Experiment #2 Paper Chromatography: Compound Seperation

Objective

To introduce the technique of chromatography by using paper chromatography to separate and identify photosynthetic pigments in spinach leaves and calculate Rf values of photosynthetic pigments.

Theory

Plants produce their own food by using the sun's energy to transform carbon dioxide and water into glucose. In this process of photosynthesis, plants convert the sun's energy into chemical energy that is stored in the bonds of the glucose molecule. This energy fuels the metabolic processes of cells and is essential for life on earth. For photosynthesis to transform light energy from the sun into chemical energy (bond energy) in plants, the pigment molecules absorb light to power the chemical reactions. Plant pigments are macromolecules produced by the plant, and these pigments absorb specified wavelengths of visible light to provide the energy required for photosynthesis. Chlorophyll is necessary for photosynthesis, but other pigments collect and transfer energy to chlorophyll. Although pigments absorb light, the wavelengths of light that are not absorbed by the plant pigments are reflected back to the eye. The reflected wavelengths are the colors we see in observing the plant. For example, green pigments reflect green light, yellow pigments reflect yellow light. Plants contain different pigments, and some of the pigments observed include: chlorophylls (greens), carotenoids (yellow, orange red), anthocyanins (red to blue, depending on pH), betalains (red or yellow).

The process of chromatography separates molecules because of the different solubilities of the molecules in a selected solvent. In paper chromatography, paper marked with a plant extract, is placed in a container with a specified solvent. The solvent carries the dissolved pigments as it moves up the paper. The pigments are carried at different rates because they are not equally soluble. A pigment that is the most soluble will travel the greatest distance and a pigment that is less soluble will move a shorter distance.

The distance the pigment travels is unique for that pigment in set conditions and is used to identify the pigment. The ratio is the Rf (retention factor) value. Standards are available for comparison.

$$Rf = \text{distance pigment travels (cm)} / \text{distance solvent travels (cm)}$$

Equipment

Fresh spinach leaves
Large test tube
Chromatography paper (precut 18 cm strips)
6 ml syringe
Plastic wrap
Chromatography solvent (9:1 petroleum ether & acetone)
Plastic pipettes
5 test tubes (20-30 mL)
Beaker

Safety

Wear goggles and aprons when working with chemicals. Petroleum ether, acetone and alcohol are volatile and flammable. Avoid breathing vapors of the reagents.

Procedure

Sample Preparation

(Important! Handle the chromatography paper as little as possible and only by the edges as oil from the skin affects the separation.)

1. Take a strip of chromatography paper approximately 18 cm long. One end is blunt and the other is pointed.
2. With a pencil lightly make a line 2 cm from the pointed end of the paper.
3. Bend the strip of paper at the blunt end and attach it to the small end of the cork with the push pin. Adjust the length of the paper so that when it is inserted into the test tube, it will touch the bottom without curling.
4. Place a ruler over the leaf so that is covers the pencil line on either end.
5. Using a penny coin, press down firmly and roll along the ruler edge several times to form a definite green line.
6. Allow the green line to dry thoroughly.
7. Use a fresh area of the leaf and repeat several times until the pencil line is covered completely with a narrow green band. Be careful not to smear this green line.

Separation of Pigments

1. Place the test tube in the test tube rack. Using the 6mL syringe, dispense 5 mL of chromatography solvent in the test tube.
2. Carefully lower the paper strip into the test tube and secure the cork in the top. The solvent must touch the pointed end of the paper but should not touch the green line.
3. Be careful not to slosh the solvent. Allow the tube to stand undisturbed.
4. Observe the solvent movement and the band separation.
5. When the pigments have separated into distinct bands (the solvent has moved approximately half the distance of the paper), lift the cork with paper attached from the test tube. Mark the

edge of the solvent front with a pencil. Remove the push pin and detach the paper from the cork.

6. Place the push pin back in the cork and place the cork back on the test tube to minimize fumes.

If the chromatography solvent has not become contaminated it can be reused each class period. Use the waste container for disposal of used chromatography solvent. To properly dispose of the solvent, dilute with three volumes of water and then pour the diluted solvent down the drain.

7. Allow the paper to dry completely.

Data Analysis

1. On the notebook, illustrate the color bands on the chromatogram. Label the band that traveled the greatest distance 1, the next 2, the next 3. Continue until all bands are labeled.
2. Describe the color of each band in Table 1.
3. Measure the distance from the first pencil line to the solvent front. Record this value in Data Table 1for each pigment.
4. Now measure the distance from the first pencil line to the average peak of each color band.
5. Record these values in Table 1.
6. Cut the different colored bands apart carefully and trim off excess paper being careful to include all the pigment for each band.
7. Label each test tube, one for each pigment in Table 1.
8. Cut each band of color into pieces small enough to fit into a 20-30 mL test tube. Insert the paper pieces in the appropriate test tubes.
9. Add 5 ml of isopropyl alcohol to each test tube and seal with a small piece of plastic wrap. Allow samples to stand overnight until the color is completely eluted from the paper. These solutions will be used in the next activity.
10. Calculate the Rf values for each pigment and record the values in Table 1.
11. Using the given value below, determine the name of each pigment and record the name in Table 1.

Rf Values: β-carotene: 0.99, chlorophyll a: 0.30, chlorophyll b: 0.13, violaxanthin: 0.40, lutein: 0.68.

Table 1

Pigment	Description of clolor	Distance the solvent travelled	Distance the color travelled	Rf value	Name of the pigment (From data analysis #11)
1					
2					
3					
4					
5					