Exercise 4B: Photosynthesis / The Light Reaction

Light is a part of a continuum of radiation or energy waves. Shorter wavelengths of energy have greater amounts of energy. For example, high-energy ultraviolet rays can harm living things. Wavelengths of light within the visible spectrum of light power photosynthesis. when light is absorbed by leaf pigments, electrons within each photosystem are boosted to a higher energy level and this energy level is used to produce ATP and to reduce NADP to NADPH. ATP and NADPH are then used to incorporate CO_2 into organic molecules, a process called carbon fixation.

Design of the Exercise

Photosynthesis may be studied in a number of ways. For this experiment, a dye-reduction technique will be used. The dye-reduction experiment tests the hypothesis that light and chloroplasts are required for the light reactions to occur. In place of the electron accepter, NADP, the compound DPIP (2,6-dichlorophenol-indophenol), will be substituted. When light strikes the chloroplasts, electrons boosted to high energy levels will reduce DPIP. It will change from blue to colorless.

In this experiment, chloroplasts are extracted from spinach leaves and incubated with DPIP in the presence of light. As the DPIP is reduced and becomes colorless, the resultant increase in light transmittance is measured over a period of time using a spectrophotometer. The experimental design matrix is presented in Table 4.3.

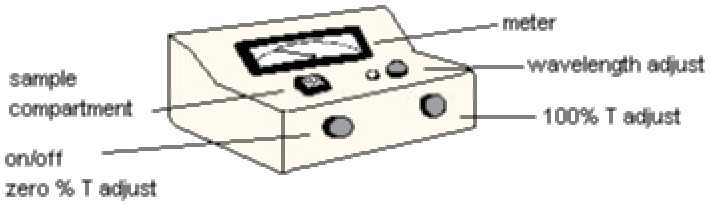

Table 4.3: Photosynthesis Setup

	Cuvettes				
	1 Blank	**2 Unboiled Chloroplasts Dark**	**3 Unboiled Chloroplasts Light**	**4 Boiled Chloroplasts 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 + 3 drops
DPIP		1 ml.	1 ml.	1 ml.	1 ml.
Unboiled Chloroplasts	3 drops	3 drops	3 drops		
Boiled Chloroplasts				3 drops	

Procedure:

1. Turn on the spectrophotometer to warm up the instrument and set the wavelength to 605 nm by adjusting the wavelength control knob.
2. While the spectrophotometer is warming up, your teacher may demonstrate how to prepare a chloroplast suspension from spinach leaves.
3. Set up an incubation area that includes a light, water flask, and test tube rack. The water in the flask acts as a heat sink by absorbing most of the light's infrared radiation while having little effect on the light's visible radiation.
4. Your teacher will provide you with two beakers, one containing unboiled chloroplasts. **Be sure to keep these on ice at all times.**
5. At the top rim, label the cuvettes 1,2,3,4, and 5, respectively. Using lens tissue, wipe the outside walls of each cuvette (Remember: handle cuvettes only near the top). Using foil paper, cover the walls and bottom of cuvette 2. Light should not be permitted inside cuvette 2 because it is a control for this experiment.
6. Refer to Table 4.3 to prepare each cuvette. **Do not add unboiled or boiled chloroplasts yet**. To each cuvette, add 1 ml of phosphate buffer.
7. Bring the spectrophotometer to zero by adjusting the amplifier control knob until the meter reads 0% transmittance. Cover the top of cuvette 1 with Parafilm@ and invert to mix. Insert cuvette 1 into the sample holder and adjust the instrument to 100% transmittance by adjusting the light -control knob. **Cuvette 1 is the blank to be used to recalibrate the instrument between readings.** For each reading, make sure that the cuvettes are inserted into the sample holder so that they face the same way as in the previous reading.
8. Obtain the unboiled chloroplast suspension, stir to mix, and transfer three drops to cuvette 2. **Immediately** cover and mix cuvette 2. Then remove it from the foil sleeve and insert it into the spectrophotometer's sample holder, read the % transmittance, and record it as the time 0 reading in Table 4.4. Replace cuvette 2 into the foil sleeve, and place it into the incubation test tube rack. Turn on the flood light. Take and record additional readings at 5,10,and 15 minutes. Mix the cuvette's contents just prior to each readings. Remember to use cuvette 1 occasionally to check and adjust the spectrophotometer to 100% transmittance.
9. Obtain the unboiled chloroplast suspension, mix, and transfer three drops to cuvette 3. **Immediately** cover and mix cuvette 3. Insert it into the spectrophotometer's sample holder, read the % transmittance, and record it in Table 4.4. Replace cuvette 3 into the incubation test tube rack. Take and record additional readings at 5, 10, and 15 minutes. Mix the cuvette's contents just prior to each readings. Remember to use cuvtte 1 occasionally to check and adjust the spectrophotometer to 100% transmittance.
10. Obtain the boiled chloroplast suspension, mix, and transfer three drops to cuvette 4. **Immediately** cover and mix cuvette 4. Insert it into the spectrophotometer's sample holder, read the % transmittance, and record it in Table 4.4. Replace cuvette 4 into the incubation test tube rack. Take and record additional readings at 5, 10, and 15 minutes. Mix the cuvette's contents just prior to each readings. Remember to use cuvtte 1 occasionally to check and adjust the spectrophotometer to 100% transmittance.
11. Cover and mix the contents of cuvette 5. Insert it into the spectrophotometer's sample holder, read the % transmittance, and record it in Table 4.4. Replace cuvette 5 into the incubation test tube rack. Take and record additional readings at 5, 10, and 15 minutes. Mix the cuvette's contents just prior to each readings. Remember to use cuvtte 1 occasionally to check and adjust the spectrophotometer to 100% transmittance.

Table 4.4: Transmittance of Chloroplast Solution (%)				
	Time (minutes)			
Cuvette	0	5	10	15
Unboiled /Dark				
Unboiled/ Light				
Boiled / Light				
No Chloroplasts				

Table 4.4: Transmittance of Chloroplast Solution (%) Class Data				
	Time (minutes)			
Cuvette	0	5	10	15
Unboiled /Dark	31.3	32.5	35.5	34.8
Unboiled/ Light	32.7	54.5	63.7	65.1
Boiled / Light	32.7	32.9	33.1	32.5
No Chloroplasts	31.3	31.3	31.3	31.3

Analysis of Results

Topics for Discussion

1. What is the purpose of DPIP in this experiment?
2. What molecule found in chloroplasts does DPIP "replace" in this experiment?
3. What is the source of the electrons that will reduce DPIP?
4. What was measured with the spectrophotometer in this experiment?
5. What is the effect of darkness on the reduction of DPIP? Explain.
6. What is the effect of boiling the chloroplasts on the subsequent reduction of DPIP? Explain.
7. What reasons can you give for the difference in the percent transmittance between the live chloroplasts that were incubated in the light and those that were kept in the dark?

DO NOT FORGET DISCUSSION, CONCLUSION, REFLECTION