LAB 08 – The Light Reactions of Photosynthesis

Introduction:

The photosystems found in the chloroplasts of palisade mesophyll leaf cells contain pigments that absorb light. These chloroplasts have two different kinds of pigment systems, Photosystem I and Photosystem II. Photosystem I (PSI) contains a specialized chlorophyll *b* molecule called P700 (its absorption spectrum peaks at 700 nm), and Photosystem II (PSII) contains a specialized chlorophyll *a* molecule called P680 (its absorption spectrum peaks at 680 nm).

When light is absorbed by leaf pigments, electrons within each photosystem are boosted to a higher energy level and the energy is captured in chemical bonds of ATP and NADPH. During the light-dependent reactions, electrons are passed from photosystem II to photosystem I and then, finally, they reduce to NADP to NADPH. The high-energy products, ATP and NADPH, are then used to incorporate CO_2 into organic molecules, a process called carbon fixation.

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-accepting compound, NADP, a compound, DPIP (2,6-dichlorophenol-indophenol), will be substituted so that when a reduction reaction has occurred, the DPIP changes 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 1**.

Procedure:

1. Review the functions and operation of the spectrophotometer with your group. Then turn the Spec-20 on to warm up the instrument and set the wavelength to 605 nm by adjusting the wavelength control knob.
2. While warming up, follow the teacher's instructions in preparing a chloroplast suspension from spinach leaves.
3. Keep chloroplast suspensions on ice.
4. Your instructor will have set up an incubation area that includes a fluorescent "Gro" light and test tube rack.
5. Use the matrix in **Table 1** as a guide. READ THE FOLLOWING PROCEDURE FIRST BEFORE USING THE MATRIX AS A GUIDE.

Table 1: Contents of our Cuvettes

	Cuvettes		
	1 Blank – (no DPIP)	2 Chloroplasts – Light	3 No Chloroplasts – Light
Phosphate Buffer	1 mL	1 mL	1 mL
Distilled H_2O	4 mL	3 mL	3 mL + 3 drops
DPIP	----	1 mL	1 mL
Chloroplast Suspension	3 drops	3 drops	----

6. At the top rim of the cuvettes, place labels numbered 1, 2, and 3 respectively. Using lens tissue, wipe the outside walls of each cuvette. REMEMBER TO HANDLE CUVETTES ONLY NEAR THE TOP! **To each cuvette**, add 1 mL of phosphate buffer. **To cuvette 1**, add 4 mL of distilled water. **To cuvette 2 and 3**, add 3 mL of distilled water. **To cuvette 3** add an additional 3 drops of distilled water. **To cuvettes 2 and 3**, add 1 mL of DPIP. Obtain the chloroplast suspensions, mix, and transfer 3 drops to **cuvette 1**.
7. **Cuvette 1** is the blank. Use this to set the spectrophotometer to 100% transmittance. Remember to occasionally check and adjust the Spec-20 to 100% transmittance throughout the lab with this cuvette.
8. Obtain the chloroplast suspension, mix, and transfer 3 drops to **cuvette 2**. Immediately mix **cuvette 2**, insert into the sample holder, read the % transmittance, and record in **Table 2**. Place **cuvette 2** in the incubation test tube rack. Take and record additional readings every 2 minutes for 16 minutes. Mix the cuvette's contents just prior to each reading.
9. **Cuvette 3** should already be prepared. Mix **cuvette 3**, insert into the sample holder, read the transmittance, and record in **Table 2**. Place **cuvette 3** in the incubation test tube rack next to **cuvette 2**. Take and record additional readings every 2 minutes for 16 minutes. Mix the cuvette's contents just prior to each reading.

Results:

Table 2 – Percent Transmittance at 605 nm

Time (minutes)	1 Blank – (no DPIP)	2 Chloroplasts – Light	3 No Chloroplasts – Light
0	100		
2	100		
4	100		
6	100		
8	100		
10	100		
12	100		
14	100		
16	100		

Questions:

1. What is the function of DPIP in this experiment?

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2. What molecule found in chloroplasts does DPIP "replace" in this experiment? What is the source of the electrons that will reduce DPIP?

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3. What was measured with the spectrophotometer in this experiment?

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4. Identify the function of <u>each</u> of the cuvettes that you used.

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5. Using the grid on the next page (or your own paper), prepare a graphical analysis of the data with the correct labeled axes. Make sure it is properly formatted!