Picking out the Pigments: Isolating Plant Pigments

Plants contain a variety of pigments that vary in color and chemical characteristics. Chlorophyll *a* and chlorophyll *B*, carotenes, and xanthophylls are four pigments found in the chloroplasts of plants. These pigments play important roles in the process of photosynthesis. Chlorophyll *a* has a blue-green appearance. The yellow-green pigment is Chlorophyll *B*. The orange-yellow pigment is carotene and the light yellow pigments are called xanthophylls. These four pigments are non-polar and are not soluble in water.

Chlorophyll *a* appears as bright green to blue green in color to the human eye while chlorophyll *B* appears to be olive green in color. The color detected by human eyes results from the green wavelength of light being reflected by this pigment. As sunlight strikes the leaf, the other wavelengths of light in the visible light spectrum (red, orange, yellow, blue, indigo, and violet) are absorbed by the leaf while the chlorophyll pigments reflect the green wavelengths. The wavelengths that are absorbed by the various pigments in the plant's leaves are collectively referred to as the absorption spectrum. These wavelengths that have been absorbed by the plant will energize the plant's photosynthetic process, setting energy transfer events into action. Measuring the amount of actual photosynthesis occurring in various colors of the light is called the plant's action spectrum. It seems logical, then, that plants should do photosynthesis best when in red or blue light and very little or none in green light. When comparing the two spectra, one finds that photosynthesis does indeed work best in red and blue light; however, it is surprising to find that some photosynthesis does occur in green light. This is due to the aid of the yellow pigments, carotenes and xanthophylls. They have the ability to capture the energy of the green photons and contribute to the light reaction.

The various pigments in a cell extract are separated and identified using a technique called paper chromatography. In this technique, the solvent moves up the chromatography paper and carries the dissolved pigments with it. The pigments move up the paper at unequal rates. One reason for this unequal rate is that the pigments vary in their solubility.

Plant leaves contain varying amounts of chlorophyll, carotene, and xanthophylls. However, most plants have a higher concentration of chlorophyll than the other pigments, giving them an overall green appearance. During the fall, deciduous plants will decrease their levels of photosynthesis in response to the reduced hours of sunlight per day and the amount of chlorophyll present will decline. This results in revealing the carotenes and xanthophylls which make the leaves appear orange and yellow. Different combinations of these pigments give us a wide range of colors each fall.

Purpose:

In this activity you will prepare a chromatogram with a spinach leaf to isolate and identify the pigments in these leaves.

Materials

40- 50 mL graduated cylinder with solvent
1 strip of chromatography paper
1 piece of aluminum foil
1 spinach leaf
1 penny
Goggles
Ruler

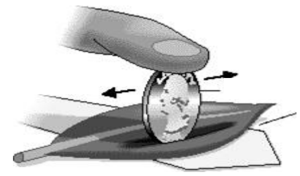
Safety Alert:

1. Goggles must be worn during this activity
2. CAUTION: solvents are flammable and evaporate quickly, keep covered at all times.
3. Avoid inhalation of fumes from solvents.

Procedure:

You will find all necessary materials at your lab bench.

1. On the piece of chromatography paper, draw a line across the width of the paper using a pencil 1.5 cm from the bottom of the paper. This will be your starting line.
2. Place the spinach leaf on top of the line. Using a coin, press the pigment into the chromatography paper by rolling the coin over the surface of the leaf several times.



3. Remove the aluminum foil from the cylinder and place the paper inside. Make sure the pigments are not touching the solvent. Cover the cylinder completely with the aluminum foil.
4. Let the paper sit in the solvent until the solvent has moved to 1 cm from the top of the paper. Do not let the solvent reach the top of the paper.
5. Remove the paper from the cylinder and quickly mark the level the solvent traveled.
6. Using a pencil mark the location of each pigment on your chromatogram. Sketch your chromatogram in the Data and Observations section of your student answer page. Include the colors of the pigments in your diagram.
7. Using a ruler, measure the distance the solvent moved from the starting line to the solvent pencil mark. Record this distance in Data Table.
8. Measure the distance traveled by each of the pigments from the starting line. Record the measurements in the Data Table.
9. Calculate the R_F value for each pigment using the formula and record it in your Data Table:

$$R_F = \frac{\text{distance pigment traveled}}{\text{distance solvent front traveled}}$$

10. Using the information provided in the introduction, determine the type of pigment found in each band on the chromatogram and record this in the Data Table.
11. Answer the Conclusion questions.

Name: ____________________

Date: ________________

Block: ________

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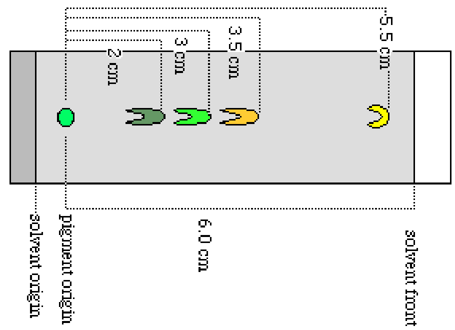
DATA AND OBSERVATIONS:

Sketch your chromatogram in the space below:



Data Table				
Band #	**Color**	**Distance Traveled**	**Pigment Type**	**R_f**
1 (top) Solvent Front	**n/a**		**n/a**	**n/a**
2				
3				
4				
5				

CONCLUSION QUESTIONS:

1. What types of pigments are typically found in leaves?

2. What causes the pigments to move up the chromatogram different distances?

3. List the pigments in order of solubility from most soluble to least.

4. Why do some pigments appear yellow to our eyes while others appear green?

5. Why are all of the pigments not visible in a typical leaf?