EXPERIMENT-9

Separation of the coloured components of the following by paper chromatography and comparison of their R_f values.

(a) Black ink or a mixture of red and blue inks

(b) Juice of a flower or grass

9.1 OBJECTIVES

After performing this experiment, you should be able to

- *separate the various constituents of inks, juice of a flower and / or grass, and*
- *calculate the R_f values of the various components of a mixture.*

9.2 WHAT YOU SHOULD KNOW

Chromatography is a technique by which a mixture of various substances can be (a) separated, (b) purified, and (c) identified.

The term chromatography is applied to separation processes based on the principle of distribution of a sample between two phases namely :

(i) a stationary phase or a fixed phase; and

(ii) a moving phase.

There are two variations in the types namely,

(i) solid-liquid chromatography

(ii) liquid - liquid chromatography.

In solid- liquid chromatography, the solid surface adsorbs the substance which is loaded (charged) on the surface and the compounds are separated on the solid surface. It can also be classified as (i) adsorption chromatography, (ii) partition chromatography, (iii) and ion-exchange chromatography. Adsorption chromatography is based on selective adsorption of substances on the surface of an adsorbent. Column chromatography, thin layer chromatography, etc. are based on the adsorption phenomenon. In column chromatography, the stationary phase is an adsorbent and the mobile phase is a solvent.

Liquid-liquid chromatography involves the distribution of a substance between two immiscible solvents to different extents. Paper chromatography is an example of liquid-liquid chromatography. The stationary phase is supported by sheet of filter paper. The filter paper, known as chromatography paper, holds water which acts as a stationary phase. The mobile phase is another liquid which carries the substance on the paper along with it.

Every substance has a characteristic R_f value at a given temperature and for a given solvent.

R_f stands for 'Ratio of Fronts' or the retention factor. It is the ratio of the distance travelled by the component to the distance travelled by the solvent, from the orign (Fig 9.1).

$$R_f = \frac{\text{distance travelled by the substance from the initial line}}{\text{distance travelled by the solvent from initial line}}$$

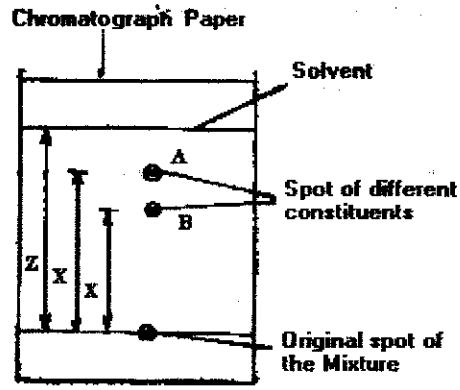

Fig. 9.1 : *Measurement of R_f values*

R_f of component $A = \frac{Y}{Z}$ and R_f of component $B = \frac{X}{Z}$

9.3 MATERIALS REQUIRED

(1) Apparatus	(2) Chemicals
Gas jar, Gas jar cover, Glass rod, Filter paper strip (whatmann No. 1), Fine capillary tube, Hook or clip for hanging the paper, Cork.	A mixture of red and blue inks, Alcohol,

9.4 HOW TO PERFORM THE EXPERIMENT

(a) To separate the components of a mixture of red and blue inks and find out their R_f values.

Take the Whatmann filter paper strip (25 × 2 cm). Draw a line with a pencil about 4 cm above the lower end as shown in Fig. 9.2.

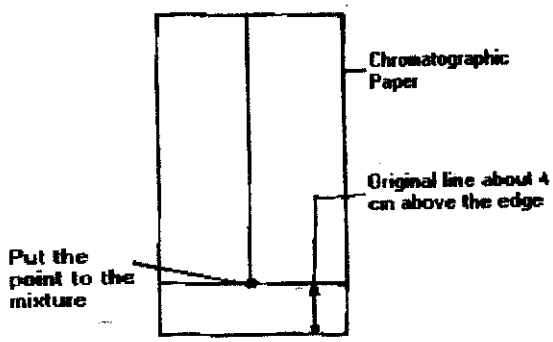

Fig. 9.2 : *Spotting the chromatographic paper*

Spotting : With the help of a fine capillary tube, put a drop of the mixture of red and blue inks at point A (Fig. 9.2) on the filter paper strip. Let the spot dry in air. Put another drop on the same spot and dry again. Repeat the process 2-3 times to make the spot rich in the mixture. Suspend the filter paper vertically in the gas jar containing the solvent mixture (50% alcohol). Hang the paper from the glass rod with the help of clips or hook in such a way that the spot remains about 2 cm above the solvent level.Alternatively cork with hook may be used.

Cover the jar with a glass cover and keep it undisturbed. Notice the rise of the solvent as it ascends the paper. After the solvent has risen about 20 cm, you will see two different spots of red and blue colours on the filter paper. Take the filter paper out of the jar and mark the level of the solvent with a pencil. Dry the paper. Mark the centres of the blue and the red spots. Measure the distance of the two spots and that of the solvent from the initial line. Fig 9.2. Record your observations in Table 9.1 and calculate the R_f values of the red and blue spots.

(b) To separate the coloured components present in grass / flower juice by paper chromatography and find the R_f values of the components separated out.

Material Required

(1) Apparatus	(2) Chemicals :
Same as in experiment 9 (a)	Flower or grass juice as the sample. Solvent mixture (17 ml of petroleum ether + 3 ml of acetone), acetone.

Note : The flower or grass juice is extracted by crushing the flowers or grass in a mortar with the help of a pastle and then extracting the juice by adding a little amount of acetone.

Perform the experiment in the same way as in experiment 9 (a)

9.5 OBSERVATIONS

OBSERVATION FOR EXPERIMENT = 96

- Record your observations and R_f in Table 0.2

Table 9.1

S.N.	Colour of the spot	Distance travelled by the spot from the initial line/cm	Distance travelled by the solvent from the initial line / cm	R_f
1.	Red	....	....	...
2.	Blue	...	...	...

9.6 PRECAUTIONS

1. Use very fine capillary tube for spotting. Otherwise the spot will become too big.
2. Allow the spot to dry before putting another drop. A good spot is small and does not contain too much of the mixture.
3. Do not press the capillary tube very hard, otherwise it may pierce the paper or break. If you make a hole in the paper then the compound will not rise.
4. Do not allow the paper to curl at the ends. It should hang straight, without touching the sides of the jar.
5. Keep the jar covered. Do not disturb the jar after hanging the filter paper till the solvent has risen up to the desired level.
6. The spot should not dip in the solvent. Otherwise the compounds will dissolve in it.
7. Use distilled water for making the solvent mixture to be taken in the jar.

Precautions

Same as in Experiment 9 (a).

Observation for Experiment 9(b)

Table 9.2

S.No.	Colour of the spot	Distance travelled by the spot from the initial line/cm	Distance travelled by the solvent from the initial line / cm	R_f
1.	Green (Chlorophyll)	....	....	...
2.	Yellow (Xanthophyll)			
3.	Red (Carotene)	...	...	...

The R_f of red ink = ...
The R_f of blue ink = ...

9.7 CONCLUSIONS

R_f of the green component (chlorophyll) = ...
R_f of the yellow component (xanthophyll) = ...
R_f of the red component (carotene) = ...

9.8 CHECK YOUR UNDERSTANDING

1. What is chromatography?

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2. What is the basic principle of chromatography?

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3. What is paper chromatography?

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4. What is meant by R_f value?

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5. What is a chromatogram?

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6. How are colourless compounds detected on a chromatogram ?

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7. What will happen if the paper strip is not vertical or it touches the side of the jar?

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8. While spotting, if a hole is created on the paper, how will it affect the separation?

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9.9 NOTE FOR THE TEACHER

(i) An exercise can be given to separate a mixture of Ni^{2+}, Co^{2+} and Zn^{2+} by paper chromatography using the solvent mixture (85 ml acetone + 5 ml water + 10 ml conc. HCl).

(ii) When students extract fruit juice or grass juice, the teacher should explain the method of extraction.

(iii) Even high moisture content in the atmosphere will interfer in the solvent extraction. Therefore, the teacher should advice the students to perform in dry condition.

(iv) The teacher should help students to use proper ratio of solvents, so that the required polarity can be maintained.

9.10 CHECK YOUR ANSWERS

Ans.1. Chromatography is a technique by which mixtures of various substances can be (a) separated (b) purified, and (c) identified.

Ans.2. Chromatography is based on the principle of distribution of a sample between two phases, namely a stationary phase and a moving phase.

Ans.3. Paper chromatography is a type of liquid - liquid chromatography. Here the water held on the cellulose of the paper acts as the stationary phase and the moving solvent acts as the moving phase.

Ans.4. R_f value is the ratio of distance travelled by the component from the original spot to the distance travelled by the solvent from the original spot.

R_f is a constant for a given substance at a particular temperature and for a given solvent.

Ans.5. The colour spots appear on the paper at different levels after the chromatographic separation is over. This is called a chromatogram.

Ans.6. The colourless compounds can be made visible by spraying a reagent on the paper. The separated component on the paper react with the reagent and produces a coloured compounds which become visible.

Ans.7. If the paper strip is not vertical or it touches the sides of the jar then solvent flow will not be uniform or separation will not be proper.

Ans.8. If a hole is created in the paper then the constituents of the mixture will not rise at all, but spread near the original point.