Plant Pigment Chemistry: Pigment Extraction and Analysis using Thin Layer Chromatography

Lesson developed by Kristi Crowe, Ph.D. Candidate, University of Maine, NSF GK-12 Fellow (2005)

Grade Level High School Chemistry (with modifications, also for middle school.)

Objectives:

- To learn about the roles of pigments present in leaves.
- To extract and isolate pigments present in autumn leaves using thin layer chromatography (TLC).

Additional Resources:


Glossary of Chromatography Terms:

Mobile phase – liquid solvent or combination of solvents chosen to allow efficient separation of sample components

Stationary phase – finely divided adsorbent material which permits competitive interaction with solute and solvent molecules

Solvent front – distance traveled by the solvent. The solvent front is measured from the line penciled in on the TLC plate where the sample is spotted prior to separation.

Retention factor – ratio of the distance traveled by the pigment relative to the distance traveled by the solvent

Materials:

Mortar & pestle
Beaker
Filter paper
Quartz sand
Ruler
Glass developing chambers with lids
TLC silica gel plates (www.carolinabiological.com)
Pasteur pipettes
Bulbs
Test tube containing 15mL acetone
Developing solvent (4:1 petroleum ether:acetone)
Assorted autumn leaves (3 per student)
Plant Pigment Chemistry Lesson & Lab
Chemistry of Plant Pigments Powerpoint

Activity:

This lab focuses on the separation of plant pigments using thin layer chromatography. The distinctive separation provided by this type of extraction helps uncover the mystery of hidden plant pigments to the chemistry of autumn.

Glass jars with lids can be used as developing chambers. Petroleum ether and acetone in a 4:1 solvent ratio were chosen as the developing solvent (mobile phase) because of the impressive separation that can be achieved. Due to flammability and health risks associated with these chemicals, the developing chambers were filled with a thin layer of mobile phase (2-3 mL) prior to class. Acetone works best as the extraction solvent, and again for safety concerns, the acetone was measured out in 15mL aliquots and transferred to capped test tubes by the instructor.

Visible pigments include carotene, pheophytin, chlorophyll a and b, lutein, xanthophylls, and anthocyanins. Due to the polarity of the silica gel plates (stationary phase), the polarity of the developing solvent (mobile phase), and the molecular shape of the analyte (pigment), the pigments separate into distinctive bands of color. In our experiments, pigment separation from the top of the TLC plate downward was as follows:

- Carotene (golden)
- Pheophytin (olive-green)
- Chlorophyll a (blue-green)
- Chlorophyll b (yellow-green)
- Lutein (yellow)
- Xanthophylls (yellow)
- Anthocyanins (red)

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The basic principles of this lab can be incorporated into a variety of units including chemical properties of bonding, solubility and concepts of polarity, plant physiology, biochemistry, and photosynthetic biology. Students should be introduced to the principles of chromatography prior to beginning this lab. One way to introduce these concepts would be through a paper chromatography lab, for example separating pigments in water-based inks. This introductory lab allows students to learn terms such as mobile and stationary phase, solvent front, and retention factor.

Figure 1. Plating extracted plant pigments on TLC plates

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This lab focuses on the separation of pigments using thin layer chromatography (TLC). TLC, like other types of chromatography, is used to separate organic molecules based on their affinity for a mobile and stationary phase. In thin layer chromatography, the stationary phase is composed of a plastic film coated with a thin layer of silica gel. The mobile phase or solvent is selected based on the degree to which it interacts with the stationary phase and the solute (or pigments). Just as in paper chromatography, the mixture to be separated is applied as a spot or line to the solid phase while the mobile phase is allowed to pass through the solute dissolving it along the way. As the solute dissolves in and moves with the solvent along the stationary phase, a chromatogram is formed.

The distance traveled by the pigment is characteristic for a specific set of conditions and this distance may be used to identify the compound. The ratio of the distance traveled by the solute to that of the solvent front is called the retention factor or Rf factor.

\[
Rf = \frac{\text{distance traveled by the compound}}{\text{distance traveled by solvent}}
\]

**Lab Materials**
- mortar & pestle
- developing chamber
- test tube w/15mL acetone
- Pasteur pipette
- TLC silica gel plate
- 100 mL beaker
- bulb
- 3 leaves (mixed colors)
- quartz sand
- filter paper
- ruler

**Procedure**
1. Add the following items to the mortar: 3 leaves torn into small pieces, 15mL acetone, and 1 gram of sand. Using the pestle, grind the mixture for 5 minutes or until the solution is dark in color. The plant cell walls are tough, so be persistent!

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2. Fold the filter paper to make a cone, place it in the beaker, and pour the solvent/leaf mixture into the filter paper. Allow the pigment/acetone solution to drain through the paper.

3. Using a pencil, draw a line on the silica side of the TLC plate 1.5 cm from the bottom. Be careful not to scratch the silica gel with the pencil!!

4. Using the Pasteur pipette, apply a small spot of pigment solution onto the center of the line. Let dry. Repeat this step 4-5 times or until the spot is a dark color.

5. Place the chromatography plate into the developing chamber and close the chamber. Allow the solvent to migrate upward until the solvent front is 1 cm from the top of the plate. Remove the plate and mark the solvent front IMMEDIATELY!

6. Allow the chromatogram to dry. Measure the distance traveled by each pigment band. Remember to measure to the center of each band. Calculate the Rf values.

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\text{Solvent Front} = \underline{\text{__________________________}}
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<th>Leaf Pigment</th>
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Chemistry of Plant Pigments – Review

1. Why was sand added to the leaves during the extraction procedure?

2. What happens to a leaf when it changes color in the fall? Be specific about each pigment.

3. Why was the developing solvent composed of 2 different solvents?

4. According to the basic definition of chromatography, what determines the partitioning or distribution of the pigments on the TLC plate? In other words, why do some pigments move further up the TLC plate than others?

5. What are the functions of carotenes and anthocyanins in the leaf?

6. Of the pigments extracted today, which ones are polar and which are non-polar?

7. Where are carotenes and anthocyanins found within a plant?