Spectrophotometers and colorimeters

You will use either a spectrophotometer or colorimeter to measure the color change of DPIP. You will prepare a sample by adding a chloroplast suspension, DPIP, and a buffer to water in a vial called a cuvette. The cuvette is the appropriate size to fit the chamber of the instrument. The instrument works by shining a beam of light of known intensity through the clear cuvette. On the opposite side from the light source is a photoresistor that detects the intensity of light that has passed through the sample. DPIP will absorb some of the light that enters the cuvette. As the light reaction continues and the DPIP is replaced by the colorless DPIPH, less light is absorbed in the cuvette, so the photoresistor detects increasing intensity. Since DPIP absorbs light most strongly at orange-red wavelengths, you will set the spectrophotometer to read the amount of light transmitted in that part of the spectrum (605 nm). If you are using a colorimeter, a data-logger device will set the amount of light transmitted, or, if connected to a computer, the software will set the light transmission. The following procedure is written for use with a spectrophotometer. Your teacher may give you additional instructions specific to the particular instrument that you use.

Pre-laboratory Questions

1. What is the reaction for photosynthesis?
2. What will happen to solutions of DPIP and chloroplast suspension if the chloroplasts are actively carrying out light reactions?

Guided Activity

Materials

- chromatography paper
- tightly capped chromatography jar, with 10 mL solvent
- small stapler or paper clips
- coin
- a fresh spinach (or other) leaf
- spectrophotometer or colorimeter
- 5 cuvettes
- aluminum foil
- heat sink (small aquarium or similar clear tank filled with water)
- lamp
- 4 dropping pipets
- vial of unboiled chloroplast suspension
- vial of boiled chloroplast suspension
- vial of 0.1 M phosphate buffer
- vial of DPIP solution
- distilled water
- lens tissue
- bucket of ice
- 4 squares of Parafilm® (if the cuvettes have no caps)
- labels
- ruler
- calculator
- clock or timer
- test tube rack (if you have round-bottomed cuvettes)

Procedure

Activity A: Leaf Pigment Chromatography

1. Get an 8-cm square of chromatography paper and one fresh spinach (or other) leaf.
2. Make two pencil marks 1.5 cm from one edge of the chromatography paper.

3. Lay the leaf across the chromatography paper between the two pencil marks. Using the marks as a guide, lay a ruler on top of the leaf so that one edge of the ruler is holding the leaf against the paper exactly 1.5 cm from and parallel to the paper's edge.
4. Roll a coin firmly along the ruler's edge, pressing the leaf pigments into the paper. If you do not produce a vivid green stripe on the paper, repeat this step at the same 1.5-cm position on the paper, but repositioning the leaf to ensure that the coin is rolling over fresh leaf tissue. Use a pencil to mark the location of the bottom of the pigment line on the paper (the edge of the green line closer to the paper's edge). This line will indicate the origin after the chromatography solvent has moved the pigment(s) up the paper.
5. Roll the chromatography paper into a cylinder with the line on the outside. Place a staple at the top and at the bottom to just hold the cylinder together, but do not allow any overlap. Stand the tube of chromatography paper in the jar so that the pigment-streaked end of the paper is barely immersed in the solvent but the stripe itself is not touching the liquid. Seal the jar tightly. **Caution:** *Avoid breathing fumes from the solvent.*
6. Tightly cap the jar. Do not disturb the jar for several minutes, but continue to observe the chromatography paper.
7. When the solvent has wicked to about 1 cm from the top of the paper, remove the paper from the jar and immediately mark the location of the solvent front before it evaporates.
8. Mark the bottom of each pigment band.
9. Beginning at the origin line, measure the distance traveled by the solvent front and by each of the pigment bands. Record the results in Table 1. Number the bands so that Band 1 is the pigment band nearest the origin line at the bottom of the paper.

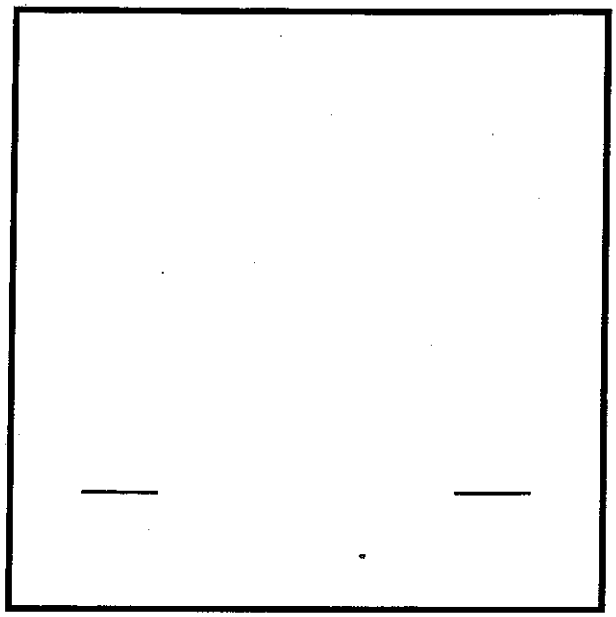


Figure 1. Model of the chromatography paper

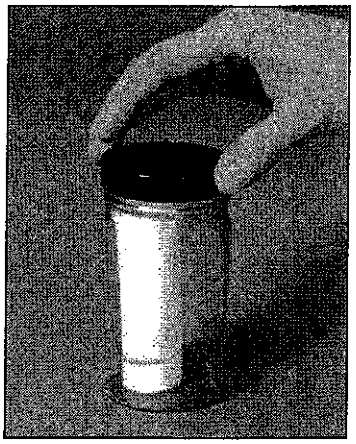


Figure 2. Place the chromatography paper into the jar containing the chromatography solvent and close the jar tightly.

Table 1. Chromatography of Plant Pigments

	Distance from origin (mm)		
Solvent front	68		
Band #		**Band Color/Identification**	**R_F Value**

Analysis

For a given solvent and substrate system (in this case, cellulose), each pigment will move a distance that is proportional to the distance moved by the solvent. This is expressed as the R_F (reference front) value, and it is a constant for the solvent/substrate/pigment. For example, suppose that a pigment moves 75 mm while the solvent moves 100 mm. Then

$$R_F = \text{distance of pigment from origin/distance of solvent front from origin}$$

$$R_F = 75 \text{ mm}/100 \text{ mm} = 0.75$$

1. Calculate R_F values for each of the pigment bands you have identified. Record this data in Table 1.
2. Using the data you have collected, make tentative identifications of the pigment band(s) on your chromatography paper. Record these in the "Band Color/Identification" column of Table 1.

Activity B: The Light Reactions of Photosynthesis

1. Turn on the spectrophotometer. Some models require a warm-up period. If so, wait the required time before proceeding. (You can perform step 3 while the spectrophotometer is warming up.)
2. Once the spectrophotometer has warmed up, set it to read light transmission. Set the wavelength to 605 nm.
3. Set up a work area as shown in Figure 3. The lamp is set up as a light source but it also produces heat. We are interested in the effect of light but not the effect of heat on the light reaction. The aquarium is set up as a heat sink to eliminate the variable of heat in the experiment. The heat (infrared radiation) given off by the lamp can damage the chloroplasts.

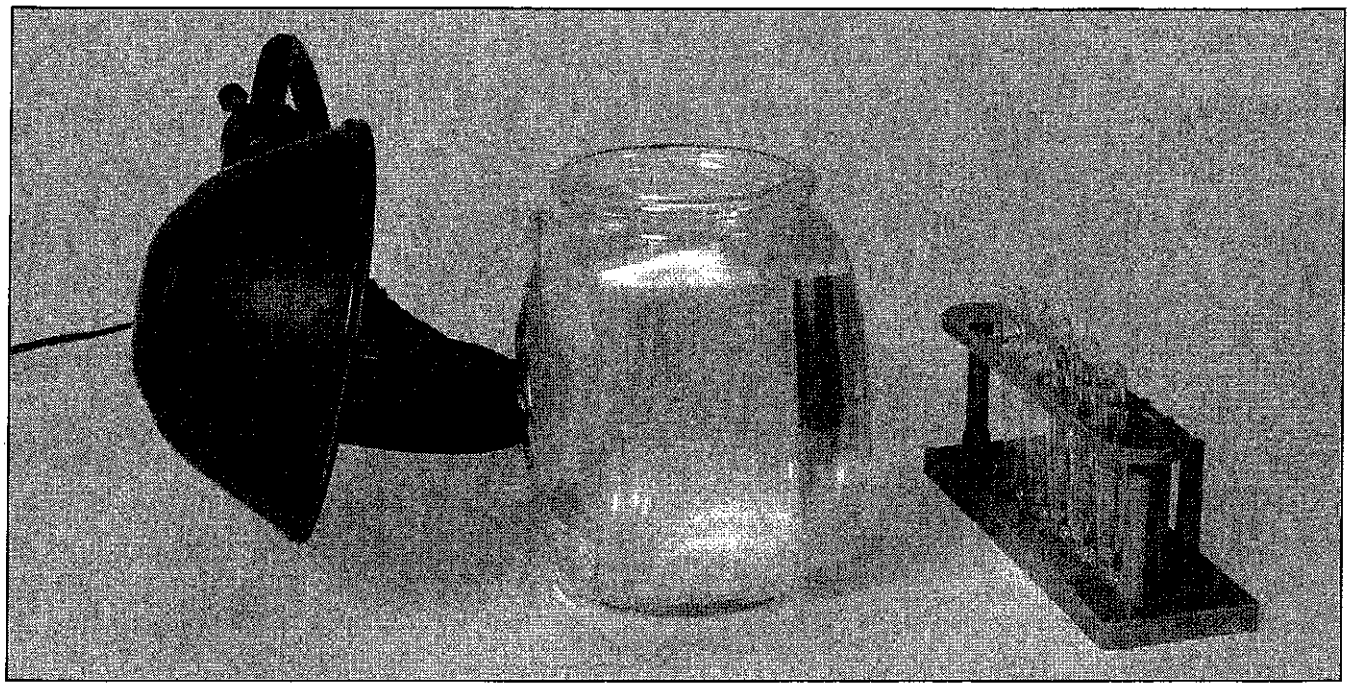


Figure 3. The cuvettes are exposed to a lamp. The aquarium filled with water serves as a heat sink to absorb infrared radiation from the lamp.

4. Number your cuvettes 1–5. If your cuvettes have caps, number those, too. Place the numbers near the tops of the cuvettes—they must not block the light used by your instrument.
5. With a permanent marker, label the pipets: "H_2O," "DPIP," "unboiled," and "boiled."

6. Add 4 mL of distilled water to cuvette 1, and 3 mL each to cuvettes 2–5.
7. Add 3 additional drops of distilled water to cuvette 5.
8. With the same pipet, add 1 mL of phosphate buffer to each cuvette (1–5).
9. Use a different pipet for DPIP. Add 1 mL DPIP to each of cuvettes 2–5. (Cuvette 1 will have no DPIP.)
10. Create an aluminum foil cover for cuvette 2. The cover must prevent light from entering the cuvette.
11. Obtain a vial of unboiled and a vial of boiled chloroplast suspension. Keep these vials on ice throughout the activity.
12. Make sure the cap is secure, and then mix the unboiled chloroplast suspension by inverting the vial several times. With a fresh pipet, add 3 drops of the unboiled chloroplast suspension to cuvette 1. (Dedicate this pipet for use only with unboiled chloroplast suspension.)
13. Follow your teacher's directions for calibrating the spectrophotometer. It should register 100% transmittance for cuvette 1. (At each time interval during the experiment, you will use cuvette 1 to ensure that the spectrophotometer remains calibrated. If it ever registers other than 100% transmittance for cuvette 1, readjust it as needed.) Remember throughout the activity to invert a cuvette to mix its contents before taking its reading.

Table 2: Contents of the Cuvettes

	Samples				
Contents	**1 Blank (Unboiled)**	**2 Unboiled/Dark**	**3 Unboiled/Light**	**4 Boiled/Light**	**5 No chloroplasts**
Phosphate buffer	1 mL	1 mL	1 mL	1 mL	1 mL
Distilled water	4 mL	3 mL	3 mL	3 mL	3 mL plus 3 drops
DPIP	–	1 mL	1 mL	1 mL	1 mL
Unboiled chloroplasts	3 drops	3 drops	3 drops	–	–
Boiled chloroplasts	–	–	–	3 drops	–

14. You will take percentage transmittance readings for each sample at 0, 5, 10, and 15 minutes. The following steps describe the optimum way to accomplish this.
15. Mix the unboiled chloroplast suspension and add 3 drops of the suspension to cuvette 2.
    a. Immediately mix the contents of cuvette 2.
    b. Remove cuvette 2 from its foil cover, insert it into the test chamber, and read its percentage transmittance. Record the results in Table 3 under "0 min."
    c. Return cuvette 2 to its foil cover and place it in the test tube rack, positioned in front of the lamp.
    d. Turn on the lamp (and leave it on throughout the 15 minutes of readings).

16. Mix the unboiled chloroplast suspension and add 3 drops of it to cuvette 3. Read the percent transmittance of cuvette 3, record the results under "0 min," and place it in the rack.
17. Mix the boiled chloroplast suspension and, with the remaining fresh pipet, add 3 drops of it to cuvette 4. Read the percent transmittance of cuvette 4, record the results under "0 min," and place it in the rack.
18. Mix the contents of cuvette 5. Read the percent transmittance of cuvette 5, record the results under "0 min," and place it in the rack.
19. Repeat readings of the cuvettes at 5, 10, and 15 minutes and record the results. (Remember to mix the contents of a cuvette before taking its reading and to place a cuvette in the machine in the same orientation for each reading.)

Table 3. Percent Light Transmittance of Chloroplast Suspensions

	Time			
Cuvette #	**0 min**	**5 min**	**10 min**	**15 min**
1. Unboiled chloroplasts, no DPIP				
2. Unboiled/Dark				
3. Unboiled/Light				
4. Boiled/Light				
5. No chloroplasts				

Analysis

1. Plot the data from Table 3. Title the graph and supply the following information. Label each plotted line.
   a. The independent variable is _____.
   b. The dependent variable is _____.

   Plot the independent variable on the x-axis and the dependent variable on the y-axis.

Laboratory Questions

1. Write a hypothesis that this experiment is designed to test.

2. Which cuvette serves as a control for this experiment? Explain your answer.

3. What variables are tested in this experiment? Describe how each variable is tested and then describe the results of your experiment.

4. Why was DPIP not added to cuvette 1?

5. What was the purpose of adding 3 drops of chloroplast suspension to cuvette 1?

6. Why were 3 drops of water added to cuvette 5?

7. What effect did boiling have on the chloroplast suspension?

Inquiry Activity

On the basis of what you learned in the Guided Activity, develop a question to test about plant pigments or photosynthesis. In developing an experimental question, consider the materials and equipment available to you. Consult your instructor for the availability of additional supplies.

Additional Materials

leaves from other plants (e.g., magnolia, geranium, mulberry)
senescing leaves (changing color)
colored light bulbs or filters
UV lamp
screens (for partial blocking of light)

Procedure

1. In your group, collaborate to come up with a testable question about plant pigments or the light reactions of photosynthesis. If you have trouble, ask your teacher for guidance.
2. Design an experiment to test your question. Consider the following as you frame your experiment:
   - **Question** - What are you testing in your experiment? What are you trying to find out?
   - **Hypothesis** - What do you think will happen? Why do you think so? What do you already know that helps support your hypothesis?
   - **Materials** - What materials, tools, or instruments are you going to use to find the answer to the question?
   - **Procedure** - What are you going to do? How are you going to do it? What are you measuring? How can you make sure the data you collect are accurate? What are the independent and dependent variables in this experiment? What is/are your control(s)? What safety practices do you need to use?
   - **Data Collection** - What data will you record, and how will you collect and present it? Show and explain any data tables and graphs that you plan to use.
3. Have your teacher approve your experimental plan before you begin the experiment.
4. After you perform the experiment, analyze your data:
   - **Data Analysis** - What happened? Did you observe anything that surprised you? Show and explain any tables and graphs that support your data.
   - **Conclusion** - What conclusions can you draw from the results of your experiment? How does this compare with your initial hypothesis? Identify some possible sources of error in your experiment. If given the opportunity, how might you conduct the experiment differently?
5. Be prepared to present the findings of your experiment to the class according to your instructor's specification.

Experimental Design Template

Part A: To be completed and approved before beginning the investigation

What question will you explore? _____

_____

On the basis of your previous laboratory exercise, background knowledge, and research, what is the hypothesis that you will test? _____

_____

_____

What will be the independent and dependent variables? _____

_____

What will be the control group(s)? _____

_____

What equipment and materials will you need (list items and quantity)? _____

_____

_____

What procedure (step-by-step) will you follow? _____

_____

_____

_____

_____

_____

What safety steps will you follow (equipment and procedures)? _____

_____

_____

How will you collect data? _____

_____

How will you analyze data? _____

_____

Teacher approval to begin your investigation: _____

Part B: To be completed during or after your investigation

What changes or modifications have you made to the investigation? _____

Attach any data collection or analysis as instructed by your teacher.

What results did you see in the experiment?

Was the hypothesis accepted or rejected? What conclusions can you draw on the basis of the data and analysis? _____

What sources of error may have existed, and how might the experiment have been conducted differently?

What additional questions arose from the experiment?

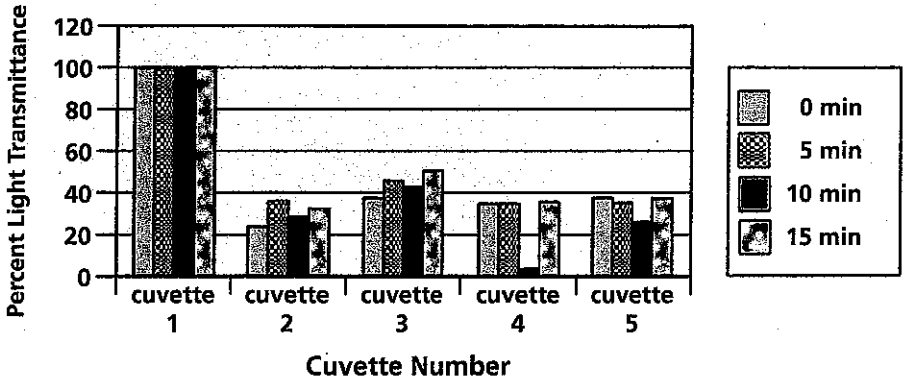
Big Idea Assessments

A class performed an experiment using spectrophotometers and DPIP to determine if both active chloroplasts and light were necessary for the light reactions of photosynthesis. Five samples were tested in the experiment. The percent light transmittance was measured using a spectrophotometer. The results are presented in this table:

Percent Light Transmittance of Chloroplast Suspensions

	Time			
Cuvette #	**0 min**	**5 min**	**10 min**	**15 min**
1. Unboiled chloroplasts, no DPIP	100	100	100	100
2. Unboiled/Dark	25.1	35.4	27.6	32.5
3. Unboiled/Light	36.3	46.7	44.8	48.9
4. Boiled/Light	33.7	34.0	3.2	34.0
5. No chloroplasts	35.0	33.3	27.4	35.3

From the data, one student produced the graph below and concluded that "unboiled chloroplasts exposed to light without the addition of DPIP provide the best conditions for photosynthesis."



a. Analyze the graph and the student's conclusion. Explain why you agree or disagree with the student's reasoning.

b. Construct an appropriate graph from the data in the table.

c. Based on the data presented, describe possible sources of error.