SBI4U Name: _____

# Paper Chromatography of Leaf Pigments

Background:
Spinach leaves contain a variety of photosynthetic pigments including chlorophylls (a/b) and carotenoids (Beta-carotene and xanthophylls).

This mixture of pigments can be separated by **paper chromatography**. The separation is based on each pigments **solubility in a solvent** which travels up the chromatography paper (chromatogram) carrying the pigments.

The **more soluble** a pigment is in the solvent, the **further up** the paper it will travel. The distance travelled is constant for a particular pigment in a particular solvent.

The distance travelled by a pigment is called the **Rf (ratio factor)**.

Diagram

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

Pigment Spot Colours on Chromatogram:

| | | |
|---|---|---|
| **Xanthophyll** | - | dull yellow |
| **Chlorophyll b** | - | yellow green |
| **Chlorophyll a** | - | blue green |
| **Beta-carotene** | - | deep yellow / orange |

Procedure: **WEAR SAFETLY GLASSES, NO OPEN FLAMES**

1. Extract the pigments from spinach leaves as described by the teacher using acetone.

2. Holding the chromatography paper by the sides only, cut one end of the paper into a "v-shape".

3. Place some chromatography solvent in a test tube so that when the chromatography paper is inserted, only the tip of the cut "v-shaped" end will be submerged.

4. Using a capillary tube, obtain a bit of the pigment extract and gently spot it on the chromatography paper a bit higher up than the "v-shaped" cut. This is the "origin".

5. Dry the extract spot using a hairdryer or by waving the chromatography paper in the air.

6. Repeat the spotting of extract several times, drying after each application, until the spot is a very dark green.

7. Place the chromatogrpahy paper in the test tube and secure it to a stopper so that the "v-shaped" cut just touches the solvent. Try to ensure that the sides of the paper don't touch the sides of the test tube.

8. The solvent will move up the chromatography paper carrying the pigments. When the solvent is close to (but not at) the top of the paper, remove the chromatogram.

9. Immediately draw a line to mark the origin and the position where the solvent front ended. Also circle each pigment spot and write the name/abbrev. of the pigment beside it.

10. Record the distance (in cm) travelled by the solvent (solvent front) and the pigments (from the origin to the middle of each coloured spot) and calculate the Rf values.

11. Wash all equipment thoroughly.

12. Attach your chromatogram to this sheet once it has completely dried.

Results:

| | Distance (cm) | Rf |
|---|---|---|
| Solvent front | | - omit - |
| Carotenoids | | |
| Xanthophyll | | |
| Chlorophyll a | | |
| Chlorophyll b | | |

Questions (attach a separate sheet):

1. In which order (from origin to solvent front) do the pigments appear on the chromatogram?

2. Which pigment was the most soluble in the solvent? Which was the least soluble?

3. Is the Rf value larger or smaller for the most soluble pigment?

4. Why are some pigment spots larger (area and volume) than others?

5. Would you expect the Rf values to be the same if you used a different chromatography solvent? Explain.

6. Light at the blue end of the spectrum penetrates most easily underwater. Why are seaweeds often yellow-brown in colour?

7. How would your chromatogram change if you used the same leaf but in the fall?