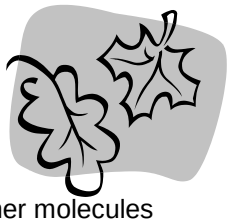
Name	Date	Period

Plant Pigments



Chromatography of Plant Pigments

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** contains oxygen and nitrogen and is 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.

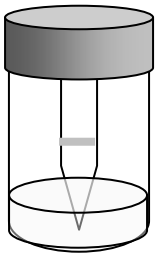
Procedure

Materials Needed

1 Vial
1 Chromatography Paper Strip
Chromatography Solvent, 10ml
Coin
Spinach

Safety: *Avoid inhaling the chromatography solvent. Avoid open flames.*

1. With a pencil, draw a line 1.5cm from the bottom of the paper.
   Note: *Touch the paper as little as possible; the oils from your fingers will interfere with the chromatogram.*
2. Place a piece of spinach over the line. Rub the ribbed edge of a quarter over the spinach leaf to extract the pigments. Repeat eight or 10 times, making sure you are rubbing the coin over the pencil line and moving the leaf each time.
3. Pour 10ml chromatography solvent into a glass vial. Place the chromatography paper in the vial so that the pigment end of the paper is barely immersed in the solvent. Cap the vial and leave it undisturbed until the solvent reaches approximately 1cm from the top of the strip.
4. Remove the paper and immediately mark the location of the solvent front; it will evaporate very quickly.
5. Mark the location of each of the four bands and record your data in Table 1.



Analysis

Table 1
Chromatography of Plant Pigments

Band Number	Pigment	Migration Distance (mm)	Rf Value
--	Solvent		--
1 (top)	Carotene (yellow to yellow-orange)		
2	Xanthophyll (yellow)		
3	Chlorophyll a (bright green to blue-green)		
4	Chlorophyll b (yellow-green to olive-green)		

1. Calculate the Rf (Relative distance traveled factor) values for plant pigment chromatography using the following formula and record your data in Table 1. (This allows you to compare your data with other groups since the distance traveled is relative.)

$$Rf = \frac{\text{Distance Substances (Pigments) Traveled}}{\text{Distance Solvent Traveled}}$$

Questions

1. Which pigment migrated the farthest and why?

2. Which of the two forms of chlorophyll is more soluble?

3. Why do leaves change color in autumn?

4. What is the function of the chlorophyll in photosynthesis?

5. What are the accessory pigments and what are their functions?

6. What does the Rf value represent?