Chromatography

Introduction

The pigments involved in photosynthesis can be separated by paper chromatography. Absorptive paper containing a concentrated spot of leaf extract is dipped into a suitable solvent (acetone). The various pigments have different solubilities in the solvent, meaning that the pigments move at different rates as the solvent ascends the absorptive paper. In this way they become separated from one another and can be identified by their different colours and relative positions.

Methodology

1. Cut a strip of filter paper of sufficient length to almost reach the bottom of a large test tube and of such a width that the edges do not touch the side of the tube.
2. Fold the end of the paper and use a pin to attach it to a rubber stopper (see figure 2 for example).
3. Using the head of a small pin as a dropper, place a drop of pigment solution roughly 30mm from the end that is not attached to the stopper (see figure 1). Allow the drop to dry and then repeat this process for about 10 minutes, building up a small area of concentrated pigment (the smaller the area, the better the results).
4. Pour some solvent (acetone) into the boiling tube to a depth of no more than 15 mm. Seal the tube while the pigment solution is being added so that the tube can become fully saturated with acetone vapour.
5. Suspend the paper strip in the tube. The bottom edge should dip into the solvent but ensure that the spot of pigment is not immersed in the solvent.
6. The solvent will ascend rapidly, and the pigments will separate in about 10 minutes. When the solvent is roughly 20 mm from the two of the paper, remove the strip and mark the solvent front while the paper dries.
7. Measure the distance of the pigments and the solvent front from their original position, in order to calculate the Rf value, using the formula listed below:

$$\mathrm{Rf} = \frac{a}{b}$$

Where: a = distance moved by the pigment
b = distance moved by the solvent

8. Compare your results to the values provided in the table to the right (pigments found in a typical leaf).

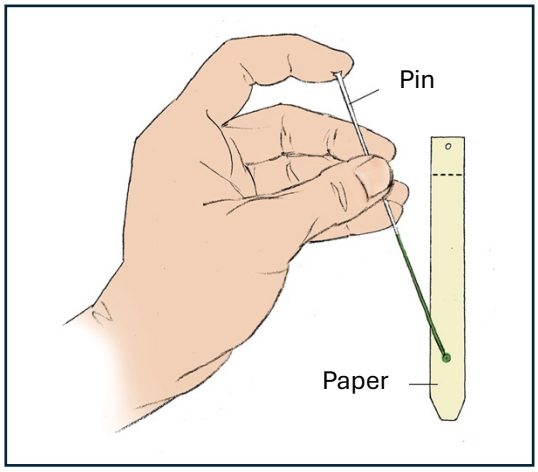

FIGURE 1: Pigment Preparation

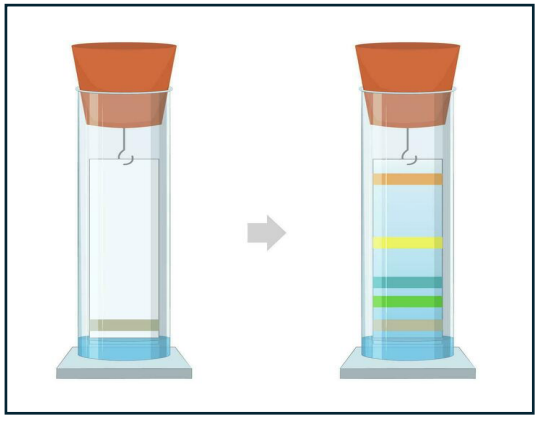

FIGURE 2: Experimental Setup

Name	Colour	Rf
Carotene	Yellow	0.95
Phaeophytin	Yellow	0.83
Xanthophyll	Khaki	0.71
Chlorophyll a	Aqua	0.65
Chlorophyll b	Green	0.45

TABLE 1: Typical Colours / Rf Data

Results

Solvent Distance (mm)	Pigment Distance (mm)	Rf value	Predicted Pigment

Discussion

1. What does the colour of a pigment suggest about its absorption spectra?

2. What could be a reason for a pigment not being visible on a chromatogram?

3. Suggest a reason why leaves from different plants may have different pigment profiles.