

## PLANT PIGMENTS AND PHOTOSYNTHESIS LAB

**OBJECTIVES:** After completing this lab you should be able to:

1. separate pigments and calculate their  $R_f$  values,
2. describe a technique to determine photosynthetic rate,
3. compare photosynthetic rates at different light intensities using controlled experiments, and
4. explain why the rate of photosynthesis varies under different environmental conditions.

### PART I: PLANT PIGMENT CHROMATOGRAPHY

Paper chromatography is a useful technique for separating and identifying pigments and other molecules from cell extracts that contain a complex mixture of molecules. The solvent moves up the paper by capillary action, which occurs as a result of the attraction of solvent molecules to the paper and the attraction of solvent molecules to one another. As the solvent moves up the paper, it carries along any substances dissolved in it. The pigments are carried along at different rates because they are not equally soluble in the solvent and because they are attracted, to different degrees, to the fibers in the paper through the formation of intermolecular bonds, such as hydrogen bonds.

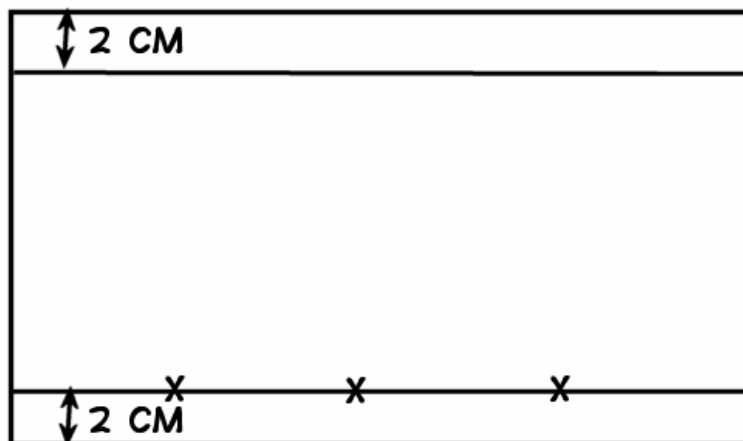
Beta carotene, the most abundant carotene in plants, is carried along near the solvent front because it is very soluble in the solvent being used and because it forms no hydrogen bonds with cellulose. Another pigment, xanthophylls, differs from carotene in that it contains oxygen. Xanthophyll is found further from the solvent front 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 are the other pigments.

Chlorophyll *a* is the primary photosynthetic pigment in plants. A molecule of chlorophyll *a* is located at the reaction center of photosystems. Other chlorophyll *a* molecules, chlorophyll *b*, and the carotenoids (that is, carotenes and xanthophylls) capture light energy and transfer it to the chlorophyll *a* at the reaction center. Carotenoids also protect the photosynthetic system from the damaging effects of ultraviolet light.

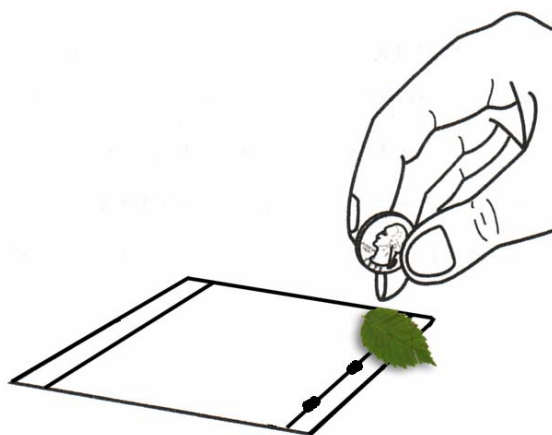
### PROCEDURE

1. Obtain a piece of chromatography paper.

2. With a pencil, draw a line 2 cm from the top of the chromatography paper. Also draw a line 2 cm from the bottom. Place three X's equal distant apart on the bottom pencil line.

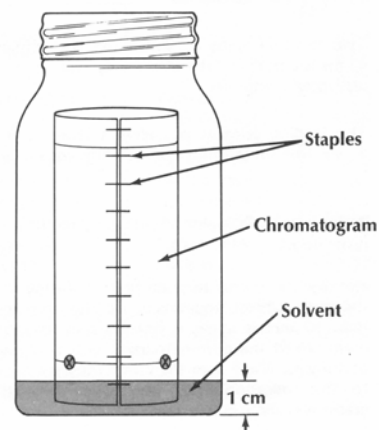


3. Use a quarter to extract the pigments from spinach leaf cells. Place a small section of leaf on top of the pencil line at the location of an "X". Use the ribbed edge of the quarter to crush the cells. Be sure that the pigment line is on top of the pencil line. You should repeat this procedure 8 to 10 times being sure to use a new portion of the leaf each time.



4. Repeat step 3 at the other "X" locations. When finished you should have a green spots on the bottom pencil line at the location of each "X".

5. Roll the chromatography paper into a cylinder, with the pencil marks to the outside, and staple the edges together. Be careful not to overlap the edges.



6. In the fume hood, pour solvent to a depth of 1 cm into the jar. The solvent level should not be higher than the lower pencil line of the paper.
7. Place the paper cylinder into the jar and put the lid on the jar. Allow the chromatogram to develop during the class period.

8. When the solvent is close to the top pencil line, remove the paper from the jar and immediately draw a pencil line to indicate the leading edge of the solvent. Use the pencil to mark the top of each pigment band.
9. Measure the distance each pigment migrated from the bottom of the pigment origin to the bottom of the separated pigment band. Record the distance that each front, including the solvent front, moved in the data table below. You should see 4 pigment bands.

<b>Band</b>	<b>Trial 1 Distance (mm)</b>	<b>Trial 2 Distance (mm)</b>	<b>Trial 3 Distance (mm)</b>
<b>Orange</b>			
<b>Yellow</b>			
<b>Blue green to bright green</b>			
<b>Yellow green to olive green</b>			
<b>Solvent line</b>			

### ANALYSIS OF RESULTS

10. The relationship of the distance moved by a pigment to the distance moved by the solvent is a constant called  $R_f$ . It can be calculated for each of the four pigments using the formula:

$$R_f = \frac{\text{distance pigment migrated (mm)}}{\text{distance solvent front migrated (mm)}}$$

Calculate the  $R_f$  values for each pigment band for each trial. Then determine the average  $R_f$  value for each pigment. Record your answers in the data table below.

### Data Table - $R_f$ Values

<b>Color</b>	<b>Pigment</b>	<b>Trial 1</b>	<b>Trial 2</b>	<b>Trial 3</b>	<b>Average</b>
<b>Orange</b>	<b>Carotene</b>				
<b>Yellow</b>	<b>Xanthophyll</b>				
<b>Blue green</b>	<b>Chlorophyll a</b>				
<b>Yellow green</b>	<b>Chlorophyll b</b>				

11. What factors are involved in the separation of the pigments?

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12. Would you expect the  $R_f$  value of a pigment to be the same if a different solvent were used? Explain.

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13. What type of chlorophyll does the reaction center contain?

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14. What are the roles of the other pigments?

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## **PART II: PHOTOSYNTHESIS – THE LIGHT REACTION**

When light is absorbed by leaf pigments, electrons within each photosystem are boosted to a higher energy level and this energy is used to produce ATP and to reduce NADP to NADPH. ATP and NADPH are then used to incorporate  $\text{CO}_2$  into organic molecules, a process called carbon fixation.

Photosynthesis may be studied in a number of ways. One experiment involves a dye-reduction technique. The dye-reduction experiment tests the hypothesis that light and chloroplasts are required for the light reactions to occur. In place of the electron acceptor, NADP, the compound DPIP (2,6-dichlorophenol-indopenol), is substituted. When light strikes the chloroplasts, electrons boosted to high energy levels reduce DPIP. It changes from blue to colorless when reduced.

In the 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 shown in the following table.

## PHOTOSYNTHESIS SETUP

	Cuvettes				
	1 Blank	2 Unboiled chloroplasts Dark	3 Unboiled chloroplasts Light	4 Boiled chloroplasts Light	5 No chloroplasts
Phosphate buffer	1 mL	1 mL	1 mL	1 mL	1 mL
Distilled water	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	--

Cuvettes were set up with the contents listed in the table above. Cuvette 2 was covered with foil to prevent exposure to light. A spectrophotometer was used to measure the initial percentage of light transmitted through each cuvette. Cuvette 1 was used to calibrate and recalibrate the spectrophotometer.

An incubation area was set up with a light source and a test tube rack. A water-filled flask was placed between the light and test tube rack and acting as a heat sink by absorbing most of the light's infrared radiation while having little effect on the light's visible radiation. The cuvettes were allowed to incubate for 15 minutes with the percent transmittance measured every 5 minutes. The measurements are reported in the data table below.

## PERCENT TRANSMITTANCE

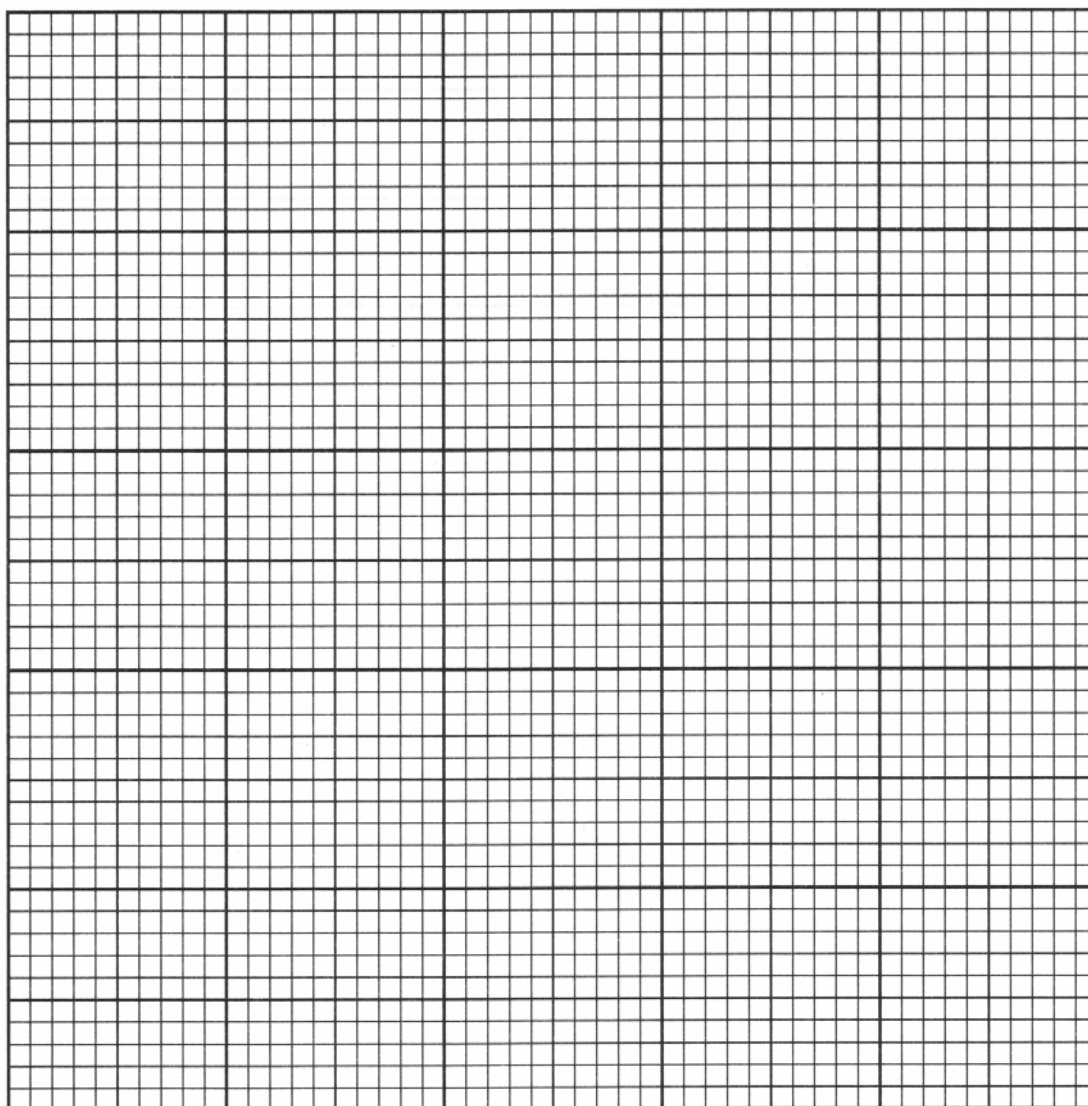
Cuvette	Time (minutes)			
	0	5	10	15
2 Unboiled/Dark	31.3	32.5	35.5	34.8
3 Unboiled/Light	32.7	54.5	63.7	65.1
4 Boiled/Light	32.7	32.9	33.1	32.5
5 No chloroplasts	31.3	31.3	31.3	31.3

15. Graph the percent transmittance from the four cuvettes on the graph below.

What is the independent variable? \_\_\_\_\_

What is the dependent variable? \_\_\_\_\_

Graph Title: \_\_\_\_\_



16. What is the purpose of DPIIP in the experiment?

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17. What molecule, found in chloroplasts, does DPIIP "replace" in this experiment?

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18. What is the source of electrons that will reduce DPIIP?

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

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20. What is the effect of darkness on the reduction of DPIIP?

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Why did this happen?

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21. What is the effect of boiling the chloroplasts on the reduction of DPIIP?

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Why did this happen?

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22. Why was there a difference in the percentage of transmittance between the live chloroplasts that were incubated in the light and those that were kept in the dark?

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23. What is the function of each of the cuvettes in this experiment?

<b>Cuvette</b>	<b>Function</b>
<b>1</b>	
<b>2</b>	
<b>3</b>	
<b>4</b>	
<b>5</b>	