THINKING AND ACTING LIKE A SCIENTIST

TEACHER'S GUIDE

Plant Pigment Chromatography

What are the photosynthetic pigments present in my leaf?

GRADES 9–12

Life Science

VAN ANDEL EDUCATION INSTITUTE

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INQUIRY-BASED SCIENCE

Life Science

Plant Pigment Chromatography

Grade Level/Content	9–12/Life Science
Lesson Summary	This is the first of two lessons providing students with opportunities to explore how plants utilize energy from the sun to create compounds that store energy. In this lesson, students learn about and investigate the types of plant pigment macromolecules used to capture energy from the light reactions of photosynthesis. The second lesson explores the Rate of Photosynthesis.
Estimated Time	1, 45-minute class period
Materials	Fresh baby spinach leaves (or other available leaves); large test tubes; chromatography paper; chromatography solvent (9:1 Petroleum ether and acetone); mortar, pestle, and ethanol (optional); additional plant structures (roots, stems, fruits, etc.) for optional follow-up investigation; colored pencils; one coin (penny, quarter, etc.); pencil; Investigation Plan; journal; Internet
Secondary Resources	Photosynthetic Pigments What Pigments are in Fruit & Flowers?
NGSS Connection	**HS-LS1-5** Use a model to illustrate how photosynthesis transforms light energy into stored chemical energy.
Learning Objectives	• Students will use paper chromatography to separate photosynthetic pigments found in spinach leaves (or leaves of their own choice). • Students will use color differences to identify photosynthetic pigments from their leaves and calculate observed R_f factors. • Students will describe the role of photosynthetic pigments in capturing the energy that initiates the light reactions of photosynthesis.

What are the photosynthetic pigments present in my leaf?

Photosynthesis is a critically important biochemical process providing the oxygen required to support respiration in nearly all non-autotrophic organisms on earth. Providing students with an opportunity to explore the chemistry of plants, along with the chemicals used in the process, provides them with a solid foundation for understanding and modeling the biochemical reactions that drive photosynthesis.

In this lesson, students use chromatography to extract the plant pigments from spinach leaves that are responsible for photosynthetic activity. Understanding that there are different pigments present in plants prepares students for a deeper look at photosynthesis. In the follow-up investigation, Rate of Photosynthesis, students determine how various factors influence the rate of photosynthesis.

Investigation is based on the Van Andel Education Institute (VAEI) Instructional Model for Inquiry-Based Science. In all investigations:

Students don't know the "answer" they are supposed to get.

Students play a driving role in determining the process for learning.

Teachers and students construct meaning together by journaling.

Students are working as hard as the teacher.

Part 1

INVESTIGATION SETUP

Collect the materials necessary for each student group to perform the investigation.

- fresh baby spinach leaves (or other available leaves)
- large test tube
- chromatography paper
- chromatography solvent (9:1 mixture of Petroleum ether:acetone)
- colored pencils
- one coin (penny, quarter, etc.)
- pencil
- mortar, pestle, and ethanol (optional method for extracting pigments for application on chromatography paper)
- Investigation Plan
- additional plant structures (roots, stems, fruits, etc.) for optional follow-up investigation
- Internet
- journal

Part 2

INVESTIGATION FACILITATION

Question

Introduce the investigation question.

What are the photosynthetic pigments present in my leaf?

Personal Knowledge

Students capture what they already know about plant pigments and plant coloration.

- Have students identify key components of the investigation question.
- Ask them to write 3–5 ideas in their journal before sharing them with their table partners. Once ideas are shared, have students record additional ideas from their table partners in their journal.
- Encourage students to review their list and circle ideas and concepts that they are not sure about.

Secondary Knowledge

Provide students with background information about how paper chromatography works, and why it is used to separate plant pigments.

- Chromatography is used to separate mixtures of substances into their components. Paper chromatography is a technique used to separate and identify plant pigments.
- Plant pigments are not equally soluble within the chromatography solvent. As a result, they will stop at different places as the solvent wicks up the chromatography paper.
- Plant pigments are colored molecules that absorb light at specific wavelengths. These molecules capture the energy of sunlight and use it to make their own food. For more information about the different types of plant pigments, provide this resource (or your own) for students to review: Photosynthetic Pigments

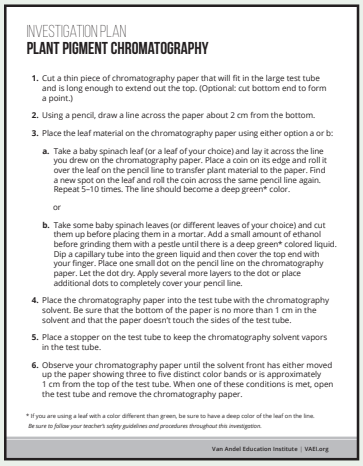
Investigation Plan

Students use paper chromatography to separate plant pigments from the spinach leaf.

- Have students work in teams of 2–3.
- Review the materials and preparations to use paper chromatography to separate photosynthetic pigments found in leaves.
- Have students follow the Investigation Plan.

CRITICAL THINKING

Use the Fair Test checklist to help students think critically about the investigation plan. Help them understand that a good investigation plan must include a test that is repeatable, generates quality data, and minimizes error. The more critically students think about their investigation plan, the more confident they can be in their results.



INVESTIGATION PLAN

PLANT PIGMENT CHROMATOGRAPHY

1. Cut a thin piece of chromatography paper that will fit in the large test tube and is long enough to extend out the top. (Optional: cut bottom end to form a point.)
2. Using a pencil, draw a line across the paper about 2 cm from the bottom.
3. Place the leaf material on the chromatography paper using either option a or b:
   a. Take a baby spinach leaf (or a leaf of your choice) and lay it across the line you drew on the chromatography paper. Place a coin on its edge and roll it over the leaf on the pencil line to transfer plant material to the paper. Find a new spot on the leaf and roll the coin across the same pencil line again. Repeat 5–10 times. The line should become a deep green* color.

   or

   b. Take some baby spinach leaves (or different leaves of your choice) and cut them up before placing them in a mortar. Add a small amount of ethanol before grinding them with a pestle until there is a deep green* colored liquid. Dip a capillary tube into the green liquid and then cover the top end with your finger. Place one small dot on the pencil line on the chromatography paper. Let the dot dry. Apply several more layers to the dot or place additional dots to completely cover your pencil line.
4. Place the chromatography paper into the test tube with the chromatography solvent. Be sure that the bottom of the paper is no more than 1 cm in the solvent and that the paper doesn't touch the sides of the test tube.
5. Place a stopper on the test tube to keep the chromatography solvent vapors in the test tube.
6. Observe your chromatography paper until the solvent front has either moved up the paper showing three to five distinct color bands or is approximately 1 cm from the top of the test tube. When one of these conditions is met, open the test tube and remove the chromatography paper.

* If you are using a leaf with a color different than green, be sure to have a deep color of the leaf on the line.

Be sure to follow your teacher's safety guidelines and procedures throughout this investigation.

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Investigation Plan

Observation

Students record their data.

- Have students create a data table to record their observations.
- Students will record the band colors and distances travelled (in cm) for each observed plant pigment band, as well as the distance travelled for the chromatography solvent.
- Remind students to record qualitative observations for each observed plant pigment band.

CURIOSITY

As students observe and record their data, encourage them to consider the mechanics of chromatography. What carries the chemicals along the paper? Why do some chemicals travel farther than others? What are the advantages and disadvantages of using paper chromatography to separate chemicals in a mixture? These questions can serve as opportunities for follow-up investigations by student groups or individuals. Providing students with a thinking space to be curious about what is taking place is crucial to creating a classroom where curiosity, creativity, and critical thinking thrive.

Part 3

INVESTIGATION ANALYSIS AND DEVELOPMENT OF CLAIM

Data Analysis

Students make sense of their data by organizing it and representing it visually.

- Have students analyze their data. They may wish to use the Data Analysis prompt as a guide.
- Have students **evaluate** their data for trustworthiness.
- Then, have them analyze their data to find patterns and trends. They may **organize** the data and/or **represent** it visually to construct meaning.
- The distance traveled by each plant pigment is another way to identify the specific pigment. The ratio of the distance traveled by a pigment to that of the solvent front is known as the R_f (retardation factor) value. Although there are R_f standards for various pigments based on the chosen chromatography solvent, students are best served in this experiment by using colors to identify pigments.
- To calculate R_f values, direct students to divide the distance the pigment traveled by the distance the solvent front traveled:

$$R_f = \frac{\text{migration distance of pigment}}{\text{migration distance of solvent front}}$$

- The general order of R_f values from largest to smallest is: carotene, pheophyton, chlorophyll a, chlorophyll b, xanthophyll 1, and xanthophyll 2.
- Have students **interpret** what the identified patterns or trends mean.
- Ensure they have enough data that it can be used as evidence to support a claim.

CRITICAL THINKING

Have students dig deeper into their data by considering possible errors or unaccounted factors impacting their trials. Remind students that these ideas should be included in their reasoning when developing their explanation.

Secondary Knowledge

Students use colors to identify the photosynthetic pigments present in their leaf.

- Photosythetic pigments are identified by color:
  - Carotene is yellow to yellow-orange
  - Pheophytin is an olive-green
  - Chlorophylls are green (Chlorophyll a is a bright green to blue-green. Chlorophyll b is a yellow-green to olive-green)
  - Xanthophylls are yellow
- This online resource helps clarify common types of pigments and where they are found: What Pigments are in Fruit & Flowers?
- Students use this information in the reasoning portion of their explanation.

Explanation

Students write a claim and provide evidence and reasoning to support it.

- Have students use what they've discovered to write an explanation that answers their investigation question.
- Students may wish to use the Explanation prompt as a guide. Have them write their explanation in their journal.
- Have students develop a **Claim** to answer the question: What are the photosynthetic pigments present in my leaf?
- Then, have them add **Evidence** (the analyzed data) to support their claim.
- Finally, have them add **Reasoning** to their claim. Reasoning should include the information obtained from this investigation as well as science principles they have learned.

Claim

There were three distinct color bands on the chromatography paper using spinach. The yellow-orange traveled the farthest, next came the dark green, and last was the yellow-green.

Evidence

The yellow-orange pigment had an R_f value of .96.The dark green pigment was second with an R_f value of .66, and the third, yellow-green pigment had a value of .28.

Reasoning

Investigation: The investigation plan was followed carefully and completed as written. We analyzed our data using the suggested data analysis steps and calculations for R_f. Therefore, we believe our data is valid and reliable.

Science: From the Secondary Knowledge we learned that the yellow-orange pigment is carotene, the dark green pigment is chlorophyll a, and the yellow-green pigment is chlorophyll b. We found some data that listed those three pigments' R_f values as .98, .59, and .42. Our R_f values were .96, .66, and .28. Our data showed the R_f values in the same order as the resource and was close numerically.

The most soluble pigment in the ether/acetone solvent traveled the farthest, and that is the carotene. The least soluble pigment traveled the shortest distance, and that was the chlorophyll b. The chlorophyll a molecule was in the middle of the other two and showed an intermediate solubility.

- Once the explanation is written, have students discuss their results using a Present and Defend.

DISCOURSE

Select two groups to conduct a Present and Defend. This helps develop presentation and audience participation skills. Research teams present a summary of their investigation to the class. The class analyzes the information presented and asks clarifying questions, challenges and/or supports the arguments made, and even presents alternative explanations as appropriate. Research teams defend their explanation with evidence and reasoning.

Evaluation

Students reflect on the investigation.

Have students discuss:

- What surprised them?
- What question would they like to investigate next?
- What alternative explanations should be considered for the data collected?

Part 4

INVESTIGATION ASSESSMENT AND EXTENSION

Application

Students conduct related experiments and continue exploring photosynthesis by moving to the second lesson in this sequence.

- Using the same paper chromatography procedures, provide time for students to develop investigation questions that explore plant pigments present in different plant structures (roots, stems, fruits, etc.) for comparison with plant pigments found in the leaves of a plant chosen by the students.
- Have students continue their exploration of how photosynthesis transforms light energy into stored chemical energy by completing the Rate of Photosynthesis investigation.

Assessment

Determine how well students:

- use paper chromatography to separate photosynthetic pigments found in spinach leaves (or a leaf of their own choice).
- use color differences to identify photosynthetic pigments from their leaf and calculate observed R_f factors.
- describe the role of photosynthetic pigments in capturing the light energy that initiates the light reactions of photosynthesis.

INVESTIGATION PLAN
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   b. Take some baby spinach leaves (or different leaves of your choice) and cut them up before placing them in a mortar. Add a small amount of ethanol before grinding them with a pestle until there is a deep green* colored liquid. Dip a capillary tube into the green liquid and then cover the top end with your finger. Place one small dot on the pencil line on the chromatography paper. Let the dot dry. Apply several more layers to the dot or place additional dots to completely cover your pencil line.

4. Place the chromatography paper into the test tube with the chromatography solvent. Be sure that the bottom of the paper is no more than 1 cm in the solvent and that the paper doesn't touch the sides of the test tube.

5. Place a stopper on the test tube to keep the chromatography solvent vapors in the test tube.

6. Observe your chromatography paper until the solvent front has either moved up the paper showing three to five distinct color bands or is approximately 1 cm from the top of the test tube. When one of these conditions is met, open the test tube and remove the chromatography paper.

* If you are using a leaf with a color different than green, be sure to have a deep color of the leaf on the line.

Be sure to follow your teacher's safety guidelines and procedures throughout this investigation.

PLANT PIGMENT CHROMATOGRAPHY

7. Place a stopper back on the test tube and follow your teacher's guidance to reuse or dispose of the chromatography solvent.

8. Place the chromatography paper on a paper towel and carefully mark how far the solvent front moved using a pencil. Continue by carefully marking the boundary of each color band. Do both of these quickly because the solvent will dry and the pigment colors are likely to fade.

9. Place the paper in a well ventilated area to dry. Choose colored pencils matching the observed colors of each band to outline and number each color band from top to bottom. Record the color of each band in your journal.

10. Record the color bands, distance travelled (in cm) for each color, and the solvent front in your data table. Add qualitative data from your observations to your journal.