

Separation of photosynthetic pigments by paper chromatography

Introduction

Chlorophyll is in fact only one pigment in a group of closely related pigments commonly found in photosynthesising plants called photosynthetic pigments. This can be demonstrated by extracting the pigments from leaves with acetone and separating them by means of paper chromatography. With a bit of luck five pigments can be identified: chlorophyll a (blue-green), chlorophyll b (yellow-green), xanthophylls (yellow), carotene (orange) and phaeophytin (grey, it is a breakdown product of chlorophyll).

Absorptive paper with a concentrated spot of leaf extract is used in this experiment. When dipping in a suitable solvent, the pigments ascend the absorptive paper at different rates because they have different solubilities in the solvent. In this way they become separated from one another and can be identified by their different colours and positions.

Requirements:

- Large test tube (24 * 150 mm);
- Stopper to fit test tube;
- Pin;
- A small glass tube to transfer pigment solution;
- Chromatography paper or filter paper;
- Rack of test tube;
- Pigment solution;
- Solvent (5 cm³).

Procedure:

- A strip of absorptive paper has been prepared. It has such a length that it almost reaches the bottom of a large test tube and such a width that the edges do not touch the sides of the tube;
- Draw a pencil line across the strip of paper 30 mm from one end. The paper has been folded at the other end through 90 degrees and attached to the stopper using a pin. Take care not to let the lower end of the paper touch the bottom of the tube or edges touch the sides;
- Remove the paper from the boiling tube and use the small glass tube provided, place a drop of the pigment solution at the centre of the pencil line. Dry the spot under the heat from a hairdryer or let it dry naturally. Place a second small drop on the first. Repeat this process for about 15 minutes and a small area of concentrated pigment has been set up. N.B. the smaller and more concentrated the spot is, the better;
- While preparing the pigment spot, pour a mixture of propanone and petroleum ether into the boiling tube to a depth of no more than 15 mm. Seal the tube with a stopper for about 10 minutes so that the inside of the boiling becomes saturated with vapour;
- Suspend the strip of paper in the boiling tube. The lower end of the paper should dip into the solvent but the pigment spot should not be immersed;
- The solvent will ascend rapidly carrying the pigments and in about 10 minutes the pigments

can be separated. When the solvent is about 20 mm from the top of the paper remove the strip, rule a pencil line to mark the solvent front and dry the paper;

- Detectable pigments can be identified by their colours and the R_f values;
- Measure the distance from the pencil line to the leading edge of each clear pigment and work out the R_f value for each one using this formulae:

$$R_f = a / b$$

Where a = distance moved by substance from its original position;

b = distance moved by solvent from the same position.

Name	Colour	R_f
Carotene	Yellow	0.95
Phaeophytin	Yellow-grey	0.83
Xanthophyll	Yellow-brown	0.71
Chlorophyll a	Blue-green	0.65
Chlorophyll b	Green	0.45

Table 1

Colours and R_f values of the pigments found in a typical leaf
(R_f values for propane/ ether mixture).

Result and calculation:

$$b = 8.4 \text{ cm}$$

For the first pigment: a = 8.1 cm $R_f = 8.1 / 8.4 = 0.965$

For the second pigment: a = 7.2 cm $R_f = 7.2 / 8.4 = 0.857$

For the third pigment: a = 5.9 cm $R_f = 5.9 / 8.4 = 0.70$

For the fourth pigment: a = 5.1 cm $R_f = 5.1 / 8.4 = 0.61$

For the fifth pigment: a = 3.8 cm $R_f = 3.8 / 8.4 = 0.452$

The colours the different pigments displayed are not distinguishable from each other but we can compare the R_f values to find out each pigment's identity.

Compare the results with the typical R_f values in Table 1, we can found that all of the pigments have been identified: pigment one is Carotene; pigment two is phaeophytin; pigment three is xanthophyll; pigment four is chlorophyll a and pigment five is chlorophyll b.

Evaluation:

The experiment can be improved if the following actions can be taken into account:

- A hair dryer was used to dry the chlorophyll. I don't recommend using it because when over heated, the pigments can be denatured thus their colours and solubilities can be affected. Instead I would recommend letting the pigments dry naturally;
- In order to obtain more favourable results we should let the solvent run as long distance as possible. We can remove the paper strip when the solvent almost reaches the top of the paper

instead of taking the paper out too early;

- An extra technique can be employed to make the pigments more concentrated thus enhance the result. Pour some of the pigment solution onto a watch glass and leave it for about half an hour. Water will evaporate during that time and leave the pigment more concentrated;
- I would recommend drawing a pigment line rather than a pigment spot. When separated, pigments will appear as bands rather than smears so the calculations can be made easier;
- The chlorophyll pigment was taken out from the aluminium paper long before it was used. Light could have affected the nature of chlorophyll thus led to inaccurate results. I would recommend not taking out the pigment until the last minute when it is needed in order to minimise the effect of light;
- An alternative technique (thin layer chromatography) mentioned in the preparation paper can be employed: instead of absorptive paper, a glass or plastic slide (or aluminium sheet) coated with silica gel is used. The gel is spotted and then the slide or aluminium sheet is placed vertically in a beaker or Coplin jar containing solvent at the bottom;
- Further experiment can employ pigments from different plants. Chlorophyll a is of universal occurrence in all photosynthesising plants but we can compare the different pigments in different plants. Because different pigments absorb light of different wavelengths, by comparing the occurrence of different pigments we can deduce the plants' habitat;
- We can do further experiment to determine the absorption spectrum of each pigment. We can make separate solution of each pigment and use a colorimeter to find out what wavelength of light each one absorbs. Then we can find out what wavelength of light a plant uses most to carry out photosynthesises.