WJEC Wales Biology A Level

SP 3.2a: Investigation into the separation of chloroplast pigments by chromatography

Practical notes

Introduction

Thin layer chromatography can be used to separate **chloroplast pigments** according to differences in their **solubility** in the **mobile phase** and their relative **attractions** to the **stationary phase**.

Equipment

- Spinach leaves
- Propanone
- Petroleum ether
- 2:1 propanone: petroleum ether *(running solvent)*
- Sand
- Distilled water
- TLC silica gel plate
- 3 5cm^3 syringes
- Pipette
- Boiling tube
- Capillary tube
- Split bung
- Specimen tube
- Pestle and mortar
- Vial
- Scissors
- Pencil
- Ruler
- Black polyethene bag

Risk assessment

Hazard	Risk	Precaution	Emergency
Broken glass	Cuts	Keep glassware away from the edge of the desk	Dispose of broken glassware carefully; elevate cuts and apply pressure; do not remove glass from cuts; seek medical assistance
Scissors	Cuts	Handle scissors with care; keep scissors away from the edge of the desk	Elevate cuts and apply pressure; wash minor cuts in cold water; seek medical assistance

Propanone	Highly flammable	Make sure that there are no naked flames in the room	Put out small fires with a damp cloth; evacuate the building
	Irritation to eyes	Wear safety goggles	Flood eye(s) with tap water; seek medical assistance
	Irritation to skin	Wear gloves when handling propanone	Remove contaminated clothing; run the affected area under cold water; seek medical assistance
Petroleum ether	Highly flammable	Make sure that there are no naked flames in the room	Put out small fires with a damp cloth; evacuate the building
	Irritation to respiratory system	Do not directly inhale; ensure the room is well-ventilated; use in a fume cupboard	Seek medical advice
	Irritation to eyes	Wear safety goggles	Flood eye(s) with tap water; seek medical assistance
	Irritation to skin	Wear gloves when handling propanone	Remove contaminated clothing; run the affected area under cold water; seek medical assistance

Method

1. Place two to three leaves into a mortar. Cut larger leaves into smaller pieces using scissors.
2. Using a **5 cm^3 syringe**, add **5 cm^3** propanone (**solvent**) into the mortar along with a pinch of sand.
3. Use the pestle to crush the leaves until a **smooth pulp** and **dark green liquid** is obtained.
4. Transfer the paste into a boiling tube. Add **3 cm^3** distilled water, shake and leave to settle for **8 minutes**.
5. Using a **5 cm^3 syringe**, add **3 cm^3** petroleum ether into the boiling tube and gently shake to mix. Leave to settle for **5 minutes** to allow the layers to separate.
6. Using a pipette, collect the upper layer (**high chloroplast concentration**) and transfer to a vial.

7. Draw a starting line on the TLC plate (around 2 cm from the end) using **graphite pencil**. *Graphite, unlike ink, does not interact with the mobile phase.*

8. Using the **capillary tube**, spot a small volume of pigment in the centre of the pencil line. **Allow to dry** and repeat until a **concentrated** dark green spot is produced.
   - *Ensure that the capillary tube does not tear the TLC plate.*
   - *Allow the spot to dry before adding the next to minimise its spread.*
   - *Avoid fingerprints on the TLC plate (oils may interact with the mobile phase).*

9. Place the TLC plate into a split bung. In a **fume cupboard**, pour the **running solvent** into a specimen tube until it is a **few mm** deep. **Immediately** insert the split bung into the specimen tube. *Ensure that the solvent wets the TLC plate but does not touch the spots.*

10. Cover the specimen tube with a **black polyethene bag** (pigments fade in the light).

11. When the solvent reaches **1 cm** from the top, remove the TLC plate and immediately mark the position of the **solvent front** and the top of each pigment as the colours may rapidly fade.

Results

Each pigment can be **identified** by calculating their retardation factor (**Rf value**) and comparing it to known values:

$$Rf = \frac{\text{distance moved by pigment}}{\text{distance moved by solvent}}$$