PLANT PIGMENT CHROMATOGRAPHY

Background Information:

The plant life around us is full of wonderfully rich and vibrant colors. From red to violet, the entire color spectrum can be found in plant materials. These colors come from a surprisingly small number of plant pigments. The three primary groups of plant pigments are anthocyanins, carotenoids, and chlorophyll. Each of these absorbs light at different parts of the visible light spectrum and has a different function in the plant.

- Anthocyanins attract insects for pollination by absorbing light in the UV spectrum, serve as a sunscreen to protect plants from sun damage, and provide protection against predators because of their foul taste.
- Carotenoids and chlorophyll both serve as light-harvesting pigments in the photosynthetic processes of plants.

It is possible to separate these pigments from one another by the use of paper chromatography. In this process, plant tissue is applied to filter paper and an organic solvent is allowed to move up the filter paper. 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.

Some pigments have a higher affinity for the solvent than others and move at different rates up the filter paper. Because of these differences, several color bands would be expected if there is more than one pigment present. Based on the bands formed on the filter paper, the *retention factor*, or R_f, value can be calculated for each pigment. This is done by dividing the distance the pigment traveled by the distance the solvent traveled.

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

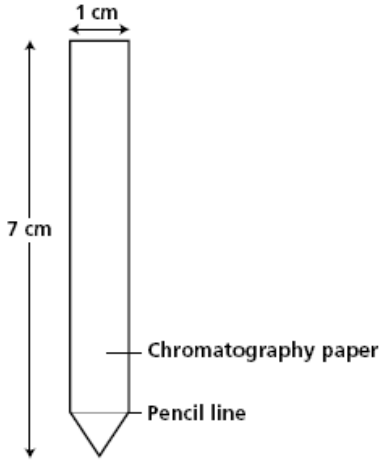
Materials:

graduated cylinder
chromatography paper
chromatography solvent (3:1:1 mixture of petroleum ether, acetone, and water)
ruler
pencil
penny coin
fresh spinach leaf
scissors
foil or plastic wrap

SAFETY

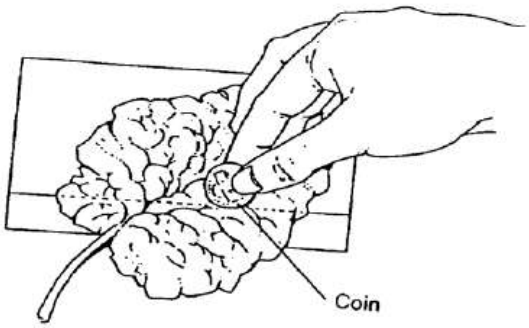
Goggles and aprons to be worn
Petroleum ether, acetone and alcohol are volatile and flammable
Avoid breathing vapors of the reagents

Procedure:



1. Cut chromatography paper into a strip that will fit into the graduated cylinder. Cut one end of the chromatography paper into a point or tip. Make a light pencil line about 1.5 cm from the end of the paper you just cut.

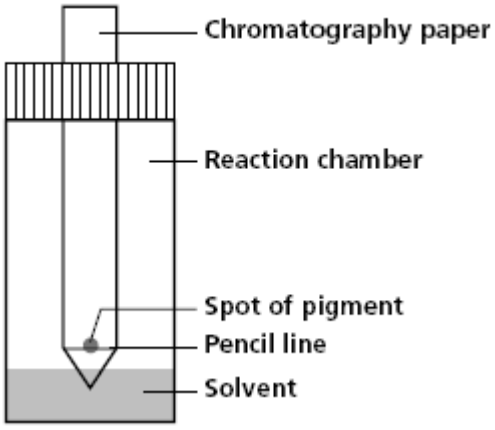
2. Use the coin to extract plant pigments from the leaf. Take a dark green spinach leaf and place it over the pencil line you have drawn. Roll the penny over the leaf at the same position as the pencil line. You should see plant pigment across the pencil line you have drawn. Move the leaf around and repeat this step 8-10 times using different parts of the leaf. Allow the pigment to dry.



3. Pour about 1.0 cm of solvent into the bottom of the cylinder. Place the tip of the filter paper in the solvent being careful not to let the solvent touch the plant pigment line you have made.

4. Cover the cylinder with the aluminum foil to prevent the vapors from evaporating. The solvent should travel up the filter paper. Watch it!

5. When the solvent is about 1-2 cm from the top of the filter paper remove the filter paper and make a pencil mark where the solvent stopped (do this quickly, before the solvent evaporates!)



6. Allow the paper to dry. Once the paper dries, draw a pencil line at the bottom of each distinguishable pigment band on the filter paper. Depending on the leaf you used, you should see 4-5 bands.

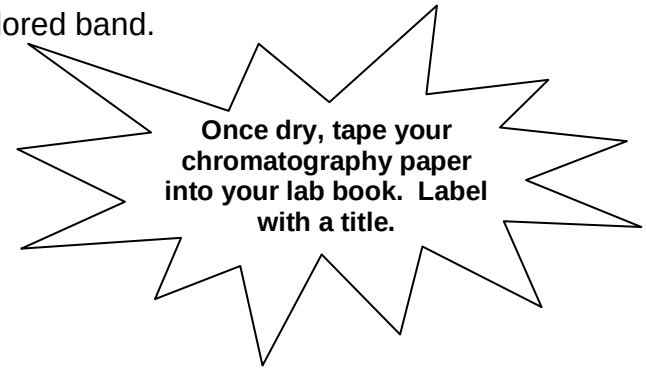
7. Make sure work area is clean and dry.

Analysis and Calculations:

1. Create a table in which you record the distance each pigment band traveled, the band color and the calculated R_f value.

2. Determine and record the pigment type for each colored band.

    - Carotenoids are yellow to yellow-orange
    - Chlorophyll a is bright green to blue-green
    - Chlorophyll b is yellow-green to olive green
    - Anthocyanins are red



Questions and Applications:

1. Did you find examples of all pigment types in your plant sample? If not, suggest a reason why the pigment may have been missing.

2. Which of the pigments migrated the farthest? Why did it move further than the other pigments?

3. Which of the chlorophyll forms is more soluble? How do you know?

4. Why is it important for plants to have more than one pigment?