Plant Pigments
Cell Energy: Photosynthesis

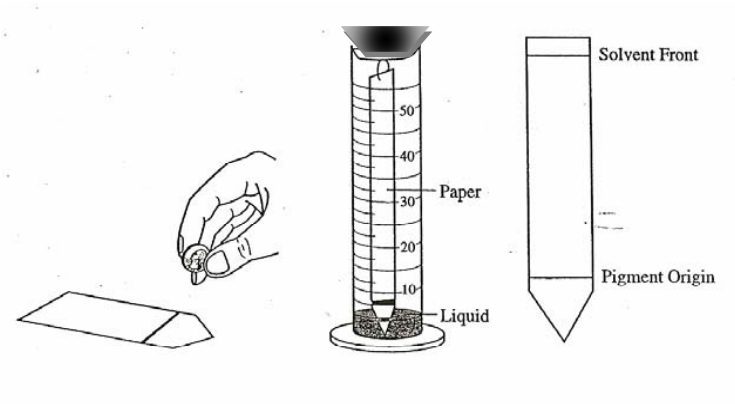
Pre-Lab Discussion
Photosynthesis begins when light is absorbed by pigments in the plant cell. One technique for separating and identifying these pigments is paper chromatography. In paper chromatography, solvent moves up the paper carrying with it dissolved substances – in this case, plant pigments. 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. 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?

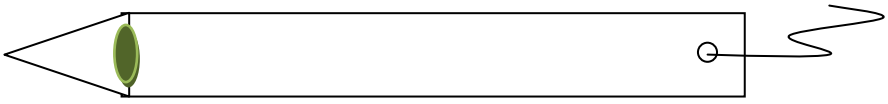
MATERIALS: (per group)

Solvent: (70% isopropyl alcohol)
2 Chromatography Paper Strips
2 Graduated Cylinders
Coin
2 Paper clips
Pencil
2 Corks
Fresh Spinach Leaf
Coleus Leaf
Metric Ruler
Paper towel
Scissors
Beaker (disposal)
Goggles
Apron

PROCEDURE:



1. Carefully measure 8 mL of solvent into a 100 mL graduated cylinder.
2. Cover the graduated cylinder with a cork or rubber stopper and let sit for 1-2 minutes.
3. Obtain a chromatography paper strip and label one end with “S” for Spinach and one sample with “C” for Coleus.
   On the back of the paper sample record your name **in pencil**.
4. Trim the opposite end of the paper to form a point.
5. On the pointed end of the paper, using a pencil, make a line 2 centimeters from the end of the strip **in pencil.**
6. Use a coin to transfer a sample of your leaf onto the pencil line, as shown in the diagram (and during the pre-lab demonstration).



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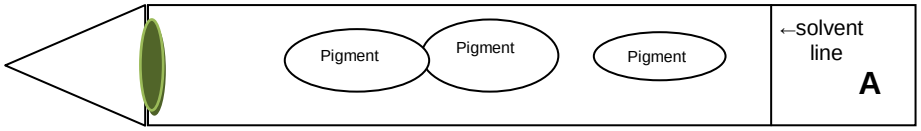
2 cm

7. Uncover the graduated cylinder and gently lower the pointed end of the paper into the solvent (making sure the paper clip is attached and hooked to the rim of the graduated cylinder). ***The leaf mark must remain ABOVE the solvent level.**
8. Cover the graduated cylinder with the cork/rubber stopper.
9. Let the solvent climb as long as possible but do not allow the solvent to run off the paper.
10. Once the solvent has reached the top quadrant of the chromatography paper remove the paper from graduated cylinder and place paper towel to blot.
11. Trace over the solvent front line and pigments with a #2 pencil before the chromatography paper dries.

DATA

12. Determine the R_f for each pigment in your leaf sample.

Example:



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(Distance each pigment moved in mm)

13. Record the Samples and the Solvent in the observation data table.
14. Measure the distance (in mm) 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 baseline to the highest point of the first pigment spot. Record this as Pigment Color and Pigment Height in your data table.
16. Repeat step 12 for other pigments in your sample.
17. Calculate the R_f value for each pigment and record in your data table.

$$R_f \text{ Value} = \frac{\text{Pigment height (mm)}}{\text{Solvent height (mm)}}$$

OBSERVATIONS

	= R_f for carotene (yellow to yellow orange)
	= R_f for xanthrophyll (yellow)
	= R_f for chlorophyll a (bright green/bright bue)
	= R_f for chlorophyll b (yellow green/olive green)
	= R_f for anthrocyanin (red)

	Pigment Line	Distance (in mm)	Color Observed	Probable Pigment
Spinach	1 (Base Line)			
	2			
	3			
	4			
	5 (front line)			
Coleus	1 (Base Line)			
	2			
	3			
	4			
	5 (front line)			

Tape your chromatography sample to your lab paper.
(If your lab partner has the sample on their paper trace the sample below and draw the pigment placements)

ANALYSIS:
Answer the questions. Use additional paper if necessary.

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 (ex: 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 color dot to the highest point of the pigment spot.

6. In addition to separating plant pigments, list some other uses for chromatography.