AQA Biology A-Level

Required Practical 7

Use of chromatography to investigate the pigments isolated from leaves of different plants, e.g. leaves from shade-tolerant and shade-intolerant plants or leaves of different colours.

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 list

- Filter paper
- Leaf sample
- Distilled water
- Pestle and mortar
- Pencil
- Ruler
- Capillary tube
- Chromatography solvent
- Acetone

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.
2. Cut a section of leaf and place it in a **mortar.** Add 20 drops of **acetone** and use the **pestle** to grind up the leaf sample and release the **pigments**.
3. Use a **capillary tube** to extract some of the **pigment** and blot it onto the centre of the pencil line you have drawn.
4. **Suspend the paper in the solvent** so that the level of the liquid does not lie above the pencil line and leave the paper until the **solvent has run up the paper** to near the top.
5. 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.
6. **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 a **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

1. **Affinity**- pigments have different affinities to the chromatography paper; those with **lower** affinities will **travel further** up the paper.

2. **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**.