Plant Pigments and Photosynthesis

Introduction
In photosynthesis, plant cells convert light energy into chemical energy that is stored in sugars and other organic compounds. Critical to the process is chlorophyll, the primary photosynthetic pigment in chloroplasts.

Exercise A: Plant Pigment Chromatography
Paper chromatography is a technique used to separate a pigment mixture into its component molecules. The pigments are dissolved in a solvent that carries them up the paper. The molecules migrate up the paper due to capillary action, which in turn occurs as a result of the attraction of solvent molecules to the paper and attraction of solvent molecules to one another. The rate of migration occurs at different rates because of differences in solubility of pigment molecules in the solvent, the molecular mass of solvent molecules, and hydrogen bonding of solvent molecules with the paper. In the **chromatogram** shown in Figure A1, the solvent for the ink mixture is water.

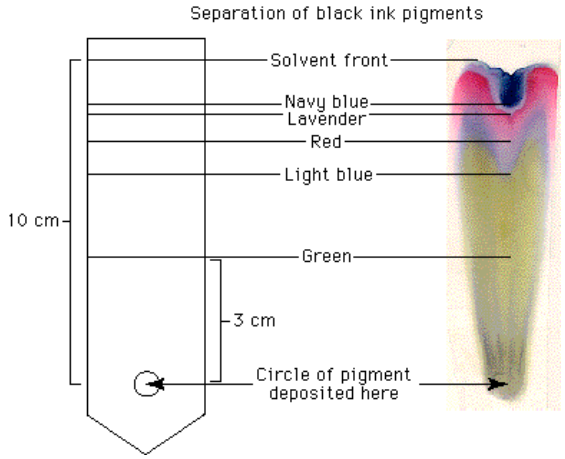


Figure A1. Chromatogram
In black ink, what appears to be a single color is actually a material composed of many different pigments—green, blue, red, and lavender. Such is the case with pigment molecules inside chloroplasts.

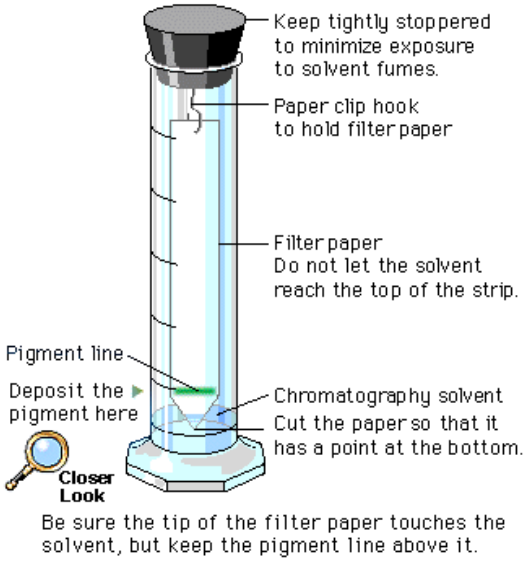


Figure A2. Chromatography setup

■ Learning Objectives
In the following activity, you will separate plant pigments in chloroplasts using an organic solvent, such as a mixture of ether and acetone. Be sure to keep the beaker tightly covered except when you are using it because the solvent is very volatile and produces fumes you should not breathe (see Figure A2).

Beta carotene, the most abundant carotene in plants, is carried along near the solvent front because it is very soluble in the solvent being used and because it forms no hydrogen bonds with the cellulose in paper. Another pigment, **xanthophyll**, differs from carotene in that it contains oxygen. Xanthophyll is found further from the solvent font because it is less soluble in the solvent and has been slowed down by hydrogen bonding to the cellulose. **Chlorophylls** contain oxygen and nitrogen and are bound more tightly to the paper than the other pigments. Chlorophyll *a* is the primary photosynthetic pigment in plants. A molecule of chlorophyll *a* is located at the reaction center of the photo systems. Other chlorophyll *a* molecules, chlorophyll *b*, and the carotenoids (carotenes and xanthophylls) capture light energy and send it to chlorophyll *a* at the reaction center. Carotenoids also protect the photosynthetic systems from damaging effects of ultraviolet light.

■ Procedure

1. Obtain a 400mL beaker with about 1cm of solvent (petroleum ether) at the bottom. Cover the beaker with aluminum foil to prevent the vapors from spreading.
2. Cut a piece of filter paper long enough to reach the solvent. Draw a line about 2cm from the bottom of the paper.
3. Use a quarter to extract the pigments from spinach leaf cells. Place a small section of leaf on the **top** of the pencil line. Use the ribbed edge of the coin, rub it back and forth over the pencil line to crush the leaf cells. **Be sure the pigment line is on top of the pencil line.** Repeat with different sections of spinach about 10 times to get as much pigment as possible onto the filter paper.
4. Place the chromatography paper in the beaker with solvent (see Figure A2). Gently fold the top of the paper over the edge of the beaker. Cover the beaker with aluminum foil. **Do not allow the pigment to touch the solvent.**
5. When the solvent is about 1cm from the top of the paper, or about 30 minutes have passed, remove the paper and *immediately* mark the location of the solvent front before it evaporates.
6. Mark the bottom of each pigment band. Identify the band color and corresponding pigment molecule:

   chlorophyll *a* – bright green to blue green
   chlorophyll *b* – yellow green to olive green
   carotene – yellow to yellow-orange
   xanthophyll – yellow

7. Measure the distance each pigment migrated from the bottom of the pigment origin to the bottom of the separated pigment band. In Table A1, record the distance that each front, including the solvent front, moved.
8. Calculate and record your R_f values for each pigment band.

Table A1. Distance moved by Pigment Band (millimeters)

Band Number	Distance (mm)	Band Color/Pigment	R_f value
1			
2			
3			
4			

Distance Solvent Front Moved _____ (mm)

Analysis of Results

The migration of pigment relative to migration of solvent is expressed as a constant, called R_f (reference front). It can be calculated for each of the pigments using the formula:

$$R_f = \frac{\text{distance pigment migrated}}{\text{distance of solvent migrated}}$$

Exercise A Questions:

1. What factors are involved in the separation of the pigments in the chromatography experiment?
2. Would you expect the R_f value of a pigment to be the same if a different solvent (more polar or less polar) were used? Explain your answer.
3. What type of chlorophyll does the reaction center in the chloroplast contain? What are the roles of the other pigments?
4. What errors may have occurred during this lab experiment?
5. What other related experiments would you propose, based on your results from this lab, or based on ideas that occurred to you as you were performing this lab?

Exercise 4B: Rate of Photosynthesis

Photosynthesis fuels ecosystems and replenishes the Earth's atmosphere with oxygen. Like all enzyme-driven reactions, the rate of photosynthesis can be measured by either the disappearance of substrate or the accumulation of product (or byproducts). The general summary equation for photosynthesis is

$$H_2O + CO_2 + \text{light} \rightarrow \text{carbohydrate } (CH_2O)_n + O_2 + H_2O$$

What could you measure to determine the rate of photosynthesis?

- Production of O_2 (How many moles of O_2 are produced for one mole of sugar synthesized?) or
- Consumption of CO_2 (How many moles of CO_2 are consumed for every mole of sugar synthesized?)

In this investigation, you will use a system that measures the accumulation of oxygen.

Because the spongy mesophyll layer of leaves (shown in Figure B1) is normally infused with gases (O_2 and CO_2), leaves — or disks cut from leaves — normally float in water. What would you predict about the density of the leaf disk if the gases are drawn from the spongy mesophyll layer by using a vacuum and replaced with water? How willthat affect whether or not the leaf floats? If the leaf disk is placed in a solution with an alternate source of carbon dioxide in the form of bicarbonate ions, then photosynthesis can occur in a sunken leaf disk. As photosynthesis proceeds, oxygen accumulates in the air spaces of the spongy mesophyll, and the leaf disk will once again become buoyant and rise in a column of water. Therefore, the rate of photosynthesis can be *indirectly* measured by the rate of rise of the leaf disks. However, there's more going on in the leaf than that! You must also remember that cellular respiration is taking place at the same time as photosynthesis in plant leaves. (Remember that plant cells have mitochondria, too!) What else could be going on that might affect this process? Aerobic respiration will consume oxygen that has accumulated in spongy mesophyll. Consequently, the two processes counter each other with respect to the accumulation of oxygen in the air spaces of the spongy mesophyll. So now you have a more robust measurement tool — the buoyancy of the leaf disks is actually an indirect measurement of the *net* rate of photosynthesis occurring in the leaf tissue.

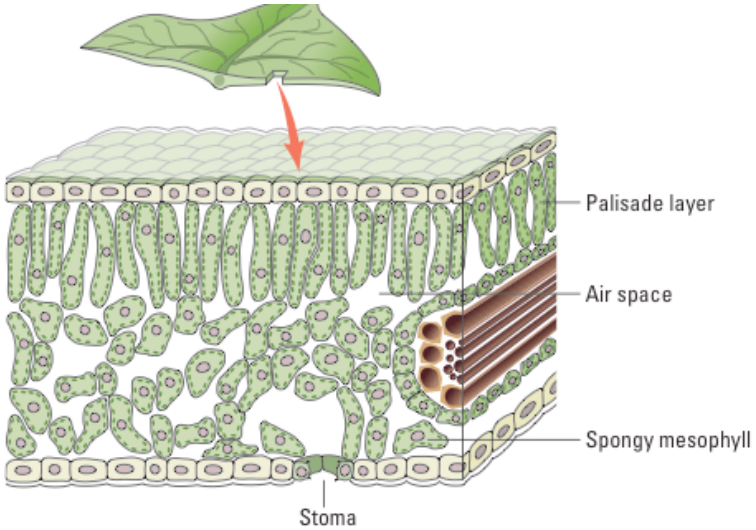


Figure B1. Leaf Anatomy

■ Learning Objectives

- To design and conduct an experiment to explore the effect of certain factors, including different environmental variables, on the rate of cellular photosynthesis
- To connect and apply concepts, including the relationship between cell structure and function (chloroplasts); strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases

■ **Procedure**

In this part of the lab, you will learn how the floating leaf disk technique can measure the rate of photosynthesis by testing a variable that you know affects photosynthesis. Later, you will apply this technique (or computer-based probes) to test a variable that you choose. It is important for you to develop a few skills during this part of the investigation in order to carry out your own investigation. For the floating disk technique, the most challenging skill is getting the disks to sink. Don't just watch someone do this; make sure you can get the disks to sink as well.

Materials (Figure B2)

- 02% baking soda solution (sodium bicarbonate)
- liquid soap (approximately 5mL of dishwashing soap in 250mL of water)
- 2 plastic syringes (10mL or larger)
- living leaves (spinach, ivy, etc.)
- hole punch
- 2 clear plastic cups
- timer
- light source

Figure B2. Leaf disk materials

When immersed in water, oxygen bubbles are usually trapped in the air spaces ofthe spongy mesophyll in the plant leaf. By creating a vacuum in this experimental procedure, the air bubbles can be drawn out of the spongy mesophyll, and thespace is refilled by the surrounding solution. This allows the leaf disks to sink in the experimental solution. If the solution has bicarbonate ions and enough light, the leaf disk will begin to produce sugars and oxygen through the process of photosynthesis. Oxygen collects in the leaf as photosynthesis progresses, causing the leaf disks to float again. The length of time it takes for leaf disks to float again is a measure of the net rate of photosynthesis. This process is shown in Figure B3.

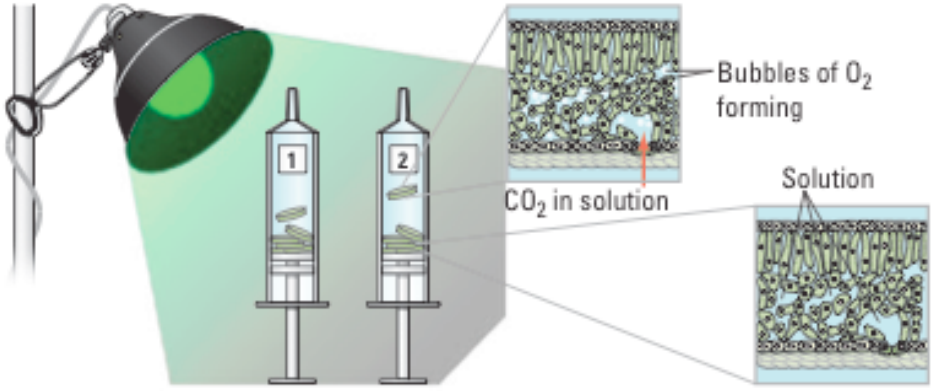


Figure B3. Photosynthesis at work

Question: If the leaf disks are treated in a way you know increases the net rate of photosynthesis, should they start to float faster or slower? Why?

1. Prior to this lab, your instructor should soak the spinach leaves in water overnight in a refrigerator. Fresh, dark green spinach leaves will work best. Your instructor will also need to prepare a 300ml of a 0.2% sodium bicarbonate solution by mixing 2g of baking soda in one liter of water.

2. Pour the bicarbonate solution into a clear plastic cup to a depth of about 3cm. Label this cup A (with CO_2). Fill a second cup with only water to be used as a control group. Label this cup B (without CO_2).

3. Using a pipette, add one drop of a dilute liquid soap solution to the solution in each cup, avoiding bubbles. If either solution generates bubbles or suds, then dilute it with more bicarbonate or water solution. The soap acts as a surfactant or "wetting agent" — it wets the hydrophobic surface of the leaf, allowing the solution to be drawn into the leaf and enabling the leaf disks to sink in the fluid. (Figure B4)

4. Using a paper punch, cut out 20 leaf disks from the freshest portions of the spinach leaves. Try to avoid veins, stems, and other non-leafy portions. (Figure B5)

5. Remove the plunger from two syringes labeled A and B. Carefully place 10 leaf disks into each syringe barrel without crushing them, and replace the plunger. Then suction up 5cc of the sodium bicarbonate plus soap solution from cup A into syringe A, and a small volume of water plus soap solution from cup B into syringe B.

6. Create a vacuum in each plunger to draw the air out of the leaf tissues: Hold your finger over the end of the syringe, pull back on the plunger as far as you reasonable can for about 10 seconds, and swirl the leaf disks to suspend them in the solution. As air is being suctioned out, sodium bicarbonate is going into the spongy layer of the leaf cells, causing the leaf disks to sink in the syringe. You may have to repeat this procedure two to three times in order toget the disks to sink. (If you have any difficulty getting your disks to sink after three tries, it is usually because there is not enough soap in the solution. Try adding a few more drops of soap to the cup and replacing the liquid in the syringe.) (See Figure B6a).

7. Pour the disks and the solution from the syringe into the appropriate clear plastic cup. Disks infiltrated with the bicarbonate solution go in cup A "with CO_2", and disks infiltrated with the water go in cup B "without CO_2". Swirl the disks to dislodge any that get stuck against the side of the cups.

8. Place both cups under the light source and start the timer. At the end of each minute, record the number of floating disks in Table B1. Continue until all of the disks are floating in cup A with the bicarbonate solution.

9. To make comparisons between experiments, a standard point of reference is needed. Repeated testing of this procedure has shown that the point at which 50% of the leaf disks are floating (the median or ET50, the Estimated Time it takes 50% of the disks to float) is reliable and repeatable, and can be used as a point of reference for this procedure. We can also obtain class data for an average.

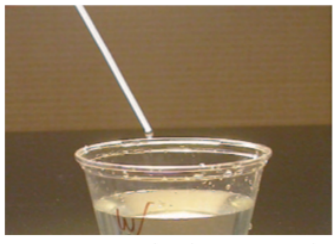


Figure 4. Dilute Liquid Soap Solution Added to Cup

Figure 5. Leaf Disks

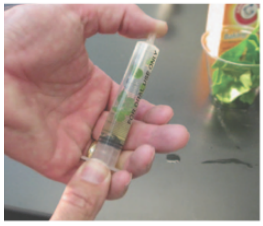


Figure 6a. Creating a Vacuum in the Plunger

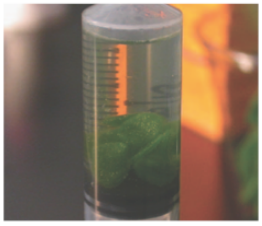


Figure 6b. Sinking Leaf Disks

Table B1. Rate of Photosynthesis

#Floating Leaf Disks (control)	Time (min)	#Floating Leaf Disks (sodium bicarbonate)	Time (min)
1		1	
2		2	
3		3	
4		4	
5		5	
6		6	
7		7	
8		8	
9		9	
10		10	

■ Designing and Conducting your Investigation

What factors affect the rate of photosynthesis in living plants? Once you have mastered the floating disk technique, you will design an experiment to test another variable that might affect the rate of photosynthesis. Discuss with your lab partners the following questions:

- What environmental variables might affect the net rate of photosynthesis? Why do you think they would affect it? How do you predict they would affect it?
- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?
- Could the way you perform the procedure affect the outcome? If the outcome changes, does it mean the net rate of photosynthesis has changed?

Some independent variables to investigate may include the following:

- light intensity
- color
- temperature
- pH

You may present your findings to the class in a mini-poster.

Exercise B Questions:

1. Why was the 2% sodium bicarbonate solution necessary? Explain by describing what specific part of photosynthesis would be affected if sodium bicarbonate had not been not present.

2. How would the following scenarios impact the rate at which the leaf disks rise? Write "faster" or "slower" next to each scenario:
   a. increased light intensity
   b. raised temperature
   c. increased sodium bicarbonate concentration
   d. lowered pH

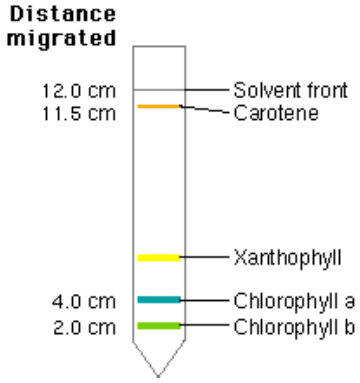
3. Create a graph to compare your three results: the control, using sodium bicarbonate, and using your independent variable of choice. Briefly explain the differences between each result.

Sample Lab Exam Questions

1. Look again at the chromatogram you completed in the previous exercise. Which of the following is true for your chromatogram?
   a. The R_f for carotene can be determined by dividing the distance the yellow-orange pigment (carotene) migrated by the distance the solvent front migrated.
   b. The R_f value of chlorophyll b will be higher than the R_f value for chlorophyll a.
   c. The molecules of xanthophyll are not easily dissolved in this solvent, and thus are probably larger in mass than the chlorophyll b molecules.
   d. If this same chromatogram were set up and run for twice as long, the R_f values would be twice as great for each pigment.

2. If a different solvent were used for the chlorophyll chromatography described earlier, what results would you expect?
   a. The distances traveled by each pigment will be different, but the R_f values will stay the same.
   b. The relative position of the bands will be different.
   c. The results will be the same if the time is held constant.
   d. The R_f values of some pigments might exceed 1.0

3. What is the R_f value for carotene calculated from the chromatogram below?



   a. 1.09
   b. 0.17
   c. 0.96
   d. 0.33
   e. 0.50

4. The pigment molecules responsible for photosynthesis are located in the
   a. cytoplasm of the cell
   b. stroma of the chloroplast
   c. thylakoid membrane of the chloroplast
   d. all of the above

5. During what stage of photosynthesis is O_2 produced?
   a. cyclic photophosphorylation
   b. the light-dependent reactions involving photosystems I and II
   c. carbon fixation
   d. the Krebs cycle

6. The oxygen that is released as O_2 during photosynthesis came from _____ molecules.
   a. carbon dioxide
   b. water
   c. glucose
   d. chlorophyll

AP Lab: Photosynthesis

(Written portion must be typed, using 12pt Times New Roman font, 1 inch margins)

Question: What is the objective and/or problem being tested?

Hypothesis: Briefly describe the experimental design, how the rate of photosynthesis can be measured and factors that influence it (Use "If ..., then ...." statement.)

Procedure: Write: *See lab handout*

Data:

- Table A1 Chromatography results
- Chromatogram
- Table B1 Floating Leaf Disk results (with sodium bicarbonate)
- Experimental design for 2nd floating leaf disk experiment
- Table B2 Floating Leaf Disk results (with 2nd variable)
- Graph B1 Floating Leaf Disk results
- Exercise A Questions #1-5
- Exercise B Questions #1-3
- Sample Exam Questions #1-6

Conclusion:

a. briefly state the overall findings (use numbers from data)
b. discuss hypothesis and variables (dependent, independent, control, constant)
c. discuss errors and future experiments (other variables to test)