

# Plant Pigment Chromatography

Paper chromatography is a technique used to separate substances in a mixture based on the movement of the different substances up a piece of paper by capillary action. Pigments extracted from plant cells contain a variety of molecules, such as chlorophylls, beta carotene, and xanthophyll, that can be separated using paper chromatography. A small sample of plant pigment placed on chromatography paper travels up the paper due to capillary action. Beta carotene is carried the furthest because it is highly soluble in the solvent and because it forms no hydrogen bonds with the chromatography paper fibers. Xanthophyll contains oxygen and does not travel quite as far with the solvent because it is less soluble than beta carotene and forms some hydrogen bonds with the paper. Chlorophylls are bound more tightly to the paper than the other two, so they travel the shortest distance.

The ratio of the distance moved by a pigment to the distance moved by the solvent is a constant,  $R_f$ . Each type of molecule has its own  $R_f$  value.

$$R_f = \frac{\text{distance traveled by pigment}}{\text{distance traveled by solvent}}$$

## OBJECTIVES

In this experiment, you will

- Separate plant pigments.
- Calculate the  $R_f$  values of the pigments.

## MATERIALS

50 mL graduated cylinder  
chromatography paper  
spinach leaves  
coin  
goggles

cork stopper  
pencil  
scissors  
solvent  
ruler

## PROCEDURE

*Obtain and wear goggles! **Caution:** The solvent in this experiment is flammable and poisonous. Be sure there are no open flames in the lab during this experiment. Avoid inhaling fumes. Wear goggles at all times. Notify your teacher immediately if an accident occurs.*

1. Obtain a 50 mL graduated cylinder with 5 mL of solvent in the bottom.
2. Cut the chromatography paper so that it is long enough to reach the solvent. Cut one end of the paper into a point.
3. Draw a pencil line 2.0 cm above the pointed end of the paper.
4. Use the coin to extract the pigments from the spinach leaf. Place a small section of the leaf on top of the pencil line. Use the ribbed edge of the coin to push the plant cells into the chromatography paper. Repeat the procedure 10 times making sure to use a different part of the leaf each time.

## LabQuest 4A

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- Place the chromatography paper in the cylinder so the pointed end just touches the solvent. Make sure the pigment is not in the solvent.
- Stopper the cylinder and wait until the solvent is approximately 1 cm from the top of the paper. Remove the chromatography paper and mark the solvent front before it evaporates.
- Allow the paper to dry. Mark the bottom of each pigment band. Measure the distance each pigment moved from the starting line to the bottom of the pigment band. Record the distance that each of the pigments and the solvent moved, in millimeters.
- Identify each of the bands and label them on the chromatography paper.
  - beta carotene: yellow to yellow orange
  - xanthophyll: yellow
  - chlorophyll *a*: bright green to blue green
  - chlorophyll *b*: yellow green to olive green
- Staple the chromatogram to the front of your lab sheet.
- Discard the solvent as directed by your teacher.

## DATA

Band number	Distance traveled (mm)	Band color	Identity
1			
2			
3			
4			
5*			
Distance solvent front moved = _____ mm			

\* The fifth band may not appear.

## PROCESSING THE DATA

Calculate the  $R_f$  values and record in Table 2.

Table 2	
Molecule	$R_f$
beta carotene	
xanthophyll	
chlorophyll <i>a</i>	
chlorophyll <i>b</i>	

## QUESTIONS

1. What factors are involved in the separation of the pigments?
2. Would you expect the  $R_f$  value to be different with a different solvent?
3. Why do the pigments become separated during the development of the chromatogram?

## EXTENSIONS

1. Repeat the paper chromatography with various species of plants. What similarities do you see? What differences are there?
2. Use colored filters around the cuvettes to test the effect of red, blue, and green light on the photosynthetic activity of spinach.
3. Vary the distance of the floodlight source to determine the effect of light intensity on photosynthesis.
4. Compare the photosynthetic activity of spinach with that of chloroplasts from other plants.
5. Investigate the effect of temperature on the photosynthetic activity of spinach.
6. Explain why the rate of photosynthesis varies under different environmental conditions.