UNIT 5

GENERAL ASPECTS OF CHROMATOGRAPHY

Structure

5.1 INTRODUCTION

This is the first unit of this Block on 'Chromatography and Ion exchange'. Here, we will cover the general aspects of chromatography. The unit will begin with a discussion on classification of chromatographic methods. This will be really an eye opener for you to realise that what a vast variation of this technique is possible .A wide variety of options has resulted in this technique with time as newer developments kept on taking place and increasing need of separation of various types of substances was felt.

We will also explain the technique of paper chromatography which is classified as partition chromatography because the *mechanism* of the separation involved in this technique is partition. Under this technique, we will focus on the principle of separation as well as the efficiency of this technique. The mechanism involved will also be discussed and the development of the chromatograms will be explained. We will also discuss the characteristics of the mobile phase and the concept of R_f value.

Expected Learning Outcomes

After studying this unit you should be able to:

- discuss the classification of various chromatographic techniques according to the different criteria;

- ❖ state the importance of paper chromatography and explain the various terms involved in it;
- ❖ explain the principle of paper chromatography;
- ❖ describe the mechanism of separation in partition chromatography;
- ❖ explain how the chromatogram is developed in paper chromatography; and
- ❖ discuss the applications of paper chromatography.

5.2 CLASSIFICATION OF CHROMATOGRAPHIC METHODS

Mikhail Tswett (1872-1919)

'Chromatography' was introduced by a Russian Botanist, Mikhail Tswett in 1906. It is now a journey of more than a century and a lot of interesting developments have been introduced in using such techniques .A lot of variations with regard to the use of equipment and materials used have taken place from time to time.

Chromatography was used by Tswett to separate the plant pigments using a simple glass column packed with finely divided calcium carbonate. He used petroleum ether to separate various pigments which appeared as bands at different heights in the column after separation. You will also study about *column chromatography* in Unit 6.

Thus, the technique of chromatography involves the separation of the components of a mixture by their *distribution between two phases*- one being the ***stationary phase*** *of large surface area* and the other being the ***mobile phase.*** Here, the mobile phase moves on the stationary phase in a definite direction. Depending upon the ***type of stationary support, nature of mobile phase*** and ***the mechanism involved in the separation***; several types of chromatographic methods are possible, see Fig.5.1.

The technique of chromatography is a physical method of separation. It is based on molecular weight of a substance and its adsorption or partition coefficient. Thus, substances with lesser molecular masses diffuse more quickly than those with higher molecular masses.

Let us see the following possible variations when each of the above mentioned aspects are taken into account:

(i) Depending upon the **type of stationary support** or more accurately the ***shape*** of the stationary support, chromatographic technique can be ***two-dimensional*** or ***three-dimensional.***

The techniques of *paper chromatography* and *thin layer chromatography* are two further subdivisions of *two-dimensional chromatography* because the stationary phases used in these two types of chromatography are, respectively *paper* and a *solid support* coated on a plate *of glass, plastic* or *metal***.** The shapes of all these supports are *two-dimensional*; hence, these two techniques are classified under two-dimensional chromatography.

The technique of *column chromatography* is considered under the *three-dimensional chromatography. Column chromatography* involves the stationary support present in a *column* which is *three-dimensional in shape.*

(ii) According to the **nature of mobile phase**-whether the mobile phase is gas, liquid or supercritical fluid, the respective chromatographic techniques are called *gas chromatography*, *liquid chromatography* or *supercritical fluid chromatography.*

(iii) We can also classify chromatographic techniques into different classes according to the **mechanism involved in separation**. Accordingly, we can name these techniques as *partition, adsorption, ion exchange* or *size exclusion chromatography.*

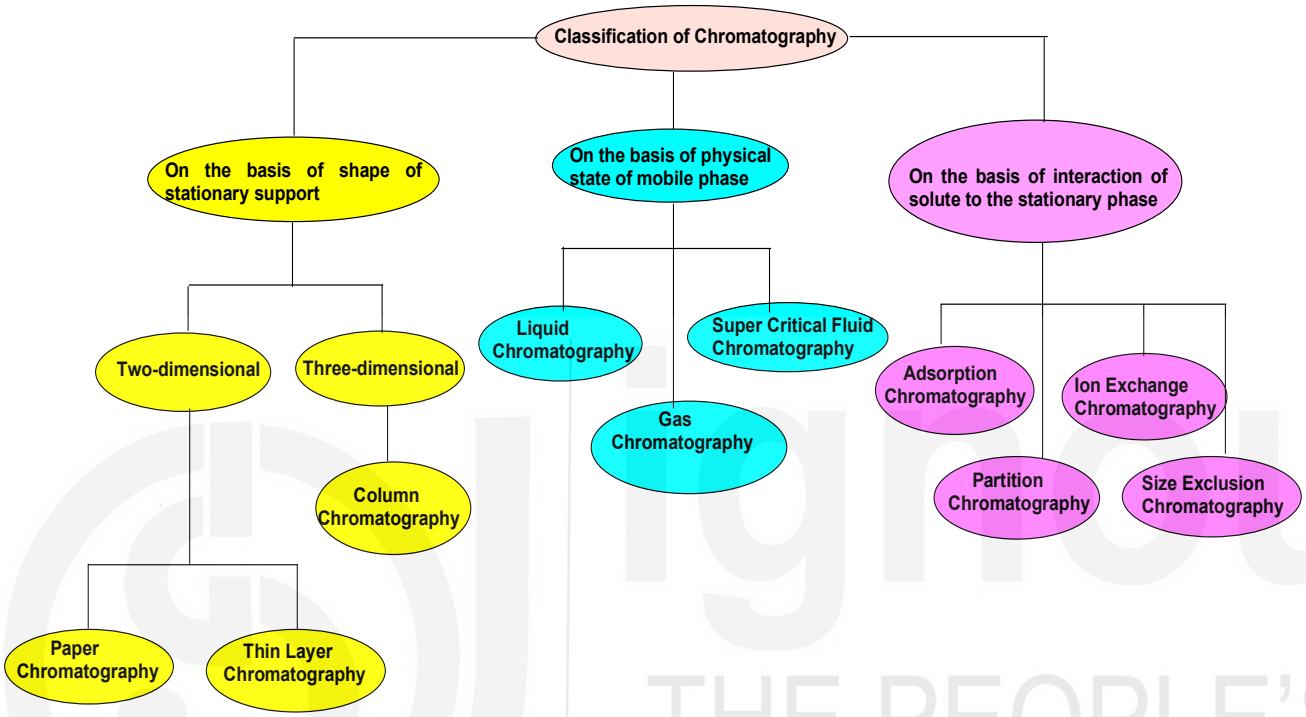


Fig. 5.1: Classification of Chromatography

We will be dealing with *partition chromatography* i.e. *paper chromatography* in this unit itself. The *adsorption chromatography* using *column chromatography* will be dealt in Unit 6 while *ion exchange chromatography* will be described in Unit 7. The *size exclusion chromatography*, however, is *not* a part of this course and will not be dealt here.

Thus, all the available chromatographic techniques cannot be classified using a single criterion, as the different combinations of stationary and mobile phases can be used and the mechanism operating in separations may also differ. Hence, it is the ***nature of mobile phase*** which is taken as main criterion to classify the technique. The other factors mentioned above are discussed as *details* or *sub-criteria* under this main class.

Thus, when the *nature of mobile phase is taken as the main criterion*, then the ***gas, liquid*** and ***supercritical fluid*** chromatographies are considered. The *gas* and *supercritical fluid chromatography* are beyond the scope of this course. Therefore, we will keep our discussion focused to the *liquid chromatography only*.

When the *mobile phase* is ***liquid,*** the *stationary phase* can be either a ***liquid supported on a solid*** or ***a solid*** and this gives rise to the **liquid-liquid chromatography** or **liquid-solid** chromatography. In *liquid-liquid chromatography*, the distribution of components of mixture takes place by their

partitioning of the components of the mixture in the two liquid phases, as was the case in the solvent extraction.

Further, two-dimensional and three-dimensional chromatographies are possible as shown in Fig. 5.2, according to the shape of the stationary phase taken. Each of these variations may further involve *upward or downward* direction of movement of the mobile phase, thereby widening the options to *ascending* and *descending* modes of operation. This is illustrated below in Fig. 5.2.

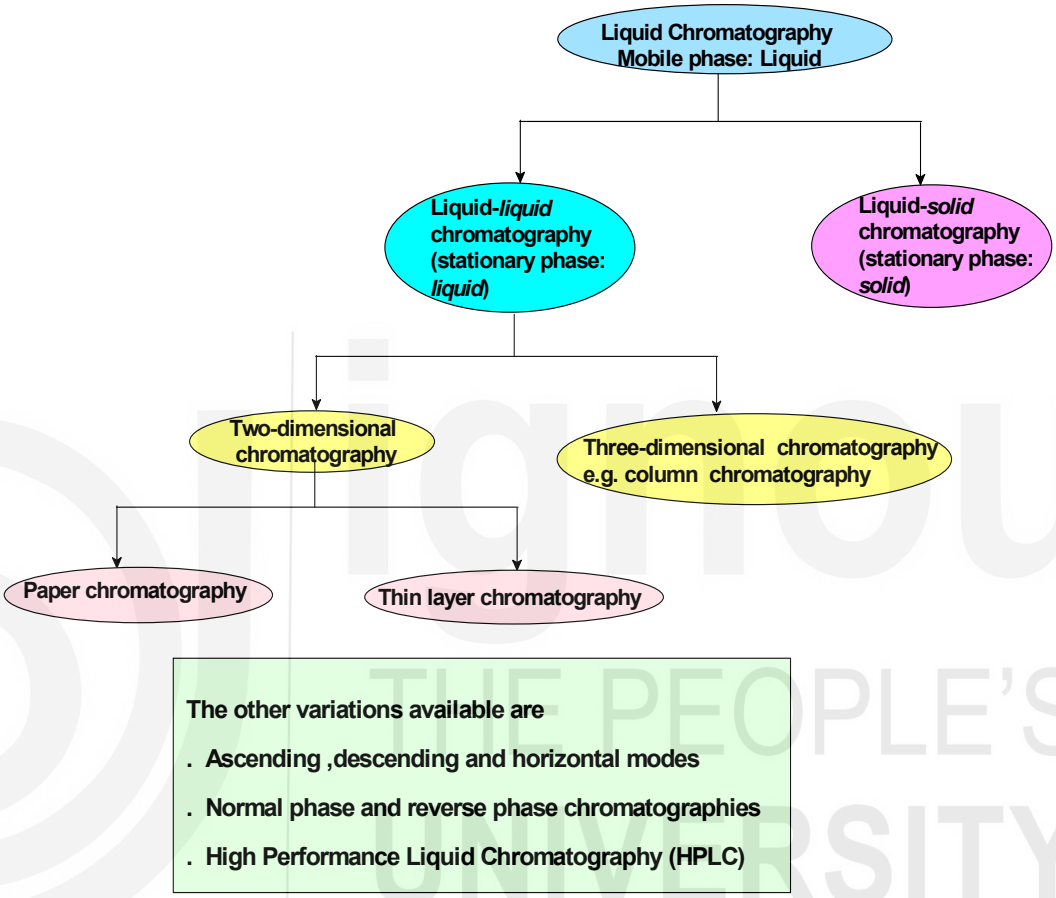


Fig. 5.2: Types of liquid chromatography

Normally, *polar support is taken as the stationary phase* and *non-polar mobile phase* is chosen. Such a combination is referred to as ***normal-phase*** chromatography. But, if the polarities of the two phases are reversed, then it is called ***reverse-phase*** chromatography.

Various forms of chromatographies explained above can also be performed under high pressure leading to **high performance** or **high pressure liquid chromatography (HPLC).**

Let us now focus our attention on paper chromatography which involves partition mechanism.

SAQ 1

Name two types of two-dimensional chromatography.

5.3 PARTITION CHROMATOGRAPHY: PAPER CHROMATOGRAPHY

The foundation of the paper chromatography dates back to 1944 when R. Consden, A.H. Gordon and A.P.J Martin reported the separation of amino acids using paper. The amino acids present in only 200 μg of wool could have been separated using paper chromatography. The importance of chromatography was recognised when Nobel prize was awarded in 1952.

The technique of the paper chromatography is very simple and useful. Let us now understand its principle.

5.3.1 Principle

Paper chromatography is a kind of liquid-liquid partition chromatography in which the substances are distributed between two liquids-the stationary phase(usually water that is held in the fibres of the paper, also called water –cellulose complex containing 20% water) and the moving liquid or the mobile phase ,also called the developing solvent.

The paper chromatography involves the use of paper which is made up of highly pure cellulose .The examples of such a paper are - Whatman papers with no. 1,2,3,3 MM, 31 ET etc. These papers are having uniform physical characteristics and are very low in organic and inorganic impurities. Some more types of modified papers are also used about which you will study later.

Here, it would be interesting to know that paper chromatography can be done in *ascending mode*, *descending mode* or in *circular fashion*. The simplest one is ascending mode paper chromatography for which the set up is shown below in Fig. 5.3.

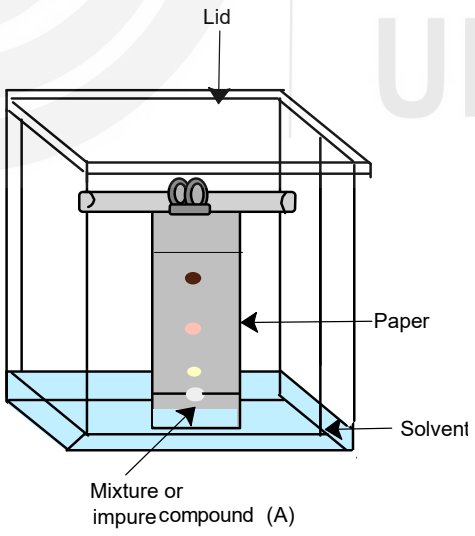


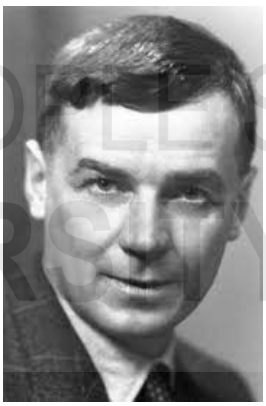
Fig. 5.3: Set up for the ascending mode paper chromatography

The sample containing the mixture of compounds to be separated is taken in a suitable solvent and using a capillary, a fine small spot is put on the line drawn on the paper. Let this spot be marked as A. The sample could also be an impure compound which could be tested by paper chromatography for how many impurities are present.

Archer John Porter Martin

(1st March 1910-28th July 2002)

He shared Nobel Prize in Chemistry for year 1952 with the Richard Synge for the invention of *partition chromatography*.



Richard Laurence Millington Synge

(28th Oct 1914-18th August 1994)

He shared Nobel Prize in chemistry for the year 1952 with A.J.P. Martin for the invention of *partition chromatography.*

The paper is then kept in the development chamber as shown in Fig. 5.3.The solvent which is the mobile phase is then allowed to rise by the capillary action over the spot of mixture of compounds . After some time, the solvent takes along with it, the different components of the mixture (of spot A) to different heights on the paper leading to their separation.

Similarly, we can have *descending paper chromatography* and *circular paper chromatography* .The experimental set ups for these two methods are shown in Fig. 5.4. a) and b) below.

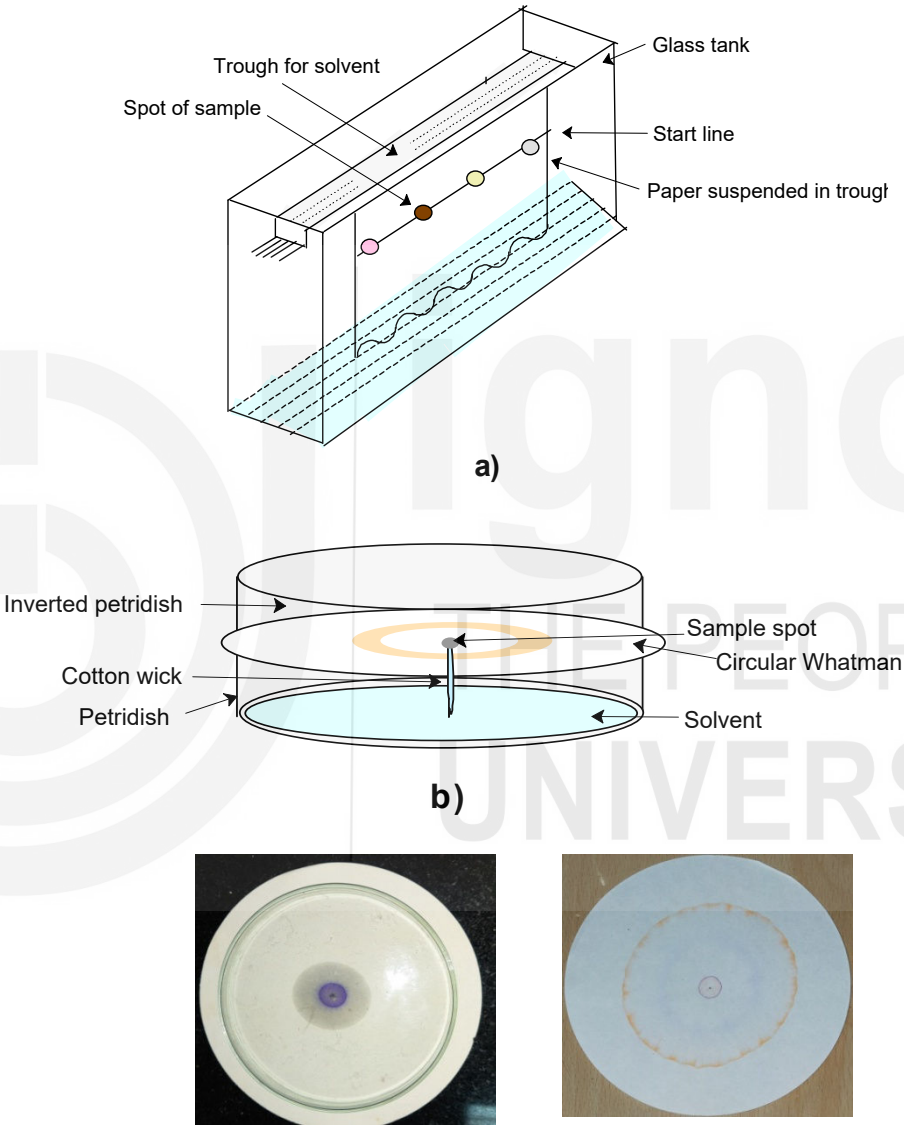


Fig. 5.4: a) Descending paper chromatography

b) Circular paper chromatography

c) Separated compounds by circular chromatography

In **descending paper chromatography**, the solvent tank contains the solvent which moves from *upside to downward* direction on the paper. The spots of the sample are put on the paper and the same is placed in the development chamber as shown in Fig. 5.4 a).

In circular paper chromatography, as shown in Fig. 5.4 b), the spots of the sample are placed on the paper in circular fashion. Then, the moving phase or the mobile phase which is a liquid (i.e. a solvent or a mixture of solvent) is taken in a Petri dish. At the centre of the paper, a very small hole is made using a pin. A wick is then placed in this hole whose other end dips in the mobile phase.

The solvent rises to the centre of the paper through the cotton wick .The mobile phase then carries along it, different components of the sample in a circular fashion. Thus the different separated components are obtained as rings on the Whatman paper and not as the spots.

Having understood the basic principle of paper chromatography, let us now study about its efficiency.

5.3.2 Efficiency of the Technique

As discussed above, the techniue of paper chromatography is not only simple and easy to perform but it is also efficient in separating the components of a mixure or in obtaining the pure compound from an impure sample.

For obtaining the pure components of a mixture or for getting a pure compound from its impure sample, *quantitative paper chromatography* has to be performed. A 30 cm x 30 cm sheet of paper is taken and several spots of the sample are put in a line near the base of the paper. The paper is then kept in the development chamber as shown in Fig. 5.5. The mobile phase is then allowed to rise by the capillary action over the spots of sample.

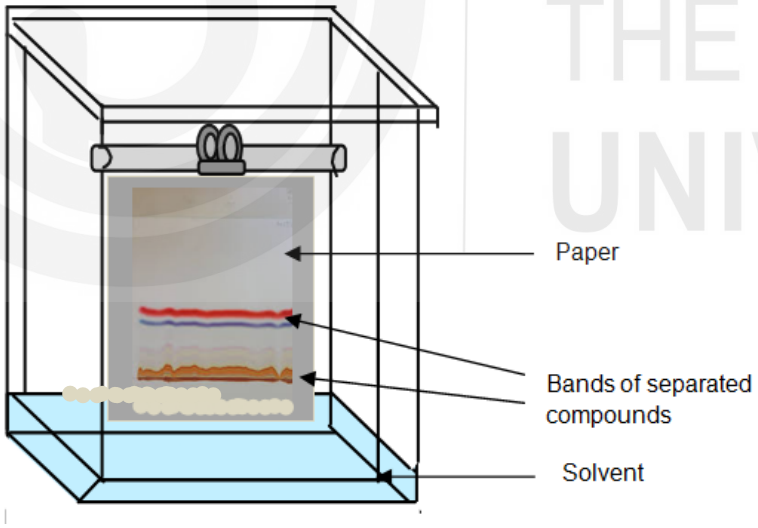


Fig.5.5: Quantitative paper chromatography

The separated components appear as separate bands at different heights on the paper. The relevant portions of the paper are cut for obtaining different components in pure form. Each of the component is then separataly extracted from the paper by dissolving it in a suitable solvent. The removal of the mobile phase or the solvent yields the pure components.

The technique of paper chromatography has been efficiently used for the *qualtative analysis* of several types of naturally occurring and synthetically obtained reaction mixtues. The separation can be effectively carried out and

the identification of different components can be done by putting the spot(s) of the known standard sample(s) alongwith the sample to be tested. The spots of the same compound (whether present in a mixture and as a pure sample) will appear at the same level on the paper after the separation has occurred. Hence,the componenets of the sample can be correlated with the standard compounds.

Paper chromatography is a very efficient technique and it can be used to separate very closely related compounds such as isomers, homologues and species with different valency or oxidation states etc.

Some examples of separations using paper chromatography are as follows:

(i) Identification of metal ions such as Ni^{2+}, Co^{3+}, Zn^{2+} and Mn^{2+}

(ii) Separation of mixture of pesticides containing heptachlor, BHC, aldrin etc. into its pure components

(iii) Checking of foods and drinks for adulterants using the standard samples of pure components and adulterents.

After understanding the efficiency of paper chromatography ,let us now study the mechanism by which such separations take place.

5.3.3 Mechanism of Separation

In the beginign of this section, we have mentioned that cellulose paper is used as solid support in paper chromatography and the mobile phase is a suitable solvent which passes over the sample carrying along with it, the different components of the sample to different extents. But how does this happen?

Which mechanism is operating in such a separation?Let us find out.

So far we have not deliberated upon the water present in the cellulose paper.But, this has the *actual role* in deciding the mechanism of separation. In fact, it is this water which is acting as the stationary phase. Here, the mobile phase is also a liquid. Thus, the components of the mixture will be separated between two liquid phaes and hence,paper chromatography is a *liquid-liquid chromatography .*

Therefore, the mechanism involved here is the ***partition*** of the componens in two different liquid phases. Every time the mobile phase moves, there is an equilibrium between the two liquid phases and the components partition themselves in the two phases.

The components which are held strongly by the stationary phase will be slow in moving alongwith the mobile phase while those which are weakly held by the stationary phase will be moving faster along with the mobile phase.

Thus,*different components of the mixture will move with different rates along with the mobile phase*. This eventually leads to their separation in due course of time.

However, this simple mechanism of partitioning is not taken as the only mode of separation in paper chromatography. It is now believed that hydrogen

bonding, interactions between solutes and cellulose support also play an important role in separation.

It is also possible to use **modified cellulose papers** in paper chromatography. The chemically modified papers such as carboxylated and acetylated papers are available. The carboxylated papers have increased carboxyl content and are suitable for the separation of amines and amino acids. The aceylated paper is more hdyrophilic and can be used for reverse phase chromatography.

The paper to be used in paper chromatography can also be loaded with cation or anion exchange resins. In such a case, the mechanism of separation would be via *excange of ions.*

It is also possible to use papers impregnated with silica or alumina, in which case, the mechanism of separation would involve adsorption of the components of the mixture on paper to different extents leading to their separation.

Hydrophilic papers can be obtained by treatment with methanol, glycerol, glycol etc.

Having understood the mechanism of separation,try to answer the following **SAQs.**

SAQ 2

What are modified cellulose papers?Give examples.

SAQ 3

What is the general mechanism of separation in paper chromatography? How does it get modified in case of papers impregnated with silica gel?

5.3.4 Development of Chromatograms

We have discussed above about the ascending, descending and circular forms of paper chromatography. Whatever form we choose from these, before starting the separation using paper chromatography ,the general procedure involves the saturation of the development tank with the vapours of the solvent used. The development tank containing the mobile phase is covered with the lid/cover and kept undisturbed for sometime to allow the vapours of the mobile phase to saturate the tank .

The substance to be analysed is dissolved in a suitable solvent and its spot of is put on the paper. Let the position of this spot be represented by the line at point A. The paper is then cap in the development chamber and it is then allowed to come in contact with the mobile phase. The system is again left undisturbed for sometime.

The components present in the substance are then carried along by the mobile phase. Their rates of movement will be different as their inteactions with the stationary and mobile phases will be different.

Here, it is also important to note that the nature of solvent used as the *mobile phase has an important role in the development of paper chromatogram.*

The solvent or the mobile phase should be pure, free from impurities and dry. The following other criteria should also be followed while chosing the mobile phase.

- The solvent system should not react chemically with the components of the substance to be analysed.
- The composition of the solvent system should not change during the course of separation. So, as far as voltility is concerned, we should prefer non-volatile solvents.
- The solvent system or the mobile phase should be so selected that it is able to separate different components present in the sample. The polarity of the solvent system can be changed for getting the good separation.

As in the case of paper chromatography, here also the water present in the cellulose acts as the stationary phase.Hence, here in paper chromatography , the starionary phase is polar in nature.Therefore.we can use a relatively less polar solvent such as ethanol, acetone, formamide, amines or a suitable mixture of such solvents as the mobile phase.

Some typical examples of such mobile phases are as follows:

- Isopropanol-Ammonia-Water (9:1:2 v/v)
- *n*-Butanol-Acetic acid-Water (4:1:5 v/v)
- Water-Phenol
- Formamide-Benzene

For the separation of cations, some of the commonly used mobile phases are as follows:

- Methanol
- Methyl ethyl ketone containing 30% (v/v) water and 1% (w/v) potassium thiocyanate
- Pyridine containing 10% (v/v) water
- Acetone containing 5% (v/v) water and 8% (v/v) hydro
- chloric acid.

The components of the substance get separated after leaving the paper in the development chamber for some time. The paper is then taken out from the development chamber and a line is marked on it with a pencil at the level to which the mobile phase has travelled. Let this line be represented by B. The solvent is then allowed to dry. Remember that the position of the original spot was represented as point A and a line can be drawn at this level, see Fig. 5.7.

If the separated components of the mixture are coloured, their spots will be clearly visible on the paper, see Fig. 5.6. Otherwise, a suitable locating agent is sprayed on the paper to detect the components.,

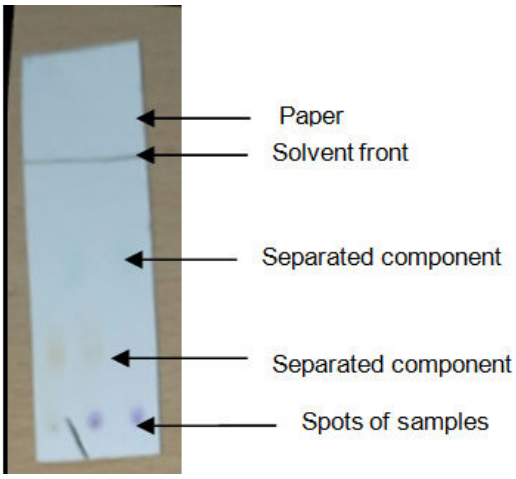


Fig. 5.6: Chromatogram

The examples of some locating agents are as follows:

- Dimethyl glyoxime (for Ni)
- Dithizone
- Potassium chromate
- Ammonium sulphide
- Iodine vapours (for organic compounds)
- Starch solution (for carbohydrates)
- Ninhydrin solution (for amino acids)

Sometimes, the spots of the components are visible under UV light.

Once the spots of the separated components are located, these then need to be characterised. One such way is to calculate their R_f values and match these values them with the R_f values of their standard or pure samples which are usually run simultaneously on the same paper under identical conditions.

For this purpose, let us first understand what is an R_f value.

The R_f value

It is the retardation factor and is defined as the following ratio:

$$R_f = \frac{\text{Distance travelled by a solute}}{\text{Distance travelled by the solvent}} \quad \ldots (5.1)$$

If we now look at the chromatogram shown in Fig. 5.6, then, we can calculate the R_f values for the separated components.

Let us understand this the calculation of the R_f values with the help of a hypothetical chromatogram shown in Fig. 5.7. Let there be two components 1 and 2 which have been separated by using the technique of paper chromatography (or TLC). Let the distance travelled by component 1 is AY and that travelled by component 2 is AX.

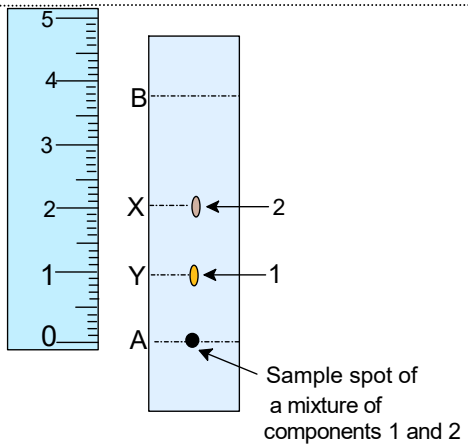


Fig. 5.7: Calculation of R_f value from chromatogram

Therefore, their R_f values can be calculated as given in the following equations:

For component 1, $$R_f = \frac{\text{Distance travelled by component 1}}{\text{Distance travelled by mobile phase}} = \frac{AY}{AB} \ldots (5.2\ a)$$

For component 2, $$R_f = \frac{\text{Distance travelled by component 2}}{\text{Distance travelled by mobile phase}} = \frac{AX}{AB} \ldots (5.2\ b)$$

It must be taken care that the distances AY and AX are measured between the line A and the centre points of spots Y and X, respectively.

Many times, the sample of the available standard substances are placed along with the mixture (or unknown substance). Then the chromatogram is obtained. The separated components of the mixture are then matched with the spots of the standard samples and the components are characterised, see Fig. 5.8.

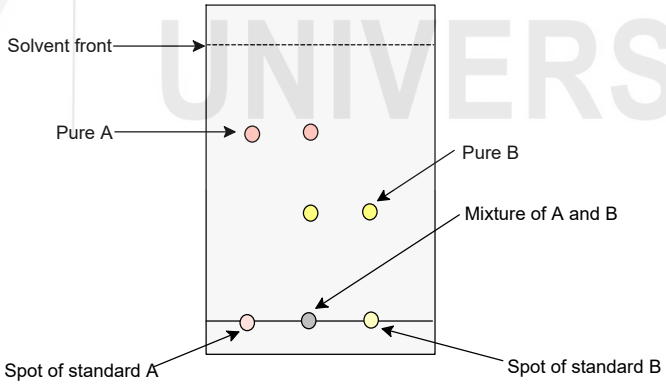


Fig. 5.8: Development of Chromatogram along with the use of standard samples

The R_f values are characteristic of the solute under a given set of conditions. They may change with the change in the solvent system, temperature, presence of impurities and sometimes with the development time. Hence, these need to be carefully compared.

Hence, we can say that paper chromatography can be used for the qualitative analysis of different samples and identification of components present in a mixture.

Now you can answer the following questions.

SAQ 4

Which component shown in Fig. 5.7 has smaller R_f value?

SAQ 5

Give any two characteristics of a mobile phase.

Let us now sum up, what we have learnt in this Unit.

5.4 SUMMARY

In this unit, we have learnt the following main aspects of chromatography:

- The chromatographic techniques can be classified in various ways according to the shape of the stationary support, nature of the mobile phase and the mechanism of separation.
- A variety of chromatographic techniques have evolved with variations in their operational aspects over the time.
- Paper chromatography is a very simple and useful chromatographic technique.
- Various modes of paper chromatographic techniques are available- ascending, descending and circular.
- The mechanism of separation using paper chromatography is mainly partition. However, other variations, i.e. adsorption and ion exchange using the modified papers, are also possible.
- The development of chromatograms is an important skill and several aspects related to placement of spots of samples, use of suitable mobile phases and proper handling of chromatogram are to be taken care of to achieve the good separation of the components. The spots of the sample and the reference substances should not dip in the solvent.
- The R_f values are characteristic of the substances but several factors may affect these values.

5.5 TERMINAL QUESTIONS

1 Name different types of mechanisms operating in the chromatographic techniques.

2. What are the polarities are two phases in reverse-phase chromatography?

3. Differentiate between ascending and descending modes of paper chromatography.