Lesson Plan 2 for Biology: Chromatography of Pigments

Objectives: Students will be able to:

- Explain what molecules make living plant leaves green and autumn leaves multicolored
- Explain how dark colors are composed of many differently colored pigments
- Perform a chromatographic experiment to separate a mixture of pigments
- Explain how chromatography works and how one identifies each compound

* It is recommended that this lesson be performed in conjunction with the Physics lesson.

California Content Standards:

Physical Sciences:

Grade 3: 1.a. Students know energy comes from the Sun to Earth in the form of light.
1.b. Students know sources of stored energy take many forms, such as food, fuel, and batteries.
2.c. Students know the color of light striking an object affects the way the object is seen.

Grade 5: 1.b. Students know all matter is made of atoms, which may combine to form molecules.
1.f. Students know differences in chemical and physical properties of substances are used to separate mixtures and identify compounds.

Grade 7: 6.a. Students know visible light is a small band within a very broad electromagnetic spectrum.
6.e. Students know that white light is a mixture of many wavelengths (colors) and that retinal cells react differently to different wavelengths.
6.f. Students know light can be reflected, refracted, transmitted, and absorbed by matter.

Grade 8: 5.a. Students know reactant atoms and molecules interact to form products with different chemical properties.

Life Sciences:

Grade 6: 5.a. Students know energy entering ecosystems as sunlight is transferred by producers into chemical energy through photosynthesis and then from organism to organism through food webs.

Investigation & Experimentation:

Grade 3: 5.c. Use numerical data in describing and comparing objects, events, and measurements.

Grade 4: 6.d. Conduct multiple trials to test a prediction and draw conclusions about the relationships between predictions and results.

Grade 5: 6.f. Select appropriate tools (e.g., thermometers, meter sticks, balances, and graduated cylinders) and make quantitative observations.

Grade 6: 7.b. Select and use appropriate tools and technology (including calculators, computers, balances, spring scales, microscopes, and binoculars) to perform tests, collect data, and display data.

Before you begin, you may want to watch the DSSC videos to prepare the lab: http://www.youtube.com/caltech and click on “Resources for Teachers” on the right.

For technical assistance please contact a scientist at Caltech at JuiceFromJuice@caltech.edu
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Background:
Photosynthesis is the process plants use to convert carbon dioxide and water into carbohydrates, using the energy of light.

\[
\text{Light} + \text{CO}_2(g) + \text{H}_2\text{O}(l) \rightarrow \text{C}_6\text{H}_{12}\text{O}_6(aq) + \text{O}_2(g) \quad (\text{Note: equation is not balanced})
\]

Special plant chemicals called pigments absorb light at specific wavelengths and use the energy to split water. As the water splits into oxygen molecules and hydrogen ions, electrons are released and begin the photosynthetic path that eventually leads to the formation of the simple sugar glucose.

\[2 \text{H}_2\text{O}(l) \rightarrow \text{O}_2(g) + 4 \text{H}^+ + 4 \text{e}^-\]

The main photosynthetic pigments that give the green color to plants are chlorophylls. They often hide the other pigments that may be present. Chlorophyll a is a bright-green to blue-green color and chlorophyll b is yellow to olive-green to the human eye. This is the color that is reflected by the leaf. All other colors are absorbed by the pigments, especially reds and blues. In addition to chlorophyll many green plants contain one or more other pigments including the yellow/orange carotenes, yellow xanthophylls, and red-to-purple anthocyanins.

Chromatography is a separation technique invented in 1910 by the Russian botanist Mikhail Tsvet for the purpose of separating plant pigments. The word chromatography is derived from the Greek words for color (chromo) and writing (graph). Chromatography has a stationary phase such as paper, or a thin layer of an absorbent substance, and a mobile phase, the solvent that will dissolve the pigment.

The formula for calculating the polarity of different pigment molecules is called the “Ratio to the Front” or \(R_f\) value.

\[R_f = \frac{\text{length to a spot}}{\text{length to the front}} = \frac{\text{distance traveled by the pigment from the origin}}{\text{distance traveled by the solvent from the same origin}}\]

For example, using a spotted chromatography paper column, the measurement from the start point to a distinct color spot is the length to a spot, or the distance to a spot. The measurement from the same start point to where the solvent stopped is the length to the solvent front. The ratio of these two values is specific for each pigment. These ratios should be fairly consistent, i.e. the ratio for chlorophyll a will be \(~0.71\) and chlorophyll b will be \(~0.42\).
TEACHER DEMONSTRATION: CHROMATOGRAPHY WITH PLANT PIGMENTS
(This will be followed by the students’ lab, using water-soluble markers.)

Materials and Supplies:
For preparation
- (optional) Some Blackberries
- Some Spinach leaves
- Spinach leaf soaking solvent (acetone)
- 2 Erlenmeyer flasks (250 mL) or medium jars
- (optional) 2 beakers (50 mL) or small jars

For demonstration
- A few chromatography paper strips, cut to ~10 cm x ~1 cm:
  - Whatman® #1 filter paper* OR
  - Coffee filters
- A few capillary tubes or coffee straws
- Chromatography solvent (petroleum ether, isopropanol, and water)
  - Some other solvent may be used alone if necessary
- 1 beaker (250 mL) or large glass jar
- 1 Pencil*
- 1 rubber band
- A few paper clips
- Aluminum foil
- 1 Metric ruler

*Provided in the Juice from Juice kit

Pre-lab Preparation: (For a video demo, go to http://www.youtube.com/Caltech, click on the “Resources for Teachers” playlist on the right, and choose the Biology videos, starting with #1.)

Cut a Whatman® filter, or coffee filter, into a few strips, approximately 10cm x 1cm.

The day before the lab, tear fresh spinach leaves and place them in the medium glass container. Submerge the leaves in acetone to extract the pigments. Be sure to cover the glass container to keep the acetone from evaporating. Also place a few blackberries in water in another medium glass container and mush them up.

SAFETY NOTE: Acetone and the developing solutions are flammable. Keep them away from sparks and open flames. Always wear goggles.
Chromatography Procedure:

1. **Optional Step:** Pour the acetone-spinach solution through a filter and collect the liquid in the small container. Do the same for the blackberry solution, but put it into the other small container.

2. Wear gloves when handling the chromatography paper strips to keep the oils of your skin off the paper. Draw a pencil line about 1.5 cm from the end of your paper strip.

3. Use a capillary tube or coffee straw to place a small drop of your green solution on the center of the line. Be sure to let the drop completely dry before you add another drop. This will help keep the spot from spreading out too much. Repeat 15 to 20 times. Be careful not to let the spot spread out too far. Do the same for the blackberry solution on another strip.

4. Pour enough chromatography solution into the larger container to barely completely cover the bottom of the container but not so high up that they will directly touch the spotted drops on the strips. Petroleum ether is best for the spinach solution; isopropanol or water work for blackberry solution.

5. Stretch the rubber band around the larger container lengthwise over the mouth and bottom of the container. This will provide a place to hang the chromatography strips. Suspend the paper strips in the container by hanging them over the rubber band. You can attach them with a paper clip or simply fold the end of the strips over the rubber band. Alternatively, gently place the chromatography strips in the container leaning nearly straight up and down against one of its walls. The end of your strips should just touch the solvent.

6. Cover the large container carefully with aluminum foil.

7. Observe as the pigments travel up the paper strips. After about 20 to 30 minutes remove your developed chromatograms, i.e. chromatography strips. Be careful to handle the strips by the paper clip or top fold only.

8. Before they dry, quickly mark with a pencil where the solvent stopped. This is called the solvent front.

9. Also mark where each pigment stopped moving.

**Analysis:**

The relationship between the distance the pigment moved to the distance the solvent moved is called the $R_f$ value.

$$R_f = \frac{\text{length to a spot}}{\text{length to the front}} = \frac{\text{distance traveled by the pigment from the origin}}{\text{distance traveled by the solvent from the same origin}}$$

Calculate the $R_f$ values for each of the pigments.
Checking for Understanding: Analysis Questions to Ask Your Students.

1. Which pigments were you able to observe in your chromatogram? How did you determine you actually had these particular ones? What were some of the differences between the pigments in spinach leaves and blackberries?

2. Why do the pigments move at different rates through the chromatogram?

3. Why is it an advantage for plants to have different colored pigments?

4. With what you have discovered about plant pigments, what conclusions can you make regarding the changing color of leaves in autumn?
STUDENT LABORATORY PROCEDURE

Materials and Supplies (per group of students (any number)):
- A few chromatography paper strips, cut to ~10 cm x ~1 cm:
  - Whatman® #1 filter paper* OR
  - Coffee filters
- A few water-soluble markers, in different colors; some dark and some light*
- Water
- 1 clear plastic cup
- A few Binder clips*
- 1 Pencil*
- 1 Metric ruler

*Provided in the Juice from Juice kit

Pre-lab Preparation:
Cut Whatman® filters, or coffee filters, into many strips, approximately 10cm x 1cm.

Chromatography Procedure:

1. Using a pencil, draw a line approximately 3 cm from the bottom of the strip.

2. Make a round dot on the line with a dark marker. Repeat on 2 other strips using different colored markers.

3. Let dry. Continue with the next step while you wait.

4. Pour water into a clear plastic cup, up to ~2 cm from the bottom. Use a ruler to measure the height of the water that you put in.

5. Place the strips into the cup, leaning nearly straight up and down against one of its walls, being careful not to let them fall into the water. If need be, use binder clips to hold the strips in place.

6. Watch closely as the water wicks up the strip via capillary action – it is a quick process.

7. Take the strips out after 10 – 15 minutes, yet before the color runs to the end of the strips.

8. Observe the color separation. Notice which pigment traveled the fastest. What does this tell you about the size of the molecules that make up that pigment relative to the others?

9. Tape your strips to a piece of paper (or in a lab book), and write about the science of chromatography, the science of the separation of pigments. This type of separation technique is used in many fields, e.g. separating DNA, crime scene investigation, pharmaceuticals. Explain how this technique is useful in those fields; also explain how it is useful in the field of solar energy conversion.

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Lesson Plan for Grades 3-8