Names:

Plant Pigment Chromatography

Background Information

The incredible variety of plant colors is produced by a surprisingly limited number of organic pigments. They can be divided into three primary groups: chlorophylls, carotenes, and anthocyanins. Each has different properties and performs different functions for the plant, including absorbing light in different parts of the spectrum. More light absorbed = more energy for a plant. Most plant parts, especially leaves, contain some combination of these three pigments, even if only one is especially obvious. It is possible to separate these pigments from each other using a technique called paper chromatography. In this process, plant tissue extract is applied to a piece of chromatography (filter) paper. A solvent is allowed to travel up the paper, and if the pigment is soluble in the solvent, it will be carried along with it. Different pigments have different affinities for the solvents and will travel at different rates. 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 measures the distance the pigment traveled versus the distance the solvent traveled, or the solubility of the pigment in the solvent. R_f values are characteristic of specific pigments and are a reliable identifier.

Chlorophylls: Chlorophylls are perhaps the most familiar plant pigment to most people. They are polar (water-soluble) pigments that act as electron-transporters in photosynthesis and provide green colors to plants. Two types of chlorophyll are usually visible with paper chromatography: chlorophyll a and chlorophyll b. Chlorophyll a is more soluble than chlorophyll b and will travel farther up the paper with the solvents we are using. **<u>Chlorophyll a produces a bright green to blue-green band</u>, and *chlorophyll b produces a dull olive to yellow-green band.***

Carotenes: Carotenes are non-polar (fat-soluble) hydrocarbons. This class of pigments ranges in color from yellow to red. They are also essential as helpers in photosynthesis. Most likely, two carotene bands will be visible with the chromatography you will be using. **<u>Carotene itself produces a yellow-orange band.</u> *Xanthophyll produces a lighter yellow band***.

Anthocyanins: Anthocyanins are polar (water-soluble) pigments that provide red, blue, and purple colors to plants. They don't have critical photosynthetic roles for the plant, but rather are considered to be metabolites. Depending on what type of plant material you use, you may or may not see anthocyanins. ***Anthocyanins tend not to travel very far up the paper and form a reddish band*** along the bottom.

Materials

- Variety of colored leaves
- Tall vial with a lid with 1 cm of chromatography solvent in bottom
- Chromatography paper
- Pencil and ruler
- penny
- Safety glasses

Procedure

1. Get a vial with a lid that has ~ 1 cm of chromatography liquid in the bottom and a green leaf.

2. Cut chromatography paper in half lengthwise.

3. Cut one end of the filter paper into a point. Make a pencil line all the way across one end of the strip, 1.0 cm up from the bottom.

4. Use a coin to extract the pigments from the green leaf. Place a small section of leaf on top of the pencil line. Use the edge of the coin to crush the cells. Be sure that the pigment line is on top of the pencil line. Repeat this about 8 to 10 times using a new portion of the leaf until you get a dark line at the bottom.

5. Place the chromatography paper into the vial so that the pointed end barely touches the solvent liquid**. Do not allow the pigment to be in the solvent.**

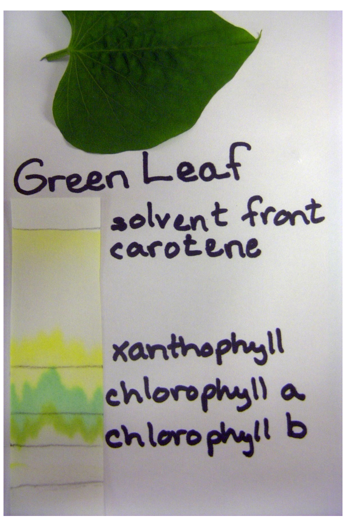
6. Put the lid on the vial. When the solvent is about 1 cm from the top of the paper, remove the paper and immediately mark where the solvent reached before it evaporates.

7. Once the paper is dry, use the light box to draw a line across the bottom of each pigment band. Depending on the species of plant used, you may be able to see 4 to 5 pigment bands.

8. Repeat the above procedure one more time with a different colored leaf or petal.

9. By measuring from the point of origin to the bottom of each pigment band, the R_f value for each pigment can be calculated using the following formula: **Do the math for 1 leaf only.**

$$R_f = \frac{\textbf{distance of pigment from origin}}{\textbf{distance of solvent front from origin}}$$



Sample Rf Problem

Band #	Distance moved (mm)	band color
1	12 mm	olive green
2	21 mm	bright green
3	27 mm	bright yellow
4	53 mm	yellow-orange

Solvent front moved 53 mm

Pigment		Rf value
Carotene	53 mm ÷ 53 mm = 1	
Xanthophyll	27mm ÷ 53 mm = 0.51	
Chlorophyll a	21 mm ÷ 53 mm = 0.40	
Chlorophyll b	12 mm ÷ 53 mm = 0.22	

Student Data Tables and Analysis

Distance Moved by Pigment Bands

Band Number	Distance (mm)	Band Color
1		
2		
3		
4		
5		

Distance in millimeters the solvent moved ______________

Calculate the R_f for each band $R_f = \frac{\text{distance moved by pigment from original position}}{\text{distance moved by solvent from original position}}$

Pigment Rf Values

Pigment	R_f Value
Carotene (yellow-orange)	
Xanthophylls (yellow)	
Chlorophyll a (bright green)	
Chlorophyll b (olive or yellow-green)	
Anthocyanins (red)	

Analysis Questions

1. Which pigments did you discover in your leaf sample?

2. Compare your Rf values to another group. Are they close? They should be. If they aren't suggest a reason why.

3. Why do you think some pigments move farther than others? (Hint: think about polarity and size)

4. Based on what you have seen in this activity, come up with an explanation for how green leaves turn such brilliant colors in the fall.

5. What is the advantage to a plant of having several different pigments rather than just one?

6. Could you use paper chromatography to separate pigments for anything else? (Hint: Think about shows like CSI, NCIS and Cold Case)

Attach your two chromatography strips to this paper when you turn in the lab.