TP 04 : Separation of Plant Pigments by Paper Chromatography

The separation of plant pigments by paper chromatography is an analysis of pigment molecules of the given plant. Chromatography refers to colour writing. This method separates molecules based on size, density and absorption capacity. In this type of Chromatography the pigments are separated using two phases:

A-Mobile phase: A liquid substance represented by organic solvents Such as the use of saturated Butanol alcohol, acetyl acetone saturated with water , methanol alcohol with hydrochloric acid or ethyl methyl keton. In this experiment we used the ethanol 70% .

B-Stationary phase: mostly be a solution that are carried on paper cellulose fibers, Hence the name paper Chromatography. In this experiment, the solution that carried on filter paper fiber is supernatant that extracted from Spinach or Chard leaf juice.

Objectives:

- Separate plant pigments in Spinach leaves
- Calculate the Rf values for the pigments

Materials

fresh spinach leaves- Ruler -Large test tube -Cork with push pin -Chromatography paper (precut 18 cm strips) -Pencil - Chromatography solvent (9:1 petroleum ether & acetone) -6 ml syringe -Colored pencils - Calculator –Scissors -Plastic wrap- 70 % Isopropyl alcohol- testes tubes (250 ml) -Plastic pipettes.

Procedure

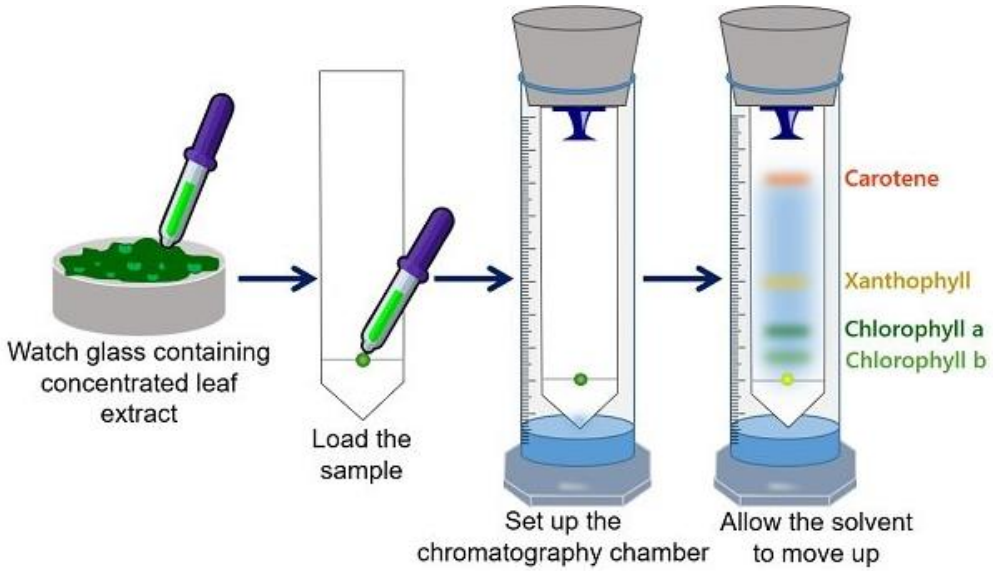


Fig 1 : Separation of plant pigments through paper chromatography.

STEP 1 : Obtain a 4 x 8 cm piece of thin layer chromatography paper and draw two pencil marks 1.5 cm from the bottom. It is important that you are exact when preparing your chromatography paper!

STEP 2: Pour 10 mL of the pre-mixed 1:1 acetone-hexane solution, along with a pinch of sand into your mortar. Add a couple healthy looking spinach leaves. Use your pestle to grind up the leaves. **DO NO DIRECTLY INHALE OVER THE MORTAR.

STEP 3: Once the leaves are sufficiently macerated, use a transfer pipette to decant the top organic layer of the solution. Place this into a 50 mL beaker and then put the beaker under the fume hood so that some of the solvent will evaporate.

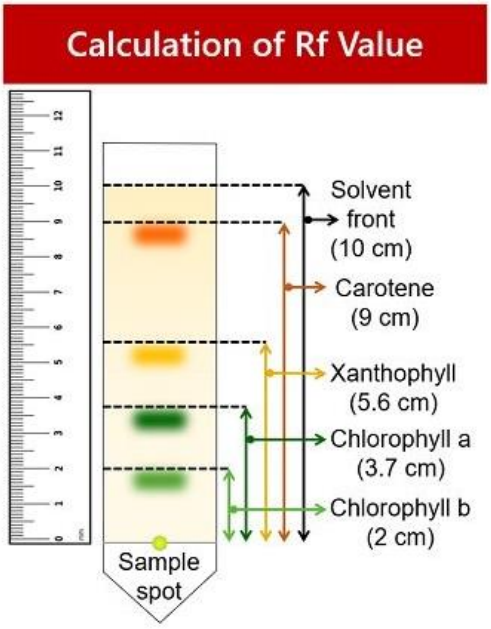
STEP 4: When your beaker appears to have about 2 mL of solution in it, use a transfer pipette to move this liquid from the beaker to the chromatography paper. Be sure to follow your 1.5 cm marks and make a straight line with the spinach solution all the way across the paper.

STEP 5: Place the chromatography paper in the jar so that the pigment-streaked end of the paper is barely immersed in the solvent. The pigment stripe itself should not be in the solvent! **DO NOT INHALE the fumes from the solvent.

STEP 6: Tightly cap the jar. Place jar on a flat surface and do not disturb for several minutes. Carefully observe the chromatography paper within.

STEP 7: When the solvent appears to be about 1 cm from the top margin of the paper, remove the paper from the jar and mark with a line the location of the solvent before it evaporates.

STEP 08: Use a ruler to measure the distance traveled by the solvent front and each pigment band, starting from the origin line. Record these results in Table 1. Number the bands so that Band 1 is the pigment band nearest the origin line.



STEP 09 : Identify each of the bands and label them on the chromatography paper.

- Beta carotene: yellow to yellow orange
- Xanthophyll: yellow
- Chlorophyll a: bright green to blue green
- Chlorophyll b: yellow green to olive green

STEP 10 : Notice that ethanol begin arise and carries with it pigments and separated depending on the molecular weight of the pigments and the degree of solubility in solvent and then applied the following equation:

$$Rf = \frac{distance\ traveled\ by\ pigment}{distance\ traveled\ by\ solvent}$$

The retention factor (Rf) may be defined as the ratio of the distance traveled by the solvent to the distance traveled by the solvent. Rf values are usually expressed as a fraction of 2 decimal places. If the Rf value is zero, the solute is immobile and not soluble in the solvent. A known Rf value in a given solvent can be used to identify a molecule. The retention factor (Rf) may be defined as the ratio of the distance traveled by the solvent to the distance traveled by the solvent. Rf values are usually expressed as a fraction of 2 decimal places. If the Rf value is zero, the solute is immobile and not soluble in the solvent. A known Rf value in a given solvent can be used to identify a molecule.

STEP 11 : Cut the chromatography paper in half length wise and tape into your lab notebook.

STEP 12 : Calculate the Rf values for each pigment and record the values in Data Table 1.

Table 2	
Molecule	R_f
Beta carotene	
Xanthophyll	
Chlorophyll a	
Chlorophyll b	