

Biology 3A Lab: Photosynthesis

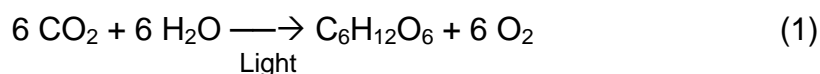
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Objectives

- To observe the spectral absorbance of a mixed chlorophyll sample
- To separate and identify several common plant pigments using paper chromatography
- To investigate the rate of oxygen production during photosynthesis
- To measure the effect of wavelength of light on photosynthetic rate
- To investigate starch production and storage in a single leaf

Introduction

Photosynthesis can be summarized in a fairly simple equation:



This reaction actually occurs in two phases. First, light energy from the sun is captured by the pigment chlorophyll. This first step is often called the light reaction. The next phase does not require light. In this phase carbon (from carbon dioxide) is fixed into glucose. Both of these reactions occur in the chloroplast of plant cells.

In the chloroplast, the chlorophyll is found in the membranes of the grana. When struck by photon of the appropriate wavelength the chlorophyll gives up outer shell electrons to the electron transport chain located in the thylacoid membrane of the chloroplast. The energy in these electrons is used to drive the reaction



by chemiosmosis. In addition high-energy electrons are also transferred to NADP, an electron carrier, to create NADPH. The byproduct of this set of reactions is oxygen gas, released from the leaf stomata into the atmosphere.

The ATP and the NADPH produced in the light reaction are used as energy sources to drive reactions that build macromolecules. In these reactions, called the Calvin Cycle (or dark reactions), carbon dioxide is fixed into organic molecules using the energy from ATP and the hydrogen atoms from NADPH. In most plants, the product of the Calvin Cycle is glyceradlhyde-3-phosphate, which is converted into many compounds including glucose and its polymers, starch and cellulose.

A. Photosynthetic “Photography”

As pointed out above, in the light dependent reactions of photosynthesis, photon energy is absorbed by pigment molecules and transferred to a reactive chlorophyll *a* molecule. In order to illustrate light as a reactant for photosynthesis, you will use geranium leaves that have been kept in the dark for seven to ten days prior to the lab (in order to deplete carbohydrate stores). Photosynthetic rates should vary as a function of light intensity, thus the production and storage of starch should also vary as a function of light. In fact, the rate may vary over the surface of a single leaf, where one part of the leaf is exposed to more light than another! In the macromolecules lab you learned the iodine test for starch. Today we'll apply that method to a whole leaf subjected to different light intensities.

Exposure Procedure:

1. Obtain the following items: a piece of black felt soaked in a 0.1M sodium bicarbonate solution, two pieces of glass, your negative, floral tube with lukewarm water and several rubber bands.
2. Place the NaHCO₃ soaked black felt and place it on the glass slide. Obtain a leaf with petiole (stalk) and place it on the black cloth sandwiching it between the two glass plates making sure the leaf is flat.
3. On the outside of the glass, on the leaf side, place the negative of the photograph. Secure the entire "sandwich" with rubber bands near the edges of the glass plates. Carefully insert the petiole into the floral tube with lukewarm water.
4. Use the slide projector to create bright light to expose the image onto the leaf for at least **1 hour** in the back room.

Development Procedure:

1. After at least one hour, carefully remove the leaf from the glass plates. Place the leaf in boiling 70% alcohol for three to five minutes to leach out the pigments. The treated leaf should be brownish-white in color.
2. Place leaf in boiling water for 3 minutes to rinse.
3. Place the leaf in a Petri dish and cover with iodine. It takes at least 2 – 3 minutes to develop, but could take up to an hour. Turn the leaf over to see the image.

B. Photosynthetic Pigments

Pigments are chemical compounds that reflect specific wavelengths of visible light. This makes them appear "colorful". Leaves, flowers, corals, and even animal skin contain pigments that give them their colors. In addition to reflecting wavelengths, pigments absorb certain wavelengths. This makes pigments are useful to plants and other autotrophs. In plants, algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. Since specific pigments absorb only a narrow range of the spectrum, there is usually a need to produce several pigments, each of a different color, to maximize the capture of solar energy.

Three Basic Groups of Plant Pigments: Chlorophylls, Carotenoids and Phycobilins

Chlorophylls are greenish pigments that are used to "capture" the energy of sunlight. There are several kinds of chlorophyll; the most important is chlorophyll *a*. This is the molecule that passes its energized electrons on to molecules that will manufacture sugars. All plants, algae, and cyanobacteria that photosynthesize contain chlorophyll "a". A second kind is chlorophyll *b* occurs only in "green algae" and in the plants. A third form, chlorophyll *c*, is found only in the dinoflagellates. The differences between the chlorophylls of these major groups were used to study their evolutionary relationships.

Carotenoids are usually red, orange, or yellow pigments. There are two major groupings of carotenoids, carotenes (orange color) and xanthophylls (yellow color). These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer light energy directly to the photosynthetic pathway; they pass their absorbed energy to chlorophyll. For this reason, they are called **accessory pigments**. The familiar β -carotene is found in many plants, and of course is responsible for the orange color of carrots. Of the xanthophylls, lutein and fucoxanthin are the most common. Lutein is

found in all of the large algal forms and fucoxanthin (brown-yellow) is found only in the brown algae (Phaeophyta) and diatoms.

Phycobilins are water-soluble pigments found in the cytoplasm or the stroma of the chloroplast. They occur only in Cyanobacteria and red algae (Rhodophyta). Phycocyanin is a blue pigment, which gives the Cyanobacteria their name ("blue-green algae"). A reddish pigment, phycoerythrin, gives the red algae their common name. The phycobilins are also used as research and medical tools. Both phycocyanin and phycoerythrin fluoresce at specific wavelengths. The light produced by this fluorescence is so distinctive and reliable, that phycobilins may be used as chemical "tags". The pigments are bonded to antibodies, which are then put into a solution of cells. This has found extensive use in cancer research, for "tagging" tumor cells.

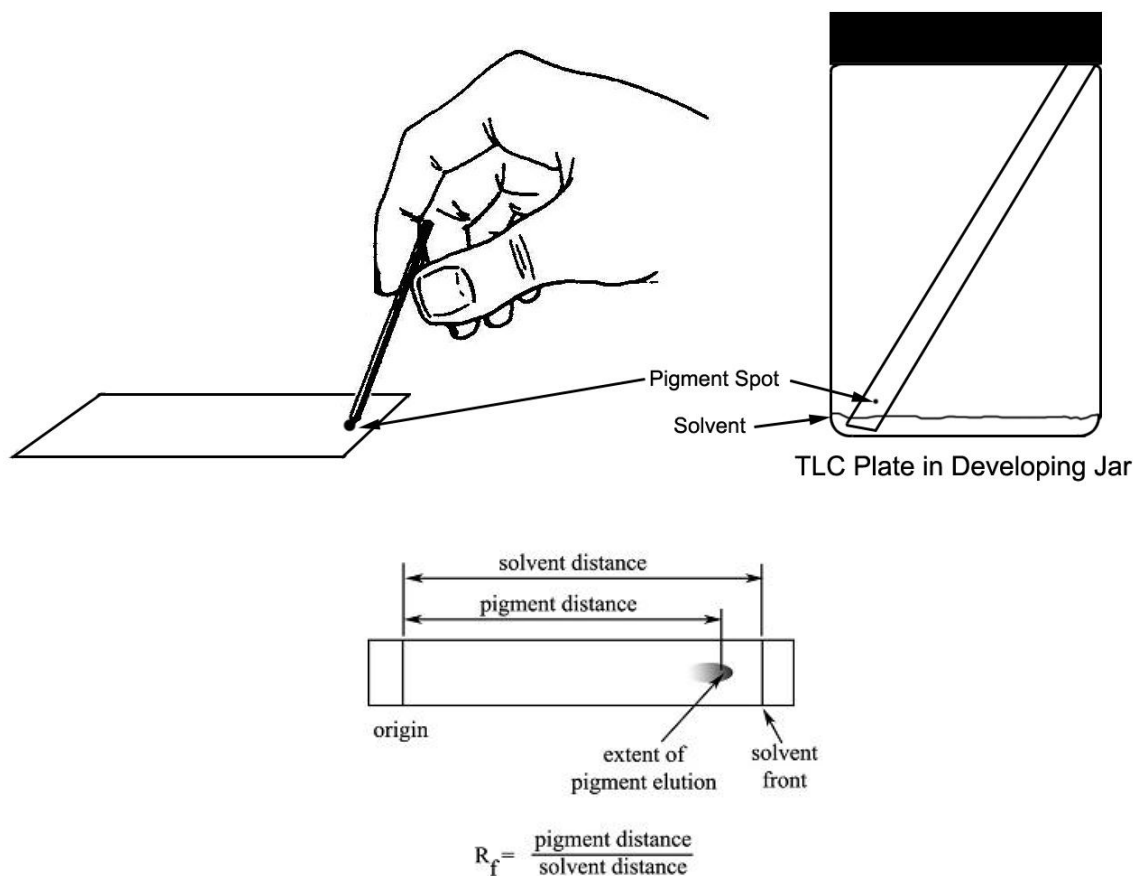


Figure1. Spotting and developing the TLC Plate and Rf calculation

Plant Pigment Procedures

Procedure B1. Pigment Chromatography

Pigment extraction

1. Extract the fluids from several spinach leaves using the juice extractor. Catch the whole juice in a beaker behind the extractor.
2. Add an equal volume of methanol to the extracted juice. Mix this solution well.
3. Add an equal volume of petroleum ether to this solution.

4. Mix well and decant into a separatory funnel.
5. Allow this mixture to rest in the funnel for at least twenty minutes. When the ether layer (on top) has separated and is colored a rich green, decant the methanol layer.
6. Retrieve the ether layer and carefully decant it so that no aqueous methanol remains. The solution should be dark green and crystal clear.
7. Allow the ether to evaporate until about 2 mLs of solution remains. Be careful not to evaporate to dryness.

Separation of Plant Pigments using Thin Layer Chromatography

1. Obtain a TLC plate and place it silica side up on a paper towel.
2. Obtain a developing jar and place about 2 mLs of developing solvent into the jar. Place the lid on the jar.
3. Work near a ventilation duct for the next part of this procedure.
4. Using a capillary tube place a spot of the pigment solution about 1 cm from one end of the plate.
5. Continue to place a spot on top of the original spot every time the last spot has dried.
 - a. The ether will evaporate very quickly, so you will be able to re-spot about every 15 seconds.
 - b. You will need to place about 20-25 spots on top of each other to get a very dense, almost black spot.
 - c. You should work to keep the spot small, no more than 4 - 5 mm in diameter.
 - d. Be sure to allow the spot to dry after each application, or you will get a very large, smeary spot.
6. Place the plate into the developing jar with the spot end at the bottom.
 - a. The end of the plate should be resting in the developing solvent, but the spot should be above the level of the solvent.
 - b. Place the lid on the jar and over the next 20 to 25 minutes the solvent will elute to the top of the plate.
7. Remove the plate just before the solvent reaches the top.
8. Place the plate on a paper towel.
9. Mark the final extent of the solvent by drawing a line (fine) in the silica layer. Allow the plate to dry.

At this point I would recommend photographing the plate. Measure the distance from the origin to the final extent of the solvent front and the distance to the center of each color band. The R_f value for each band is the distance to the center of the band divided by the distance from the origin to the final extent of the solvent. Record all data in Figure 3.

Research your plant's pigments on the internet. Look up known R_f values. Using what you can find, suggest what pigment each of the bands may represent.

B2. Chlorophyll Spectral Analysis

1. In this procedure you will extract the pigment present in spinach and use the spectrophotometer to determine which visible spectrum wavelengths this plant utilizes.
 1. Obtain a large fresh spinach leaf and a 150 ml beaker.
 2. Rip the leaf into quarters and crush with your hand (avoid over handling the leaf)
 3. Place the crushed leaf parts into the beaker and add 30 ml of ethanol (**CAUTION** ethanol is flammable)

4. Place the beaker on a hot plate and heat until the solution acquires a clear green color. DO NOT BOIL. Use caution when handling the hot beaker.
5. When the solution is cool, use it to fill 1/4 of a cuvette tube. Fill the remaining 3/4 of the cuvette with ethanol.
6. Set the spectrophotometer on TRANSMITTANCE at 550 nm.
7. Set the spectrophotometer to 100% Transmittance using a “blank” tube filled with only ethanol.
8. Insert your pigment extract into the spectrophotometer and read the transmittance. The reading should be between 65% and 85%. If it is not, adjust the density using more ethanol or more extract until it falls into this range.
9. You are now ready to record your spectral data. Set the spectrophotometer on ABSORBANCE.
10. Set the wavelength to 400 nm. Using the “blank” tube, set the absorbance to zero. Read the absorbance of the pigment extract. Record the absorbance at this wavelength in Table 1.
11. Change the wavelength to the as indicated in Table 1. You must recalibrate with the blank each time you change the wavelength. Read your pigment extract at each of the indicated wavelengths.
12. Using Excel, graph the absorbance spectrum of the spinach extract.

C. Oxygen production in spinach leaf disks

Leaf tissue is filled with intercellular spaces. Gases (oxygen and carbon dioxide) diffuse through these spaces on their way to and from the chloroplasts. As a result of these gas-filled spaces, leaves float. However, if a leaf is placed in a solution and subjected to a vacuum, the gas will be pulled from these spaces and replaced by the solution, causing the leaf to sink. If the leaf is exposed to light, photosynthesis will produce oxygen, which will fill these spaces and cause the leaf to float again. In this experiment, sodium bicarbonate solution will be used to infiltrate the leaf (because it will provide a carbon dioxide source). The time until the leaf disk floats will be a measure of photosynthetic rate.

Procedure

1. Place approximate 100 ml of 0.2% NaHCO_3 into a 250 ml flask. Fill three Petri dishes approximately 2/3 full with the same solution.
2. Cut 40 to 50 leaf disks from fresh spinach using a cork borer against a layer of paper towels. You can stack several leaves together and cut multiple disks. Make sure the disks are not ragged, however.
3. As they are cut, place the disks into the 250 ml flask.
4. Place the cork assembly into the flask and use the vacuum to sink the disks. Note: You will apply the vacuum, then release while swirling the flask. The disks will continue to float while under the vacuum. They will only sink when the vacuum is released. It may take several cycles to sink the disks. It is acceptable if a few disks remain floating
5. Pour the disks and the solution into an evaporating dish. With forceps gently transfer 10 - 15 disks to each Petri dish. Place the lid onto each of the Petri dishes.
6. Place one dish under a 1000 ml beaker of water with a lamp set above. Place one dish into a dark place, such as a cabinet or drawer. Place one dish on the upper lab bench where it will receive only room light.

After 10 minutes record how many disks are floating (or turned up standing on edge) in each treatment. After 20 minutes record how many disks are floating? Record your data in Table 2.

D. Wavelength and Photosynthetic Rate

Thus far we have investigated the increase in the rate of photosynthesis as a function of light intensity. However, from our spectral analysis it is clear that wavelength of incident radiation will also affect photosynthetic rate. In this experiment we will estimate the photosynthetic rate by measuring the rate of oxygen production of the plant. It should be noted, however, that this is not an exact measure of photosynthetic rate because some of the oxygen produced used by the plant cells is used in the process of aerobic respiration.

Procedures:

GLX Setup

1. Plug the end of the Dissolved Oxygen probe cable into the connector on the top of the PASPORT Dissolved Oxygen Sensor.

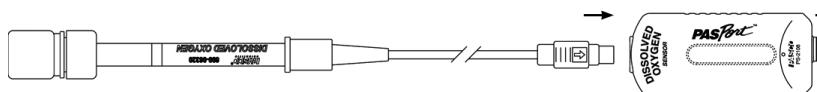


Figure 1. Dissolved O₂ probe & DO sensor

2. Connect the Dissolved Oxygen Sensor into Port 1 on the top of the Xplorer GLX (Figure 2).
3. The Graph Screen will automatically open with Dissolved Oxygen (mg/L) versus Time (s).



Figure 2: Connect sensor to GLX

Equipment Setup


1. NOTE: Student groups in the front of the class will shine the light towards the whiteboard. Students in the back of the class will shine the light towards the back wall (where the microscope cabinet is located). This is to ensure that another groups light sources does not have an effect on your data. NOTE: The overhead room lights also need to be turned off.
2. Place the photosynthesis tank on the magnetic stirrer (Figure 3) and attach the light source to a ring stand.
3. Mix 4 drops of green food coloring with 1200 mL of DI water. Place the green colored water into the outer chamber of the tank. The water helps to maintain the temperature of the inner tank and will only allow a particular wavelength of light to be absorbed by the plant
4. Place the light meter into the inner chamber making sure that light sensor is facing the light source and is not tilted upward or downward. You can wedge some paper towels to make the light sensor stay vertical.
5. Turn on the light and adjust the distance so that the light meter reads 1000 lux.
6. Put a stir bar into the photosynthesis tank that contains 325 mL of 0.1 M NaHCO₃ solution and the 12 cm sprig of *Elodea* plant. Put the large two-hole stopper into the top of the tank.
7. Remove the storage bottle from the end of the Dissolved Oxygen (DO) probe and *carefully* insert the end of the probe through the larger opening in the two-hole stopper.
8. Make sure that the metal band on the Dissolved Oxygen probe is below the surface of the water in the tank and that no air bubbles are trapped on the end of the probe.



Figure 3: Setup

- Put a #3 rubber stopper into the other hole in the two-hole stopper.
- Allow the plant to stabilize for 5 minutes (There is not set distance for each different color of light. The distances are based upon the illuminance in lux. Please see the instructor if you have any questions).

Record Data

- Turn on the magnetic stirrer to a moderate speed so the water circulates in the tank.
- After the 5 minute stabilization, press the Start key  on the GLX and record data for 10 minutes.
- After 10 minutes, turn the lamp off and carefully change the green colored water to red by removing. Yes, you will need to remove the stir bar, *Elodea* and water.
- Mix 7 drops of red food coloring with 1200 mL of DI water. Place the red colored water into the outer chamber of the tank. The water helps to maintain the temperature of the inner tank and will only allow a particular wavelength of light to be absorbed by the plant
- Place the light meter into the inner chamber making sure that light sensor is facing the light source and is not tilted upward or downward.
- Turn on the light and adjust the distance so that the light meter reads 1000 lux.
- Put a stir bar into the photosynthesis tank that contains 325 mL of 0.1 M NaHCO₃ solution and the 12 cm sprig of *Elodea* plant. Put the large two-hole stopper into the top of the tank.
- With the light on, allow the plant to stabilize to the red colored solution for 5 minutes. Record data with the plant in behind for 10 minutes.
- After 10 minutes, turn the lamp off and carefully change the red colored water to blue as before.
- Mix 7 drops of blue food coloring with 1200 mL of DI water and complete the same procedures as #4 above for the blue dyed water.
- After 10 minutes, turn the lamp off and carefully change the blue colored water to DI water. Do not pour out the NaHCO₃ in the inner chamber. Remove the DO probe and use the other #3 stopper.
- Place the light meter into the inner chamber making sure that light sensor is facing the light source and is not tilted upward or downward.
- Turn on the light and adjust the distance so that the light meter reads 1000 lux.
- With the light on, allow the plant to stabilize to the DI water solution for 5 minutes. Record data with the plant in behind for 10 minutes. Turn off the magnetic stirrer.

RECORD YOUR DATA ON THE CLASSROOM COMPUTER

- Collect all class data (O₂ production for green, red, blue and white light). Using Excel, graph the effects of wavelength on photosynthetic rate.

2. Your instructor will provide absorbance spectra for each of the colored water. Create another graph in which you overlay the chlorophyll spectral absorbance data (from Part B2) with the photosynthetic rates at three different wavelengths (from Part D).
3. Clean up the equipment as instructed.
4. Do not let the magnetic stir bars go down the sink!
5. Wash and dry the photosynthesis tank.

A. Photosynthetic Photography

1. Do you see the image in the leaf? Is the image a positive or negative of the original? Explain your answer to this question.

B1. Chromatography of Photosynthetic Pigments

Draw the results of your chromatograms

Species _____

Pigments present				
R_f values				

Figure 3. Results of paper chromatography on spinach leaves

2. How many pigments were present in the spinach sample?

3. How many of the pigments can you identify? Explain how you did this. What is the function of each of these groups of pigments?

B2. Spectral Analysis of Spinach Extract

Complete the following table

Instructor initials _____

Table One. Spectral Absorbance of a Spinach Extract

Wavelength (nm)	Absorbance	Color
400		
420		
440		
460		
480		
500		
520		
540		
560		
580		
600		
620		
640		
660		
680		
700		

4. What colors of light are absorbed by the spinach pigments? _____
5. What colors do living spinach leaves reflect? _____

C. Oxygen production during Photosynthesis.

Table 2. Number and percentage of leaf discs floating after 10 and 20 minutes **Instructor initials** _____

Light Source	No. Floating at 10 minutes	% Floating at 10 minutes	No. Floating at 20 min	% Floating at 20 minutes
Dark				
Room				
Lamp				

6. State a reasonable hypothesis that was tested in this experiment.
7. Does the data collected support this hypothesis?
8. Construct an Excel graph that would be used as figure to support this hypothesis
9. If you repeated this experiment with using different colors of light (other wavelengths), what would you predict as outcomes for blue, green and red light.

D. Wavelength and Photosynthetic Rate

Instructor initials _____

Table 3. Oxygen production of *Elodea* under different wavelengths of light

	Color of Light				
Total O ₂ Production (mg/L)	Green	Red	Blue	White	Dark
Initial O ₂					
Final O ₂					
Change in O ₂					

10. In general, what is the effect of wavelength on the rate of oxygen production? Include this in the figure caption for #11C.
11. Using the whole class data set (available on the web) create an Excel graph that relates light color to oxygen production and wavelength.
- a) First let's express oxygen production as a rate. Divide the **mean** (from all sections) oxygen production for each colored water by the total time for the experiment, 10 minutes. What are the units for this rate?

- b) Now, examine the spectral analyses provided for each of the colored water used in this experiment. For each color choose a major transmitted wavelength range downloaded from the website.
- c) Finally, create an Excel graph in which you show the absorbance spectrum of a typical green plant pigment extract (from exercise B2 above) and the rate of oxygen production vs. wavelength.

Suggestions for 11C above:

- White light and darkness don't fall on this graph, so do not use those data
- Graph the data from B2 using a line graph (Wavelength will be on the x-axis and on one y-axis place the absorbance values)
- Use Oxygen Production in ml/min which you calculated in 11a.
 - On the other y-axis place the mean oxygen production values calculated from 11a.
 - You will need to have these three values at a particular wavelength associated with the wavelengths from B2 in order to be able to graph them correctly. Please take notes on how to do this.
 - You will fit the three bars to the oxygen production