

Photosynthesis Lab

We will be doing 4 main exercises in this lab. It doesn't matter much what order you do them in but keep this in mind:

- Chromatography will take 10-20 minutes while it draws the pigments out of the extract
- The Elodea leaves need to 'sit' for at least 1 hour to produce enough oxygen to measure. So...you may want to tackle these early on.

Objectives:

1. To extract pigments from spinach chloroplasts to a) see the difference in molecular weight between the major pigments and b) see the actual colors *reflected* by these pigments (contrast that to the spectrophotometry where you will be measuring the wavelengths *absorbed*)
2. To become familiar with the techniques of chromatography and using a spectrophotometer.
3. To observe the effect of light intensity on photosynthetic rates
4. To observe the production of oxygen as an indicator of photosynthesis
5. To measure wavelengths absorbed by different pigments in spinach, and to relate that to the chromatography.

Questions, terms and concepts to know:

- Why do we measure the oxygen production?
- What effect does light intensity have on photosynthesis? Why?
- Why does the carotene line migrate up the paper the farthest?
- How do the Chromatography results relate to the spectrophotometer results?
- What are the primary wavelengths of light used by spinach for photosynthesis? How do you know?
- What are the 4 major pigments you were able to identify?
- Know the spectrum of white light and the range of wavelengths
- Know the major wavelengths used in photosynthesis
- Know how to predict the absorption spectrum of a plant if you were given its color

Exercises. Please follow the instructions set out by the Laboratory technician

Work in pairs, and team up (eg. 4 to a team) for the spectrophotometer. All of you should have a chance to use it though!

I. Chromatography to separate pigments (from spinach) by their molecular weight

On the lab bench are strips of chromatography paper, a container of capillary tubes, a mortar and pestle for smashing spinach up, and some spinach leaves, and a chromatography cylinder with a cork stopper. In the bottom of the cylinder is about

1 cm of chromatography solution (acetone-petroleum ether). The cylinder will be under the hood. Please prepare your paper with the spinach but put the apparatus under the hood while it is drawing the pigments out.

****NOTE:** you will need to check your paper frequently to make sure the stopper has not become displaced due to increased pressure in the tube.

- Obtain a strip of the chromatography paper.
- Smash up a bunch of the spinach. You are aiming to get some of the green goo you are able to create. Make as much as possible
- Make a line of extract approximately 2 cm above the pointed end of the paper using a capillary tube to apply the extract. You will need to make several applications so that it is nice and dark, but not soaked and messy
- Dip one end of the capillary tube into the extract.
- Touch the end of the capillary tube to the chromatography paper and drag it across the paper.
- Allow the paper dry slightly and then reapply.
- Repeat this procedure until there is a dark Grass Green line of leaf pigment extract across the paper strip. Usually 10 to 20 applications are needed.
- Attach the non-pointed end of the paper to the clip on the
- cork stopper using the thumb tacks.
- Carefully insert the cork into the cylinder until the tip of the paper touches the chromatography solution. Only 1 cm or less of the paper strip should be covered by the solution. The solution does NOT come all the way to your green line of spinach extract
- The pigment extract line should be ABOVE the solution.
- Leave the paper in the column until the solvent front has moved about 3/4 of the way up the paper strip.
- Remove the strip from the solution. BE SURE TO PUT THE CORK BACK IN THE CYLINDER, and put it back under the hood
- Sketch the paper strip, noting the colors of the bands and the approximate distances between the bands.
- Measure the R_f values (distance of the pigment from the start line divided by the distance of the solvent front

1. How many different types of pigments were in the leaf?

2. What colors were they?

3. What are the names of what you extracted?

4. Which molecule was the smallest?

5. Why did the pigments move at different speeds, causing them to separate?

(Molecular Weight: Ch-a : 892.5; Ch -b : 906.7; Xanth= 568.4; Car = 536.4)

II. Spectrophotometer

You will be using spinach extract and will be measuring the wavelengths of light absorbed by the pigments in the chloroplasts. Follow the instructions next to the machine carefully. Take measurements at the following wavelengths: Start at 400 nm and go all the way through 700 nm. Take a reading every 20 nm, so 400, 420, 440, 460 etc.

- Make an organized chart for your readings
- Graph the results with absorption on the Y axis and wavelength on the X (use your notebooks to make an approximate graph, no need for a computer graph unless you want to)
- 1. Where were the peaks (what wavelengths?)
 2. How does that correspond with the color of spinach (green)?
 3. Relate these results with the chromatography: on one you saw the wavelengths of the colors the pigments *reflected*, on the other you saw the opposite (the wavelengths *absorbed*).
 4. What would the absorption spectrum of a purple plant likely look like? **Keep in mind that purple is a mixture of red and blue!

III. Elodea and oxygen production

We will be looking at the production of oxygen by the aquatic plant elodea. Follow the steps outlined in the lab. Elodea will be immersed in water with bicarbonate (source of CO₂), and it will produce little oxygen bubbles which will float to the top of the test tube and be measureable after a period of time. We'll be putting some in the dark, some in the sun etc. to compare photosynthesis rates.

IV. The effect of light intensity on photosynthetic rate

This is the mystery exercise...We'll be measuring dissolved oxygen (DO) at various distances from a fixed light source to look at light intensity effect on photosynthesis