Lab 7- Photosynthesis

I. PHOTOSYNTHESIS OVERVIEW

- Summary Equation:

$$6\ CO_2 + 12\ H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6\ H_2O + 6\ O_2$$

- Photosynthesis can be divided into two stages:

  (1) **Light-Dependent Phase (=Light Reaction)**- energy is captured from sunlight
  (2) **Light-Independent Phase (=Dark Reaction)**- energy captured during light reaction is used to fix CO_2, converting it into sugars and other compounds

Question:
Name four limiting factors that influence the rate of photosynthesis.

II. PHOTOSYNTHETIC PIGMENTS

- Plants contain <u>pigments</u> (molecules that absorb specific wavelengths of light) to capture sunlight. These include . . .

  (1) **Chlorophyll** a
  (2) **Chlorophyll** b
  (3) **Carotenoids**
  (4) **Xanthophylls**

  } Accessory Pigments (capture different wavelengths of light and pass energy on to chlorophyll a molecules)

- In order to separate and visualize these pigments, one technique known as **paper chromatography** is used.

- In paper chromatography a solution containing several different chemicals with different molecular weights and varying degrees of polarity is absorbed by filter paper.

- The technique utilizes the polar properties of chromatography paper (cellulose) and the nonpolar properties of organic solvents (petroleum ether and acetone) to separate pigments. Each pigment migrates up the paper at different rates depending upon its solubility in the nonpolar solvent and its molecular weight. The smaller and more soluble the pigment, the faster it will migrate up the paper. Conversely, the larger and less soluble pigments will migrate at a slower rate.

Photosynthetic Pigments Exercise

Safety Note: *The solvents used in the lab today are extremely volatile and flammable!!! Do not light a flame in the lab today and avoid breathing fumes as much as possible.*

Procedure:

1. Obtain a test tube and rubber stopper. Cut a piece of chromatography paper so that it fits in the test tube and is ½ inch from the stopper inside the closed tube (see illustration below). [**Caution**: do not touch the surface of the chromatography paper, handle from the edges; oils from your fingers interfere with the process]

2. Cut one end of the chromatography paper to make a point by making two 45° angle cuts.

3. Draw a light pencil line across the chromatography paper 2.5 cm from the pointed end. Next, use the blunt end of a forcep to rub a leaf along the pencil line to transfer the plant extract. Repeat rubbing plant extract onto the chromatography paper14 more times.

4. After you have applied the plant extract, pour a small amount of chromatography solvent (90% petroleum ether, 10% acetone) into your test tube. There should be enough solvent to just submerge the pointed end of the chromatography paper.

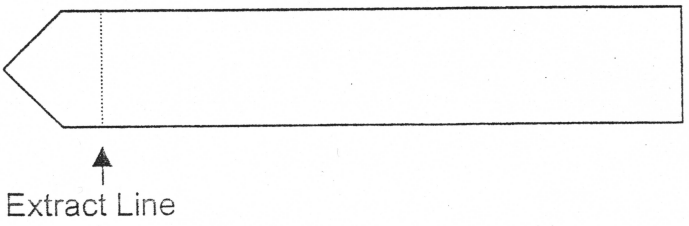
5. Insert the chromatography paper into the test tube containing solvent pointed end down and replace the stopper tightly. Place the test tube upright in a jar, beaker or test tube rack

6. When the solvent front has almost reached the top of the filter paper remove the strip and let it dry. Mark the four pigment fronts and the solvent front.

- **Analysis of Chromatogram**- Identify the positions of the different pigments based upon the information below:

Pigment	Color	Solubility
Chlorophyll a	blue-green	more than Chlorophyll b
Chlorophyll b	pale or olive green	low
Carotenes	yellow	high
Xanthophylls	pale yellow-brown	less than Carotenes

1. Indicate the relative positions of each pigment on the illustrated chromatography paper:



7. Use a metric ruler to measure the average distance traveled by each pigment and the solvent from the point where the pigments were applied to the chromatography paper. Calculate the R_f values (rate of migration) for each pigment using the following formula and enter the values in the table below:

$$R_f = \frac{\text{Distance traveled by pigment}}{\text{Distance traveled by solvent}}$$

Pigment	Pigment Distance	Solvent Distance	R_f Individual	R_f Class (average)

Questions:

1. **Which pigment is least soluble? Which pigment is most soluble? Defend your conclusions.**

2. **Why do leaves of deciduous trees change colors from green to yellows and reds in the fall?**

Staple your chromatogram here (cut in two to share with partner):

'I. PHOTOSYNTHESIS AND CARBON DIOXIDE

- Carbon dioxide is utilized during the **dark** or **light-independent reaction** of photosynthesis.

Uptake of Carbon Dioxide Demonstration

Phenol red is a pH indicator. When a solution containing phenol red is alkaline (pH>7), it is red. When the solution is acidic (pH<7), it is pale yellow. When you take a soda straw and gently blow (not inhale!) into a beaker containing phenol red, the solution eventually turns yellow. The carbon dioxide from your breath dissolves in water, forming carbonic acid (H_2CO_3).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Two beakers containing a dilute phenol red solution indicating an acid condition ($H^+ > OH^-$) were placed under lights. A sprig of *Elodea* was added to one of the tubes. Note any color change.

Question:
Why is there a color change in the test tube with the *Elodea* but not in the control?

.V. STOMATA AND GAS EXCHANGE

- Plants must "breathe" in carbon dioxide to allow the CO_2 to diffuse into photosynthetic cells within the leaf. Likewise oxygen, a byproduct of photosynthesis, will travel from the leaf to the external environment. How do they do this? Plants have small openings on the leaf surface known as **stomata** (**stoma** singular).

- The stoma is bordered by two specialized epidermal cells, known as **guard cells**. Guard cells are the only cells on the epidermis that have chloroplasts. The guard cells act as border guards, allowing gases to freely flow into and out of the leaf when there is sufficient water within the leaf. At certain times of the day or night, or when there is insufficient levels of water inside the leaf, the guard cells will change shape and cause the stoma to close. This is a first line of defense to prevent excessive water loss when the plant is losing more water than it is gaining from the roots.

Epidermal Peel Exercise

Procedure:

1. Make a wet mount of a thin epidermal peel of the plant provided. Fold the leaf and tear away the epidermis. [instructor will illustrate this technique]

2. Mount the leaf so that the outer surface faces up on the slide. [the instructor will illustrate] Using the microscope under high power, view the epidermal peel and notice the pattern of stomata on the epidermis

3. Locate and draw on a separate sheet of unlined paper a stoma under high power and label the following: **epidermal cell, guard cell, chloroplasts, stoma**

Questions:

1. **Are the guard cells surrounding the stoma open or closed shut?**

2. **If they are or were open, what would happen to the guard cells if you added salt water to the wet mount? Would the stoma remain open? [hint: you are creating a hypertonic medium surrounding the guard cells]**

V. STARCH SYNTHESIS

- Starch, a storage form of carbohydrate, is a product of the photosynthesis process. Simple sugars are combined to form this complex carbohydrate.

Coleus Leaf Pigmentation Exercise

Procedure:

1. The previous day a 2 mm wide strip from a leaf of a variegated Coleus (green and white patches) was obtained, including both a green and white area. The cut strips were placed in iodine overnight.

2. Remove a strip and drain off excess iodine; make a wet mount and observe the stain pattern under the compound microscope.

Question:

Starch granules stain a dark color. From which region of the leaf, the green or white region, did you observe the presence of starch? Why?