

# Cornell Institute for Biology Teachers

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**Title:**

## Photosynthesis and Spectrophotometry

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(adapted from the *Advanced Placement Laboratory Manual*, 1989)

**Appropriate  
Level:**

Regents, Honors, or AP.

**Abstract:**

The first part of this lab makes use of a spectrophotometer to study the rate of photosynthesis in a suspension of spinach chloroplasts. The chloroplasts are subjected to varying light and temperature conditions. As the blue dye, DPIP, is reduced, it becomes colorless, a change which can be measured quantitatively by following the % transmittance of the suspension.

The second part of the lab measures the absorption spectrum of a solution of pigments isolated from spinach leaves.

**Time Required:**

Allow one period for explanations, discussion of controls, and a demonstration of spectrophotometer operation. The experimental portions can each be run in single periods on different days if the teacher prepares the chloroplast suspension and pigment extract ahead of time. A final period is required for data analysis and discussion. Note: the two parts of the lab are independent of each other.

An honors class or AP class might enjoy doing the preparation work themselves. The experimental portions could then be divided among lab groups the following day, with groups reporting their findings to the rest of the class on the third day.

**Special Notes:**

Special Equipment: This lab is designed to be used with the Fisher Scientific Model 415 Spectrophotometer. These spectrophotometers and cuvettes are available to CIBT participants as part of a lending library kit through the Cornell Biotechnology Program. Other spectrophotometers will work just as well, but the operating instructions in this lab may require modification.

The Fisher Model 415 can input data directly to a Mac Classic II or higher level computer. This requires Mac SpectroMaster<sup>®</sup> and Hypercard software, and a connecting cable.

Flood lights are required. They may be assembled using 100 watt bulbs and inexpensive electrical equipment.

# Additional Teacher Information

## Introduction

### The Physics:

A spectrophotometer measures the degree to which a solution absorbs light. It may be used to identify unknown substances and to determine concentrations.

The spectrophotometer contains a light bulb, a reflector, and a detector. When a sample is in place and the chamber lid is closed, light emitted by the light bulb passes through the sample. The detector measures the amount of light transmitted. The light bulb is of the same type used in a high intensity study lamp. The white light produced by the bulb may be separated into different wavelengths by reflecting the beam off of a diffraction grating, creating a “rainbow” or spectrum. By controlling the angle of deflection with a mirror, light of a single wavelength can be passed through the sample.

In Part 1 of this experiment, the change in color of a solution is measured by exposing the sample to the same wavelength of light at 5 minute intervals as the reaction progresses. The spectrophotometer is set to the wavelength at which blue (oxidized) DPIP exhibits maximum absorbance. The device is being used to monitor the concentration of the DPIP. In Part 2, a pigment solution is exposed to varying wavelengths of light in order to determine which wavelengths are absorbed maximally. The absorbance is then plotted against wavelength to obtain the absorption spectrum.

The spectrophotometer scale gives two measurements - absorbance and transmittance. Transmittance refers to the amount of light that passes unchanged through the sample and is measured by the detector. Absorbance refers to the amount of light absorbed by the sample. They are mathematically related [ $A = \log(1/T)$ ]. In Part 1, transmittance is used, while absorbance is used in Part 2. It will be important for students to think carefully about which measure they are using during data analysis.

It is important to note that the sample is not the only thing in the system that will affect the beam of light. The cuvettes are made of special optical glass. Care should be taken to avoid scratching them. Cuvettes should be wiped clean of fingerprints before readings are taken.

### The Chemistry:

In Part 1, DPIP (2,6-dichlorophenol-indophenol) replaces NADP<sup>+</sup> in the light reactions of photosynthesis. As the light reactions progress, molecules of DPIP are reduced, that is, they accept electrons and protons that originated from water. The reduced form (DPIPH) is colorless. At the beginning of the experiment, the experimental sample transmits none (0%) of the incident light. As reduction progresses, the % transmittance increases to approximately 100% when all of the DPIP is reduced.

In Part 2, the extract from spinach contains several pigments, including chlorophylls a and b, as well as carotenes and xanthophylls. (Many high school biology classes do simple paper chromatography to demonstrate the presence of these pigments in the mixture.) Because the sample contains more than one pigment, the absorption spectrum will be the “sum” of the absorption spectra of the individual pigments. Although sharp peaks are not obtained, there are clear absorption maxima at around 450 and 650 nm.

**The solvents used in preparing the samples themselves affect absorption of light. It is essential to place a sample of solvent in the chamber and set the spectrophotometer to read “0% transmittance.” This step instructs the meter to “ignore” contributions made by the solvent and to read only the portion of the sample (DPIP or pigments) that is of interest. Because the absorption characteristics of the sample change in a wavelength dependent manner, the blanking procedure must be repeated at each wavelength in Part 2.**

## **Objectives of experiment**

In Part 1, students will set up an experiment which investigates the effects of light and temperature on the light reactions of photosynthesis. They will use a spectrophotometer to measure the change in concentration of DPIP as the reactions progress. They will then graph the results and calculate the rate of the reactions. They will draw conclusions about the effects of the experimental variables.

In Part 2, students will measure and graph the absorbance of spinach pigments at different wavelengths. From the resulting absorption spectrum, they will make suggestions for the design of lights which will have the maximum effect on plant growth. They will also relate the wavelengths of light to color and draw conclusions about the colors which are least and most effective in promoting photosynthesis.

## **Level of course:**

Regents, honors, or AP.

Running both parts of the lab will be somewhat time-consuming. While this should not be a problem for honors or AP classes, for Regents classes you may choose to do only Part 1 and then refer to the Part 2 procedure when discussing the fact that different pigments absorb different wavelengths of light. Having already used a spectrophotometer to do Part 1, the students should find it easy to understand how an absorption spectrum is obtained.

## **Class time required**

Allow one period for discussion of the procedure and demonstration of spectrophotometer operation. One class period will be required for each part of the lab, unless lab groups are assigned to do the parts separately and report back to the class during the third period. A third period will be required to analyze and graph data and discuss the results. (Note: this time frame assumes that the teacher will prepare chloroplast suspensions and pigment solutions ahead of time. Honors or AP students may prefer to do this themselves. If so, add additional time. The teacher may also choose to include this preparation in the pre-lab discussion/demonstration if time allows.)

## **Preparation time required**

Prior to class, allow about one hour to mix the buffer solution and make the chloroplast suspension and pigment solution. The best results are obtained when these materials are freshly made, but they may be prepared the day before, and then be refrigerated until needed for the lab. To get the best results from

the chloroplasts, incubate spinach leaves under a light for a few hours before use. (Do not allow them to become hot.) The solvent used in part 2 will evaporate unless kept in a sealed container. The buffer lasts indefinitely and may be made well in advance.

Additional time will be needed to set out materials and clean up afterwards.

Be sure to turn the spectrophotometers on **before** the beginning of class, b. They will need to warm up for at least 20 minutes.

## **Information with which students must be familiar**

### **Part 1:**

- a. Photosynthesis involves a series of light reactions in which electrons are passed from water, through chlorophyll molecules, to an electron acceptor. The electron acceptor, NADP<sup>+</sup> in live plants or DPIP in the experiment, becomes reduced.
- b. The spectrophotometer measures the absorption of light at a particular wavelength by a solution. It may be used to monitor the progress of a reaction or to determine concentrations of colored compounds.

### **Part 2:**

- a. Plants contain a mixture of pigments - both chlorophylls a and b and accessory pigments like carotenes and xanthophylls.
- b. Different pigments absorb different wavelengths of light. Having a mixture of pigments allows plants to harvest the energy from a wide range of light wavelengths for photosynthesis.
- c. The spectrophotometer can be used to measure the absorption spectrum of a colored compound like a pigment.

## **Instructor's Materials**

### **Part 1:**

- 0.5 M sucrose, cold
- 0.1 M phosphate buffer
- blender
- funnel
- test tube
- hot plate and 250 ml beaker
- DPIP solution
- spinach leaves
- cheesecloth
- ice
- test tube clamp

## Preparation Instructions:

**Sucrose:** Add 85.5 grams of sucrose to 500 ml of distilled water. Store in refrigerator.

**DPIP (2,6-dichlorophenol-indophenol):** Add 0.036 grams of DPIP (Sigma Chemical Co.) to 500 ml of distilled water. Store in the refrigerator in an amber bottle or a bottle wrapped in foil.

**Buffer:** Make a solution of 87 grams  $K_2HPO_4$  (dibasic) brought to 500 ml with distilled water. Make a second solution of 68 grams  $KH_2PO_4$  (monobasic) brought to 500 ml with distilled water. To make a stock 1.0 M buffer, mix 345 ml of the monobasic solution with 160 ml of dibasic. Adjust the pH to 6.5. If the pH is too high, add more monobasic. If the pH is too low, add more dibasic. In this lab, you will need 0.1 M buffer, so take 10 ml of the 1.0 M stock buffer and add 90 ml of distilled water. The stock keeps well at room temperature.

## Part 2:

- acetone solution (80% acetone, 20% distilled water)
- filter paper
- blender
- spinach leaves
- water aspirator
- Buchner funnel
- sidearm flask

## Preparation Instructions:

Homogenize 5 grams of spinach in 100 ml of acetone solution. Filter through filter paper in a Buchner funnel. Use a water aspirator to speed up the process.

## Student Materials (per group)

### Part 1:

- spectrophotometer
- ring stand or other support for lamp
- test tube rack
- 4 test tube cuvettes
- 1 ml pipettes
- 5 ml pipettes
- Kimwipes or tissues
- 100 watt flood lamp
- large (1-2 liter) flask or beaker for heat sink
- aluminum foil
- ice bucket with ice
- Pi pump or other pipetting device
- 2 eyedroppers or Pasteur pipettes
- stopwatch or access to clock with second hand

- graph paper
- 10 ml of 0.1 M phosphate buffer in small beaker or other container
- small beaker of chloroplast suspension, wrapped in foil and placed on ice
- 10 ml DPIP solution in small beaker or other container, wrapped in foil

### Part 2:

- spectrophotometer
- test tube rack
- 2 cuvettes
- Kimwipes or tissues
- pigment solution
- acetone
- graph paper
- references that give visible light wavelengths and corresponding colors

### Special directions for the instructor

1. Read directions accompanying spectrophotometer.
2. Be sure to turn the spectrophotometers on to warm up at least 20 minutes before using them.
3. The wavelength setting on the spectrophotometer must always be set between 330 nm and 999 nm. Otherwise, damage may occur.
4. Instruct students to handle cuvettes carefully. Avoid scratching and wipe with Kimwipes or tissues before taking each reading. Wash immediately after using. Rinse thoroughly.
5. The boiled chloroplasts have a tendency to clump. Students should mix them and wipe off the tube before each reading.
6. The acetone solvent used in Part 2 may be hazardous if spilled. It will dissolve or damage plastics. Instruct students to be particularly careful when handling their tubes near the spectrophotometer.
7. In Part 2, it takes a while to take all of the recommended readings. You may instruct students to take readings every 20 nm when they are finding little change in absorbance. They should return to 10 nm intervals when they start to see a change in readings.

### Answers to Pre-Lab Questions

#### Part 1:

1. How is red light different from green light?  
*Red light has a different wavelength than green light. (Red light has a longer wavelength.)*

2. What color is DPIP? What new substance does it become after it takes part in the light reaction? What color is this final compound?

*DPIP is blue before it takes part in the light reactions. During the reactions, it is converted to DPIP<sub>H</sub>, a new substance which is colorless.*

3. Why must you use a blank when you make measurements with a spectrophotometer?  
*A blank is used to adjust the spectrophotometer so that it will read 100% transmittance when the experimental substance is not present. If a blank is not used, the spectrophotometer will give a reading based on all substances in the mixture, not just the one of interest.*

## **Part 2:**

1. What color of light is reflected by the pigments in spinach leaves? What colors are absorbed?  
*Leaves reflect green light and absorb the majority of the other visible light colors - red, orange, yellow, blue, indigo, violet. (Auxiliary pigments will absorb some of these other colors.)*
2. Which light is important for photosynthesis, the reflected light or the absorbed light?  
*The absorbed light is important for photosynthesis. This is the light that the plant uses.*
3. Why is it an advantage for a plant to have a mixture of several pigments instead of just one?  
*A plant with pigments that have different preferred absorption wavelengths can harvest energy from more than one wavelength of light.*

## **Answers to Post-Lab Questions**

### **Part 1:**

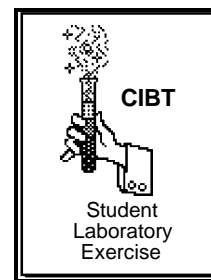
1. Graph your results.  
*The graph for tube 2 should be more or less horizontal. It may show some increase in transmittance, but very little compared with tube 3. The graph for tube 3 should show a rapid increase in transmittance followed by a leveling off as it approaches 100% transmittance. The graph for tube 4 should be horizontal. Any deviation from this is most likely due to poor mixing, as boiled chloroplasts tend to clump and settle to the bottom of the tube.*
2. Describe the change in appearance, if any, in each tube.  
*Tube 2 - very little change, probably not detectable by the unaided eye  
Tube 3 - tube should be colorless, or very nearly so  
Tube 4 - no change, chloroplasts may be settling to the bottom of the tube*
3. Many students find that tube 2 shows some increase in transmittance, even though it is kept in the dark. What result would you have predicted for tube 2? If you found an increase in transmittance for this tube, how might you explain your result?  
*You would expect tube 2 to show no change in transmittance because the light reactions cannot occur in the absence of light. This tube is exposed to light for short periods of time while readings are taken. During this brief time, some reduction of DPIP will occur.*

4. Examine your graph for tube 3. During which time interval was the change most rapid?  
*Change is usually most rapid during the first time interval.*
5. Calculate the initial rate of the light reaction. The rate of any reaction is the change in the concentration divided by the change in time. Your measure of concentration is % transmittance.  
*This will vary from team to team. Have them compare results. Their results should be expressed as "change in % transmittance/min."*
6. You had two controls in this experiment, tubes 2 and 4. Explain the significance of each control. How is the function of the blank different from that of the controls?  
*Tube 2, containing chloroplasts incubated in the dark, showed that the light reactions do not occur in the dark. Tube 4, containing boiled chloroplasts, showed that boiling prevents the light reactions. This is because it disrupts the chloroplast membrane and the carrier proteins that transport electrons. The blank is simply used to set the spectrophotometer, it does not tell us anything about the effects of the variables.*

## **Part 2:**

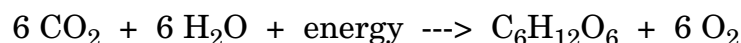
1. Graph your results.  
*The graph should show two peaks, one in the mid 400's and the other in the high 600's.*
2. Does your graph show one **absorption maximum** or more than one? At what wavelength(s) are these maxima observed?  
*Two absorption maxima as described above.*
3. What colors of light are most strongly absorbed? To determine this, you will have to find a chart of the visible spectrum that shows both colors and wavelengths.  
*The absorption maximum in the mid 400's corresponds to light in the blue to violet region. The peak in the high 600's corresponds to light in the red region.*
4. If you were an engineer designing the lighting for a greenhouse, what wavelengths of light should your light fixtures emit?  
*You would choose fixtures that emit wavelengths of light that correspond to the absorption maxima. They would have a high output in the blue and red wavelengths.*

# Photosynthesis Part I: Measuring the Light Reactions



## Background Information

Photosynthesis is the process by which plants absorb energy from sunlight and use it to convert carbon dioxide and water to glucose and oxygen. The glucose molecule which forms contains the energy from the sunlight converted to a new form, chemical energy. The overall chemical equation for these reactions is shown below:



The energy that plants trap is essential, both for their own growth and for other organisms that rely on plants for food.

Although the overall chemical equation for photosynthesis seems simple enough, it is actually the net result of a long series of reactions. Biochemists divide the reactions into two processes: the **light reactions** and the **dark reactions** (also known as the **Calvin cycle**). As the name implies, the light reactions require light energy. The dark reactions don't require light. They use chemicals produced in the light reactions. When a plant is exposed to light, the light and dark reactions will occur at the same time. If the light is removed, the light reactions stop immediately, but the dark reactions keep going until the chemicals produced in the light reactions are used up. At that point, photosynthesis stops until light is again available.

In this lab, you will measure the rate of the light reactions. You will take a suspension of chloroplasts from spinach leaves and actually measure the rate at which light is converted into chemical energy.

During the light reactions, a small packet of light energy called a **photon** knocks an electron out of a molecule of water. The electron is now a high-energy electron that is passed from molecule to molecule within the chloroplast until it is finally captured by an electron acceptor molecule called  $\text{NADP}^+$ . (Actually, the  $\text{NADP}^+$  captures two of these high-energy electrons and, in the same process, a proton or  $\text{H}^+$ . The electrons and the  $\text{H}^+$  come from water.) The  $\text{NADP}^+$  is converted to  $\text{NADPH}$  by a chemical process called **reduction**.  $\text{NADPH}$  is then used in the dark reactions to produce glucose.

In a chloroplast, you cannot see the light reactions occur.  $\text{NADPH}$  is produced, but your eyes are not capable of detecting it. In this experiment, you will use a substitute that you can see. It is called DPIP (dichlorophenol-indophenol). DPIP is a dark blue color until it is reduced, that is, until it captures an electron and a proton ( $\text{H}^+$ ). After capturing the

electron and proton, it becomes colorless. Thus, if the light reaction is occurring, you will be able to see a change in the color of the blue DPIP to the colorless DPIP<sub>H</sub>.

To measure the change in color more precisely, you will use a device called a **spectrophotometer**. Inside the spectrophotometer, there is a light bulb which can be made to shine a beam of light of just one wavelength through your sample tubes. (Remember that normal white light is a mixture of all the colors of the rainbow. Each of these colors is different because it has a different wavelength.) There is also a **detector** which will measure how much light passes through your sample.

You will adjust the bulb to emit light that is absorbed by the unreduced DPIP. At the beginning, when the DPIP is blue, it will absorb all the light and will not allow any to pass through. As the DPIP is reduced to DPIP<sub>H</sub> and becomes colorless, more and more light will pass through the sample. You will measure this change at different time intervals. The amount of light that passes through the sample is known as **transmittance**.

When you use DPIP in this experiment, it will be part of a mixture. The other parts of the mixture are water, buffer, and chloroplasts. They also transmit and absorb light. If you want the spectrophotometer to read the concentration of **just** the DPIP, and not the other substances, you must adjust the meter so that it will “ignore” everything except the DPIP. You will do this by using a **blank**. A blank is a sample that contains all the substances in the experimental sample except the one you will be measuring. The blank is inserted into the spectrophotometer before any experimental readings are taken. When the blank is in the chamber, the meter will be adjusted to read 100% transmittance. In other words, the meter will “ignore” buffer, water, and chloroplasts. When an experimental sample is placed in the chamber, the meter will record only the amount of light transmitted by the DPIP.

## Pre-Lab Questions

1. How is red light different from green light?
2. What color is DPIP? What new substance does it become after it takes part in the light reaction? What color is this final compound?
3. Why must you use a blank when you make measurements with a spectrophotometer?

## Materials

- spectrophotometer
- ring stand or other support for light
- test tube rack
- 4 test tube cuvettes
- 1 ml pipettes
- 5 ml pipettes
- large (1-2 liter) flask or beaker for heat sink
- 10 ml of 0.1 M phosphate buffer in small beaker or other container
- stopwatch or access to clock with second hand
- 100 watt flood light
- aluminum foil
- ice bucket with ice
- Pi pump or other pipetting device
- 2 eyedroppers or Pasteur pipettes
- Kimwipes or tissues
- small beaker of chloroplast suspension, wrapped in foil and placed on ice
- 10 ml DPIIP solution in small beaker or other container, wrapped in foil

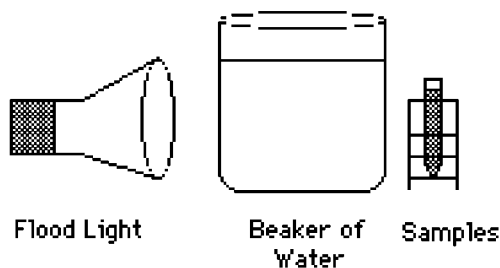
## Procedure

### 1. Preparing the chloroplast suspension

Unless otherwise directed, watch your teacher prepare the suspension in the following manner:

- Pour enough 0.5 M cold sucrose solution into the blender to cover the blender blades.
  - Pack fresh spinach into the blender until it is about one inch above the blades.
  - Set up a beaker in ice with 2 layers of cheesecloth folded over a funnel.
  - Blend spinach (three 10-second pulses) and squeeze it through the cheesecloth into a beaker. Keep the suspension on ice and cover it with foil.
  - Place about 20 ml of the suspension in a test tube and heat in a boiling water bath for 5 minutes.
2. Place four test tube cuvettes in your test tube rack. These are special tubes for use in the spectrophotometer. **Take care not to scratch them.** Label the tubes by placing a small piece of tape near the top of each tube. Number the tubes 1-4.

3. Follow your teacher's instructions as you set up your **heat sink** and flood light. The beaker of water will serve as a heat sink. It will absorb heat from the flood light so that the temperature of the experimental samples will not change. Your test tube rack will be put in place after the samples are prepared.



4. Fill the tubes as shown in the table below.
- Add 1 ml of phosphate buffer to each tube.
  - Add 4 ml distilled water to tube 1 and 3 ml to tubes 2, 3, and 4.
  - Add 1 ml of DPIP to tubes 2, 3, and 4. Do not add DPIP to tube 1.
  - Add 3 drops of **normal** chloroplasts to tube 1, mix it well by rolling it between your hands until it is evenly mixed. Place the tube in your test tube rack.
  - Add 3 drops of **normal** chloroplasts to tube 2 and mix. Immediately wrap the tube in foil. Make a foil cap cover for the tube. Place the tube in your test tube rack.
  - Add 3 drops of **normal** chloroplasts to tube 3 and mix. Place the tube in your test tube rack.
  - Add 3 drops of **boiled** chloroplasts to tube 4 and mix. Place the tube in your test tube rack.

	Tube #1 - Blank	Tube #2 - Dark	Tube #3 - Light	Tube #4 - Boiled
Phosphate Buffer	1 ml	1 ml	1 ml	1 ml
Distilled Water	4 ml	3 ml	3 ml	3 ml
DPIP	-	1 ml	1 ml	1 ml
Normal Chloroplasts	3 drops	3 drops	3 drops	0
Boiled Chloroplasts	-	-	-	3 drops

5. Measure the amount of light transmitted by each sample at “0 minutes.” It is helpful if one partner handles the tubes while the other records the data.
  - Handling the tubes by the top, wipe off any liquid or fingerprints with a Kimwipe or tissue.
  - Set the wavelength on the spectrophotometer to 660 nm using the knob on the right side of the meter.
  - Place tube 1 in the sample chamber, close the lid, and adjust the meter to 100% transmittance. Do this by pressing the TRANS/ABS button. Then press the 100%T/OA button. The display should now read 100.0 for TRANS mode or 0.000 for ABS mode. Remove tube 1 and put it back in your test tube rack.
  - Remove the foil from tube 2, place it in the sample chamber, and close the lid. Read and record the value in the TRANS display. Remove the tube, re-cover it, and put it back in your test tube rack.
  - Place tube 3 in the sample chamber and close the lid. Read and record the value in the TRANS display. Remove the tube and put it back in your test tube rack.
  - Place tube 4 in the sample chamber and close the lid. Read and record the value in the TRANS display. Remove the tube and put it back in your test tube rack.
6. Place your test tube rack in position next to the heat sink, **on the side opposite the flood light.**
  - Repeat the procedures in step 5 after 5, 10, and 15 minutes of exposure to light. Be careful to keep the “dark” tube covered with foil whenever you are not taking a reading. Record your readings each time.

Tube #	0 minutes	5 minutes	10 minutes	15 minutes
2 - Normal/Dark				
3 - Normal/Light				
4 - Boiled/Light				

## Post-Lab Questions

1. Graph your results. Plot time on the X-axis and % transmittance on the Y-axis. Draw all three graphs on the same set of axes. Use colored pencils or different types of lines (solid, dashed, etc.) to distinguish between the tubes. Make sure you include a legend that identifies each graph.

2. Describe the change in appearance, if any, in each tube.

Tube 2

Tube 3

Tube 4

3. Many students find that tube 2 shows some increase in transmittance, even though it is kept in the dark. What result would you have predicted for tube 2? If you found an increase in transmittance for this tube, how might you explain your result?

4. Examine your graph for tube 3. During which time interval was the change most rapid?

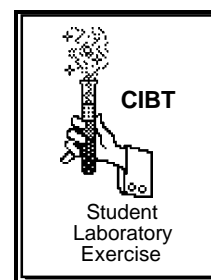
5. Calculate the initial rate of the light reactions. The rate of any reaction is the change in the concentration divided by the change in time. Your measure of concentration is % transmittance.

$$\text{Initial rate} = \frac{\text{transmittance at 5 min} - \text{transmittance at 0 min}}{5 \text{ min}}$$

Initial rate = \_\_\_\_\_

6. You had two controls in this experiment, tubes 2 and 4. Explain the significance of each control. How is the function of the blank different from that of the controls?

# Photosynthesis Part II: Measuring an Absorption Spectrum



## Background Information

Colored substances appear colored because they absorb some of the wavelengths of light that strike them and reflect other wavelengths. For example, a red apple appears red because it reflects the red wavelengths that strike it. The other visible light wavelengths are absorbed by the apple. You see a red apple because only the reflected red light reaches your eyes.

The colored substances in the leaves of a plant are called **photosynthetic pigments**. The main pigments are chlorophyll a and chlorophyll b. They give the plant its green color. Most plants also have other pigments, like xanthophyll (yellow) and carotene (orange). These pigments are usually not present in large enough amounts to be seen. In the fall, when cold temperatures cause the green chlorophyll to break down, these yellow and orange pigments become visible.

Photosynthetic pigments play an important role in photosynthesis. They are responsible for trapping energy from the sunlight that strikes the leaves. Each type of pigment traps energy from a different wavelength of light.

In this experiment, you will determine the wavelengths or colors of light that are most strongly absorbed by the photosynthetic pigments in spinach leaves. It is very easy to extract the pigments from spinach leaves by grinding them up in acetone. When acetone is mixed with the ground-up leaves, the pigments dissolve in the acetone. After a few minutes of mixing, you can just pour off the liquid. It will contain acetone and the pigments. All of the debris from the plant tissues will be left behind.

In this experiment, you will use a device called a **spectrophotometer**. Inside the spectrophotometer, there is a light bulb that can be made to shine a beam of light of just one wavelength through your pigment sample. (Remember that normal white light is a mixture of all the colors of the rainbow. Each of these colors is different because it has a different wavelength.) There is also a **detector** that will measure how much light is absorbed by your sample.

You will start by adjusting the bulb to emit light with a wavelength of 400 nm. The detector will display a value known as **absorbance**. This value represents the amount of light absorbed by the sample in the sample chamber. You will then adjust the bulb to emit light of 410 nm and check the absorbance of your sample at that wavelength. After taking readings every 10 nm until you get up to 750 nm, you will notice a definite pattern.

At some wavelengths, absorbance is very high. At others, absorbance is very low.

A graph of the absorption data is called an **absorption spectrum**. The spectrum for photosynthetic pigments is entirely different from that of hemoglobin or any other substance. The absorption spectrum of a substance can be used like a fingerprint for identification of unknown substances.

In order to extract the spinach pigments, you will have to use the solvent acetone. The solvent has its own absorption characteristics. In order to distinguish between the solvent and the pigments, you will use a **blank**. A blank is a sample that contains all substances in the experimental sample except the one you will be measuring. Your blank will be a tube of acetone. It is inserted into the spectrophotometer before any experimental readings are taken. When the blank is in the chamber, the meter will be adjusted to read 0.000 absorbance. In other words, the meter will “ignore” the acetone solvent. When an experimental sample is placed in the chamber, the meter will record the amount of light absorbed by the spinach pigments alone.

## Pre-Lab Questions

1. What color of light is reflected by the pigments in spinach leaves? What colors are absorbed?
2. Which light is important for photosynthesis, the reflected light or the absorbed light?
3. Why is it an advantage for a plant to have a mixture of several pigments instead of just one?

## Materials

- acetone
- pigment solution
- 2 cuvettes
- Kimwipes or tissues
- test tube rack
- spectrophotometer
- graph paper

## Procedure

1. Place two test tube cuvettes in your test tube rack. These are special tubes for use in the spectrophotometer. **Take care not to scratch them.**
2. Fill the first tube halfway with acetone. This is your blank. Fill the second tube halfway with the pigment solution.
3. Begin to take absorbance readings.
  - Set the wavelength to 400 nm using the knob on the right side of the meter.
  - Place the blank in the sample chamber and close the lid. Adjust the meter to 100% transmittance. Do this by pressing the TRANS/ABS button. Then press the 100%T/OA button. The display should now read 100.0 for TRANS mode or 0.000 for ABS mode. Remove the blank and put it back in your test tube rack.
  - Place the pigment tube in the sample chamber and record the value in the ABS display. Remove the pigment tube and put it back in the test tube rack.
  - Reset the wavelength to 410 nm and repeat the procedure, using the blank first to set absorbance to 0.000, and then the pigment tube to obtain a reading.
  - Continue to take readings, resetting the wavelength 10 nm higher each time until you get to 750 nm.

Wavelength	ABS	Wavelength	ABS	Wavelength	ABS	Wavelength	ABS
400 nm		500 nm		600 nm		700 nm	
410 nm		510 nm		610 nm		710 nm	
420 nm		520 nm		620 nm		720 nm	
430 nm		530 nm		630 nm		730 nm	
440 nm		540 nm		640 nm		740 nm	
450 nm		550 nm		650 nm		750 nm	
460 nm		560 nm		660 nm			
470 nm		570 nm		670 nm			
480 nm		580 nm		680 nm			
490 nm		590 nm		690 nm			

## Post-Lab Questions

1. On a separate piece of graphing paper, graph the results of your experiment. Plot wavelength on the X-axis and absorbance on the Y-axis.
2. Does your graph show one **absorption maximum** or more than one? At what wavelength(s) are the maxima observed?
3. What colors of light are most strongly absorbed? To determine this, you will have to find a chart of the visible spectrum that shows both colors and wavelengths.
4. If you were an engineer designing the lighting for a greenhouse, what wavelengths of light should your light fixtures emit?