Separation Techniques – Paper Chromatography

Background: Chromatography is a separation technique used to separate the components in a given chemical mixture. Contrary to popular belief, paper chromatography is a type of liquid-liquid chromatography, i.e., partition chromatography, meaning that the stationary and mobile phases are liquids. The components of the mixture spotted onto the paper get separated by partitioning between the cellulose-bound polar water molecules (the stationary phase) trapped as moisture within the paper's pores and the non-polar solvent system (the mobile phase).

Requirements:

Category	Particulars
Specimen/Biological sample	Green leaf
Chemicals	• Petroleum ether (40–60°C) • Acetone
Apparaus/Facilities	• Glass capillary tube • Glass test tube • 250 ml glass beaker or Coplin jar (as chromatography chamber) • Microcentrifuge vial • Mortar pestle • Pipettes • Pre-cut Whatman paper (No. 3, 10×2 cm) strip • Test tube stand
Miscellaneous	• Aluminum foil • Forceps • Pencil • Scissors

Procedure:

Part 1: Preparing the chromatography chamber

1. Pour 15 ml solvent system (Petroleum ether : Acetone (90%) :: 100 : 12) into the beaker and cover it with aluminium foil.
2. Keep it aside for 15–20 minutes to allow the chamber to get saturated with the solvent vapours. *(For good chromatographic separation, the solvent must uniformly saturate the chamber.)*
3. Meanwhile, proceed with the next part of the experiment.

Part 2: Preparing the leaf extract

1. Cut the leaf into fine pieces and put them in the mortar.
2. Add 5 ml of 100% acetone and macerate well to extract the pigments into the solvent.
3. Add another 5 ml acetone and macerate further. *(Remember to be quick, as acetone is volatile.)*
4. Pour the prepared extract into a test tube and allow the leaf debris to settle.
5. After some time, you will see that the leaf debris has settled down.
6. Now carefully collect the supernatant in a vial and use it as the leaf extract.

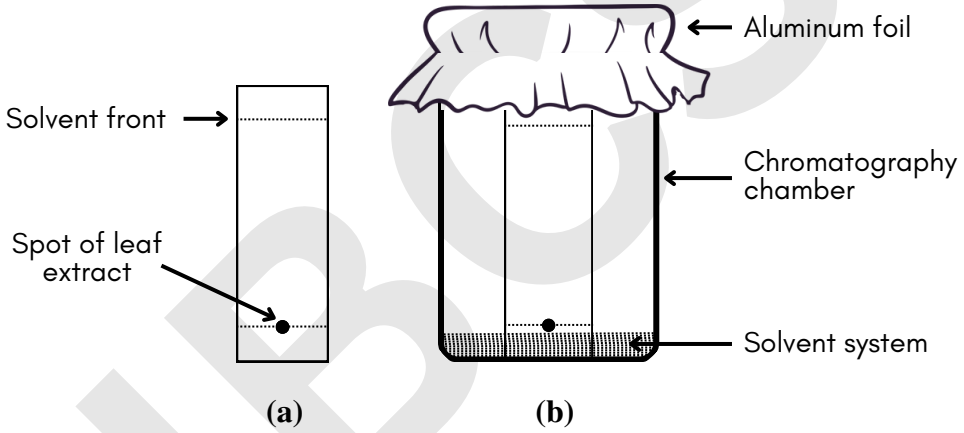

Fig. 1: Paper chromatography setup

Part 3: Spotting the leaf extract

1. Take a strip of Whatman filter paper No. 3.
2. With a pencil, make a faint line 1 cm from the base of the Whatman paper strip, as shown in Fig.1a. (*Care to be taken while handling the paper strip: Always hold the paper strip at the edges to avoid any contamination of the strip.)*
3. Now take the capillary and dip it in the extract.
4. The extract will rise in the capillary due to capillary action.
5. Now briefly touch the capillary containing the plant extract at the centre of the line on the strip to make a small spot, as shown in Fig.1a.
6. Allow the spot to dry.

7. Repeat the same process 10–15 times. *(Ensure that the diameter of the spot remains the same until spotting is complete. Take care that no particulate matter is loaded.)*

Part 4: Separation of plant pigments

1. Carefully remove the foil slightly to ensure that the chamber does not desaturate.
2. Quickly place the strip in the chamber and immediately cover it with the foil, as shown in Fig.1b.
3. Ensure that the sides of the paper don't touch the walls of the beaker.
4. Allow the solvent to rise to 1 cm below the upper edge, as shown in Fig.1a.
5. Then, remove the strip from the chamber and immediately mark the solvent front using a pencil. *(Do not use an eraser or a pen on the paper strip.)*
6. Let the chromatogram dry completely, and then mark the positions of the separated plant pigments on the paper.

Part 5: Identifying the plant pigments

Using the formula,

R.f. value = Distance travelled by the pigment / distance travelled by the mobile solvent

calculate the R.f., i.e., retardation factor values of the various plant pigments.

(Since the pigments fade with time, one must calculate the R.f. values immediately.)

Points for Discussion:

1. Comment on the chemical nature of the solvent used as the mobile phase.
2. List out the pigments observed on the chromatography paper after separation.
3. Identify the predominant pigments separated on the chromatogram.
4. Comment on the polarity of chlorophylls (a & b), carotenoids and anthocyanins.
5. Perform chromatography using extracts from different leaves and compare the separation patterns obtained on the chromatograms.