

Paper Chromatography

Separating Plant Pigments

Lesson Plan and Teacher Notes

OBJECTIVE

To introduce the process of chromatography and why it is used by scientists in many fields. Students will separate the pigments in fresh spinach leaves. Based on color, the student will identify the pigment in each color band. For each pigment, the student will calculate the R_f value, which can be compared with other students' results and/or with chromatography experiments using other materials. (At more advanced levels, the students can discuss the polarity of the molecules in the mixture and the paper, and predict which solvents will best separate the constituents in various mixtures.)

LEVEL

Middle School: pre-AP Biology or Chemistry

TIME FRAME

Three (3) hours (divided) with some preparation a few days in advance.

MATERIALS

(for 8 groups of students)

SAFETY NOTES



Methyl alcohol is poisonous if swallowed. Hexane is stable but highly flammable, and it is harmful if inhaled. Personal protection requires safety goggles and good ventilation. A **Material Safety Data Sheet** (MSDS) for hexane is available at <http://www.vngas.com/pdf/g38.pdf>. An MSDS for methyl alcohol is available at http://www.sciencelab.com/xMSDS-Methyl_alcohol_Absolute-9924660.

To make spinach juice:

- (1) 1000mL beaker
- (1) 500mL graduated cylinder
- methyl alcohol
- distilled water
- ½ bag fresh spinach leaves
- plastic wrap and rubber band
- 8 centrifuge tubes
- transfer pipette

To conduct lab activity:

- hexane (solvent)
- (8) 1000mL beakers
- (8) centrifuge tubes containing spinach juice
- (8) pieces of chromatography paper
- plastic wrap
- rubber bands
- (8) paintbrushes
- (8) pencils
- (8) rulers
- (8) pairs of scissors
- scotch tape
- paper towels

TEACHER NOTES

Several days before the class activity, prepare spinach solution as follows:

- In a 1000mL beaker, combine 210mL of methyl alcohol with 90mL of water to make 300mL of a 70% methanol solution.
- Place spinach leaves into the methanol solution. Use a stir bar or spoon to press the leaves down into the liquid. Cover the beaker with plastic wrap and allow it to sit overnight (or longer). Stir periodically.
- Strain the liquid, and allow it to evaporate under a hood or ventilated area until the liquid is reduced to approximately 75mL. (If it is not completely reduced to this volume, it will still work, but the resulting bands may not be as brilliant as with a more concentrated spinach solution.)
- Aliquot spinach juice into small centrifuge tubes. Store leftover liquid in freezer.

Day 1 – Engage students

- Ask engaging questions about plant pigments; give interesting examples and present new topics
 - Why do different objects have different colors? What makes one flower pink and another yellow? What makes a tomato turn from green to red on the vine? What is changing in the tomato's skin when this happens?
 - What are pigments? What are dyes? What is the difference between them?
 - pigments – substances in animal tissues or cells that give them color; they are dry substances that do not dissolve in water
 - pigments are mixed with liquids to make dyes
 - Where are dyes found?
 - dyes can be found in fabrics, plastics, paint, food
 - When do you think people first used dyes? Pigments? – talk about the history of human uses of dyes and pigments (examples: fabrics, food coloring)
 - Ask about or present the four major plant pigments and their colors – chlorophyll a (medium olive green), chlorophyll b (blue-green), carotene (orange), and xanthophyll (yellow). Anthocyanin (red) is considered a minor pigment, and will not be seen in this experiment.
 - Talk about how different pigments (and dyes) stick to fabrics differently – How could a scientist use this knowledge to design an effective stain remover?
 - Depending on time available and the level of knowledge of the students, the discussion could expand into chemistry and attraction between variously charged molecules of pigment and paper.
 - Depending on time available and the level of knowledge of the students, the discussion could also touch on the topics of photosynthesis, the electromagnetic spectrum, absorption of light energy at various wavelengths, and reflection of light.
- Present concepts about paper chromatography
 - What is chromatography?
 - the separation of mixtures into individual components (or parts)
 - in paper chromatography with plants, pigments appear as streaks on the chromatography paper forming a pattern called a chromatogram
 - A Russian botanist named M.S. Tswett invented the first chromatography technique in 1903 while studying the coloring materials in plant life.

- How do scientists use chromatography? To find out what components are in a mixture, such as:
 - pollution in a stream
 - inorganic ions in wine
 - Linus Pauling used chromatography in his study of sickle-cell anemia
 - drugs in a urine sample
- How can paper chromatography be used to identify plant pigments?
 - R_f values – scientists calculate R_f values to determine what molecules are in a given substance

$$R_f = \frac{\text{distance traveled by pigment}}{\text{distance traveled by solvent}}$$
 - The resulting value can be compared to a chart of known values using the same stationary and mobile components to determine the constituents of the mixture
 - In discussing the “front”, which is the point to which the pigment has traveled, ask probing questions to help students understand. How is a pigment front like a weather front? How is it different?
- Preview the lab activities
 - Steps 1-5 may be completed by the students at this point, if time allows
- Instruct the students to read the lab handout for additional background information and clarification of steps in the activity.

Day 2 – Allow students to Explore

- One hour before students arrive, under a hood or in a room with good ventilation, pour approximately 50mL of hexane into each 1000mL beaker; cover with plastic wrap and secure with a rubber band. This allows the air inside the developing chamber to become saturated.
- Lab activity
 - Removing the plastic wrap in order to insert the chromatography paper should be very quick to avoid inhalation exposure. This step should be done in a well-ventilated area such as a hood or outdoors.
 - Tell students before they begin that they should observe all the steps carefully, think about why they are doing each step, and write down all observations, and things that go differently than the instructions tell them or than they expected. Tell the students that they are making a manual for the experiment (perhaps that will be judged by other students for its usefulness, or that will be used to design an additional chromatography experiment with different pigments).
 - Working in pairs or groups of no more than 4, students conduct the lab according to the lab handout. Allow time for the students to try and figure out on their own how to go through all of the steps.
 - Instructor must watch carefully that all students keep their safety glasses and gloves on while handling the solvent.
 - Students observe the development of the chromatography paper, and discuss with each other what they are seeing, and which pigments they think they have separated just based on color. Later the students will calculate the R_f values. These can be compared to a

known chart of values (if available), with other students in the classroom, and/or with other chromatography experiments with different mixtures and different stationary and mobile components.

- **Explain**
 - Students discuss their results with each other, calculate R_f values, and answer the questions at the end of the lab activity. If there is time, this can be done in class. If not, it can be assigned for homework before the post-lab discussion in the next meeting.

Day 3 – Discuss results

- **Explain** – Review results with the students. Ask probing questions to evaluate student understanding.
 - What does your chromatogram reveal?
 - Why do you think some pigment bands are larger/darker than others?
 - Why did some pigments travel farther than others?

Elaborate

- In a separate lab activity, the students can conduct the same experiment with different vegetable pigments such as those from carrots or tomatoes.
- If the students understand the concept of variously charged particles and their attractions to the chromatography paper, they can design an investigation to separate and determine the pigments or dyes in other paper chromatography experiments with substances such as food coloring, kool-aid, or black felt-tip markers, and using various solvents such as water or salt water or acetone. They can predict relative R_f values for the constituents. A simpler option is to give the students the experimental design, have them conduct the experiment and discuss the similarities and differences with the plant pigment chromatography results.

Evaluate

- Observe students conducting chromatography in the “Elaborate” situations discussed above and evaluate their new experimental designs or their discussion of differences with the plant pigment chromatography results. Suggestions to ask students:
 - How would you expect your chromatogram to look different with the pigments from carrots, sweet potatoes, squash, or tomatoes? Why?
 - Will Kool-aid and black felt-tip markers contain pigments or dyes? Which colors do you expect to see when you separate the constituents of these substances?
 - Advanced: Which chemical did you choose to conduct chromatography with Kool-aid? with black felt-tip markers? Why?
- Determine if student clearly states lab procedures, discusses why he/she is doing each step, and includes some possible mistakes to watch for.
- Determine if student performs the given lab procedure, takes notes as the experiment progresses, correctly analyses the results, and explains why they do or do not make sense.