LAB #8: Plant Pigments and Photosynthesis

Objectives:

- To identify light as a requirement for NADP reduction.
- Describe how light intensity can affect photosynthetic rates.
- Form inferences as to how light intensity, light wavelength, and temperature can affect photosynthetic rates.

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 contains a specialized chlorophyll *a* molecule called P700 (its absorption spectrum peask at 700 nm), and photosystem II contains a specialized chlorophyll *a* molecule caleed 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 both boiled and unboiled chloroplast suspensions on ice and in the dark.
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

	Cuvettes				
	1 Blank (no DPIP)	2 Unboiled/ Dark	3 Unboiled/ Light	4 Boiled/ Light	5 no chloroplasts Light
Phosphate Buffer	1 mL	1 mL	1 mL	1 mL	1 mL
Distilled H_2O	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	----

6. At the top rim of the cuvettes, place labels numbered 1, 2, 3, 4, and 5 respectively. Using lens tissue, wipe the outside walls of each cuvette. REMEMBER TO HANDLE CUVETTES ONLY NEAR THE TOP! Cover the walls and bottom of cuvette 2 with foil and make a foil cap cover for the top. Light should not be permitted inside cuvette 2. **To each cuvette**, add 1 mL of phosphate buffer. **To cuvette 1**, add 4 mL of distilled water. **To cuvette 2, 3, 4, and 5**, add 3 mL of distilled water. **To cuvette 5** add an additional 3 drops of distilled water. **To cuvettes 2, 3, 4, and 5**, add 1 mL of DPIP. Obtain the unboiled 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 unboiled chloroplast suspension, mix, and transfer 3 drops into cuvette 2. Immediately mix cuvette 2, remove from the foil sleeve, insert into the spectrophotometer, read and record in **Table 2**. Re-wrap cuvette 2 in foil, turn on the incubation light, and place the cuvette in 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 reading.
9. Obtain the unboiled chloroplast suspension, mix, and transfer 3 drops to cuvette 3. Immediately 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 at 5, 10, and 15 minutes. Mix the cuvette's contents just prior to each reading.
10. Now obtain the boiled chloroplast suspension, mix, and transfer 3 drops to cuvette 4. Immediately mix cuvette 4, insert into the sample holder, read the transmittance, and record in **Table 2**. Place cuvette 4 in the incubation test tube rack next to cuvette 3. Take and record additional readings at 5, 10, and 15 minutes. Mix the cuvette's contents just prior to each reading.
11. Cuvette 5 should already be prepared. Mix cuvette 5, insert into the sample holder, read the transmittance, and record in **Table 2**. Place cuvette 5 in the incubation test tube rack next to cuvette 4. Take and record additional readings at 5, 10, and 15 minutes. Mix the cuvette's contents just prior to each reading.

Results:

Table 2— Transmittance %

Time (minutes)	Cuvettes			
	2 Unboiled/ Dark	3 Unboiled/ Light	4 Boiled/ Light	5 no chloroplasts Light
0				
5				
10				
15				

For Your Lab Report:

The Abstract section of your lab should have (but not be limited to) your hypotheses for each cuvette (not the blank, of course) of this investigation. You may graphically show this…

The Methods section of your lab report will include (but not be limited to):

- A cuvette matrix for all components to complement your written procedure.

The Results section of your lab report will include (but not be limited to):

- Your data table (properly labeled).
- Graph of the spectrophotometer results on one grid.
  - Remember, the **I**ndependent variable is what **I**, the **I**nvestigator is measuring against (x-axis)—in this case, the time of each run. The dependent variable is the results—what you are measuring as a result of your change (y-axis).
  - Also, **DO NOT USE REGRESSION ANALYSIS!** You are plotting % T vs. time, not absorbance!

The Discussion section of your lab report will include the answers to the following questions (among lots of other information—see your Lab Guide):

1. What is the function of DPIP in this experiment?
2. What molecule found in chloroplasts does DPIP "replace" in this experiment? What is the source of the electrons that will reduce DPIP?
3. What was measured with the spectrophotometer in this experiment?
4. What is the effect of darkness on the reduction on DPIP? Explain.
5. What is the effect of boiling the chloroplasts on the subsequent reduction of DPIP? Explain.
6. Identify the function of each of the cuvettes.
7. Why did you not use a regression analysis in your graph and did the old fashioned "connect the dots" time of graphing?