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School of Maths & Science Science Practical

Separation of photosynthetic pigments by paper chromatography

◆ Aim

To extract chlorophyll pigments and separate this extract into its constituent pigments.

◆ Introduction

Absorptive paper containing a concentrated spot of chlorophyll extract is dipped into a suitable solvent. The various pigments have different solubility in the solvent with the result that as the solvent ascends the paper it carries the pigments with it at different rates. In this way they become separated from one another and can be identified by their different colours and positions.

◆ Safety

Control Measures



- The wearing of **safety glasses** and a **laboratory coat** at all times will be sufficient to take account of most hazards and significant risks.
- The organic solvents used are highly flammable and their vapours may be harmful.
- Keep stoppers on bottles as much as possible.
- Keep flammable liquids away from sources of ignition.
- Avoid inhalation of vapour by ensuring good ventilation provision.
- All waste is to be placed in the labelled container immediately after use.
- You are reminded of the need for good laboratory practice in order to maintain a safe working environment.



Hazards

Highly Flammable

Acetone, Petroleum ether



Irritant

Acetone

Harmful

Petroleum ether

◆ Procedure

1. Prepare chlorophyll solution by grinding up fresh leaves of e.g. ivy (killed by rapid immersion in boiling water) with pure acetone.
2. Cut a strip of chromatography paper of sufficient length to almost reach the bottom of a boiling tube, and of such width that the edges do not touch the sides of the tube.
3. Rule a pencil line across the strip 30mm from one end. Fold the other end through 90° and, by means of a pin, attach it to the stopper (see figure 1).
4. Using the head of a small pin as a dropper. Place a drop of chlorophyll solution at the centre of the pencil line. Let the drop dry, then place a second drop on the first. Repeat this process for about 15 minutes, building up a small area of concentrated pigment (the smaller, the better).
5. While preparing the spot, pour some solvent (1 part 90% acetone : 9 parts petroleum ether) into a boiling tube to a depth of not more than 15mm. Seal the tube with a stopper for about 10 minutes so the atmosphere inside becomes saturated with vapour.
6. Suspend the strip of paper in the boiling tube. The bottom edge of the paper should dip into the solvent but the chlorophyll spot should not be immersed.
7. The solvent will ascend rapidly and the pigments will separate in about 10 minutes. When the solvent is approximately 20mm from the top of the paper, remove the strip, rule a pencil line to mark the solvent front and dry the paper.
8. Identify the pigments by their colours and R_f values.
9. Compare your results with the information in the table.

| 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 |

Figure 1.

One possible arrangement for the chromatographic separation of photosynthetic pigments.

