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:

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

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 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.

OBJECTIVES

In this experiment, you will

- Use a Colorimeter or Spectrometer 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.

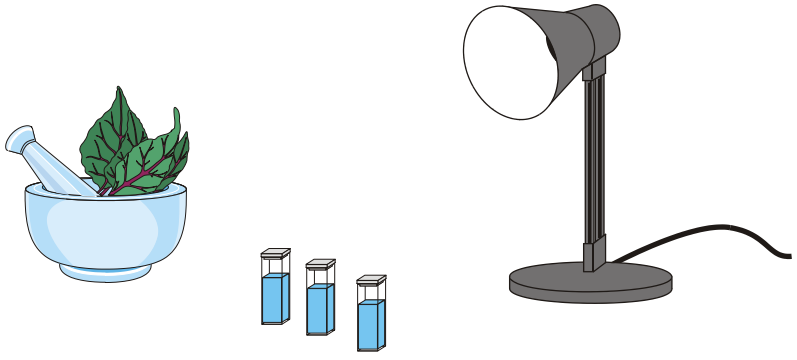

Figure 1

MATERIALS

LabQuest
LabQuest App
Vernier Colorimeter or Spectrometer
four cuvettes with lids
aluminum foil
100 watt floodlight
stopwatch
600 mL beaker
250 mL beaker
distilled water
two small test tubes
5 mL pipet
pipet pump or bulb
two Beral pipets
10 mL DPIP
phosphate buffer solution
unboiled chloroplast suspension
boiled chloroplast suspension
ice

PROCEDURE

Both Colorimeter and Spectrometer Users

1. Obtain and wear goggles.

2. Mark one Beral pipet and one test tube with a U (unboiled). Mark the other Beral pipet and test tube with a B (boiled).

3. Fill the 250 mL beaker with ice and place the test tubes in the beaker.

4. Mark the four cuvette lids with a BL (blank), a U (unboiled), a D (dark), and a B (boiled).

5. Cover all four sides and the bottom of one of the cuvettes with aluminum foil. This will be the Dark cuvette.

6. Use the information in Table 1 to add the phosphate buffer, distilled H_2O, and DPIP to each cuvette. **Important:** Do not add chloroplasts at this time.

Table 1

	BL Blank (no DPIP)	U Unboiled light	D Unboiled dark	B Boiled light
Phosphate Buffer	1 mL	1 mL	1 mL	1 mL
Distilled H_2O	2 mL	1 mL	1 mL	1 mL
DPIP	—	1 mL	1 mL	1 mL
Unboiled chloroplasts	3 drops	3 drops	3 drops	—
Boiled chlorophlasts	—	—	—	3 drops

7. Locate the unboiled and boiled chloroplast suspension prepared by your instructor. Before removing any of the chloroplast suspension, gently swirl to resuspend any chloroplast that may have settled out. Using the pipet marked U, draw up ~1 mL of unboiled chloroplast suspension and dispense into the U test tube. Using the pipet maked B, draw up ~1 mL of boiled chloroplast suspension and dispense into the B test tube. Both test tubes should remain in the ice.

8. Finish preparing the Blank cuvette by adding three drops of unboiled chloroplasts. Place the lid marked with BL on the cuvette and gently invert three times to mix. To correctly use a cuvette, remember:
   - Wipe the outside of each cuvette with a lint-free tissue.
   - Handle cuvettes only by the top edge of the ribbed sides.
   - Dislodge any bubbles by gently tapping the cuvette on a hard surface.
   - Always position the cuvette so the light passes through the clear sides.

Spectrometer Users Only (Colorimeter users proceed to the Colorimeter section)

9. Calibrate the Spectrometer.
   a. Use a USB cable to connect the Spectrometer to your computer. Choose New from the File menu.
   b. Choose Calibrate from the Sensors menu.
   c. When the warm-up period is complete, place the Blank in the Spectrometer. Make sure to align the cuvette so that the clear sides are facing the light source of the Spectrometer.
   d. Tap Finish Calibration, and then select OK.
10. Determine the optimum wavelength for examining the DPIP solution.
   a. Empty the Blank cuvette. Fill it with 1 mL phosphate buffer, 1 mL distilled water, 1 mL DPIP, and 3 drops of unboiled chloroplast. Place it in the spectrometer.
   b. Start data collection. A full spectrum graph of the solution will be displayed. Note that one area of the graph contains a peak absorbance. Stop data collection. The wavelength of maximum absorbance (λ max) is automatically identified.
   c. Tap the Meter tab. On the Meter screen, tap Mode. Change the mode to Events with Entry. Select OK.
   d. Proceed to Step 11.

Colorimeter Users Only

9. Connect the Colorimeter to LabQuest and choose New from the File menu. If you have an older sensor that does not auto-ID, manually set up the sensor.
10. Calibrate the Colorimeter.
   a. Open the Colorimeter lid.
   b. Holding the Blank cuvette by the upper edges, place it in the cuvette slot of the Colorimeter. Close the lid.
   c. Press the < or > button on the Colorimeter to select a wavelength of 635 nm (Red) for this experiment. **Note:** If your Colorimeter has a knob to select the wavelength instead of arrow buttons, ask your instructor for calibration information.
   d. Press the CAL button until the red LED begins to flash, then release. When the LED stops flashing, the calibration is complete. Proceed to Step 11.

Both Colorimeter and Spectrometer Users

11. 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 on the lamp until Step 14.

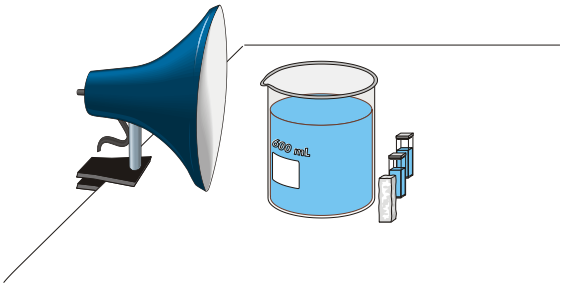

Figure 2

12. Finish preparing the cuvettes. **Important**: Perform the following steps as quickly as possible and proceed directly to Step 13.
    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 that it can be placed back in the same spot.
13. 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 device (close the lid if using a Colorimeter). Allow 10 seconds for the readings displayed in the meter to stabilize. Record the absorbance value in Table 2. 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 device (close the lid if using a Colorimeter). Wait 10 seconds and record the absorbance value in Table 2. 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 device (close the lid if using a Colorimeter). Wait 10 seconds and record the absorbance value in Table 2. Remove the cuvette and place it in its original position in front of the lamp.
14. Turn on the lamp and note the time.
15. Repeat Step 13 after 5, 10, 15, and 20 minutes have elapsed.

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.

1. Prepare LabQuest for data entry.
   a. Disconnect the Colorimeter or Spectrometer and choose New from the File menu to prepare the program for manual entry.
   b. Tap Table to display the data table.
   c. Choose Data Column Options►X from the Table menu.
   d. Enter the Name (Time) and Units (min). Select OK.
   e. Choose Data Column Options►Y from the Table menu.
   f. Enter the Name (Absorb) and leave the Units field blank. Select OK
2. Enter the absorbance and time data pairs from the *Unboiled* column of Table 1.
   a. Tap the first cell in the Time column and enter the **0** for the initial time at which data was taken.
   b. Move to the first cell in the Absorbance column and enter the initial absorbance value from the *Unboiled* column of Table 1.
   c. Continue to enter the data pairs using the same process until you have entered the 5 time and absorbance data pairs for the *Unboiled* chloroplasts.
   d. When the data for the *Unboiled* column have been entered, tap Graph to view a graph of absorbance *vs.* time.
3. Perform a linear regression to calculate the rate of photosynthetic activity.
   a. Choose Curve Fit from the Analyze menu.
   b. Select Linear as the Fit Equation. The linear-regression statistics for these two data columns are displayed for the equation in the form
   $$y = mx + b$$
   where x is concentration, y is absorbance, m is the slope, and b is the y-intercept.
   c. Enter the absolute value of the slope, m, as the rate of photosynthetic activity in Table 2.
   d. Select OK.
4. Tap Table. Choose Clear All Data from the Table menu.
5. Repeat Steps 2–3 of Processing the Data for the *Dark* and *Boiled* data.

DATA

Table 2			
Time (min)	Absorbance unboiled light	Absorbance unboiled dark	Absorbance boiled light
0			
5			
10			
15			
20			

Table 3	
Chloroplast	Rate of photosynthesis
Unboiled	
Dark	
Boiled	

QUESTIONS

1. Is there evidence that chloroplasts were able to reduce DPIP in this experiment? Explain.
2. Were chloroplasts able to reduce DPIP when 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?