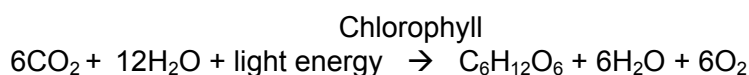


## The Light-Dependent Reactions of Photosynthesis

The process of photosynthesis transforms light energy to chemical energy during the light-dependent reactions. Light energy is used to "excite" electrons of chlorophyll molecules in the reaction centers of photosystems I and II. Some electrons from chlorophyll are used to reduce  $\text{NADP}^+$  to NADPH. Other electrons are passed from chlorophyll to the electron transport system in the thylakoid membranes, ultimately providing energy to synthesize ATP via chemiosmosis. The electron transfers in the light dependent reactions are oxidation-reductions.

The ATP and NADPH produced in the light-dependent reactions provide the energy (and hydrogen) to reduce carbon dioxide and synthesize glucose in the Calvin cycle of photosynthesis. Carbon dioxide from the atmosphere provides the carbon source for photosynthesis and water provides the electrons and hydrogen needed to reduce the carbon dioxide in the formation of carbohydrate molecules.

The overall chemical reaction for photosynthesis is:



In this laboratory exercise you will study the light-dependent reactions of photosynthesis by measuring the rate of oxidation of chlorophyll molecules.

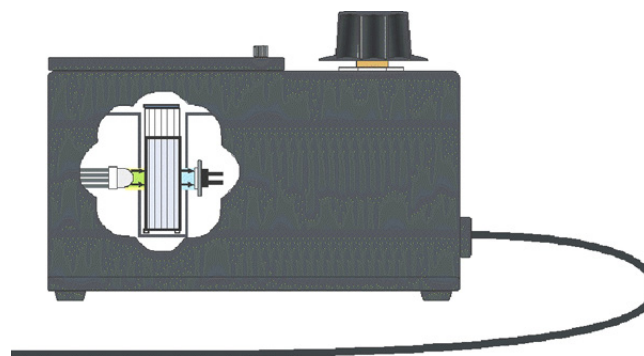
During experimental conditions, the electrons removed from chlorophyll can be transferred to alternative electron acceptors, such as 2,6-dichlorophenol indophenol (DPIP). DPIP in its oxidized state is blue. It loses color as becomes reduced. We can measure the loss of color using a colorimeter. The rate of decolorization provides a means of measuring the light reactions of photosynthesis.

### Materials Needed

Data Recorder (Computer)	Phosphate buffer
Vernier LabPro Box and Power Supply	(for calibrating the colorimeter)
Vernier Colorimeter	DPIP /Phosphate buffer solution
4 cuvettes with lids	Fresh spinach leaf chlorophyll suspension* (kept on ice)
Aluminum foil	Boiled spinach chlorophyll suspension (kept on ice)
100 – 200 watt flood lamp	Ice
Timer with second hand	Thermometer
600 ml beaker	Goggles
250 ml beaker	China marker
2 Small test tubes	
5 ml pipette	
2 pipettes or medicine droppers	

### Procedure

1. Remove the data recorder from the data recorder cubicles. You may have to ask your instructor to unlock the cubicles.
2. Connect the colorimeter to the Vernier LabPro box and plug the Vernier LabPro box into the appropriate data recorder port. Start your data recorder.



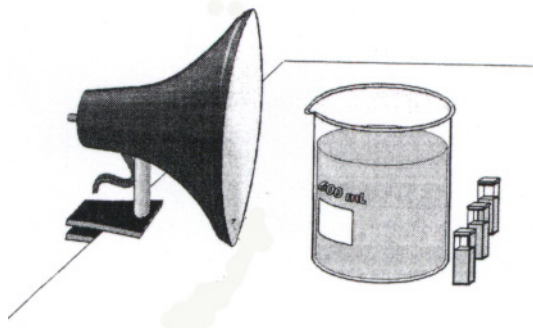
Vernier Colorimeter

3. Open the Biology 211 folder that is located on the computer desktop screen, and open "Exp 07 – Colorimeter" from the *Biology with Computers* experiment files of *Logger Pro*.
4. Be sure that you have a meter window with absorbance readings for the colorimeter. If you do not, ask your instructor for assistance in configuring the computer.
5. Prepare a blank (needed to calibrate the colorimeter) by filling a cuvette 3/4 full with Phosphate buffer solution. Cuvettes must be used properly. To do so:
  - Clean the surface with a kimwipe to ensure that the cuvette is clean and dry.
  - Handle the cuvette only by the top edge of the ribbed sides.
  - Solutions in the cuvette should be free of air bubbles
  - Position the cuvette with its reference mark facing the white reference mark at the right of the cuvette slot on the colorimeter
6. To calibrate your colorimeter, place the blank in the cuvette slot of the colorimeter.

If your colorimeter has an AUTO CAL button, set the wavelength of the colorimeter to 635nm and press the AUTO CAL button.

If your colorimeter does not have an AUTO CAL button, do the following:

- Choose "Calibrate" from the Experiment menu and then click .
  - Turn the wavelength knob on the colorimeter to the "0% T" position.
  - Type "0" in the box.
  - When the displayed voltage for Input 1 stabilizes, click .
  - Turn the knob of the colorimeter to the Red LED position (635nm).
  - Type "100" in the box.
  - When the displayed voltage for Input 1 stabilizes, click , and then click .
7. Position your flood lamp and 600ml water-filled beaker as shown below. The beaker is used as a heat shield in the experiment. Do not turn on the light until you are ready to do the experiment.



8. Obtain about 1ml of fresh chloroplast suspension in a small test tube and 1 ml of boiled chloroplast suspension in a small test tube. Put both test tubes on ice (Use a 250 ml beaker for your ice container.)
9. Label 3 cuvettes 1, 2 and 3. Cover cuvette #2 with aluminum foil.
10. Add 2.5ml of DPIP/Phosphate Buffer to each cuvette
11. This step must be completed as rapidly as possible! You may need to gently swirl the chloroplast suspensions before adding suspension to the cuvettes.
  - Add 3 drops of fresh chloroplast suspension to tube 1. Place the lid on the cuvette and gently mix. Place the cuvette in front of the flood lamp as illustrated above. Mark the cuvette position for future reference. (Use a china marker.)
  - Add 3 drops of fresh chloroplast suspension to tube 2 (the foil-wrapped tube. Place the lid on the cuvette, cover the lid with aluminum foil, and gently mix. Place the cuvette in front of the flood lamp as illustrated above. Mark the cuvette position for future reference. (Use a china marker.)
  - Add 3 drops of boiled chloroplast suspension to tube 3. Place the lid on the cuvette and gently mix. Place the cuvette in front of the flood lamp as illustrated above. Mark the cuvette position for future reference. (Use a china marker.)
12. You are now ready to take absorbance readings. Invert each cuvette 2 times to re-suspend the chloroplasts before taking readings. Be sure there are no air bubbles in the cuvettes. Synchronizing readings is important to the success of this experiment.
  - Place cuvette 1 in the cuvette slot of the colorimeter and close the lid. Wait 10 seconds for the readings to stabilize and then record the absorbance value in Table 1 below. Remove the cuvette and return it to its position in front of the flood lamp.
  - Remove the aluminum foil from cuvette 2 and place cuvette 2 in the cuvette slot of the colorimeter and close the lid. Wait 10 seconds for the readings to stabilize and then record the absorbance value in Table 1 below. Remove the cuvette, rewrap it with aluminum foil and return it to its position in front of the flood lamp.
  - Place cuvette 3 in the cuvette slot of the colorimeter and close the lid. Wait 10 seconds for the readings to stabilize and then record the absorbance value in Table 1 below. Remove the cuvette and return it to its position in front of the flood lamp.
13. Turn on the flood lamp.
14. Repeat Step 12 at 5-minute intervals for 5, 10, 15 and 20 minutes.

Table 1: Absorbance Readings

Time (min)	Absorbance		
	Fresh Chloroplasts	Fresh Chloroplasts in Dark	Boiled Chloroplasts
0			
5			
10			
15			
20			

### Processing the Data



1. Open the file "Exp 07B Data File in the "Exp 07 – Colorimeter" folder located in the Biology 211 folder.
2. Enter your data from Table 1. (The table may use "U" for Tube 1, "D" for tube 2 and "B" for Tube 3.) to input data you must have the cursor in the data cell. To enter data in a table cell you must hold the Ctrl key when you click on the table cell with the cursor. The cell should enlarge and you should see a blinking cursor. Type in your data and click ENTER. The cursor should move automatically down to the next cell. The graph automatically updates with each data entry.
3. Calculate the rate of light reactions for each of the three experimental conditions.
  - Click the Regression button  to perform a linear regression. A dialog box will appear. Select the three data sets for which you wish to perform a linear regression and click , A floating box will appear with the formula for a best fit line for each data set selected.
  - Record the slope of the line,  $m$ , as the rate of light reactions in Table 2 below. Note: You will record the "m" number displayed.
  - Close the linear regression floating box.

Table 2: Rate of Light-Dependent Reactions

Treatment	Reaction Rate
Fresh Chloroplasts	
Fresh Chloroplasts in Dark	
Boiled Chloroplasts	

### Discussion Questions

1. What evidence, if any, did you obtain to indicate that chloroplasts reduced DPIP in any of the experimental conditions?
  
  
  
  
  
  
  
  
  
  
2. What effect did keeping the chloroplast suspension covered with foil have on the rate of light reactions?

