

# Plant Pigments and Photosynthesis

Cell Energy: SC.912.L.18.7, SC.912.N.1.1

## Pre-Lab Discussion

Photosynthesis begins when light is absorbed by pigments in the plant cell. One technique for separating and identifying these pigments is paper chromatography. In paper chromatography, solvent moves up the paper carrying with it dissolved substances – in this case, plant pigments. The pigments are carried along at different rates because they are not equally soluble in the solvent and are attracted in different degrees to the paper call affinity. Many green leaves contain pigment colors that are not seen until autumn because they are hidden by the chlorophyll. A few plants have leaves that are red, orange, or yellow all year long.

In this investigation, you will use paper chromatography to determine what differences exist in the plant pigments of various colors of leaves. You will also determine which leaves or which parts of leaves contain the chlorophyll necessary to carry out photosynthesis.

## Problem

What plant pigments can be found in different colored leaves?

## MATERIALS: (per group)

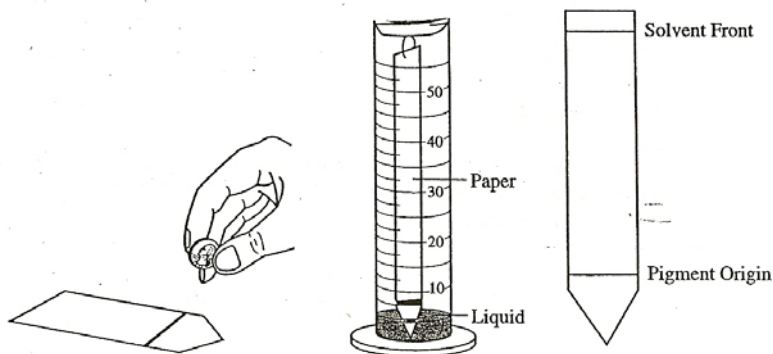
Solvent  
Chromatography Paper Strips  
Graduated Cylinder  
Fresh Spinach Leaf  
*Coleus* Leaf

Metric Ruler  
Scissors  
Apron  
Goggles

Cork ensemble  
Large Paperclip  
Coin

## PROCEDURE:

1. Make the cork hanger by inserting an elongated paperclip into the cork to use as a hook.
2. Gently poke a hole in the top of the chromatography paper with the paperclip. **Test** to see if the paper will hang properly in the graduated cylinder **before** adding solvent.
3. Remove paper from the graduated cylinder and from paperclip, set aside on paper towel.
4. Carefully measure 4 mL of solvent into a graduated cylinder.
5. **Cover** the graduated cylinder with the cork. Solvent is a volatile liquid and will evaporate quickly.
6. Trim the one end of the paper to form a point.
7. On the pointed end of the paper, using a pencil, make a line 1.5 centimeters from the end of the strip.

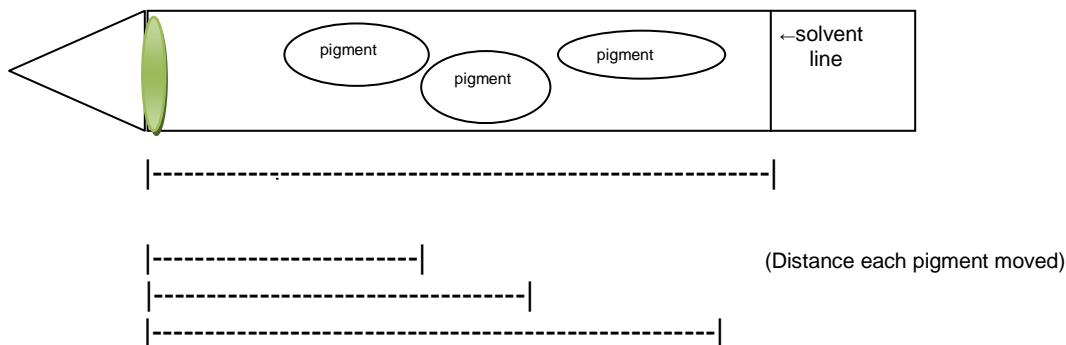


8. Place your leaf over the pencil line and gently but firmly roll the coin over the leaf to extract pigment. Make sure the pigment line is on top the pencil line. Roll the coin over the leaf several times to be certain you have sufficient pigment on the line.
9. Hook the paperclip through the top of the chromatography paper.
10. LOWER the pointed end of the paper into the solvent (it will remain upright on its own). **The pigment line must remain ABOVE the solvent level.** Let the solvent rise until it reaches almost the end (top) of the paper. This is called the *Solvent Front*. Do not allow the solvent to run off the paper.
11. After the solvent has risen up the paper, remove the strip of paper from the graduated cylinder. Place on a paper towel. Using a pencil, quickly mark the final solvent height (front line) before the paper dries and the solvent line evaporates.
12. Allow the chromatography paper to dry, and then make a sketch of the chromatogram. Some possible colors and the pigments they represent are:

faint yellow- carotenes; yellow- xanthophyll; bright green – *chlorophyll a*;  
yellow-green – *chlorophyll b*; red- anthocyanin

### DATA

13. Determine the  $R_f$  for each pigment in your sample.



14. Measure the distance (in millimeters) from the baseline to the final point the solvent traveled. Record this as Solvent Height in your data table.
15. Measure the distance (in mm) from the original pigment to the highest point of the first colored pigment spot. Record this as Pigment Spot Color and Pigment Spot Height in your **data table**.
16. Repeat step for other pigment colors in your sample.
17. Calculate the  $R_f$  value for each pigment color using the formula below and record in your data table.

$$R_f \text{ Value} = \frac{\text{distance pigment migrated (mm)}}{\text{distance solvent front migrated (mm)}}$$

18. Record the data from your lab group in your data table.

Name \_\_\_\_\_ Period \_\_\_\_\_

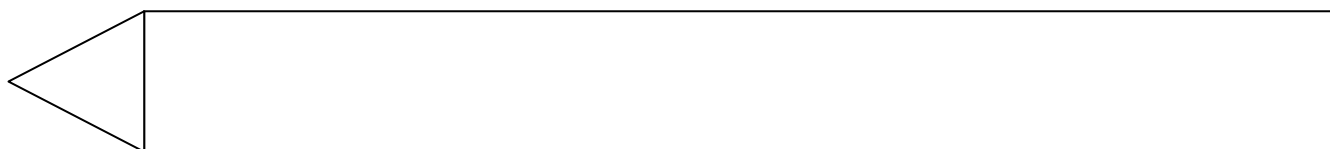
**OBSERVATIONS**

Distance moved by pigment band (millimeters)

|                | Band Number    | Distance (mm) | Band Color |
|----------------|----------------|---------------|------------|
| <b>Spinach</b> | 1 (Base Line)  |               |            |
|                | 2              |               |            |
|                | 3              |               |            |
|                | 4              |               |            |
|                | 5 (front line) |               |            |
| <b>Coleus</b>  | 1 (Base Line)  |               |            |
|                | 2              |               |            |
|                | 3              |               |            |
|                | 4              |               |            |
|                | 5 (front line) |               |            |

**Tape your chromatography sample here** (or draw exactly what it looked like, duplicating placement of pigments using color pencil)

Leaf sample #1 \_\_\_\_\_  
 Distance Solvent Front Moved \_\_\_\_\_ mm



Leaf sample #2 \_\_\_\_\_  
 Distance Solvent Front Moved \_\_\_\_\_ mm



**Observations**

$R_f \text{ Value} = \frac{\text{distance pigment migrated (mm)}}{\text{distance solvent front migrated (mm)}}$

|  |   |
|--|---|
|  | = $R_f$ for carotenes (faint yellow)            |
|  | = $R_f$ for xanthophyll (yellow)                |
|  | = $R_f$ for <i>chlorophyll a</i> (bright green) |
|  | = $R_f$ for <i>chlorophyll b</i> (yellow-green) |
|  | = $R_f$ for anthocyanin (red)                   |

**ANALYSIS:**

1. How many pigments were separated in each type of leaf?
2. How did the pigments in the spinach leaf compare with the pigments in the *Coleus* (red) leaf?
3. Which of these leaves can carry on photosynthesis? Explain your answer.
4. Would changing the solvent (for example: to water), change the  $R_f$  value for the sample? Why or why not?
5. When calculating the  $R_f$  value, discuss why it is important to measure from the original pigment to the highest point of the pigment.
6. In addition to separating plant pigments, what are some other uses for chromatography?

**Use additional paper if necessary.**