

# Exercise 23

**Aim:** Separation of plant pigments (chloroplast pigments) by paper chromatography.

**Principle:** The chloroplasts contain photosynthetic pigments - Chlorophyll a, Chlorophyll b, Carotenes and Xanthophylls. Pigments absorb solar radiation at different wavelengths of the visible spectrum for photosynthesis. These pigments differ in their chemistry, and hence in their physicochemical properties, such as molecular weight, solubility in the solvent etc.

Paper chromatography is a popular technique widely used for separating various chlorophyll pigments from a mixture. In chromatography, pigments move to different distances, depending on their solubility in the solvent system, on a fine quality cellulose paper (Whatmann No.1 chromatography paper). Movement of pigments on the chromatography paper is governed by the principle of adsorption and capillary action. The solvent system components vary in density and thus move at different rates due to wick action through the chromatography paper. Lighter components move faster than the heavier components. Differential solubility of pigments in the solvent system and the differential rates of mobility of solvent system components is used for separation of pigments.

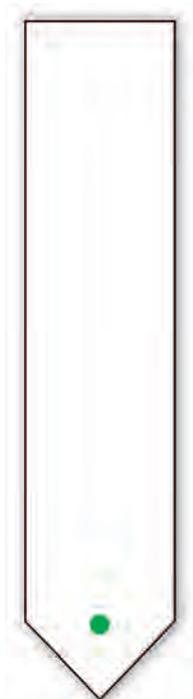
**Requirement:** Mucilage-free leaves of locally available herbaceous plants, test-tubes (6"x1"), boiling tube with split rubber cork, chromatography chamber or wide mouth test tube, 100 ml and 10 ml measuring cylinders, 50 mL beaker, capillary tube, mortar and pestle, acetone, petroleum ether, muslin cloth, sand, scissors, pins, Whatmann No. 1 filter paper, ordinary blotting sheet.

## Procedure

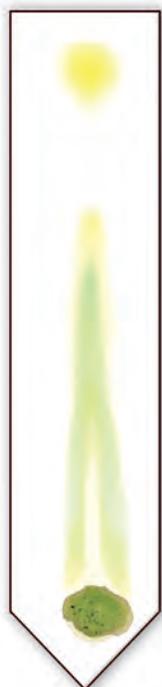
### Preparation of pigment extract

- Select tender, herbaceous, mucilage free leaves for the experiment. Remove the petiole and mid rib from 20-30 mature leaves.
- Cut the leaves into smaller bits and transfer them into a mortar. Add a pinch of sand, a few mL of acetone and grind thoroughly until the leaf extract becomes dark green in colour.
- Filter extract through a muslin cloth and collect the filtrate in 50 ml beaker.
- Prepare 9:1 solution of petroleum ether and acetone. Transfer 3-5 ml of this mixture into the chromatography chamber or wide mouthed test tube. Close the chamber or the test-tube with the split cork tightly as the solvents are volatile and keep it in standing position.

## Exercise 23



**Fig. 23.1** Loading of pigment extract



**Fig. 23.3** A chromatogram of chlorophyll

- Cut a strip of Whatmann No. 1 chromatography paper (6" X 1/2"), which can easily fit into the chamber or the test-tube.
- Using scissors make one end of the chromatography paper into a triangular shape with a pointed end.
- Using the capillary tube, load the pigment extract on the lower side of the chromatography paper in the spot (labelled S in Fig. 23.1) till the spot is dark green in colour. Make sure that the diameter of the loaded spot is not more than 3-5mm. Dry the spot by blowing warm air on it. Never use ink/ ball pen for marking on chromatography paper.
- Suspend the loaded chromatography paper into the boiling tube with the help of a split rubber cork or in chromatography chamber (Fig. 23.2) or wide mouth test tube.
- Make sure that the loaded spot is clearly above the solvent.
- Keep the apparatus straight and leave the set up in a test tube stand undisturbed for 40 minutes. Note the rise of solvent and pigments.
- Remove the chromatography paper after the four different coloured pigments appear.
- Cover the chromatogram with black paper to prevent photo oxidation.



**Fig. 23.2** Experimental setup of the chromatography

### Observation

Identify the pigments on the basis of colours. Sequentially from the bottom, the four colours visible are: greenish yellow (chlorophyll b); bluish green - (chlorophyll a), yellow (carotene) and orange (xanthophyll) (Fig. 23.3).

### Discussion

The four major photosynthetic pigments have been separated by chromatography and identified. This process of separation is called monodimensional ascending chromatography since the solution and pigments move upwards in one direction only.

## Questions

1. Which photosynthetic pigment moves farthest and why?
2. Leaves in general appear green although they possess yellow and orange pigments. Comment.
3. Why do we add sand while preparing the pigment extract?
4. Among the two solvent system components in chromatography, which moves farthest and why?

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