

Plant Pigments/Chromatography

Introduction

In this laboratory you will separate plant pigments using chromatography.

Paper chromatography is a useful technique for separating and identifying pigment and other molecules from cell extracts that contain a complex mixture of molecules. The solvent moves up the paper by capillary action, which occurs as a result of the attraction of solvent molecules to the paper and the attraction of the solvent molecules to one another. As the solvent moves up the paper, it carries along any substances dissolved in it. The pigments are carried along at different rates because they are not equally soluble in the solvent and because they are attracted, to different degrees, to the fibers of the paper through the formation of intermolecular bonds, such as hydrogen bonds.

Beta carotene, the most abundant carotene in plants, is carried along near the solvent front because it is very soluble in the solvent being used and because it forms no hydrogen bonds with cellulose. Another pigment, **Xanthophyll** differs from carotene in that it contains oxygen. Xanthophyll is found further from the solvent front because it is less soluble in the solvent and has been slowed down by hydrogen bonding to the cellulose. **Chlorophyll's** contain oxygen and nitrogen and are bound more tightly to the paper than the other pigments. **Chlorophyll a** is the primary photosynthetic pigment in plants. A molecule of chlorophyll *a* is located at the reaction center of the photo systems. The pigments collect light energy and send it to the reaction center. Carotenoids also protect the photosynthetic systems from damaging effects of ultraviolet light.

Procedure

1. Obtain a 250 mL beaker which has about 2 cm of solvent at the bottom. Cover the beaker with aluminum foil to prevent the vapors from spreading.
2. Cut a piece of filter or chromatography paper which will be long enough to reach the solvent. Draw a line about 1.0 cm from the bottom of the paper.
3. Use a quarter to extract the pigments from spinach leaf cells. Place a small section of leaf on the **top** of the pencil line. Use the ribbed edge of the coin to crush the leaf cells. **Be sure the pigment line is on top of the pencil line.** Use a back and forth movement exerting firm pressure through out.
4. Place the chromatography paper in the cylinder. **Do not allow the pigment to touch the solvent.**
5. Cover the beaker. When the solvent is about 1 cm from the top of the paper, remove the paper and **immediately** mark the location of the solvent front before it evaporates.

6. Mark the bottom of each pigment band. Measure the distance each pigment migrated from the bottom of the pigment origin to the bottom of the separated pigment band. Record the distance that each front, including the solvent front, moved in the table below. Depending on the species of plant used, you may be able to observe 4 or 5 pigment bands.

Distance moved by Pigment Band (millimeters)

Band Number	Distance (mm)	Band Color
1		
2		
3		
4		
5		

Distance Solvent Front Moved _____

Analysis of Results

The relationship of the distance moved by a pigment to the distance moved by the solvent is a constant called R_f . It can be calculated for each of the pigments using the formula:

$R_f =$	$\frac{\text{distance pigment migrated (mm)}}{\text{distance solvent front migrated (mm)}}$
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Record your R_f values in the table below.

	= R_f for carotene (yellow to yellow -orange)
	= R_f for xanthophyll (yellow)
	= R_f for Chlorophyll a (bright green to blue green)
	= R_f for Chlorophyll b (yellow green to olive green)

Topics for Discussion

1. What factors are involved in the separation of the pigments?

2. Would you expect the R_f value of a pigment to be the same if a different solvent were used? Explain.

3. What type of chlorophyll does the reaction center contain? What are the roles of the other pigments?
