by gravity).

(a) Ascending Technique

In ascending technique, the apparatus used are shown in Figure 2.38 (a-b). The paper is placed vertically in a closed jar without touching its sides as shown in the figures. As in TLC, here also, the precaution that the paper edge with starting line at bottom should be dipped into the solvent while spots must be far above the solvent level.

(b) Descending Technique

In descending technique, the apparatus used is shown in Figure 2.39. It consists of a special designed jar having a trough sufficiently supported at the top of the jar for the moving phase. The paper is held within the trough with the help of a glass rod whose one of the end is angled for the suitable handling. Another glass rod is placed close to the trough edge as shown in the above Figure 2.39 to pass the paper over it to protect the paper by the glass edge of the trough and for regular and evenly flow of solvent down the

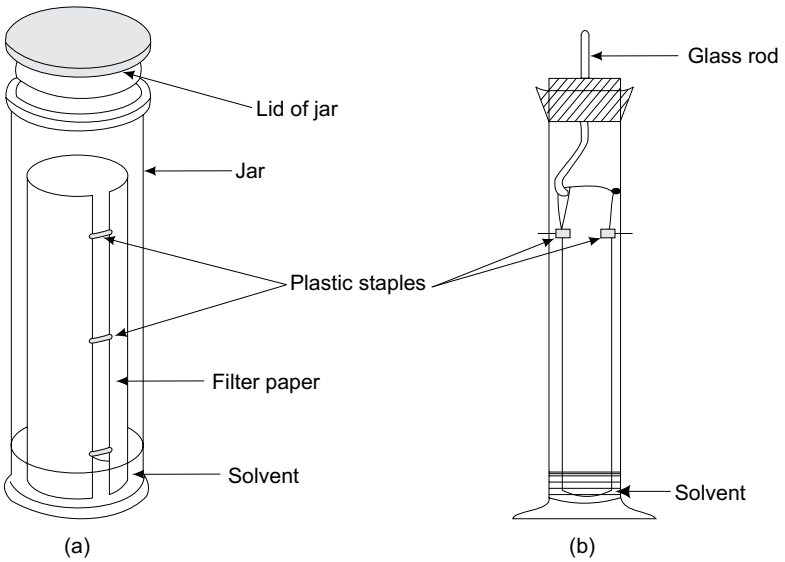


Fig. 2.38 (a-b) Apparatus for development of chromatogram in ascending technique

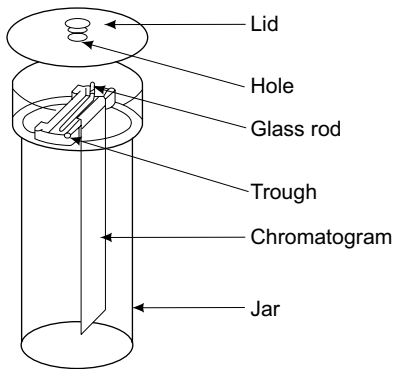


Fig. 2.39 Apparatus for development of chromatogram in descending technique

paper. The two-phase solvent system may be used in this technique, by placing the aqueous phase in the bottom of the jar and when the atmosphere becomes saturated with it, the paper to be chromatographed is then supported in the empty trough. The jar is then closed again and the paper is allowed to stand for 1-2 hours to get equilibrate with the

atmosphere of the jar. The upper layer (organic layer) of the solvent system is added into the trough through the hole in the cover. The solvent is allowed to move down for 12-13 hours. Then the cover of the jar is removed and the paper is cut down along the supporting rod and is removed from the jar by lifting the supporting rod. The solvent front is marked with a pencil and paper is dried.

2.13.2.3 Visualization of spots

If the substances are coloured, the spots can be visualized directly. Sometimes, ultraviolet light can be used to visualize the spots. Spraying agents can also be used to locate these spots. Disadvantage of this technique is that the specific reagents should be used which cannot attack the paper. This can be overcome by TLC and so corrosive reagents can be used for spraying.

2.13.2.4 R_f values

As in TLC, here also, R_f values are quite sensitive to the exact experimental conditions. For the comparison of experimental R_f values of unknown substance with the most probable known substances, samples of all these substances should run side by side on the same strip of the paper.

2.13.2.5 Exercises on paper chromatography

(a) Separation of amino acids by descending technique

Separation of glycine, DL- α -alanine and L-Leucine.

Dissolve 5 mg of each of the amino acids separately in 1 ml of distilled water. Prepare developing solvent by mixing 40 ml of n-butanol, 10 ml of glacial acetic acid and 50 ml of water in a separatory funnel. Allow the two-phase mixture to settle, separate the two layers. Use fresh solvent as it undergoes esterification on standing.

Use the container for this technique as shown in Figure 2.38(b). Cut Whatman paper No. 1 in 50×10 cm strips. Touch the strips as minimum as possible and handle them by the edges only. Mark a starting line with pencil lightly 2.5 cm from one end. Then mark the points equidistant from each other with the pencil. Apply spots of each amino acid solution on these points carefully with the help of a capillary tube [diameter of these spots should be 4-5 mm].

Add the aqueous (lower) layer of the developing solvent in the bottom of the jar. Locate the paper in the trough vertically and cover with the lid.

Procedure is same as in example(a). In case of monosaccharides, R_f values are low and therefore the solvent is allowed to move down and drip from the paper end. And as there is no solvent front position, the D-glucose is taken as a standard and the chromatographic behaviour of the individual monosaccharide is compared with it. This gives a new constant, R_g .

$$R_g = \frac{\text{distance travelled by monosaccharide}}{\text{distance travelled by D-glucose}}$$

(e) **Separation of Anthranilic acid and N-Methyl anthranilic acid**
sample solution

20 mg of pure anthranilic acid in 20 ml of methanol and 10 mg of pure Methylanthranilic acid in 20 ml of methanol.

Developing solvent: 10 ml of conc. aqueous ammonia (sp. gr. 0.88) in 40 ml of redistilled n-butanol.

Spraying agent: These acids give intense blue fluorescent zones in ultraviolet light. Ethanolic ferric chloride solution can be used as spraying agent:- N-methylanthranilic acid gives a purple brown spot and anthranilic acid gives a very faint pink colouration.

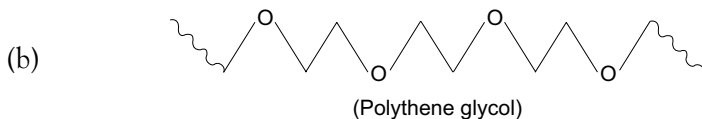
2.13.3 Gas Liquid Chromatography (GLC)

It is a rapid and convenient technique to analyse the composition of the mixtures of sufficiently volatile organic compounds. In this chromatography, stationary phase is a high boiling liquid supported on a suitable granulated solid medium, and the mobile phase is gaseous phase [mixture of components and an inert gas]. Here, the separation is carried out by partition of substances between these two phases.

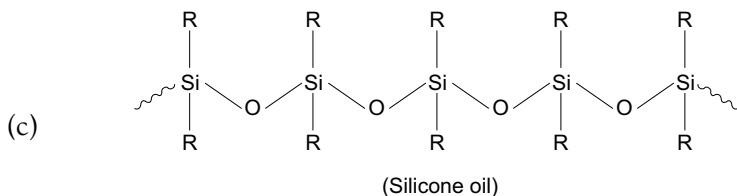
Large number of stationary phases are available commercially. Some of the examples are:



They separate molecules in order of their boiling points.



It is good for polar compounds e.g. alcohols, ketones, esters etc. It is better than the hydrocarbons.



These are generally useful and separate the molecules according to their volatility.

The solid supporting material generally used is Kieselguhr or firebrick, which are available commercially in various particle sizes.

The apparatus required for GLC is more complex and expensive than that for the other chromatographic methods. The principal features of the apparatus employed for GLC is shown in the Figure 2.40.

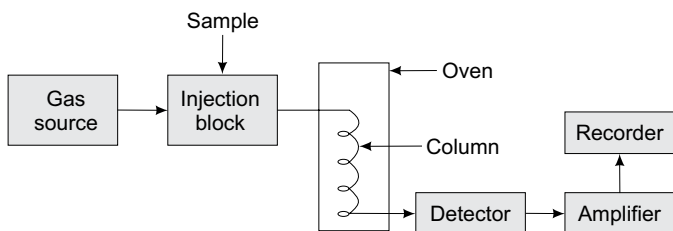


Fig. 2.40 Principal features of apparatus for GLC

It consists of a source of carrier gas, an injection block for the introduction of the sample, a column (made up of metal or glass, 2-3 m length and 2-4 mm internal diameter and is in the form of a circular spiral) in which the supporting medium along with the stationary phase is packed. The components of the sample are carried down the column where they are separated depending upon their partition coefficient between the stationary and mobile phase and comes into a detector. The detector senses the presence of the material in the carrier gas. The signal from the detector is suitably amplified by an amplifier and the recorder records the signal as a function of time.

Liquid samples are injected directly whereas solid samples are dissolved in a volatile solvent, e.g. ether, before injection. A very small amount (0.001-0.005 mL) of a sample is required which is drawn into the syringe without any air bubbles. If the air bubbles appear in the syringe,

they can be removed by either dipping the needle into the sample and pumping the plunger for 3-4 times or holding the syringe with the needle up and tapping the barrel with the finger until the bubbles rise to the needle end and then pushing the plunger in to force the bubbles out.

The recorder is started before the injection of the sample. The sample is injected by inserting the needle of the syringe into the rubber septum and pulling the needle straight back out after sliding in the plunger.

A chromatogram may take one minute to half an hour. Recorder is turned off when the chromatogram is complete. If peaks are too small or pen goes off the scale, the chromatogram may be obtained again by changing the sensitivity setting.

The area under the peak should be a measure of the amount of the given compound present in the sample. Higher is the concentration of a given compound in the carrier gas, the larger is the amount of it that comes off the column.

The qualitative and quantitative assessment for the composition of the mixture can be carried out by the examination of the graph produced by recorder. No. of peaks in the chromatogram indicates the number of components present in the given mixture. And the percentage of each component can be calculated by measuring the area under each peak* and taking this as a proportion of the total area. And for a mixture of two substances, the relative amounts of the substances can be calculated by measuring the ratio of the areas of the two peaks.

If the peaks are not resolved completely, but there is somewhat overlapping among them, estimation of the area will be difficult and in such cases, resolution can be increased by using a longer or narrower column, smaller sample size and lower operating temperature.

The identification of the components of the mixture can be made by first comparing the retention times with that of the pure components. And it can be confirmed by adding each of the suspected components in the

*Area under each peak can be determined by:

- Counting squares
- Measuring the peak height from the extrapolated base line and width at half-height. The product of the two is the area.
- Tracing each peak carefully on another paper and weighing the area enclosed by cutting these trace peaks.

$$\% \text{ of the component} = \frac{\text{Weight of the component}}{\text{Total weight of all the components}} \times 100$$

- (c) Separation of mixtures of acids and neutral compounds by the removal of acids.
- (d) Separation of mixtures of bases and neutral compounds by the removal of bases.

2.13.4.2 Exercises in ion-exchange chromatography

Examples of each of the application discussed in the above section are:

(a) Conversion of sodium citrate into citric acid

This conversion cannot be carried out easily on a semi-micro scale by ordinary chemical methods. Dilute acids can be used to liberate an acid from its salts provided the organic acid is insoluble in water e.g. aromatic acids or it is volatile e.g. acetic acid. Citric acid is water-soluble and non-volatile and therefore ion-exchange method is used in this case.

Prepare a slurry of a strong acid cation resin by stirring it with 100-120 ml of 10% hydrochloric acid in a breaker and allowing it to settle. Decant the supernatant liquid. Put a small plug of glass wool at the base of a 50 ml burette. Now pour the slurry of resin to the burette to produce a column of 25-30 cm long. Open the tap of the burette and pass 100-120 ml of 10% hydrochloric acid through the resin at the rate of 5 ml per minute. Wash the resin with distilled water until the washings of the burette are neutral to methyl orange. Close the tap of the burette. Now column is ready for use.

Prepare a solution of 0.05 g of sodium citrate in 100 ml of water and pour it down the column at the rate of 5 ml per minute. Collect the runnings in a conical flask and wash the column with 100-150 ml of distilled water. Add these washings to the above runnings. Evaporate the combined runnings to obtain pure citric acid, having m.p. 100°C.

For the regeneration of the column, pass excess of 10% hydrochloric acid and wash with water as described above. This column is now ready for reuse.

(b) Conversion of aniline hydrochloride into aniline

Prepare a column of a strong base anion resin of 25-30 cm long in a 50 ml burette as described in the above example. Wash the resin with distilled water and then pass 100-120 ml of N-sodium carbonate solution through column. Wash the column again with 200 ml distilled water. Now column is ready for the use.

flow of the liquid and thus high pressures (1000-6000 p.s.i.) are required to force the eluant through the columns. The pressures are maintained by suitable pumps and can be preset at the required value.

2.14 ELECTROPHORESIS ON FILTER PAPER (FILTER PAPER ELECTROPHORESIS)

This technique is closely associated with the chromatography. Here separation is due to the difference in the electrical properties of the components of a mixture. Under the influence of an electric field, organic ions move towards an electrode (anode or cathode) with different rates and so results in the separation of mixtures. The pH of an electrolyte is very important and is maintained by a buffer solution.

The apparatus used in this technique is shown in the Figure 2.41. It consists of two terminals for the connection to power supply, shoulder piece for supporting the ends of the paper strip, paper strip holder to hold the paper and cotton wool plug, soaked in buffer solution, to make electrical connection between the compartments.

For the separation of mixtures by filter paper electrophoresis, first the compartments are filled by buffer solution (up to 10 mm depth). In each compartment, the level of the buffer solution must be same and if not so, tilt the tank and return to the original position. The filter paper connections, of the length of strip holders and width to dip well into the

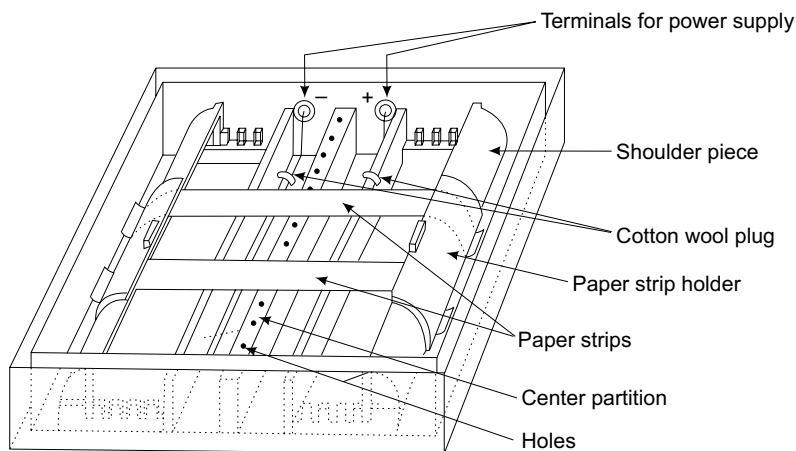


Fig. 2.41 Apparatus for electrophoresis on filter paper