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## A-level Biology example for required practical 7

**Use of chromatography to investigate the pigments isolated from leaves of different plants eg leaves from shade-tolerant and shade-intolerant plants or leaves of different colours:**

### **An investigation of pigments present in leaves**

#### **Student sheet**

#### **Introduction**

In plants, chlorophyll is the main pigment that absorbs light during photosynthesis. Most plants have other photosynthetic pigments as well and these are not green. You will be using a technique called chromatography to separate chlorophyll and other pigments from two different leaves, A and B.

#### **Method**

You are provided with the following:

- boiling-tube rack
- two boiling tubes with bungs
- small glass measuring cylinder
- solvent
- chromatography paper
- glass rod
- two leaves, A and B
- cork borer
- tile on which to use cork borer
- ruler
- pencil
- drawing pins
- marker pen
- sticky tape.

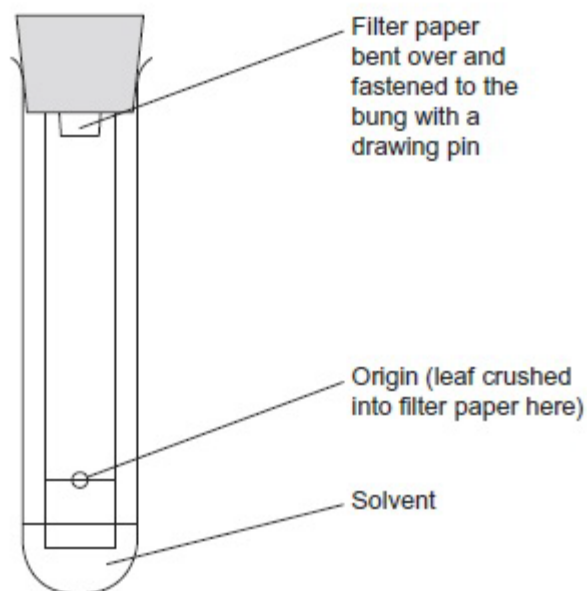
#### **Safety**

Wear eye protection and work in a well-ventilated room or fume cupboard.

You should read these instructions carefully before you start work.

1. Set up two boiling tubes at the start of the investigation. Add 3cm<sup>3</sup> of solvent to each of the two boiling tubes. Put a bung in the top of each tube and stand them upright in a rack. Label the tubes A and B.
2. Take a piece of chromatography paper that fits into the boiling tube, as shown in the diagram. Rule a pencil line 2cm from the bottom of the filter paper. This line is called the origin. Write leaf A at the top of the chromatography paper in pencil.
3. Cut a disc from leaf A with a cork borer. Avoid the veins and midrib of the leaf when you do this.
4. Place the leaf disc on the chromatography paper at the centre of the line marking the origin. Crush the disc into the paper with the end of a glass rod. The crushed leaf disc should leave a stain on the chromatography paper.
5. Pin the chromatography paper to the bung with a drawing pin, and then put the chromatography paper into the tube labelled A as shown in **Figure 1**. Make sure the end of the chromatography paper is in the solvent and that the solvent does not come above the origin. Put the tube carefully back into the rack and do not move it again.

**Figure 1**



6. Let the solvent run up the chromatography paper until it almost reaches the top of the paper. Remove the chromatography paper from the tube and immediately draw a pencil line to show how far the solvent moved up the paper. This line marks the solvent front.
7. Replace the bung in the tube.
8. The filter paper with its coloured spots is called a chromatogram. Let the chromatogram dry. Using a pencil, draw round each coloured spot on the chromatogram.
9. Repeat step two with the second piece of paper but write B at the top of the chromatography paper.
10. Repeat steps 3–8 with leaf B.

Calculate the R<sub>f</sub> value for each of the pigment spots on each chromatogram.

$$\text{Rf value} = \frac{\text{Distance moved by pigment from origin to centre of pigment spot}}{\text{Distance from origin to solvent front}}$$