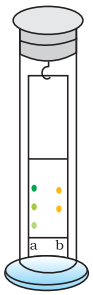
UNIT-5

Chromatography



The technique of chromatography is vastly used for the separation, purification and identification of compounds. According to IUPAC, chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.

The stationary phase is usually in the form of a packed column (column chromatography) but may take other forms such as flat sheet or a thin layer adhering to a suitable form of backing material such as glass (thin-layer chromatography). In **column chromatography**, mobile phase flows through the packed column, while in **thin layer chromatography**, mobile phase moves by capillary action. In this the thin film stationary phase may be either a liquid or a solid and the mobile phase may be a liquid or a gas. Different possible combinations of these phases give rise to principal techniques of chromatography. Two of these are described below.

In **partition chromatography**, stationary phase is thin film of liquid adsorbed on an essentially inert support. Mobile phase may be a liquid or a gas. **Paper chromatography** is an example of partition chromatography in which liquid present in the pores of paper is stationary phase and some other liquid is movable phase. Separation depends upon partition of substance between two phases and the adsorption effects of inert support on compounds undergoing chromatographic separation.

In **adsorption chromatography**, the stationary phase is a finely divided solid adsorbent and the mobile phase is usually a liquid. Process of separation depends upon selective adsorption of components of a mixture on the surface of a solid.

In chromatography, substance equilibrates between a mobile and a stationary phase. The more the interaction of substance with the stationary phase, slower is its movement.

In this unit you will learn about the technique of separating the components of a mixture by using paper chromatography.

Experiment 5.1

Aim

Separation of pigments present in the leaves (spinach) and flowers (rose, marigold) by paper chromatography and determination of R_f value of components.

Theory

In paper chromatography, water molecules present in the pores of the filter paper act as the stationary phase and the moving phase can be a solvent like hexane, toluene, acetone or a mixture of solvents such as methanol-water mixture etc. As the moving phase passes through the spot on which sample has been adsorbed, it dissolves the components more or less readily; depending upon the solubility and carries them along with it while moving on the support.

Methanol 

Acetone 

Petroleum ether 

Chloroform 

At a given temperature and for a given solvent, it is possible to determine the characteristic rate of movement of each substance on the chromotographic paper, as the moving phase moves. This is represented by relative front or **retardation factor also called R_f value.** R_f values of different compounds are different even if the mobile phase (solvent) is same. Furthermore, R_f value of a compound may be different in different solvents. R_f values can be calculated by using the following expression:

$$R_f = \frac{\text{Distance travelled by the substance from reference line (cm)}}{\text{Distance travelled by the solvent front from reference line (cm)}}$$

Since solvent front moves faster than the compounds, the R_f value of a substance will always be less than one. Also note that R_f value has no unit.

If the compound is coloured then its position on the chromatographic paper may be easily located. However, if the substance is colourless, it may be treated with a reagent, which imparts it a characteristic colour. This reagent is given the name **developer**. Iodine is the most commonly used developer in paper chromatography. Several other techniques are available for locating the spots.

Material Required

- Whatman's filter paper No.1 of size 4 cm × 17 cm : One
- Gas jar of size 5 cm × 20 cm: One
- Rubber cork fixed with hook in the centre : One
- Test tubes : As per need

- Flower extract and extract of leaves : As per need
- Distilled water : As per need
- Methanol/Acetone : As per need
- Petroleum ether boiling range (60–80°C) : As per need
- Chloroform /Acetone : As per need

Procedure

(i) Grind flowers/leaves in a mortar and transfer the paste into a test tube.

(ii) Add small amounts of methanol or acetone in the crushed material. Close the test tube with an appropriate cork and

shake it well. Filter it and collect the filtrate in a test tube and cork the test tube.

(iii) Procure a Whatman filter paper No.1 of size 4 cm × 17 cm and mark a line at a distance of 3 cm from one of the ends of the paper with the help of a pencil [Fig. 5.1(a)].

(iv) Using a finely drawn capillary, put one spot 'a' for the extract of leaves and one spot 'b' for the extract of flowers. Allow these spots to dry as shown in Fig. 5.1 (a).

(v) Hang the filter paper in a jar containing 20 mL mixture of petroleum ether (boiling range 60–80°C) and chloroform containing 19 mL petroleum ether and 1 mL chloroform or a mixture of petroleum ether (boiling range 60–80°C) and acetone in the ratio 9:1 (18 mL petroleum ether + 2 mL acetone) so that the solvent does not touch the reference line as given in Fig. 5.1 (b).

(vi) Keep this jar as such till the mobile phase (solvent) rises up to 2/3 of the length of the paper [Fig. 5.1(c)].

(vii) Remove the filter paper from the jar, mark the solvent front, outline the spots with the help of a pencil and allow the filter paper to get dry.

(viii) Measure the distance travelled by the solvent front and the centre of different spots with respect to the reference line as given in Fig. 5.1 (d).

(x) Ascertain the number of pigments, which are present in the extract of leaves and flowers.

(xi) Calculate the R_f value of different spots with the help of the expression mentioned earlier.

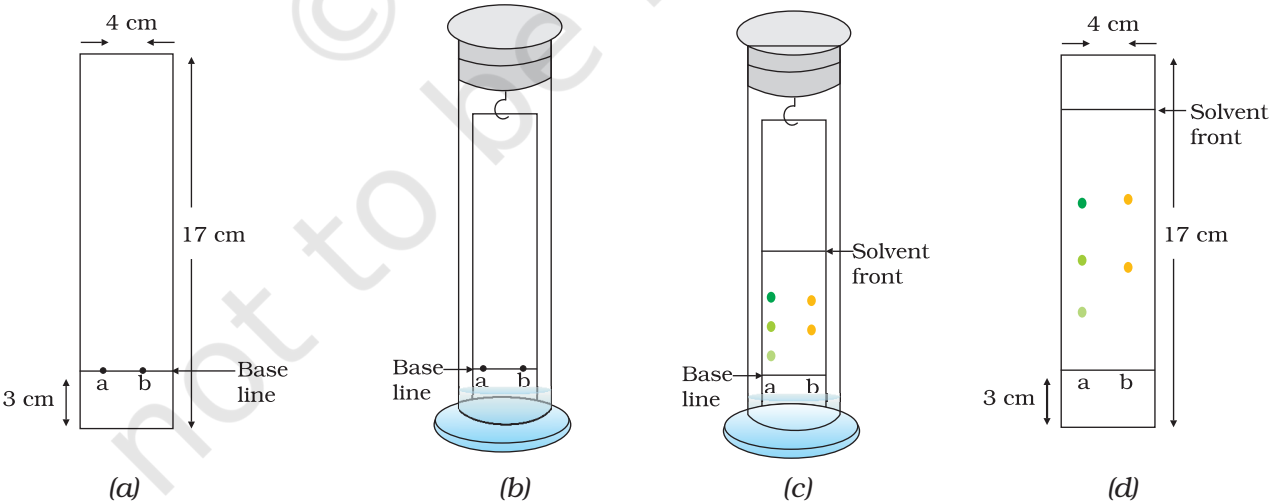


Fig. 5.1 : *(a) Marked paper; (b) Dipping the filter paper in the solvent; (c) Developing chromatogram; and (d) Developed chromatogram*

(xii) Record your observations as in Table 5.1.

Table 5.1: Separation of pigments of leaves and flowers

Sl. No.	Name of the extract	Colour of the spot	Distance travelled by the components of the spots 'a' or 'b' from the reference line in cm	Distance travelled by the solvent from reference line in cm	R_f value
1.					
2.					
3.					
4.					

Result

(i) R_f values of components of flower are _____.
(ii) R_f values of components of leaves are _____.

Precautions

(a) Use good quality pencil for drawing the reference line so that the mark does not dissolve in the solvent in which TLC is run.
(b) Dip the paper strip in the solvent in such a way that the spot of the mixture is above the solvent level and the movement of the solvent front is not zig-zag.
(c) While spotting the test solution on the paper, do not allow the spots to spread. Use finely drawn capillary to put the spot on the paper.
(d) Ensure that the filter paper strip hangs freely in the jar.
(e) Once the experiment is set, do not disturb the jar as long as the chromatogram is being developed.
(f) Keep the jar covered with the lid when the chromatogram is being developed.
(g) Make the paper strip perfectly dry before developing the spots.
(h) Handle the organic solvent/solvents, with care.

EXPERIMENT 5.2

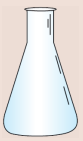
Aim

Separation of the constituents of a mixture of inorganic compounds containing two cations, Pb^{2+} and Cd^{2+}, using chromatographic technique.

Theory

Principle for the separation of cations is same as has been explained in Experiment 5.1. In this case the two cations to be separated are colourless. therefore, a developer is needed. In the present case, ammonium sulphide $(NH_4)_2S$*, can be used to locate the position of these ions on chromatographic paper or plate.

Material Required



- Whatman's filter paper No. 1 of size 4 cm × 17 cm : One
- Gas jar of size 5 cm × 20 cm : One
- Rubber cork fixed with hook in the centre : One
- Test tubes : As per need

- 1–2% solution of $Pb(NO_3)_2$ and $Cd(NO_3)_3$: As per need
- Ehthanol : As per need
- 6.0 M HNO_3 : As per need

Procedure

Ethanol 

Lead nitrate 

Cadmium nitrate 

(i) Procure a Whatman No. 1 filter paper of size 4 cm × 17 cm. With the help of a pencil, mark a line at a distance of 3 cm from one of the ends of this paper.

(ii) Put a spot of the mixture on the marked line with the help of a fine capillary.

(iii) Hang the filter paper in a jar containing a mixture of ethanol, 6.0 M HNO_3 and distilled water, in the ratio 8:1:1.

(iv) Keep the jar as such till the mobile phase (solvent) rises up to two third of the length of the paper.

(v) Remove the filter paper from the jar, mark the solvent front.

(vi) Spray ammonium sulphide solution on the chromatography paper to obtain spots of yellow and black colour. Mark the position of spots with a pencil and allow the paper to dry.

(vii) Measure the distance moved by the solvent front and the different spots of the cations with respect to the reference line. This distance is the shortest distance between the reference line and the centre of different spots.

(viii) Record the observations in tabular form as in Table 5.2. Calculate the R_f value for each cation.

* *Ammonium sulphide is prepared by passing H_2S gas through the mixture containing 100 mL water and 10 mL liquor ammonia for about 45 minutes.*

Table 5.2 : Separation of Pb^{2+} and Cd^{2+} ions by paper chromatography

Sl. No.	Colour of the spot	Distance travelled by components from reference line/cm	Distance travelled by the solvent from reference line/cm	R_f value
1.				
2.				
3.				

Result

(i) R_f values of Pb^{2+} ions is _____.
(ii) R_f values of Cd^{2+} ions is _____.

Precautions

(a) Use good quality pencil for drawing the reference line so that the mark does not dissolve in the solvent in which TLC is run.
(b) Dip the paper strip in the solvent in such a way that the spot of the mixture is above the solvent level and movement of solvent front is not zig-zag.
(c) While spotting the test solution on the paper, do not allow the spots to spread. Use finely drawn capillary to put the spot on the paper.
(d) Ensure that the filter paper strip hangs freely in the jar.
(e) Once the experiment is set, do not disturb the jar as long as the chromatogram is being developed.
(f) Keep the jar covered with the lid when the chromatogram is being developed.
(g) Make the paper strip perfectly dry before developing the spots.
(h) Handle the organic solvent/solvents, with care.

Discussion Questions

(i) What is a chromatogram? Explain the principle on which the technique of chromatography is based.

(ii) What are the essential characteristics of the substance used as a developer?

(iii) How is the phenomenon of 'adsorption' applied in the separation of compounds by chromatography?