EXPERIMENT 8

Aim

Separation of plant pigments through paper chromatography.

THEORY

Chloroplast contains photosynthetic pigments such as chlorophyll-a,b, carotenes and xanthophylls. These pigments absorb solar radiation at different wavelengths of the visible spectrum for photosynthesis. They differ in their chemical structures and hence, their physiochemical properties, such as molecular weight, solubility in the solvent, etc. are also different. Paper chromatography is a useful technique for separating various chlorophyll pigments because it is relatively quick and requires small quantity of material. Movement of pigments on the chromatographic paper is governed by the principle of adsorption and capillary action. The solvent system components vary in their densities and thus, move at different rates on the chromatographic paper. Lighter components move faster than the heavier components.

Paper chromatography is based on the principle of partition chromatography, which involves two phases:

Stationary phase: Chromatographic paper is made up of cellulose fibre which holds water between its fibres. The hydrated paper acts as stationary phase.

Mobile phase: Organic solvent acts as mobile phase, which moves by capillary action of paper. The components of the mixture get separated depending on competition of the molecules of the sample material between stationary and mobile phases. Temperature and nature of organic solvent are the factors which affect the movement of the solvent. The specific time taken by a particular solute (analyte) to pass through the chromatographic paper or a column under the set of conditions is called retention time. R_f value or retention factor or relative front value is defined as the ratio of distance moved by the solute (pigment) to the distance moved by the solvent on the chromatogrophic paper.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute (pigment)}}{\text{Distance travelled by the solvent}}$$

MATERIAL REQUIRED

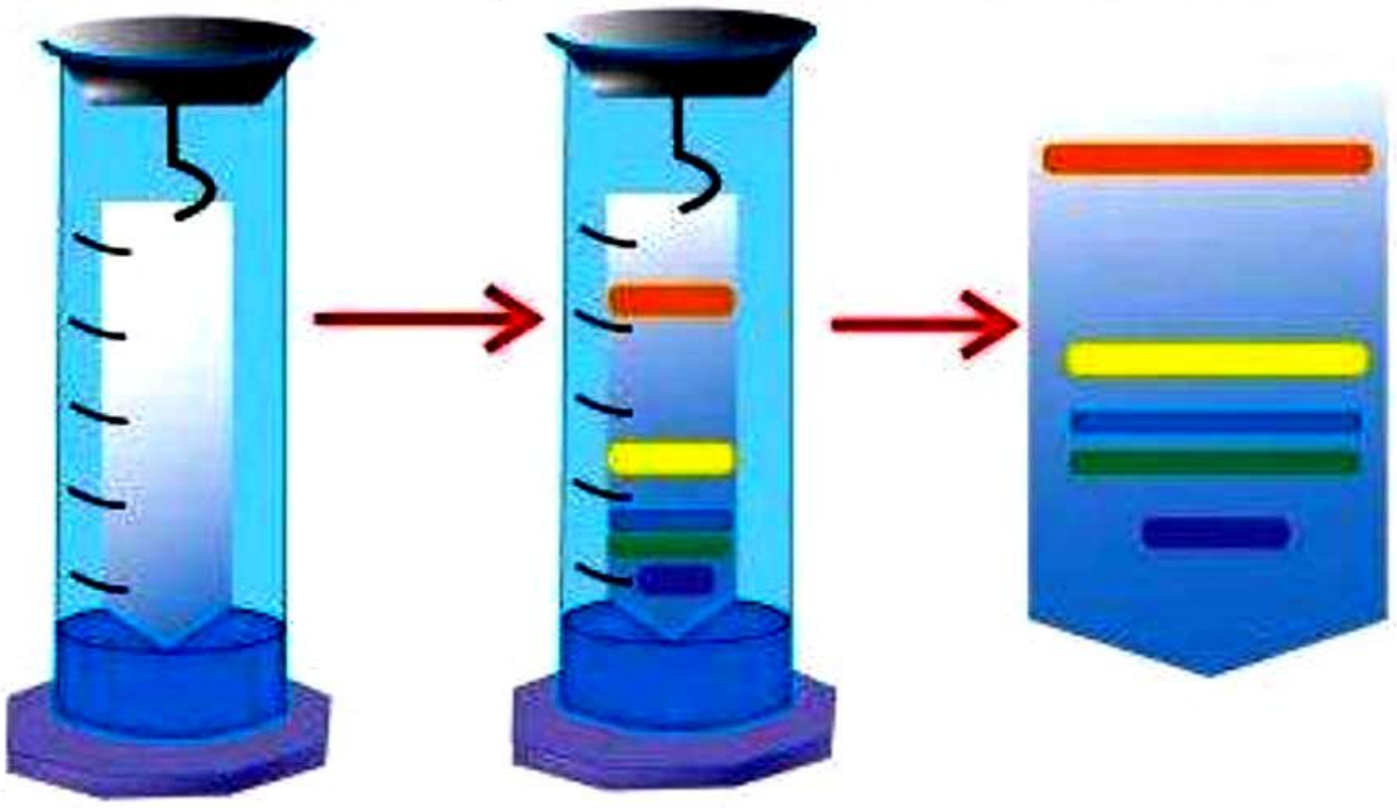
Fresh green spinach leaves, blotting sheet, chromatographic paper/ Whatman no. 1 filter paper, mortar and pestle, a wide long test tube or chromatographic chamber, 100 ml and 10 ml measuring cylinders, 50 ml beaker, split cork, capillary tube, petroleum ether, acetone scissors, sand, test tube and, funnel and muslin cloth.

PROCEDURE AND OBSERVATIONS

1. Take 10-15 fresh spinach leaves and remove their petiole and mid rib.
2. Cut the leaves into small pieces and grind them in mortar and pestle with few drops of acetone and pinch of sand, until leaf extract becomes dark green coloured.
3. Now, filter this extract through muslin cloth and collect the filtrate in a 50 ml beaker or a test tube.
4. Make a solution of petroleum ether and acetone in the ratio of 9: 1 and pour little solution into the wide long test tube. Close the wide mouth test tube by placing the split cork tightly over the mouth of the test

tube. Keep the tube in standing position in a test tube stand.

5. Cut a strip of Whatman no.1 chromatography paper according to the size of the test tube.
6. Make one end of this chromatographic paper into a triangular shape with a pointed end using scissors.
7. Apply the leaf extract on the loading spot (a point marked in the centre of the chromatographic paper strip at a distance of about 2 cm from the notch) with a very fine capillary or tip of a pine toothpick. Allow it to dry and repeat the procedure for about 4-5 applications.



Pigment	Rf value
Carotene	0.95
Xanthophyll	0.71
Chlorophyll a	0.65
Chlorophyll b	0.45
Leaf stain	

8. Suspend the chromatographic paper vertically in the wide, long test tube with the help of a drawing pin fixed on the cork. The paper should wall of the tube and make sure that the loaded spot is clearly above the solvent.
9. Leave the apparatus undisturbed till the solvent rises to about three-fourth of the strip.
10. Take out the strip and cover the chromatogram with black paper to prevent photo-oxidation.
11. With the help of pencil, mark the bands.
12. Calculate

OBSERVATION

The dried chromatographic paper shows four distinct bands as shown in the figure.

Pigments	Colour of the band	R_f (Standard value at room temp.)
Chlorophyll-b	Green	0.43
Chlorophyll-a	Bluish-green	0.6
Xanthophyll	Yellow	0.72
Carotene	Yellow-orange	0.94

RESULT

The different type of photosynthetic pigments can be identified with the help of colour of their bands and their R_f values.

PRECAUTIONS

1. Loading of extract must be done at the same point each time.

2. Let the paper dry after each loading.
3. The solvent should not touch the loading spot.
4. The leaf used should be fresh and green.
5. Only lamina of the leaf should be used.
6. Paper should be dried in air, not in the sunlight.
7. Mark the solvent front and bands immediately after taking the paper out from the tube.
8. The test tube containing petroleum ether and acetone mixture should be tightly closed with the split cork, else the chemicals will volatilize in air.

VIVA VOCE

Q1. Name the pigments which carry out photosynthesis in plants

Ans. Chlorophyll- a and b, carotene. Xanthophyll.

Q2. What is the site of location of chlorophyll in plant cells?

Ans. Chlorophyll is present in all green parts of the plant. In leaves it is present mostly in mesophyll cells.

Q3. Which pigment is called universal photo synthetic pigment?

Ans. Chlorophyll-a. It is because, it is present in all photosynthesising cells.

Q4. In which part of the cell, the photosynthetic pigments present in a plant?

Ans. The photosynthetic pigments are present in the chloroplasts of plant cells.

Q5. Why the chromatographic paper should not be dried in sunlight?

Ans. The chromatographic paper should not be dried in sunlight because the pigments may get oxidised in sunlight and may lose colour, making it difficult to identify them.

Q6. Give a reason as to why acetone is used in paper chromatography.

Ans. Acetone is an organic solvent, which dissolves the pigments so that they can be easily separated through chromatography technique Therefore, acetone has an important role in paper chromatography.

Q7. Why do we add sand while preparing the pigment extract?

Ans. While preparing the pigment extract, we add sand because it facilitates the fine crushing of leaves and accurate separation of pigments.

Q8. What is the colour of the following pigments?

(i) Chlorophyll-a

(ii) Chlorophyll-b

(iii) Xanthophyll

Ans. The colour of the given pigments are as follows:

(i) Chlorophyll-a – Bluish-green.

(ii) Chlorophyll-b – Green.

(iii) Xanthophyll – Yellow.

Q9. Which photosynthetic pigment moves farthest on a paper chromatogram and why?

Ans. Carotene moves farthest because it is light and has highest R_f value among other photosynthetic pigments.

Q10. Leaves in general appear green although they possess yellow and orange pigments.

Ans. Leaves in general appear green although they possess other pigments like carotene and xanthophyll because chlorophyll-b and chlorophyll-a is present in larger amount than any other pigment in leaves. Combination of different pigments make different shades of green.

Q11. Among the two solvent system in chromatography which moves farthest and why?

Ans. Mobile phase moves farthest in two solvent systems in chromatography because it percolates over the stationary phase carrying components along with the direction of flow. Components dissolved in the mobile phase also move with the flow.

Q12. How can you identify different compounds by paper chromatography?

Ans. The different compounds separated by paper chromatography can be identified by calculating their R_f values and matching the same with the standard R_f values of the compounds.

Q13. Why is the chromatographic paper hanged in the centre of the tube or chromatographic chamber?

Ans. Chromatographic paper in a chromatographic chamber is hanged in the centre because touching the strip with the walls of the chamber will interfere with the running of the solvent and pigment will not separate accurately.