Edexcel B Biology A-Level

Core Practical 11

Investigate the presence of different chloroplast pigments using chromatography

Chromatography is used to **separate** out different components in a sample. In this experiment, the **photosynthetic pigments** of a plant sample are separated into **bands of colour** by paper chromatography. The rates of migration of individual pigments will depend on their **solubility**, **mass** and **affinity to the paper**.

Equipment

- Chromatography paper
- Leaf sample
- Distilled water
- Pestle and mortar
- Boiling tube with bung
- Boiling tube rack
- Measuring cylinder
- Spatula
- Pencil
- Ruler
- Capillary tube
- Chromatography solvent
- Propanone

Method

1. Draw a straight line in pencil approximately **1cm** above the bottom of the filter paper being used. Do not use a pen as the ink will obscure the results.
1. Cut a section of leaf and place in a **mortar.** Add 20 drops of **propanone** and use the **pestle** to grind up the leaf sample and release the **pigments**.
2. Use a **capillary tube** to extract some of the **pigment** and blot it onto the centre of the pencil line drawn. Allow to dry and then blot again.
3. **Suspend the paper in the solvent** so that the level of the liquid does not lie above the pencil line, and leave the paper for approximately 10 minutes/until the **solvent has run up the paper** to near the top.
4. Remove the paper from the solvent and draw a pencil line marking where the solvent moved up to. The pigment should have separated out and there should be **different spots on the paper at different heights** above the pencil line.
5. **Calculate the Rf value** for each spot (distance travelled by solute/distance travelled by solvent). Always measure to the **centre** of each spot.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Biohazard	Allergies; soil bacteria; contamination	Wash hands after use	Seek assistance	Low
Chromatography solvent	Flammable; causes irritation to eyes and skin; harmful by inhalation	Avoid contact with solvent; wear eye protection; keep solvent in fume cupboard; make sure room is well ventilated; keep away from naked flame	Wash from skin and eyes using cold water; put out fires; seek medical assistance	Low
Acetone	Flammable;causes irritation to eyes; inhalation may lead to dizziness and drowsiness	Avoid contact; wear eye protection; make sure room is well ventilated; keep away from naked flame	Wash from skin and eyes using cold water; put out fires; seek medical assistance	Low

Conclusion

- Rf values should be **compared to the Rf known values** in database to **identify pigment**. When looking at the databases, ensure that they are for **paper chromatography** and **use the same solvent** as these variables will make results differ.
- Factors that affect the rate of mobility:
    - **Affinity**- pigments have different affinities to the chromatography paper; those with **lower** affinities will **travel further** **up** the paper.
    - **Solubility**- pigments that are **more soluble** **travel faster** **up** the paper and will end up **closer to the top** at the **solvent front**.
- Pigments that travel further up the paper will have a **higher Rf value**.