22A - CHLOROPHYLL EXTRACTION

INQUIRY

What colors make up the green that we see in leaves? How can we use the absorption of light to determine the colors of pigments present in spinach leaves?

MATERIALS

- Device with SPARKvue software
- Colorimeter (with 2 cuvettes)
- Coffee filter
- 1-mL pipet (2)
- Filter paper cut into 2.5 cm x 10 cm strips
- Mortar and pestle
- Coin with wide, ridged edge (such as a quarter)
- Colored pencils
- Isopropyl alcohol (rubbing alcohol)
- Spinach leaves (4)
- Beaker, 250-mL
- Beaker, 50-mL
- Plastic cup
- Scissors
- Ruler
- Rubber band

BACKGROUND

Chlorophyll is the primary pigment found in green plants. It absorbs light energy from the sun for photosynthesis that is then used by the plant to synthesize glucose from carbon dioxide and water. Chlorophyll in the leaves of plants can be extracted, separated, and analyzed using chromatography and colorimetry.

Green leaves contain a mixture of two or more of the following pigments: chlorophyll a, chlorophyll b, xanthophylls, carotene, and phaeophytin. Due to their molecular structure, each type of pigment molecule will bond to the solvent at different positions as it "climbs" the filter paper. This technique is called a chromatography. In the chromatography strip you will see different colors that correspond to specific pigments. Chlorophyll a is dark blue-green, chlorophyll b is light yellow-green, carotene appears bright yellow, and xanthophyll is pale yellow-green.

In this investigation, you will measure the absorbance of red, orange, green, and blue in a sample of chlorophyll extracted from spinach leaves. By passing light through a sample of extracted chlorophyll, you will determine which colors are absorbed the most and, therefore, which ones are transmitted to our eyes.

Chlorophyll Extraction

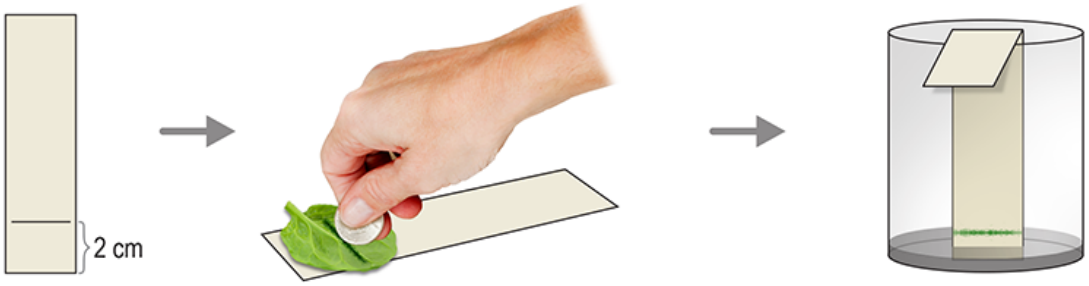
SAFETY

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Do not use alcohol near an open flame

PROCEDURE

Part 1 – Paper chromatography



1. Use a pencil and ruler to mark a line 2 cm from one end of a paper strip. The end of the strip with the pencil line is the bottom of the strip.

2. Set a spinach leaf over the pencil line on the paper. Use a coin on top of the leaf to create a dark smear of leaf pigments over the pencil line.

3. Pour 10-15 mL of rubbing alcohol into the plastic cup.

4. Stand up the strip in the cup so the pigment line is near the alcohol. Do not let the pigment line directly touch the alcohol. Allow the alcohol to rise up the filter paper to meet the pigment line. Bend the top of the strip over the rim of the cup to help it stay upright.

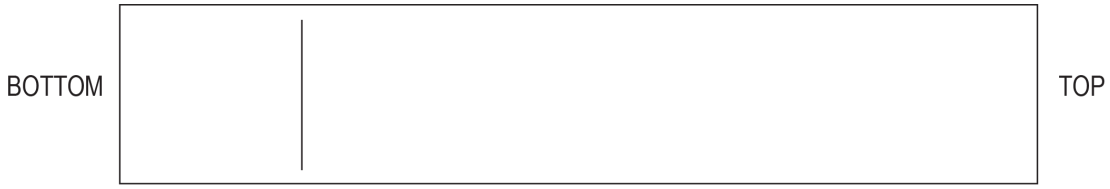
5. Observe the pigment line carefully over the next 10 minutes.

6. Use colored pencils to sketch your observations on the filter paper diagram in the Analysis section.

ANALYSIS

Part 1 – Paper chromatography

Sketch your paper chromatography observations on the filter paper diagram below.



QUESTIONS

Part 1 – Paper chromatography

1. Both chlorophyll-a and chlorophyll-b are present in spinach leaves. What evidence supports this statement, and how can you tell each type of chlorophyll apart?

2. Are there other pigment molecules in the leaf? How can you tell?

3. When leaves change color in the fall, they are less green. A chromatography experiment performed on a sample of leaves in the summer reveals 4 pigments: chlorophyll a, chlorophyll b, carotenes and xanthophylls. What changes would you expect to see if the chromatography experiment was carried out in the fall?

PROCEDURE

Part 2 – Colorimetry

1. Use scissors to cut 3 spinach leaves into small pieces. Discard the stem of the leaves. Place the leaf pieces into a mortar and soak the leaves in 20 mL of alcohol.

2. Use a pestle to grind the spinach leaves for 3 to 5 minutes.

3. Place a coffee filter over the top of a 250-mL beaker. Secure the filter with a rubber band.

4. Use a pipet to squirt the spinach mixture into the filter. Do not allow the filter to fall into the beaker.

5. Open SPARKvue.

6. Connect the colorimeter.

7. Calibrate the colorimeter with a blank cuvette that is ¾ full of alcohol.

8. Open the 22A Chlorophyll Extraction lab file in SPARKvue under Experiments > Essential Chemistry.

9. Fill a clean cuvette about ½ full with alcohol. Use a clean pipet to add 10-15 drops of filtered pigment extract to the alcohol. Seal the cuvette and gently rotate the cuvette to mix. The mixture should appear light green and transparent. Place the cuvette into the colorimeter.

10. Record the absorbance of light for each of the colors listed in Table 1. Absorbance values at or below 1.5 are acceptable; values at or near 3.0 are unacceptable. Dilute the sample with more alcohol solvent if absorbance is too high.

ANALYSIS

Table 1 – Absorbance measurements

Color	Absorbance
Red	
Orange	
Yellow	
Green	
Blue	
Violet	

QUESTIONS

Part 2 – Colorimetry

1. For the pigment extract, which color of light has the greatest absorbance? Which color of light has the lowest absorbance in the sample? Provide an explanation for these results.

2. Spinach appears as green leaf because it has a high concentration of chlorophyll. What colors of light do you think are absorbed by the green chlorophyll pigments? Explain your reasoning.

3. Which pigments in spinach do you think absorb green light?

EXTENSION

Design experiments to investigate how chlorophyll production can vary depending on the conditions of the plant and surrounding environment. You may be asked to investigate one of the following:

- The differences between old leaves (at the base of the stem near a branch) and new leaves (near the tip of a stem).
- The difference between different colored leaves on different species of trees.
- The differences between different colored leaves on the same tree during the fall season.

CHLOROPHYLL EXTRACTION

Analysis: Paper chromatography

Sketch your paper chromatography observations on the filter paper diagram below.

Questions: Paper chromatography

1. Both chlorophyll-a and chlorophyll-b are present in spinach leaves. What evidence supports this statement, and how can you tell each type of chlorophyll apart?

2. Are there other pigment molecules in the leaf? How can you tell?

3. When leaves change color in the fall, they are less green. A chromatography experiment performed on a sample of leaves in the summer reveals 4 pigments: chlorophyll a, chlorophyll b, carotenes and xanthophylls. What changes would you expect to see if the chromatography experiment was carried out in the fall?

Analysis – Colorimetry

Table 1 – Absorbance measurements

Color	Absorbance
Violet (450 nm)	
Blue (500 nm)	
Green (550 nm)	
Yellow (570 nm)	
Orange (600 nm)	
Red (650 nm)	

Questions: Colorimetry

1. For the pigment extract, which color of light has the greatest absorbance? Which color of light has the lowest absorbance in the sample? Provide an explanation for these results.

2. Spinach appears as green leaf because it has a high concentration of chlorophyll. What colors of light do you think are absorbed by the green chlorophyll pigments? Explain your reasoning.

3. Which pigment(s) in spinach do you think absorb(s) green light?