Photosynthesis

Photosynthesis is the conversion of light energy to chemical energy and its subsequent use in the synthesis of organic molecules.

In its simplest form the process can be expressed as:

$$\underset{(CO_2)}{\text{Carbon Dioxide}} + \underset{(H_2O)}{\text{Water}} \xrightarrow[\text{Chlorophyll}]{\text{Sunlight}} \underset{(C_6H_{12}O_6)}{\text{Sugar}} + \underset{(H_2O)}{\text{Water}} + \underset{(O_2)}{\text{Oxygen}}$$

Photosynthesis is the principle way in which light energy is converted into chemical bond energy in organic molecules and stored for use in growth, development, metabolism and reproduction. The energy storage molecules, along with others, serve as nutrients for the photosynthetic organisms that produce ("producers") them as well as the non-photosynthetic organisms that eat them ("consumers").

Experiment I Uptake of Carbon Dioxide

Bromothymol blue is a chemical indicator that appears *blue in a basic solution (pH above 7.0)* and *yellow-green in an acid solution (pH below 7.0).* If a sufficient amount of carbon dioxide (CO_2) is present it forms carbonic acid which dissociates releasing hydrogen ions (H^+). The presence of the acid causes the solution to turn yellow-green. If the CO_2 is removed the production of acid is reversed and the solution containing the Bromothymol blue becomes more basic causing the solution to turn blue. The series of reactions leading to the acid condition is summarized as:

$$CO_2 + H_20 \leftrightarrow \underset{\text{(Carbonic Acid)}}{H_2C0_3} \leftrightarrow HCO_3 + H^+ \leftrightarrow \underset{\text{(Carbonium ion)}}{CO_3^{++}}$$

* Note: many chemical reactions are reversible (↔)

Materials:

Bromothymol Blue solution	Elodea
Clear plastic vials with lids	Soda straws
Light source (overhead projector)	Elerenmeyer flask (125 mL)

Objectives:

- Demonstrate the uptake of carbon dioxide (carbon fixation) and release of oxygen by plants during the process of photosynthesis.
- Demonstrate the energy-absorption properties of chlorophyll and other pigment molecules involved in photosynthesis

Methods:

1. Place 50 mL of bromothymol blue solution in an Erlenmeyer flask.
2. Gently blow breath (CO_2 source!) into the bromothymol blue solution through a soda straw until the solution turns *yellow-green*.
3. Place a 9 cm length of *Elodea* sprig in one vial and gently fill the vial with the *yellow-green* bromothymol solution. Fill a second vial (*no sprig* = control) with the bromothymol blue solution. Cap both vials tightly and place them near a bright light. Note the general appearance of the vials at the beginning of the experiment (color, presence of bubbles or not, etc).
4. Periodically observe the vials during the lab period and note any changes you observe.

Results:

Describe any changes you observed while the vials were exposed to the light.

_____

_____

Were there differences between the vial that contained the living plant material and the control vial with no plant? Describe your observations.

_____

In terms of chemical changes *explain the different responses* received from the separate vials. What *caused* the changes?

_____

_____

Why are the principles demonstrated by this simple experiment so important to essentially all life on Earth?

_____

_____

Experiment II. Photosynthesis and Fluorescence

Among the products of photosynthesis are carbohydrates and oxygen. Included among photosynthetic organisms are some bacteria (Cyanobacteria), algae and, of course, plants. The principle storage form of carbohydrates in plants is starch, and oxygen (O_2) is released as a byproduct of the process of photosynthesis in most, but not all, photosynthetic organisms.

Fluorescence involves molecules that absorb light energy of one wavelength (or "color"), which in turn excites electrons that eventually release energy emitted as light at a lower wavelength (different "color"). Chlorophyll absorbs light in the red and blue/violet ranges, but *green* light is *not absorbed*, rather it is reflected or transmitted. As a result leaves are seen as green. Leaves contain other light-absorbing pigments, but chlorophyll is responsible for the vast majority of solar energy captured.

Objectives:

1. Demonstrate the presence of starch as the storage product of photosynthesis.
2. Demonstrate the effect of depriving photosynthetic plant tissue of light energy for a period of time.
3. Demonstrate the phenomenon of chlorophyll fluorescence.

Materials:

Coleus plant leaves
(1) 600 mL beaker
(2) 250 mL beakers
Petri dishes
Forceps
Iodine solution
hot plate
Pipettes
95% ethyl alcohol (ethanol)
colored pencils
graph paper / paper clips

Methods:

1. Pour 350 mL of water in a 600 mL beaker. Bring the water to a boil on a hot plate under the ventilation hood.
2. Get one of the *Coleus* leaves that have been kept wrapped in paper to prevent exposure to light.
3. Remove the paper from the leaf and draw a diagram depicting the arrangement of the color patterns in the leaf.
4. Boil the leaves for *two minutes* to remove the waxy cuticle and disrupt the cell walls.
5. Place the leaf in a 250 mL beaker and cover it with 50 mL of ethanol. CAUTION – **alcohol is highly flammable!** Place the 250 mL beaker in the 600 mL beaker forming a hot water bath. Turn the *hot plate* temperature to low and boil the leaf in the alcohol in the water bath until the leaf turns pale from loss of chlorophyll. .
6. Remove the pale leaf from the alcohol and place it in a Petri dish. Cover it with a minimal amount of water to help unfold the leaf. **Retain the** chlorophyll-containing **alcohol** for subsequent use
7. Pipette iodine solution over the leaves and draw the stain pattern. Compare the iodine-stained drawing to your original drawing.
8. Carefully (HOT!) remove the chlorophyll-containing alcohol from the water bath and allow it to cool under the ventilation hood.
9. Place the chlorophyll-containing alcohol in the white light of the overhead projector with the room light dimmed or off. Describe the fluorescence observed as the result of the light that is absorbed and then emitted as the result of the "excited electrons" having no place to go except back into their orbitals – releasing the energy they absorbed as light energy at a different wavelength (color).

Lab Questions:

1. What were the colors of the leaf that contained the chlorophyll?
   _____
2. What storage product was used to locate where photosynthesis occurred in the leaf?
   _____
3. Explain why the results from leaves exposed to light differed from those deprived of light exposure?
   _____
4. What color is not absorbed by the chlorophyll? _____
5. What color did chlorophyll fluoresce? _____

Experiment III. Absorption and Action Spectrum of Chlorophyll

What is the difference between the absorption spectrum and the action spectrum of chlorophyll?

_____

How is each measured? _____

Objectives:

- Measure the absorption spectrum of chlorophyll extracted from living plant tissue.

Materials:

Elodea, Lettuce, Spinach Leaves or Swiss Chard
Ethanol
Spectrophotometer
Centrifuge
Mortar and pestle
Calibrated test tubes
Cuvettes

Method:

1. Place a generous sample of the dried spinach leaves in a mortar, add 5 to 6 mL of ethanol and grind the leaf material to a fine pulp. The alcohol extracts the chlorophyll from the ground leaf material.
2. Decant some of the ethanol extract into a centrifuge tube. Place the centrifuge tube in one of the slots in the centrifuge head. **NOTE**: it is *very important* that the centrifuge head be balanced because it will be operating at high rpm. Prepare a ballast (balance) tube by filling a second tube with water to a *level equal* to that of your alcohol extract sample and *place the tubes opposite each other* in the centrifuge head.
3. Centrifuge the sample for 5 to 6 minutes to clarify the chlorophyll extract by pelleting the leaf solids.
4. Decant the clarified chlorophyll extract into a Spectrophotometer cuvette. The cuvette is a special tube of high quality optical properties made especially for spectrophotometry. Note the white line near the lip of the cuvette. This serves as a marker to assure correct alignment of the tube while it is in the reading chamber of the spectrophotometer
5. Carefully read the instructions for use of the spectrophotometer (provided at the end of this experiment). Don't hesitate to ask for help from the instructor, especially when you first begin using the instrument. "Zero" the instrument with a *control cuvette* containing *only ethanol* (the solvent used to extract the chlorophyll), followed immediately by *reading the chlorophyll sample*. Record the absorbance from the digital readout on the instrument. Repeat the reading and recording process at 20 nanometer (nm) intervals (see table 1) beginning with 400 nm through 700 nm. Record each reading by its respective wavelength in Table 1 (p. 30).

Results:

1. Plot the absorbance readings (y-axis) by their respective wavelengths. If available, use the lab computer (Excel Program) to build a table and graph your group's results. Make a copy for each student in the group to be included in their lab report.

2. Use the following formulas to *estimate the amounts (mg/L) of each pigment* in your sample you extracted with alcohol:

Chlorophyll a (in mg/L) = 11.85 (abs_{664}) - 1.54(abs_{647})
Chlorophyll b (in mg/L) = 21.03(abs_{647})- 5.43(abs_{664})
Chlorophyll c (in mg/L) = 24.52(abs_{630}) - 7.60(abs_{647})

Table 1. Chlorophyll Extract Absorption Spectrum. Results of readings of the absorption spectrum of the alcohol extractions of pigments from green leaves. ***NOTE**: the instrument must be "zeroed" on the solvent each time a different wavelength is used* (in order to assure accurate readings).

Wavelength (nm)	Absorbance
400	
420	
440	
460	
480	
500	
520	
540	
560	
580	
600	
620	
630	
640	
647	
660	
664	
680	
700	

Experiment IV: Separation of Plant Pigments by Ascending Paper Chromatography

Paper chromatography uses the differences in the *size and solubility* of molecules to separate them for further analysis.

Objectives:

- Demonstrate the use of ascending paper chromatography to separate the different pigments in the alcohol extraction leaves of a representative green plant.

Materials:

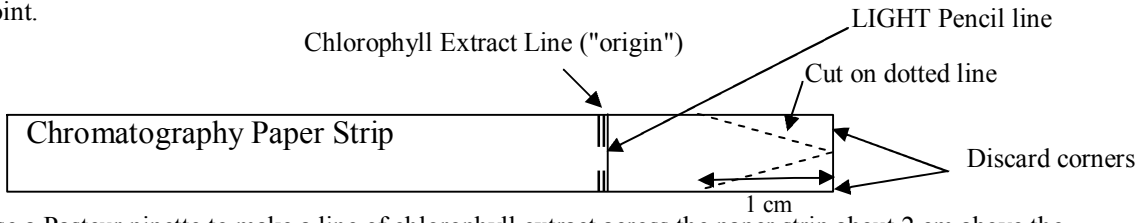
Chlorophyll extract (retained from experiment III)
Bottle with small amount of petroleum ether
Chromatography paper
Scissors
Centimeter ruler
Pasteur pipette

Method:

CAUTION: Volatile flammable liquid. The bottle containing the petroleum ether is to be kept *under the fume hood at all times*. Also, *avoid breathing the fumes.*

Using a lead pencil and a straight edge lightly mark a line across a strip of chromatographic paper 1 cm from the end.

1. Use a pair of scissors to make cuts from the 1 cm mark to the center of the end of the strip, forming a point.



2. Use a Pasteur pipette to make a line of chlorophyll extract across the paper strip about 2 cm above the point. Make the line as straight and uniform as possible. Allow the chlorophyll line to dry (it will dry quickly because of the alcohol solvent evaporation). Repeat the process several times until there is a *significant deposit of pigment* along the line across the paper strip. Handle the chromatography strip by the edges to prevent contamination of the paper with oils from your skin.
3. Place the "loaded" chromatography paper strip into a bottle ("chromatography chamber") containing a small amount of petroleum ether solvent such that only the tip of the point is immersed in the solvent. Bend the top of the paper over the lip of the bottle and lightly cap the bottle to slow the evaporation of the ether and hold the strip in place. *Do not disturb the bottle* while the solvent "migrates" up the paper.
4. *Before the solvent* "migrating front" *reaches the top* of the paper remove the paper strip from the bottle. *Mark the solvent front* (with a light pencil line) at the time of removal of the strip form the solvent chamber. Due to *differences in solubility* the pigment molecules in the alcohol extract from the leaves will *separate out as bands* along the chromatography strip.
5. Outline each colored band with a lead pencil and number the bands from the bottom (origin) up. Record the number and color of each band, respectively.
6. Measure the distance, in centimeters, from the origin at the chlorophyll extract line to the center of each band and record these distances by their respective numbers.
   The pigments separate in a particular order because of differences in their solubility in petroleum ether. The band closest to the origin contains *xanthophylls* (pale yellow), next is *chlorophyll b* followed by *chlorophyll a.* The top band contains *carotenes* (golden yellow).

Results:
How many different bands could you detect in your groups chromatograph? _____.
List the band *numbers* with their respective *migration distances* (in mm) and *colors*

_____
_____

Spectrophotometer Operating Instructions
Spectro Master™ Fisher Scientific
THE SPECTROPHOTOMETER SHOULD BE WARMED UP
FOR AT LEST 30 MINUTES BEFORE USE

If you have any questions do not hesitate to ask your instructor for assistance!

1. The spectrophotometer should be in the "ABS" (absorbance) mode.
2. Locate the wavelength selection knob (wheel-like knob inside the lid behind the sample holder). Turn the wheel to the setting for the wavelength range required for the sample to be measures, e.g. "2" is the proper setting to take an absorbance reading at a wavelength of 400 nm (See the settings and their respective ranges on the Table on the inside of the lid of the sample chamber) e.g. *setting 2* used the filter to measure within the wavelength range of *381 - 481 nm*, *setting 3* is for readings in the range of *482-736* nm, etc).
3. Set the wavelength within the range indicated by turning the *round knob* (on the left of the instrument) until the digital readout (0n the font of the instrument) displays the desired setting.
4. Note: fingerprints interfere with obtaining proper readings. Handle the cuvettes by the lip, wipe the cuvette sides with lens paper before placing it in the holder in the sample chamber. Place the *blank* (alcohol only control) cuvette in the holder with the *white line* on the tube *aligned with the notch* on the tube holder.
5. Press the **middle square button (%T/O A) to "zero" the absorbance**, i.e. to established the pure solvent as the "zero" absorbance reference point for the sample to be measured. Wait until the Lighted digital readout reads "0.000". ***NOTE****: the instrument must be "zeroed" on the solvent each time a different wavelength is used*, in order to assure accurate readings.

6. Remove the blank cuvette and immediately place sample cuvette in the holder aligned as instructed in #4 above. Close the lid.
7. Read the Absorbance Value displayed by the lighted digital readout, and record it in its proper place on Table 1, and remove the sample cuvette from the holder.
8. Repeat steps 2 through 7 for the blank cuvette and the sample cuvette for each of the wavelengths listed in Table 1.