

In this laboratory, you will investigate the effects of environmental factors (wavelengths of light) and cellular structures (pigments) on photosynthesis. In two experiments, you will determine photosynthetic activity by testing for the presence of starch (the storage product of photosynthesis) by using iodine potassium iodide (I_2KI), which stains starch purple-black.

Exercise 1. The Wavelengths of Light for Photosynthesis

Materials

- | | |
|----------------------|---|
| 1 1000-mL beaker | 1 small petri dish |
| 1 400-mL beaker | Distilled water bottle |
| I_2KI solution | Scissors |
| Hot plate | <i>Geranium</i> leaves pre-treated with various filters |
| Forceps | |
| 2 large petri dishes | |

Introduction

The solar radiation that reaches the Earth consists of a wide range of wavelengths, some of which support photosynthesis. The range of wavelengths in the electromagnetic spectrum that plants can fall within the “visible spectrum” (Figure 2). Plants produce a variety of pigments, molecules that can absorb specific wavelengths of light. Light that is not absorbed by these pigments is either reflected or transmitted. The colors we see are a result of the wavelengths reflected by the pigments. Plants utilize certain pigments to capture light energy, which is transformed into chemical energy during the **light dependent reactions** of photosynthesis. These pigments are bound to proteins in the thylakoid membranes of chloroplasts.

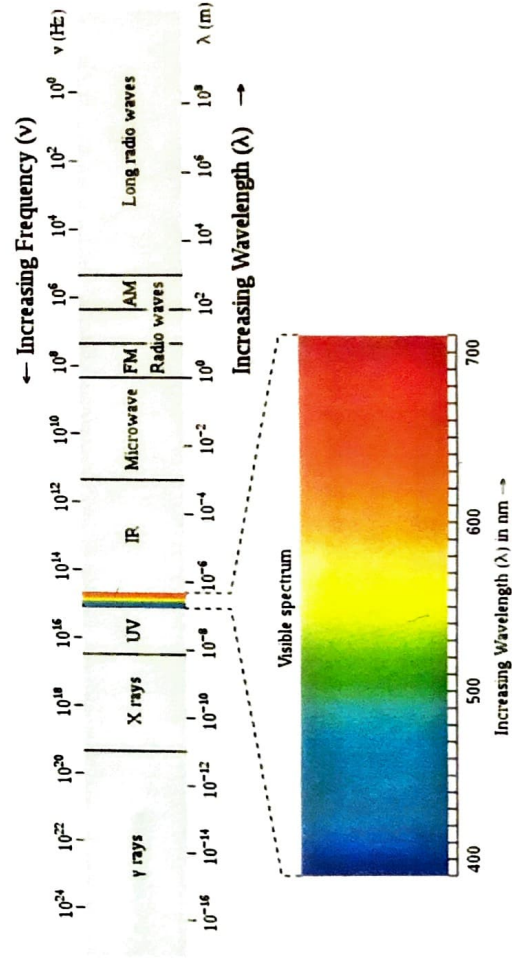


Figure 2. The electromagnetic spectrum showing the colors of the visible spectrum.

In this exercise, you will investigate the photosynthetic activity of different wavelengths of light using leaves of a *Geranium* plant that have been covered with small pieces of different colored plastic filters for several days. You will determine if products of photosynthesis are present in the leaves by testing for the presence of starch under the filters. Starch is synthesized from glucose molecules and is an indirect indication that photosynthesis has occurred. A change from the yellow-amber color of I_2KI to a purple-black color is a positive (+) test for the presence of starch.

Note: *The plastic filters used in this exercise are designed to reflect and transmit the appropriate wavelengths of light that correspond to the visible light spectrum. For example, green filters reflect green light and transmit green light to the leaf surface.*

Research Question: What wavelengths of light support photosynthesis in *Geranium*?

Hypothesis: Formulate a hypothesis about the effect of blue, red, and green wavelengths on photosynthetic ability of *Geranium*.

blue light:

red light:

green light:

Prediction: Predict the results you expect to see based on your hypothesis using Figure 2 to determine specific wavelengths for each color of light.

Procedure

1. Fill the 1000-mL beaker with 300 mL of tap water. Fill the 400-mL beaker with 250 mL of 80% ethyl alcohol. Nest the smaller beaker of alcohol inside the larger beaker. Check to make sure the smaller beaker is floating in the tap water of the larger beaker. Place the nested beakers on the hot plate and turn on the heat to a high temperature to bring the nested beakers to a boil. Once your alcohol boils, adjust the temperature to maintain a slow boil.

Note: It is important to keep the alcohol away from direct contact with the hot plate. If your smaller beaker is not floating, add more tap water to the larger beaker. Do not leave boiling alcohol unattended.

2. Obtain treated *Geranium* leaves from your instructor. With your lab group, devise a way to distinguish the treatments applied to each leaf and draw or photograph the positions of the filters before removing them from the leaves. To distinguish similarly sized and shaped leaves or the positions of filters on leaves, you can cut notches into the leaf tissue. Do not cut into the treatment areas. *Otherwise, leaves should remain intact because the untreated portion of the leaf serves as a control.*
3. Remove the filters from each leaf, saving them. With forceps, carefully drop the leaves into the boiling alcohol to extract the pigments.
4. After a few minutes, use forceps to remove the leaves and check their color. When the leaves are almost white, remove them from the alcohol. Place them in separate petri dishes and rinse them with distilled water.
5. Add enough distilled water to each petri dish to cover the leaf.
6. Add enough I_2KI to the water in the petri dish to create a pale yellow-amber color. Gently swirl the leaves in the solution.
7. After about 5 minutes, examine the leaves and report results in the results section, indicating which areas of the leaf tested positive for starch.

Table 1. Evidence of starch in the leaves of *Geranium* plants that were covered with plastic or opaque materials.

Filter	Wavelength of light transmitted	Starch under filter? Yes/No	Starch in control area? Yes/No
Green		No	No
Red		Yes	Yes
Blue		No	No
Clear		Yes	Yes
Foil		No	No

Results

1. Record your results in **Table 1** above.

Discussion

1. Evaluate your hypothesis and explain your results in terms of your hypothesis.

Exercise 2. Pigments in Photosynthesis

Materials

1 1000-mL beaker
1 400-mL beaker
I₂KI solution
Hot plate

Forceps
Distilled water bottle
Coleus leaves
Large petri dish

Introduction

Plants synthesize various pigments – substances that absorb light. If a pigment absorbs all wavelengths of light, it appears black. Other pigments absorb some wavelengths and reflect others. Yellow pigments, for example, reflect light wavelengths in the yellow portion of the visible light spectrum, green pigments in the green spectrum, and so on.

Green colors in plants are produced by the pigments **chlorophyll *a* and *b***, which are located in the chloroplasts. Yellow, orange, and bright red colors are produced by **carotenoids**, also in chloroplasts. Blues, violets, purples, pinks, and dark reds are usually produced by a group of water-soluble pigments called **anthocyanins** that are located in vacuoles and do not contribute to photosynthesis. Additional colors may be produced by mixtures of these pigments in cells.

Working with your lab group, you will use the I₂KI test for starch to determine which pigment(s) in a *Coleus* leaf support photosynthesis.

Research Question: Where in a colored *Coleus* leaf does photosynthesis occur?

Hypothesis: Examine the *Coleus* leaf and formulate a hypothesis based on the leaf colors.

Prediction: Predict the results of your experiment, based on your hypothesis.

Procedure

1. Make sure the alcohol used in Exercise 1 is at a slow boil in the water bath.
2. Record the pigments present in your *Coleus* leaf in Table 2 below, based on the descriptions provided in the introduction.
3. Predict the results of the I_2KI test for starch in each area of the leaf with a "+" (positive for starch) or "-" (negative for starch) in Table 2.
4. Take a photo or sketch an outline of your leaf, noting the color distribution. Using forceps, carefully drop the *Coleus* leaf in the boiling alcohol to extract the pigments.
5. Remove the leaf when it is almost white, rinse it with distilled water, and perform the I_2KI test as you did in the previous experiment.

Table 2. Predicted and observed results for starch presence in colored regions of the *Coleus* leaf.

Color	Pigment name	Prediction for presence (+) or absence (-) of starch	Actual results for presence (+) or absence (-) of starch
Green		Yes	Yes
Purple		yes	Yes
Pink		No	No
White		No	NO

Results

1. Record your results in Table 2 above.

Discussion

1. Evaluate your hypothesis and explain your results in terms of your hypothesis.

3. *Chromatography paper is composed of cotton cellulose, which is a polar polysaccharide.*
4. *The solvent, petroleum ether and acetone is relatively nonpolar.*

Use this information and the molecular structure of the four photosynthetic pigments to hypothesize about solubilities and predict the separation pattern for the pigments. Look at the structure of the four pigments (**Figure 3**) and rank them according to polarity in the space provided. To determine polarity, count the number of oxygen atoms present in each molecule.

Research Question: What is the relative solubility of the chlorophyll molecules and the carotenoids?

Hypothesis: Form a hypothesis about the relative solubility of the four pigments, based on their relative polarity.

Prediction: Predict the order of the four pigments on the chromatography paper (after exposure to petroleum ether), from starting line to front.

Procedure

1. Add several fresh spinach leaves to a mortar, along with a pinch of sand and enough acetone to just cover the bottom of the mortar.
2. Use the pestle to grind the spinach leaves into the acetone (the acetone extracts pigments from the tissue).
3. Continue to grind the tissue until you see a bright green, non-translucent liquid accumulate at the bottom of the mortar.

Note: Avoid over-grinding the leaves. The liquid should be relatively free of plant debris.

4. Draw a pencil line just above the pointed tip on the chromatography paper.
5. Using a capillary pipette, draw up some of the pigment extract and streak the extract across your pencil line. Allow the paper to air-dry.
6. Repeat step 5 four or five times, until you have a single band of bright green pigment on your chromatography paper.

Note: The darker your band of pigment, the better your results will be.

7. Obtain a large test tube and a cork stopper. Attach the top of your paper to the cork's protruding hook. Insert your paper into the test tube and apply the cork stopper.
8. Use a wax pencil to mark the outside of the test tube just above the pointed tip, but below your pigment band to indicate how much solvent to add in your tube.

Note: It is important that the pigment band is not immersed in the petroleum ether. Immersion will elute the pigments.

The separation of pigments will take about 15-20 minutes.

9. When the leading pigment at the front is near the top of the paper, remove the paper from the test tube and lay it on the bench to dry. **Re-stopper your test tube immediately to avoid inhaling fumes. The solvent should be returned to the flask.**

Results

1. **Draw a diagram or take a photo of your results** and include it below with a figure number (Figure 4) and caption.

Figure 4. *(Add your caption here.)*

Discussion

Evaluate your hypothesis and interpret your results in terms of the hypothesis.

Hypothesis: Refer to Figure 2 to formulate a hypothesis about the general relationship of each pigment to the color of light it absorbs.

Predictions: Predict the wavelengths of light at which absorption will be greatest for each pigment.

Procedure

1. Your instructor will provide you with pre-cut pieces of chromatography paper on which a particular pigment has been separated (chlorophyll a, chlorophyll b, beta carotene, or xanthophyll).
2. Dilute the pigments as follows: Cut up the chromatography paper with your assigned pigment into a small (20-mL) beaker. Add 10 mL of acetone to the beaker and swirl the contents to elute the pigment.
3. Fill one cuvette 2/3 full with the pigment extract from step 2. Fill a second cuvette 2/3 full with clear acetone (this will be your reference material).
4. Wipe both cuvettes with a Kimwipe to remove fingerprints, and handle cuvettes only with Kimwipes from this point forward.
5. Measure the absorption spectrum. Record your measurements in Table 3.

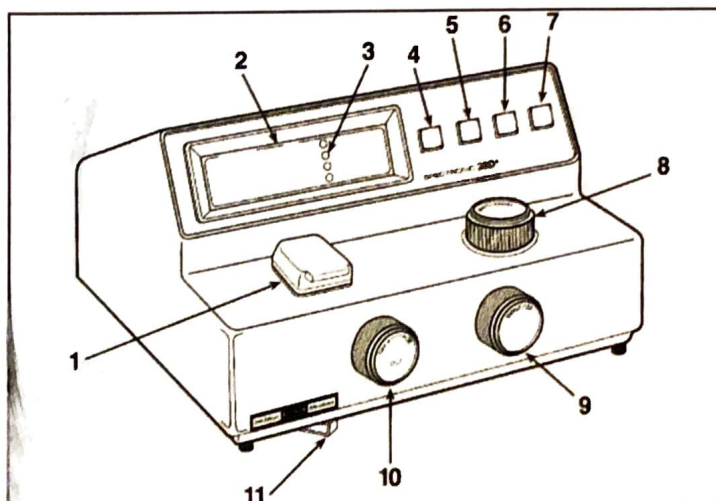


Figure 9. SPECTRONIC 20D spectrophotometer

KEY:

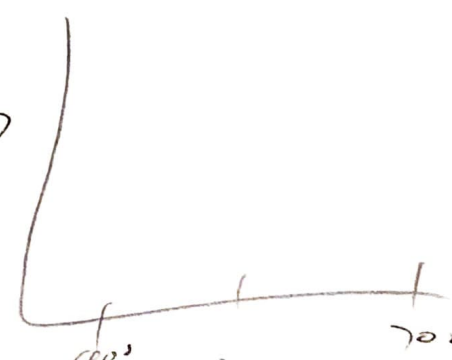
- | | |
|-----------------------|--|
| 1. Sample compartment | 7. Print |
| 2. Digital readout | 8. Wavelength control |
| 3. Mode indicators | 9. Transmittance/Absorbance control (100%T/0A) |
| 4. Mode selection | 10. Power switch/Zero Control |
| 5. Decrease | 11. Filter lever |
| 6. Increase | |

Figure 5. Spectrophotometer with labeled parts.

Table 3. Absorbance of photosynthetic pigments extracted from fresh spinach leaves.

Wavelength nm	Chlorophyll a	Chlorophyll b	Xanthophyll	Beta Carotene	All pigments
400					
420					
440					
460					
480					
500					
520					
540					
560					
580					
600					
620					
640					
660					
680					
700					
720					

Scatter
plot
X



① Average wavelength

Results

1. Using the pooled class data provided by your instructor, plot the absorption spectrum for each pigment in an Excel graph.
 - a. Complete Table 4 below.
 - b. **Create a single graph that displays results of the five treatments** (data from the four individual pigments + the “all pigments” data set). Be sure to label your axes correctly and provide a legend. Use the scatterplot function and select the format that connects the data points.
 - c. Copy and paste your completed graph in the space below and add a figure label (Figure 6) and caption below the graph.

Table 4. *Summary of the wavelengths (in nm) that were maximally and minimally absorbed by each photosynthetic pigment.*

Pigment	Predicted absorption peaks (nm)	Wavelength(s) of Maximum absorption (nm)	Wavelength(s) of Minimum absorption (nm)
Chlorophyll A			
Chlorophyll B			
Xanthophyll			
Beta carotene			

Figure 6. *(Add your caption here.)*

Discussion

1. Interpret your results in terms of your hypothesis. Was your hypothesis supported? Explain.

General conclusions

1. Make some generalized conclusions about photosynthesis. What did you learn about pigments and light energy? Consider all the exercises. For example: *What is the role of chlorophylls, carotenoids and anthocyanins in leaves? How do they contribute to photosynthesis? Why might plants have more than one kind of pigment? How do different wavelengths of light support photosynthesis?*