

PLANT PIGMENTS AND PHOTOSYNTHESIS



Westminster College

STANDARDS

- 3.2.10A, 3.2.10B, 3.2.12A, 3.2.12B
- 3.3.10.B, 3.3.12B

INTRODUCTION

Isolation of Plant Pigments

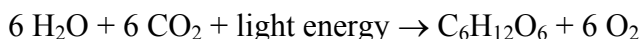
Paper chromatography is a technique that can be used to separate a complex mixture of molecules. In green plants, there is a mixture of four basic pigments found in their leaves. These pigments are shown in Table 1.

Pigment	Color	Function
Carotene	Red, orange, yellow	Capture light energy and transfer it to chlorophyll a; protect photosynthetic system from UV-light damage
Xanthophyll	Yellow	Capture light energy and transfer it to chlorophyll a
Chlorophyll a	Bright green to blue-green	Primary photosynthetic pigment
Chlorophyll b	Yellow to olive-green	Capture light energy and transfer it to chlorophyll a

The chromatography solvent used in this experiment is a mixture of acetone and petroleum. The solubility of each pigment, plus its affinity for the cellulose paper will determine how quickly certain pigments migrate with the solvent as it is drawn up the chromatography paper.

Photosynthesis (The Light Reaction)

The process of photosynthesis involves the use of light energy to convert carbon dioxide and water into sugar, oxygen, and other organic compounds. This process is often summarized by the following reaction:



This process is an extremely complex one, occurring in two stages. The first stage, called the *light reactions of photosynthesis*, requires light energy. The products of the light reactions are then used to produce glucose from carbon dioxide and water. Because the reactions in the second stage do not require the direct use of light energy, they are called the *dark reactions of photosynthesis*.

In the light reactions, electrons derived from water are “excited” (raised to higher energy levels) in several steps, called photosystems I and II. In both steps, chlorophyll absorbs light energy that is used to excite the electrons. Normally, these electrons are passed to a cytochrome containing an electron transport chain. In the first photosystem, these electrons are used to generate ATP. In the second photosystem, excited electrons are used

to produce the reduced coenzyme nicotinamide adenine dinucleotide phosphate (NADPH). Both ATP and NADPH are then used in the dark reactions to produce glucose.

In this experiment, a blue dye (2,6-dichlorophenol-indophenol, or DPIP) will be used to replace NADPH in the light reactions. When the dye is oxidized, it is blue. When reduced, however, it turns colorless. Since DPIP replaces NADPH in the light reactions, it will turn from blue to colorless when reduced during photosynthesis.

GUIDING QUESTIONS:

- What are the different plant pigments found in leaves and how do you separate them?
- What is the effect of light on the photosynthetic process?
- What environmental conditions can affect the rate of photosynthesis?
- What photosystem is being used in this experiment?
- What organic molecule does the DPIP replace?

MATERIALS

Chromatography of Plant Pigments

Chromatography vial with 1 mL solvent	Chromatography paper strip
Fresh spinach leaf	Quarter or dime
Pencil	Scissors
Forceps	Metric ruler

Photosynthesis/The Light Reaction

Vernier spectrometer	Disposable pipets
Vernier LabQuest with power supply	1 L square bottle
Graphing program or graph paper	10 mL DPIP/phosphate solution
5 disposable cuvettes	Unboiled chloroplast suspension
Aluminum foil	Boiled chloroplast solution
100 W light source	Timer or clock with second hand
ice	100-1000 μ L pipettor & tips
2-20 μ L pipettor & tips	Parafilm™
Permanent markers	Tape

PROCEDURES

I. Chromatography of Plant Pigments

1. Obtain a chromatography vial from your teacher and label it with your initials using tape and a permanent marker. There should be 1 mL of chromatography solvent in the bottom of the vial. Keep the vial closed until you are ready to perform the experiment to ensure that the atmosphere within the vial is saturated with solvent vapors (equilibration).


2. Obtain a chromatography strip from your instructor. Handle the chromatography strip by the edges only, as the oils from your fingers can interfere with the chromatogram.
3. Measure 1.5 cm from one end of the chromatography strip and draw a pencil line across the width of the strip.
4. Use a pair of scissors to cut two small pieces below the pencil line to form a pointed end (Fig. 1). The pointed end is the bottom end of the chromatogram.
5. Obtain a fresh piece of spinach and place it over the line on the chromatography strip. Rub the ribbed edge of a coin (dime or quarter) over the spinach leaf to extract the pigments. Repeat 5 to 10 times with different portions of the spinach leaf, making sure you are rubbing the coin over the pencil line (Fig. 2).
6. Remove the cap from your chromatography chamber. Using forceps, carefully place the chromatography strip into the vial so that the pointed end is barely immersed in the solvent (Fig. 3). **Do not immerse the pigments in the solvent!**
7. Cap the vial and leave it undisturbed. Watch as the solvent is drawn up the chromatography strip by capillary action. It is possible to observe the different plant pigments separating along the strip, as each one has a distinct color.
8. When the solvent front is approximately one centimeter from the top of the chromatography strip, remove the cap from the vial. Using forceps, remove the strip from the vial. Immediately mark the location of the solvent front, as the solvent evaporates quickly.
9. Place the cap back on the vial and set it aside. **Do not pour the solvent down the sink!** The solvent is an organic chemical which needs to be disposed of properly by your instructor.
10. The strip containing the separated pigments is a chromatogram. In the Chromatography of Plant Pigments (Table 1), list the pigment colors observed. There may be 3 or 4 pigment bands visible. Once the strip dries, mark the bottom of each pigment band on the chromatography strip.
11. Using a metric ruler, measure the distance from the original pencil line with the spinach extract to the solvent front and record this, in millimeters, in the Chromatography of Plant Pigments (Table 1). Then record in millimeters each mark you have made for the different pigment bands (Fig. 4).
12. Calculate the R_f value for each pigment on your chromatogram using the following formula:

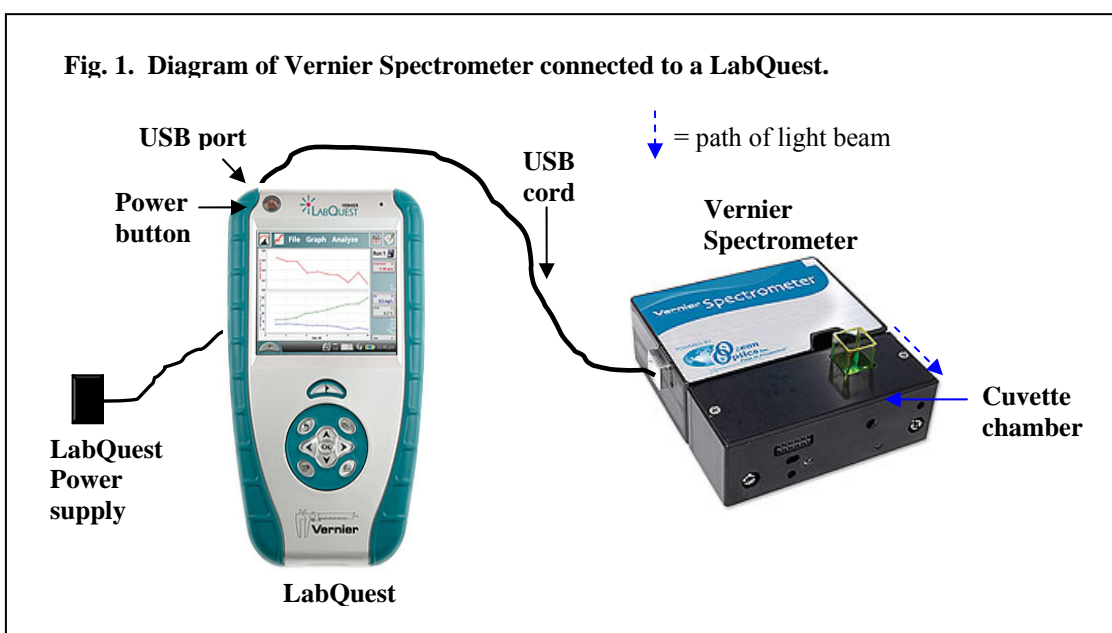
$$R_f = \frac{\text{Distance pigment traveled}}{\text{Distance solvent traveled}}$$


13. Follow your teacher's instructions for the proper disposal of all materials.

II. Photosynthesis/The Light Reaction

LabQuest and Spectrometer Set up

1. Use Figure 1 below to connect the spectrometer to the LabQuest.
2. Once the LabQuest and spectrometer are attached, turn on the LabQuest by pushing the power button at the top left corner. During the warm up, the LabQuest logo will appear on the screen. This will be replaced by the meter mode  which will display "USB:Abs" (Fig. 2A) if it is recognizing the Vernier spectrometer. Check to see that the spectrometer light is on before proceeding.



3. To the right of the screen, tap the gray box that says Mode. A Data Collection screen displaying experimental options will appear (Fig. 2B). At the top of the screen, select the Mode box drop-down menu. Select "Events with Entry" from this list. The Data Collection keyboard screen will appear (Fig 2C). You do not need to fill in any of the other boxes (Number of Columns or Volume/mL). Select when you are finished.
4. This experiment must be performed at a specific wavelength, 605 nm. While still in the meter mode , tap on the red USB: Abs box. A small white box will appear; select "Change Wavelength...". Use the keypad to type in 605 (in nm) as the "Selected Wavelength". The LabQuest may reset to a default of 604 nm; this difference will not affect your results. Select to set this wavelength.
5. This experiment is conducted using % Transmittance. Tap on the red USB: Abs box again and select "Change Units". Choose % Transmittance from the list. The red box should reflect the

wavelength and units changes by displaying “USB: Trans @ 605 nm”. At this point, the LabQuest screen may be reading a large and unusual number. This will be corrected when the spectrometer is calibrated with the blank for the experiment.

6. Let the spectrometer warm up for at least 3 min. While the spectrometer is warming up, prepare the chloroplast samples to be analyzed.

Experimental Set-Up

7. Set up an incubation area (Fig. 3) with a floodlight, a container of water for a heat shield and a cuvette holder or similar sample holder. If an appropriate sample holder is not available, place small pieces of tape on the bench top, numbered 2-5. This will provide consistency in the amount of light each sample receives over the course of the experiment. Do not turn on the light source at this time.

8. Label five cuvettes 1-5 using a permanent marking pen. Each number corresponds to a sample listed in Table 1. **Wrap cuvette #2 with tinfoil and make a tinfoil cap for the cuvette BEFORE filling this cuvette with reagents.** Be sure that the tinfoil covering is loose enough to slide on and off easily for spectrometer. This sample reaction will occur in the dark.

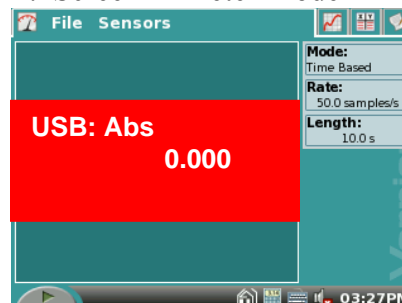
9. Prepare cuvettes 1-5 by adding the reagents listed in Table 1. **Add all the reagents EXCEPT the chloroplasts!** These will be added to cuvette 2-5 AFTER the calibration of the spectrometer (see section below), immediately before initiating the light reaction.

10. Obtain two microcentrifuge tubes containing prepared chloroplasts from your instructor. One is labeled “U” for unboiled, the other “B” for boiled.

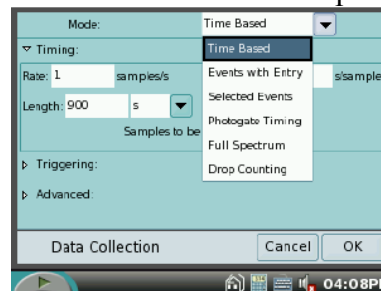
11. Add 20 μL of the unboiled chloroplasts to cuvette #1 (blank for calibration), cap it with Parafilm™ and invert it several times to mix the contents thoroughly. Proceed directly to the calibration of the spectrometer in the next step. **Do not add chloroplasts to cuvettes 2-5!**

Figure 2. LabQuest Screen Displays

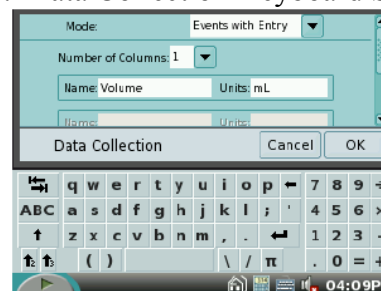
A. Screen in Meter Mode



B. Data Collection Set-Up Screen



C. Data Collection Keyboard Screen

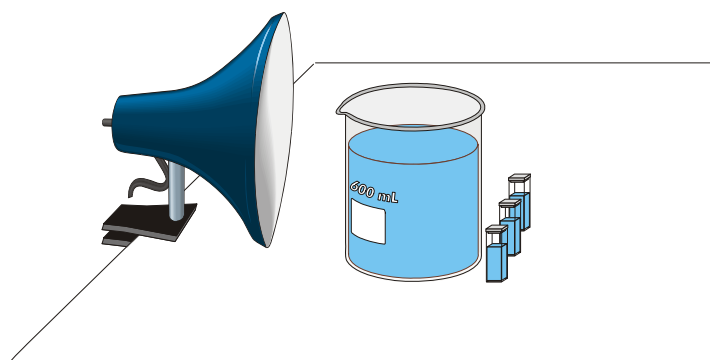


Calibration of Spectrometer and Data Collection

12. To calibrate the spectrometer, tap on the red “USB: Trans @ 605” box and select Calibrate. The next screen will give you a 60 second count down which is an important part of the spectrometer warm up. **Do not skip this step.**

13. When the warm up is finished, place cuvette #1, containing the chloroplasts, phosphate buffer and water, into the chamber. Have the side of the cuvette with the ▼ face forward; this orients the cuvette so that it is in the path of the light beam.

Figure 3. Experimental Set-up for Photosynthesis Reaction



14. Select “Finish Calibration”. If the calibration is successful, a % Transmittance of ~100% should be displayed directly over the data collection columns.

15. Now prepare cuvettes 2-5. Perform the following steps as quickly as possible. Add 20 µL of the correct chloroplast solution (**Table 1**) to cuvettes 2-5 containing reaction buffers. Cover the top of the cuvettes with Parafilm™ and invert them several time to mix the contents.

Tube #	1	2	3	4	5
Tube Contents	Blank	Unboiled Chloroplasts	Unboiled Chloroplasts	Boiled Chloroplasts	No Chloroplasts
Light/Dark	---	Dark	Light	Light	Light
Buffer (µL)	200	200	200	200	200
Water (µL)	800	600	600	600	620
DPIP (µL)	---	200	200	200	200
Unboiled Chloroplasts (µL)	20	20	20	---	---
Boiled Chloroplasts (µL)	---	---	---	20	---

16. Remove the tinfoil from cuvette #2 and place it into the spectrophotometer to obtain the 0 minute time point. Record the % transmittance under Time 0 for cuvette 2 (% Transmittance Table on the Data Sheet). Remove the cuvette, replace the tinfoil, and place it in the appropriate spot on the cuvette holder (or numbered piece of tape) at the incubation area. Repeat this procedure for cuvettes 3-5 (minus the tinfoil). Remember to note the time, as you will have to take subsequent readings of all the samples.

17. **Turn on the light source.** Place cuvettes 2-5 in front of the light source and heat shield (Fig. 3). Take % transmittance readings of samples 2-5 at 5 minute intervals for a total of 15 minutes.

18. Record the data for each time point in the % Transmittance Table provided in the Data and Analysis section.

REFERENCES

Carolina Laboratories Student Guide. AP Biology Laboratory 4: Plant Pigments and Photosynthesis. (2006). Carolina Biological Supply Company. Burlington, NC.

The College Board Advanced Placement Program. Student Manual Lab 4: Plant Pigments and Photosynthesis. (2001). pp. 45-53.

Pearson Lab Bench Activity: Plant Pigments and Photosynthesis
http://www.phschool.com/science/biology_place/labbench/lab4/intro.html

Kelly Redding and David Masterman. Advanced Biology with Vernier. (2008) Plant Pigment Chromatography (4A). Vernier Software & Technology; 13979 S.W. Millikan Way, Beaverton, OR pp. 4A-1 to 4A-2T.

Kelly Redding and David Masterman. Advanced Biology with Vernier. (2008) Photosynthesis (4B). Vernier Software & Technology; 13979 S.W. Millikan Way, Beaverton, OR pp. 4B-1 to 4B-3T.

Vernier LabQuest™ Reference Guide, Version 1.1; Vernier Software & Technology; 13979 S.W. Millikan Way, Beaverton, OR; Revised April 29, 2008.

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Name: _____

Date: _____

DATA AND ANALYSIS

CHROMATOGRAPHY OF PLANT PIGMENTS

Band Number	Pigment	Band Color	Migration Distance (mm)	R _f Value
1 (top)	Solvent front	---		
2	Carotene	Yellow to yellow-orange		
3	Xanthophyll	yellow		
4	Chlorophyll a	Bright green to blue green		
5 (bottom)	Chlorophyll b	Yellow green to olive		

QUESTIONS

1. What factors are involved in the separation of the pigments?
2. Which pigment migrated the farthest? Why?
3. Would you expect the R_f value of a pigment to be the same if a different solvent were used? Explain.
4. During summer, leaves are generally bright green. What would you hypothesize that this indicates about the role of different light wavelengths, chlorophyll and the photosynthetic process?

% TRANSMITTANCE AT DIFFERENT TIME POINTS

Cuvette	0 min	5 min	10 min	15 min
2 Unboiled/Dark				
3 Unboiled/Light				
4 Boiled/Light				
5 No Chlrplsts/ Light				

ANALYSIS OF RESULTS

1. Use a graphing program or graph paper to plot the % transmittance data for each of the four samples. Be sure to label the X-axis and Y-axis with the appropriate units.
2. If possible, use a linear portion of the curve to determine the rate of photosynthesis.
3. In this experiment, what is a) the dependent variable, b) the independent variable?
 - a)
 - b)

QUESTIONS

1. What molecule found in chloroplasts does DPIP "replace" in this experiment? Why does the color of the DPIP solution change?
2. What is the evidence that chloroplasts were able to reduce DPIP in this experiment? Under what experimental conditions did this occur? Explain.

