Lab 7. Use of DPIP Color Changes to Monitor the Rate of Photosynthesis

Prelab Assignment

Before coming to lab, read carefully the introduction and the procedures for each part of the experiment, then answer the prelab questions at the end of this lab handout. Hand in the prelab assignment just *before* the start of your scheduled lab period.

Goals of this Lab

After completing this lab exercise you should be able to.....

- Use a computer and a colorimeter to measure color changes due to photosynthesis.
- Explain the effect of light on the rate of photosynthesis.
- Explain the effect that the boiling of plant cells has on the rate of photosynthesis.
- Compare and explain the rate of photosynthesis for a chlorophyll extract with a chloroplast extract.
- Compare and explain the rates of photosynthesis for plants in different light conditions.

Introduction

The process of photosynthesis involves the use of light energy to convert carbon dioxide and water into sugar, oxygen, and other organic compounds.

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

Equation 1. The net equation summarizing the overall process of photosynthesis leading to the formation of glucose.

This process is an extremely complex one, occurring in two stages. The first stage, called the ***light dependent reactions*** (or the "light reactions") of photosynthesis, requires light energy. The products of the light dependent reactions are then used to reduce carbon dioxide to produce sugars such as glucose. Because the reactions in the second stage do not require the direct use of light energy, they are called the ***light independent reactions*** or the ***Calvin Cycle***.

In the ***light dependent reactions***, electrons derived from water are "excited" (raised to higher energy levels) in several steps, involving photosystems I and II. Light energy is absorbed by ***Chlorophyll*** molecules in each photosystem to excite electrons. Normally, these electrons are passed to a cytochrome containing ***electron transport chain***. In ***photosystem II***, these electrons are used to generate ***ATP***. In ***photosystem I***, excited electrons are used to produce the reduced coenzyme nicotinamide adenine dinucleotide phosphate (***NADPH***). Both ATP and NADPH are then used in the ***Calvin cycle*** to produce glucose. ATP is used as an energy source to drive the endothermic Calvin cylce, while NADPH is used as a source of hydrogen atoms and energy to reduce carbon dioxide to form sugars such as glucose.

Note: During the light reactions, the processes involving photosystem II occur *before* those involving photosystem I. The numbers are reversed simply because photosystem I was discovered before photosystem II.

$$2NADP^{+}_{(oxidized)} + H_2O + \text{light energy} \rightarrow 2\ NADPH_{(Reduced)} + 2\ H^{+} + O_2$$

Equation 2. The light dependent reactions use light energy to reduce $NADP^{+}$ to form NADPH by adding a pair of electrons and a hydrogen ion that were generated by the spliting of a water molecule. ATP, not shown in the equation above, is also produced in the process. Oxygen is released as a by-product.

In this experiment, a blue dye (2,6-**d**ichloro**p**henol-**i**indo**p**henol, or ***DPIP***) will be used to replace $NADP^{+}$ in the light reactions. When the dye is oxidized, it is blue. When reduced, however, it turns colorless. Since DPIP replaces $NADP^{+}$ in the light reactions, it will turn from blue to colorless when reduced during photosynthesis. This color change will allow you to monitor the rate of photosynthesis.

DPIP $_{(oxidized)}$ + **Water** + **light energy** → **DPIP**$_{(Reduced)}$ + **Oxygen**
(blue) (colorless)

Equation 3. The color change of DPIP from blue to colorless will be used to monitor the rate of photosynthesis

In order to allow the DPIP to come into contact with chloroplasts, the plant cells will need to be carefully disrupted to release the chloroplasts into the buffer solution. Placing spinach leaves along with a buffer solution in a blender to gently break open the cells easily produces a chloroplast suspension. This suspension will then be used to test for photosynthetic activity. The intensity of color, measured as absorbance, will be detected by a computer-interfaced ***colorimeter.*** The amount of ***red light*** (wavelength, λ = ***635 nm***) absorbed by the DPIP - chloroplast suspension indicates the amount of oxidized DPIP present. According to ***Beer's Law***, the concentration of solute (oxidized DPIP in this case) is proportional to the amount of light absorbed by that solute. *The more red light absorbed, the more oxidized DPIP present, and vice versa*.

Beers Law: Absorbance α Solute Concentration

But why use ***red light*** to determine the relative amount of oxidized DPIP present? DPIP in the oxidized state has a blue color because oxidized DPIP molecules *absorb* almost all of the wavelengths of visible light *except* the *blue* wavelengths. Hence, the *blue* wavelengths are *transmitted* and reflected from the solution, thus producing its blue color. Since DPIP in the oxidized state absorbs wavelengths in the red end of the spectrum, the amount of red light (e.g. λ = ***635 nm***) absorbed will, according to Beer's Law, be an indication of the amount of oxidized DPIP present. But as photosynthesis occurs DPIP becomes reduced and the blue color of the solution starts to fade as more and more of the colorless reduced form is generated.

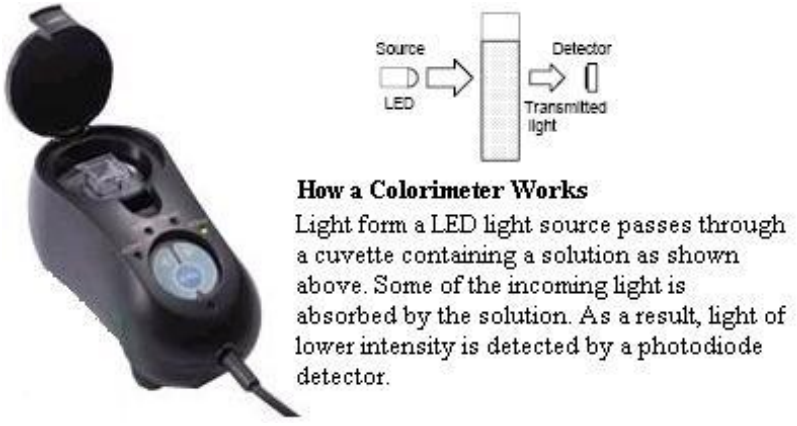

Figure 1. The Vernier colorimeter measures the percentage of light of a specific wavelength that passes through the cuvette (i.e. The % Transmittance is measured). Absorbance, the amount of light absorbed by the cuvette and its contents, is then calculated from the %T by the LoggerPro software by using the relationship: Absorbance = - log (% T / 100).

MATERIALS

Per Group of Two Students:

Goggles
GoLink
Vernier Colorimeter
2-Cuvettes with lids
1-Cuvette covered with foil with lid
One 5-mL pipette
Pipette pump or bulb
2-Beral pipettes
250 mL beaker (filled with ice)
Two small test tubes
600 or 1000 mL beaker (for heat shield)
100-watt floodlight
Watch or clock with second hand

Per Class:

DPIP (2,6-dichlorophenol-indophenol)/buffer solution
Chloroplast Suspensions (Shielded from light)
- Unboiled Chloroplast suspension
- Boiled Chloroplast suspension

Chlorophyll extract (in ethanol)
Aluminum foil
Ice

Procedure (Perform in Groups of two)

Important Note !! To get an overview of the this laboratory activity and to use your lab time efficiently read the following procedure *before* attending lab. If you and your group members are not familiar with the procedure before coming to lab you will have great difficulty completing this exercise during the lab period.

1. Obtain and wear goggles and perform the following procedure in *groups of two*.
2. Obtain two plastic *Beral pipettes*, *three cuvettes with lids*, and enough *aluminum foil* to cover one of the three cuvettes.
   - Mark one Beral pipette with a **U** (for unboiled) and one with a **B** (for boiled).
   - Cover one of the cuvettes with aluminum foil and mark the lid of the cuvette with a **D** (for dark).
   - For the remaining two cuvettes, mark one lid with a **U** (for unboiled) and one with a **B** (for boiled).
3. Connect the Colorimeter to the *GoLink*. Prepare the computer for data collection by opening the file "07 Photosynthesis" from the *Biology with Computers* folder of Logger *Pro*.
4. You are now *almost* ready to calibrate the Colorimeter. Prepare a blank by filling a cuvette 3/4 full with D.I. water. *To correctly use a Colorimeter cuvette, remember*:
   - All cuvettes should be wiped clean and dry on the outside with a ***Kimwipe*** tissue. Do *not* use a paper towel because it will scratch the cuvette resulting in the scattering of light, thus giving incorrect absorption readings.
   - *Handle cuvettes only by the top edge of the ribbed sides*.
   - All solutions should be free of bubbles. Bubbles will scatter light, thus giving incorrect absorption readings
   - Always position the cuvette with its reference mark facing toward the white reference mark at the right of the cuvette slot on the colorimeter.

5. ***Calibrate the Colorimeter***.
   a. Open the Colorimeter lid.
   b. Holding the cuvette with D.I. water by the upper edges, place it in the cuvette slot of the Colorimeter. Close the lid.
   c. Select a wavelength of ***635 nm*** (Red) for this experiment by pressing either < or > on the Colorimeter. Now press the CAL button on the Colorimeter until the red LED begins to flash. Then release the CAL button. When the LED stops flashing, the calibration is complete.
6. Obtain a *100 watt flood lamp* and a *600 or 1000 mL beaker* and fill it with DI water. 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 10*.

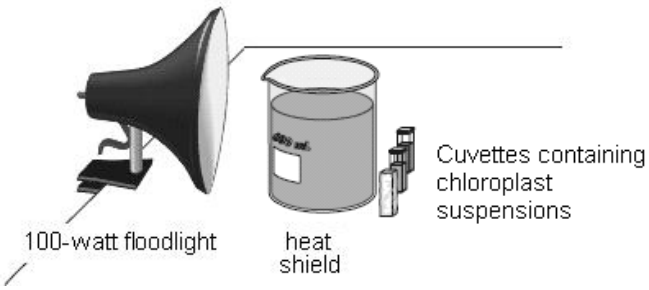

Figure 2. Illumination of a chloroplast suspensions

7. Locate the *unboiled and boiled chloroplast* suspension prepared by your instructor. Before removing any of the chloroplast suspension, gently swirl to resuspend any chloroplasts which may have settled out.
   - Using the Beral *pipette marked U*, draw up ~1 mL of *unboiled* chloroplast suspension.
   - Using the Beral *pipette marked B*, draw up ~1 mL of *boiled* chloroplast suspension.
   - To keep the chloroplasts cool, set each Beral pipette in a test tube and then place each test tube in a 250 mL beaker filled with ice at your lab table.
8. Add 2.5 mL of DPIP/phosphate buffer solution to each of the cuvettes.
   Important: perform steps a – c as quickly as possible and proceed directly to step 9
   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.

9. **Take absorbance readings for each cuvette:** Invert each cuvette two times to resuspend the chloroplasts 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. Allow10 seconds for the readings displayed in the meter 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 in the meter 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 in the meter to stabilize, and then record the absorbance value in Table 1. Remove the cuvette and place it in its original position in front of the lamp.
10. Turn on the lamp.
11. Repeat Step 9 when *5 minutes* have elapsed.
12. Repeat Step 9 when *10 minutes* have elapsed.
13. Repeat Step 9 when *15 minutes* have elapsed.
14. Repeat Step 9 when *20 minutes* have elapsed.

Processing the Data

15. Go to Page 2 of the *experiment file* and enter the data recorded in Table 1 into the appropriate column in the *LoggerPro* table. The graph will update after each data point is entered into the *LoggerPro* table.
16. Calculate the ***rate of photosynthesis*** for each of the three cuvettes tested.
    a. Click the Linear Fit button,  , to perform a linear regression. A dialog box will appear. Select the three data sets you wish to perform a linear regression on and click. OK. A floating box will appear with the formula for a best-fit line for each data set selected.
    b. In Table 1, record the slope of the line, *m*, as the rate of photosynthesis for each data set.
    c. Print a copy of the graph for each member of your team and staple it between pages 7 and 8 of the report pages.
17. Record on the class data sheet the data obtained by your group for the rate of photosynthesis.
18. Before leaving the lab record the *class* data in table 2 of your report sheet.
19. Dispose all DPIP containing solutions in the waste container under the hood and rinse the cuvettes with DI water.

"Middle age is when your age starts to show around your middle."
Bob Hope

"I never think of the future—it comes soon enough."
Albert Einstein

"I'm an excellent housekeeper. Every time I get a divorce, I keep the house."
Zsa Zsa Gabor

"Thanks, you don't look so hot yourself."—after being told he looked cool.
Yogi Berra

"A word to the wise ain't necessary—it's the stupid ones that need the advice."
Bill Cosby

"Two things are infinite: the universe and human stupidity; and I'm not sure about the universe."
Albert Einstein

Lab 7 Report Sheet
Photosynthesis Lab
Biol 201

Your Name:
Group Number:

Date:

Results

Table 1. Absorbance and Rate of Photosynthesis Data obtained by your team.

Time (min.)	**Absorbance**		
	Unboiled Chlorplasts in Light	Unboiled Chlorplasts in Dark	Boiled Chlorplasts in Light
0			
5			
10			
15			
20			
Rate of Photosynthesis (min^{-1})			

Table 2. Class data for the rate of photosynthesis

Team Number	**Rate of photosynthesis** (min^{-1})		
	Unboiled Chlorplasts in Light	Unboiled Chlorplasts in Dark	Boiled Chlorplasts in Light
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
Average Rate of PSN			

(Staple the graph of your team's data as a page between pages 7 and 8)

Analysis

1. Is there evidence that spinach chloroplasts were able to perform photosynthesis in this experiment? *Explain and use specific numerical experimental evidence to support your response*.

2. a. Were chloroplasts able to perform photosynthesis in the dark? *Explain and use specific numerical experimental evidence to support your response*.

   b. Use your knowledge of photosynthesis to explain fully (i.e. discuss the role of light in photosynthesis) *why* the chloroplasts were or were not able to perform photosynthesis in the dark.

3. a. Were boiled chloroplasts able to perform photosynthesis? *Explain and use specific numerical experimental evidence to support your response*.

   b. Use your knowledge of photosynthesis to explain fully *why* the boiled chloroplasts were or were not able to perform photosynthesis.

4. Suppose a *chlorophyll extract* (i.e. a solution that only contained chlorophyll) was used in place of the unboiled chlorplasts. Would the chlorophyll extract when exposed to light to be able to reduce DPIP?

   Yes or *No* *Circle your choice* and then use your knowledge of photosynthesis to explain fully *why* the chlorophyll extract would or would not be able to reduce DPIP.

Summary

5. Based on the data obtained in this lab exercise, summarize the conclusions that can be made about the photosynthetic activity of spinach.

Possible Extensions and Special Projects

Constact your instructor if you wish to pursue any of the following options for independent study outside of class time

1. Use colored filters around the cuvettes to test the effect of red, blue, and green light on the photosynthetic activity of spinach.
2. Determine the effect of light intensity on the photosynthetic activity of spinach.
3. Compare the photosynthetic activity of spinach with that of chloroplasts from other plants.
4. How does temperature affect the photosynthetic activity of spinach?

Lab 7. Prelab Questions **Name:**
Photosynthesis Lab **Group Number:**
Biol 201 **Date:**

Note: Do the prelab reading at the beginning of this lab handout *before* attempting to answer the questions that follow! Hand in this assignment just *before* the start of your scheduled lab period.

1. Explain why DPIP can be used to monitor the rate of photosynthesis.

2. When DPIP is used as the electron acceptor during the light reactions, as photosynthesis proceeds the absorption readings will.... (Circle the correct response)
   a. Increase. b. Decrease. c. Remain constant.

   Briefly explain your reasoning:

3. Suppose an experimenter holds a cuvette on its sides during the experiment. This will result in the absorption readings being..... (Circle the correct response)
   a. Too high. b. Too low. c. Unaffected.
   Briefly explain your reasoning:

4. Suppose an experimenter does not remove air bubbles from a cuvette in this experiment. This will result in the absorption readings being.. (Circle the correct response)
   a. Too high. b. Too low. c. Unaffected.
   Briefly explain your reasoning:

5. Read the procedure for this experiment and develop an "If ..., then ..." hypothesis that predicts how the absorbance will change in the cuvettes labeled U, D and D. Record your hypotheses in the appropriate spaces below. (e.g. If photosynthesis occurs in the cuvette containing the unboiled chloroplasts in the light, then.....)

   a.) Cuvette U: Unboiled Chloroplasts in the Light

   Hypothesis:

   b.) Cuvette D: Unboiled Chloroplasts in the Dark

   Hypothesis:

   c.) Cuvette B: Boiled Chloroplasts in the Light

   Hypothesis: