Photosynthesis

The process of photosynthesis involves the use of light energy to convert carbon dioxide and water into sugar, oxygen, and other organic compounds. This process is often summarized by the following reaction:

$$6\ H_2O + 6\ CO_2 + \text{light energy} \longleftrightarrow C_6H_{12}O_6 + 6\ O_2$$

This process is an extremely complex one, occurring in two stages. The first stage, called the *light reactions of photosynthesis*, requires light energy. The products of the light reactions are then used to produce glucose from carbon dioxide and water. Because the reactions in the second stage do not require the direct use of light energy, they are called the *dark reactions of photosynthesis*.

In the light reactions, electrons derived from water are "excited" (raised to higher energy levels) in several steps, called photosystems I and II. In both steps, chlorophyll absorbs light energy that is used to excite the electrons. Normally, these electrons are passed to a cytochrome containing an electron transport chain. In the first photosystem, these electrons are used to generate ATP. In the second photosystem, excited electrons are used to produce the reduced coenzyme nicotinamide adenine dinucleotide phosphate (NADPH). Both ATP and NADPH are then used in the dark reactions to produce glucose.

In this experiment, a blue dye (2,6-dichlorophenol-indophenol, or DPIP) will be used to replace NADPH in the light reactions. When the dye is oxidized, it is blue. When reduced, however, it turns colorless. Since DPIP replaces NADPH in the light reactions, it will turn from blue to colorless when reduced during photosynthesis. This will allow you to monitor the rate of photosynthesis. In order to allow the DPIP to come into contact with chloroplasts, the cells will need to be carefully disrupted. You will test for photosynthetic activity in blended spinach leaves. The intensity of color, measured as absorbance, will be detected by a Colorimeter.

OBJECTIVES

In this experiment, you will

- use a Colorimeter to measure color changes due to photosynthesis.
- study the effect of light on photosynthesis.
- study the effect that the boiling of plant cells has on photosynthesis.
- compare the rates of photosynthesis for plants in different light conditions

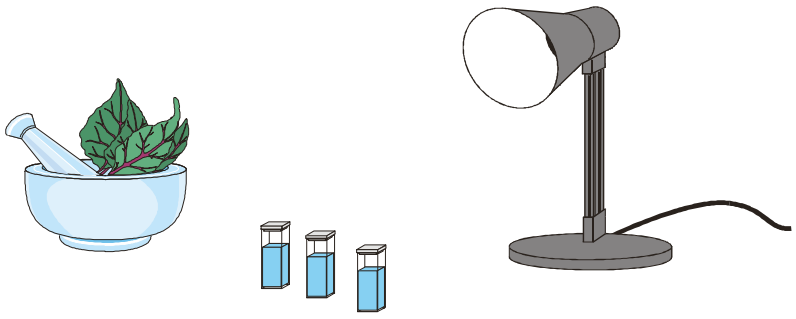


Figure 1

MATERIALS

LabPro or CBL 2 interface
TI Graphing Calculator
DataMate program
Vernier Colorimeter
two cuvettes with lids
aluminum foil covered cuvette with lid
100-watt floodlight
watch or clock with second hand
600-mL beaker
250-mL beaker
two small test tubes
5-mL pipet
two eyedroppers or Beral pipets
10-mL DPIP/phosphate buffer solution
unboiled chloroplast suspension
boiled chloroplast suspension
ice

PROCEDURE

1. Obtain and wear goggles.

2. Obtain two plastic Beral pipets, two cuvettes with lids, and one aluminum foil covered cuvette with a lid. Mark one Beral pipet with a U (unboiled) and one with a B (boiled). Mark the lid for the cuvette with aluminum foil with a D (dark). For the remaining two cuvettes, mark one lid with a U (unboiled) and one with a B (boiled).

3. Plug the Colorimeter into Channel 1 of the LabPro or CBL 2 interface. Use the link cable to connect the TI Graphing Calculator to the interface. Firmly press in the cable ends.

4. Turn on the calculator and start the DATAMATE program. Press CLEAR to reset the program.

5. Prepare a blank by filling an empty cuvette ¾ full with distilled water. Seal the cuvette with a lid. To correctly use a Colorimeter cuvette, remember:

   All cuvettes should be wiped clean and dry on the outside with a tissue.

   Handle cuvettes only by the top edge of the ribbed sides.

   All solutions should be free of bubbles.

   Always position the cuvette with its reference mark facing toward the white reference mark at the right of the cuvette slot on the Colorimeter.

6. Set up the calculator and interface for the Colorimeter.

   a. Place the blank in the cuvette slot of the Colorimeter and close the lid.
   b. If the calculator displays COLORIMETER in CH 1, set the wavelength on the Colorimeter to 635 nm (Red). Then calibrate by pressing the AUTO CAL button on the Colorimeter and proceed directly to Step 7. If the calculator does not display COLORIMETER in CH1, continue with this step to set up your sensor manually.
   c. Select SETUP from the main screen.
   d. Press ENTER to select CH 1.
   e. Select COLORIMETER from the SELECT SENSOR menu.
   f. Select CALIBRATE from the SETUP menu.
   g. Select CALIBRATE NOW from the CALIBRATION menu.

   First Calibration Point

   h. Turn the wavelength knob of the Colorimeter to the 0% T position. When the voltage reading stabilizes, press ENTER. Enter "0" as the percent transmittance.

Second Calibration Point

i. Turn the wavelength knob of the Colorimeter to the Red LED position (635 nm). When the voltage reading stabilizes, press ENTER. Enter "100" as the percent transmittance.
j. Select OK to return to the setup screen.
k. Select OK to return to the main screen.

7. Obtain a 600-mL beaker filled with water and a flood lamp. Arrange the lamp and beaker as shown in Figure 2. The beaker will act as a heat shield, protecting the chloroplasts from warming by the flood lamp. Do not turn the lamp on until Step 11.

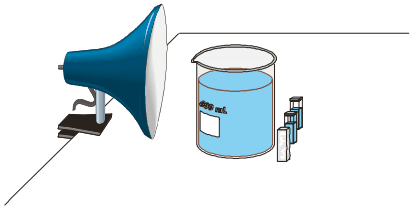


Figure 2

8. Locate the unboiled and boiled chloroplast suspension prepared by your instructor. Before removing any of the chloroplast suspension, gently swirl to resuspend any chloroplast which may have settled out. Using the pipet marked U, draw up ~1-mL of unboiled chloroplast suspension. Using the pipet marked B, draw up ~1-mL of boiled chloroplast suspension. Set both pipettes in the small beaker filled with ice at your lab station to keep the chloroplasts cooled.

9. Add 2.5 mL of DPIP/phosphate buffer solution to each of the cuvettes. **Important**: perform the following steps as quickly as possible and proceed directly to Step 10.
   a. Cuvette U: Add 3 drops of *unboiled* chloroplasts. Place the lid on the cuvette and gently mix; try not to introduce bubbles in the solution. Place the cuvette in front of the lamp as shown in Figure 2. Mark the cuvette's position so that it can always be placed back in the same spot.
   b. Cuvette D: Add 3 drops of *unboiled* chloroplasts. Place the lid on the cuvette and gently mix; try not to introduce bubbles in the solution. Place the foil-covered cuvette in front of the lamp as shown in Figure 2 and mark its position. Make sure that no light can penetrate the cuvette.
   c. Cuvette B: Add 3 drops of *boiled* chloroplasts. Place the lid on the cuvette and gently mix; try not to introduce bubbles in the solution. Place the cuvette in front of the lamp as shown in Figure 2. Mark the cuvette's position so it can always be returned to the same spot.

10. Take absorbance readings for each cuvette. Invert each cuvette two times to resuspend the chloroplast before taking a reading. If any air bubbles form, gently tap on the cuvette lid to knock them loose.
    a. Cuvette U: Place the cuvette in the cuvette slot of the Colorimeter and close the lid. Allow 10 seconds for the readings displayed on the calculator screen to stabilize, then record the absorbance value in Table 1. Remove the cuvette and place it in its original position in front of the lamp.
    b. Cuvette D: Remove the cuvette from the foil sleeve and place it in the cuvette slot of the Colorimeter. Close the Colorimeter lid and wait 10 seconds. Record the absorbance value displayed on the calculator screen in Table 1. Remove the cuvette and place it back into the foil sleeve. Place the cuvette in its original position in front of the lamp.
    c. Cuvette B: Place the cuvette in the cuvette slot of the Colorimeter and close the lid. Allow 10 seconds for the readings displayed on the calculator screen to stabilize, then record the absorbance value in Table 1. Remove the cuvette and place it in its original position in front of the lamp.

11. Turn on the lamp.
12. Repeat Step 10 when 5 minutes have elapsed.
13. Repeat Step 10 when 10 minutes have elapsed.
14. Repeat Step 10 when 15 minutes have elapsed.
15. Repeat Step 10 when 20 minutes have elapsed.
16. When all readings have been taken, select QUIT from the main screen.

DATA

Table 1			
Time (min)	Absorbance unboiled	Absorbance in dark	Absorbance boiled
0			
5			
10			
15			
20			

Table 2	
Chloroplast	Rate of photosynthesis
Unboiled	
Dark	
Boiled	

PROCESSING THE DATA

Calculate the rate of photosynthesis for each of the three cuvettes tested by performing a linear regression on each of the data sets. Enter the data from Table 1 into the calculator using one of the methods described below. Proceed to Step 2 when data have been entered.

TI-73

1. Enter the data from Table 1 into the calculator.
   a. Enter the time values from Table 1 into list L1.
   b. Enter the absorbance values from the *Unboiled* column of Table 1 into L2.
   c. Enter the absorbance values from the *Dark* column of Table 1 into L3.
   d. Enter the absorbance values from the *Boiled* column of Table 1 into L4.
   e. When all data have been entered press, 2nd QUIT.

TI-82/83/83 Plus

1. Enter the data from Table 1 into the calculator.
   a. Enter the time values from Table 1 into list L1.
   b. Enter the absorbance values from the *Unboiled* column of Table 1 into L2.
   c. Enter the absorbance values from the *Dark* column of Table 1 into L3.
   d. Enter the absorbance values from the *Boiled* column of Table 1 into L4.
   e. When all data have been entered press, 2nd QUIT.

TI-86

1. Enter the data from Table 1 into the calculator.
   a. Enter the time values from Table 1 into list L1.
   b. Enter the absorbance values from the *Unboiled* column of Table 1 into L2.
   c. Enter the absorbance values from the *Dark* column of Table 1 into L3.
   d. Enter the absorbance values from the *Boiled* column of Table 1 into L4.
   e. When all data have been entered press, 2nd QUIT.

TI-89/92/92 Plus

1. Enter the data from Table 1 into the Data/Matrix.
   a. First, enter the time values into L1. To do this, press CLEAR 2nd [{] 0 , 5 , 10 , 15 , 20 2nd [}] STO▶ L 1 ENTER. **Note:** Use ALPHA [L] instead of L on a TI-89 calculator.
   b. Enter the absorbance values from the *Unboiled* column of Table 1 (shown here as u1, u2, ...) into L2. To do this, press CLEAR 2nd [{] u1 , u2 , u3 , u4 , u5 2nd [}] STO▶ L 2 ENTER.
   c. Enter the absorbance values from the *Dark* column of Table 1 (shown here as d1, d2, ...) into L3. To do this, press CLEAR 2nd [{] d1 , d2 , d3 , d4 , d5 2nd [}] STO▶ L 3 ENTER.
   d. Enter the absorbance values from the *Boiled* column of Table 1 (shown here as b1, b2, ...) into L4. To do this, press CLEAR 2nd [{] b1 , b2 , b3 , b4 , b5 2nd [}] STO▶ L 4 ENTER.
   e. When all data have been entered, press 2nd QUIT.

All Calculators

2. Start the DATAMATE program.

3. Perform a linear regression to calculate the rate of photosynthetic activity.
   a. Select ANALYZE from the main screen.
   b. Select CURVE FIT from the ANALYZE OPTIONS menu.
   c. Select LINEAR (CH 1 VS TIME) from the CURVE FIT menu.
   d. The linear-regression statistics for these two lists are displayed for the equation in the form:

$$Y=A*X+B$$

   e. Enter the absolute value of the slope, *A*, as the rate of photosynthetic activity in Table 2.
   f. Press ENTER to view a graph of the data and the regression line.
   g. Press ENTER to return to the CURVE FIT menu.

4. Repeat Step 3 to calculate the rates for the Dark and Boiled data.
   a. Select LINEAR (CH 2 VS TIME) for the Dark data.
   b. Select LINEAR (CH 3 VS TIME) for the Boiled data.

QUESTIONS

1. Is there evidence that spinach chloroplasts were able to reduce DPIP in this experiment? Explain.
2. Were chloroplasts able to reduce DPIP while kept in the dark? Explain.
3. Were boiled chloroplasts able to reduce DPIP? Explain.
4. What conclusions can you make about the photosynthetic activity of spinach?

EXTENSION - PLANT PIGMENT CHROMATOGRAPHY

Paper chromatography is a technique used to separate substances in a mixture based on the movement of the different substances up a piece of paper by capillary action. Pigments extracted from plant cells contain a variety of molecules, such as chlorophylls, beta carotene, and xanthophyll, that can be separated using paper chromatography. A small sample of plant pigment placed on chromatography paper travels up the paper due to capillary action. Beta carotene is carried the furthest because it is highly soluble in the solvent and because it forms no hydrogen bonds with the chromatography paper fibers. Xanthophyll contains oxygen and does not travel quite as far with the solvent because it is less soluble than beta carotene and forms some hydrogen bonds with the paper. Chlorophylls are bound more tightly to the paper than the other two, so they travel the shortest distance.

The ratio of the distance moved by a pigment to the distance moved by the solvent is a constant, R_f. Each type of molecule has its own R_f value.

$$R_f = \frac{\text{distance traveled by pigment}}{\text{distance traveled by solvent}}$$

OBJECTIVES

In this experiment, you will

separate plant pigments
calculate the R_f values of the pigments.

MATERIALS

50-mL graduated cylinder
chromatography paper
spinach leaves
coin
goggles
cork stopper
pencil
scissors
solvent
ruler

PROCEDURE

Obtain and wear goggles! ***Caution:*** *The solvent in this experiment is flammable and poisonous. Be sure there are no open flames in the lab during this experiment. Avoid inhaling fumes. Wear goggles at all times. Notify your teacher immediately if an accident occurs.*

1. Obtain a 50-mL graduated cylinder with 5 mL of solvent in the bottom.
2. Cut the chromatography paper so that it is long enough to reach the solvent. Cut one end of the paper into a point.
3. Draw a pencil line 2.0 cm above the pointed end of the paper.
4. Use the coin to extract the pigments from the spinach leaf. Place a small section of the leaf on top of the pencil line. Use the ribbed edge of the coin to push the plant cells into the chromatography paper. Repeat the procedure 10 times making sure to use a different part of the leaf each time.
5. Place the chromatography paper in the cylinder so the pointed end just touches the solvent. Make sure the pigment is not in the solvent.
6. Stopper the cylinder and wait until the solvent is approximately 1 cm from the top of the paper. Remove the chromatography paper and mark the solvent front before it evaporates.
7. Allow the paper to dry. Mark the bottom of each pigment band. Measure the distance each pigment moved from the starting line to the bottom of the pigment band. Record the distance that each of the pigments and the solvent moved, in millimeters.
8. Identify each of the bands and label them on the chromatography paper.

   beta carotene: yellow to yellow orange
   xanthophyll: yellow
   chlorophyll *a*: bright green to blue green
   chlorophyll *b*: yellow green to olive green

9. Staple the chromatogram to the front of your lab sheet.
10. Discard the solvent as directed by your teacher.

DATA

Table 1

Band number	Distance traveled (mm)	Band color	Identity
1			
2			
3			
4			
5*			
Distance solvent front moved = _____ mm			

* The fifth band may not appear.

PROCESSING THE DATA

Calculate the R_f values and record in Table 2.

Table 2	
Molecule	R_f
beta carotene	
xanthophyll	
chlorophyll *a*	
chlorophyll *b*	

QUESTIONS

1. What factors are involved in the separation of the pigments?
2. Would you expect the R_f value to be different with a different solvent?
3. Why do the pigments become separated during the development of the chromatogram?

ADDITIONAL EXTENSIONS

1. Repeat the paper chromatography with various species of plants. What similarities do you see? What differences are there?
2. Use colored filters around the cuvettes to test the effect of red, blue, and green light on the photosynthetic activity of spinach.
3. Vary the distance of the floodlight source to determine the effect of light intensity on photosynthesis.
4. Compare the photosynthetic activity of spinach with that of chloroplasts from other plants.
5. Investigate the effect of temperature on the photosynthetic activity of spinach.
6. Explain why the rate of photosynthesis varies under different environmental conditions.

TEACHER INFORMATION

Photosynthesis

1. It is necessary to suspend the spinach chloroplasts in a 0.5 M sucrose solution. To prepare the 0.5 M sucrose solution, add 171 g of sucrose to distilled water to make a total volume of 1 liter. Store this solution in the refrigerator overnight before preparing the chloroplast solution. Place the blender and beaker in the freezer overnight also.

2. To prepare the phosphate buffer solution:
   a. Add 174 g of K_2HPO_4 (dibasic) to distilled water to make a total volume of 1 liter.
   b. Add 136 g of KH_2PO_4 (monobasic) to distilled water to make a total volume of 1 liter.
   c. Combine the two solutions until the pH is 6.5. About 685 mL of monobasic should be added to 315 mL of dibasic to obtain a solution with a pH of 6.5.

3. To prepare the DPIP (2,6-dichlorophenol-indophenol) solution, add 0.072 g of DPIP to distilled water to make a total volume of 1 liter. Store this solution in a dark bottle and refrigerate.

4. Prepared DPIP and phosphate buffer solution are available from *Carolina Biological Supply Company*. Prepared solutions should be mixed in a 1:1:3 ratio of DPIP solution to phosphate buffer solution to distilled water.

5. Just prior to doing the experiment, prepare the DPIP solution students will use in Step 9 of the student procedure. Combine the DPIP solution with phosphate buffer and distilled water in the following ratio:
   - 100 mL of 0.1 % DPIP solution
   - 100 mL phosphate buffer
   - 300 mL distilled water

6. To prepare a chloroplast suspension,
   a. Place fresh spinach leaves in the light for a few hours. Be sure they remain cool and hydrated.
   b. Cover the blades of a blender with cold 0.5 M sucrose.
   c. Pack the spinach leaves into the blender until they are about three centimeters above the blades.
   d. Blend the spinach with three 10-second bursts. Wait 30 seconds between bursts.
   e. Filter the mixture through cheesecloth into a cold beaker. Keep the beaker on ice. You will need to squeeze the cheesecloth to release as much liquid as possible.
   f. Split the chloroplast suspension into two equal parts. Set one part aside for Item 7 below.
   g. Distribute 2-mL portions into covered, cooled vials. The vials should be light-tight. Black electrical tape works well to cover the vials.
   h. Keep the chloroplast suspensions on ice.

7. Obtain the spinach solution that was set aside in Item 6 and boil it for 5 minutes. Distribute 2-mL portions of boiled chloroplasts into darkened, taped vials. Keep these suspensions on ice.

8. Test the chloroplast suspension prior to use. If the DPIP is reduced too rapidly, further dilute the suspension before distributing it into the vials.

9. The success of this lab is greatly dependent on the ability of the students to synchronize taking absorbance readings. Encourage the students to read over the procedure carefully before beginning.

10. The procedure used to calibrate at 0% and 100% transmittance is similar to that used with most spectrophotometers. The blank cuvette can be in the colorimeter for the 0% calibration (as well as the 100% calibration). This is because the LED is turned off when the wavelength knob is in the 0%T position.

11. The cuvette must be from 55% to 100% full in order to get a valid absorbance reading. If students fill the cuvette 3/4 full, as described in the procedure, they should be in this range.

12. Since there is some variation in the amount of light absorbed by the cuvette if it is rotated 180°, you should use a water-proof marker to make a reference mark on the top edge of one of the clear sides of all cuvettes. Students are reminded in the procedure to align this mark with the reference mark to the right of the cuvette slot on the colorimeter.

13. Prepare the solvent for chromatography by mixing 9 parts (by volume) petroleum ether with 1 part acetone. Distribute 5 mL of solvent to each of the cylinders **Warning:** *Be very careful with the petroleum ether as it is highly flammable*. Extinguish all flames in the room.

    HAZARD ALERT: Petroleum Ether: Flammable liquid; flammable; dangerous fire risk. Hazard code: C—Somewhat hazardous.

    HAZARD ALERT: Acetone: Dangerous fire risk; flammable; slightly toxic by ingestion and inhalation. Hazard code: C—Somewhat hazardous

    The hazard information reference is: Flinn Scientific, Inc., *Chemical & Biological Catalog Reference Manual, 2000*, P.O. Box 219, Batavia, IL 60510. See *Appendix F* of this book, *Biology with Calculators*, for more information.

14. Remember to handle paper strips by the edges only

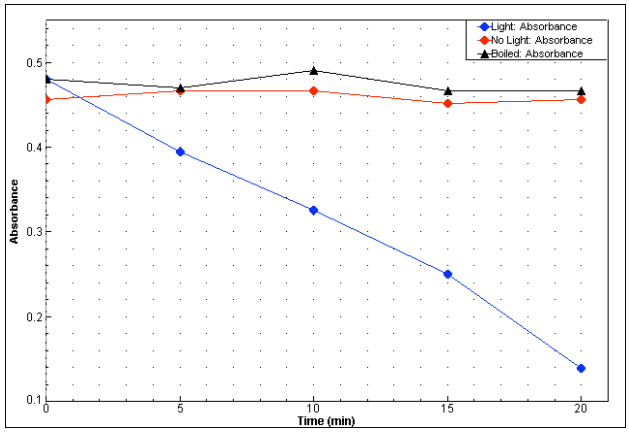
15. Remind students to wear goggles and to keep the tubes stoppered

16. Refer to the MSDS for proper disposal techniques.

SAMPLE RESULTS

Table 1			
Time (min)	Unboiled absorbance	Dark absorbance	Boiled absorbance
0	0.480	0.456	0.480
5	0.394	0.466	0.470
10	0.326	0.466	0.490
15	0.250	0.451	0.466
20	0.139	0.456	0.466

Table 2	
Chloroplast	Rate of photosynthesis
Unboiled	0.0165
Dark	0.0003
Boiled	0.0007



ANSWERS TO QUESTIONS

1. Yes, there is evidence that spinach chloroplasts were able to reduce DPIP in this experiment, as the color of the solution changed when the light intensity changed.

2. No, chloroplasts were not able to reduce DPIP while kept in the dark, as evidenced by the lack of color change of DPIP and the low rate of photosynthesis. The reduced form of DPIP is colorless, and since the solution remained blue, the DPIP did not participate in the light reactions of photosynthesis.

3. Boiled chloroplasts were not able to reduce DPIP, as the heat destroyed the photosynthetic machinery of chloroplasts. The chloroplasts were inactive, even in the presence of light.

4. Based upon student data, chloroplasts should reduce DPIP when exposed to light. Chloroplasts should not be able to reduce DPIP when placed in the dark, nor when destroyed. The amount of reduced DPIP is proportional to the amount of light the chloroplasts are exposed to. The implication is that the amount of sugar produced in photosynthesis depends upon the duration of exposure to light.

EXTENSION SAMPLE RESULTS

Table 1			
Band number	Distance traveled (mm)	Band color	Identity
1	141	faint yellow	carotene
2	60	yellow	xanthrophyll
3	30	bright green	chlorophyll *a*
4	15	olive green	chlorophyll *b*
5*			
Distance solvent front moved = 150 mm			

* band 5 may not appear

Table 2	
Molecule	R_f
beta carotene	.94
xanthophyll	.40
chlorophyll *a*	.20
chlorophyll *b*	.10

ANSWERS TO EXTENSION QUESTIONS

1. The distance that each pigment migrates up the paper is determined by its solubility and its attraction to the fibers in the paper. Pigments with the greatest solubility and least attraction to the fibers in the paper travel the greatest distance up the paper.

2. Since the R_f factor depends on a molecule's ability to dissolve in the solvent, the pigment may not travel as far up the paper.

3. The pigments become separated because they are not equally soluble in the solvent. They are attracted, to different degrees, to the fibers in the paper through the formation of intermolecular bonds, such as hydrogen bonds.