Part 1: Analysis of Plant Pigments

Objectives:
1. Learn about chromatography and how it works
2. Analyze a solution of plant pigments by paper chromatography.
3. Compare the chromatograms prepared by the class by using Rf measurements.

Materials:

Plant	test tube rack	ruler
solvent mixture	paperclip	scissors
test tube	strip of filter paper 1	paper towel
	cork stopper	pencil

Procedures:

1. Make sure your hands are clean before touching the chromatography paper strip. Cut one end of the strip to a point 1 cm long. Then make a small pencil dot 3 cm j from the tip of the point. Be careful not to bend the paper.
2. With a paperclip attach the straight end of the strip to the center of the bottom of the j stopper. Adjust the length of the strip so that the pointed end just reaches the bottom of the test tube when the stopper is in place. Make sure that the pointed end hangs free and is not bent of touching the sides of the test tube at any point.
3. Carefully lay the cork with the attached paper strip on a paper towel. Keep the strip clean and unbent.
4. Measure and mark 1.5 cm from the round end of the test tube. Put test tube in the test tube rack.
5. Apply plant pigment to strip as demonstrated by instructor.
6. Obtain solvent mixture and pour solvent to your 1.5 cm mark in your test tube - try not to let any solvent hit the sides of the test tube. Place test tube back in the test tube rack so that it stands upright.
7. Carefully lower the pointed end of the chromatography strip into the test tube until the cork is in place. Check these points: (1) the point of the paper is not bent; (2) the surface of your solvent does not reach the pigment dots; (3) The sides of the strip do , not touch the sides of the test tube; (4) the cork is snugly in place; (5) the test tube is upright. I

   Record the time.
8. Do not disturb the test tube while the solvent is moving up the paper. Observe the progress of the solvent.
9, When the solvent front is about 2 cm from the top of the strip, remove the cork and strip from the tube.

   Record the time

Table 1. Chromatography of Plant Pigments

Position of Band	Color	Plant Pigment
Top	Yellow-orange	Carotene
Second (from top)	Yellow	Xanthophyll
Third	Blue-Green	Chlorophyll a
Fourth (closest to origin)	Yellow-green	Chlorophyll b

Rf **values of Plant Pi ments** SAMPLE DATA
Pigment Distance Solvent Distance Pigments R, value Traveled cm Traveled (cm)
Carot 7.6 8 7.6/8 = .95 Xanth 7.0 8 7.0/8 = .88 Chloro 4.8 8 4.8/8 = .60
Chloro ' 1.7 8 1.7/8 = .21

10. The strip will dry quickly. With a pencil, immediately mark the line of the solvent front on the chromatogram. Also mark the top of each of the colored bands, since the color may fade.

11. Tape your chromatogram to a piece of white paper. Label each colored band with the correct pigment name. Then fill in the solvent used and the time required for the run at the bottom of the page.
12. Measure the distance (in cm) from the pencil dot at the bottom of the strip to the line of the solvent front at the top. *Record this distance in* a *table*
13. Measure the distance that each pigment traveled, from the starting point to the top of each pigment band *Record in table*
14. Study the formula for the Rf value shown in Table 2. Then calculate the Rf values for each pigment, rounding off the quotients to 2 decimal places. *Record in table*
15. Record your Rf values on the board and copy the class values into a class data table.

Data:

Group Data Table: solvent front distance, pigment distance, pigment description, Rf values for each pigment

Class Data Table: Rf values for each pigment for each group & average .

Data Analysis:

Group & Class Rf values vs. pigment

Sample Calculation

Conclusion:

1. Why was it necessary to apply a concentrated amount of pigment to the strip?
2. Why should the paper strip not touch the sides of the test tube during a solvent run? Why should it not be bent?
3. Which pigment traveled the fastest on the paper? How do you know?
4. Were your Rf values similar to those in the class? How can you explain any differences?
5. Explain the separation process involved in this chromatography lab. Which pigment was the most nonpolar? How could you tell?
7. What is the relationship between the size of the pigment band and the amount of pigment in the plant?
8. What pigments give leaves their green color? What happens in the autumn when leaves turn yellow or orange?
9. Which part of photosynthesis do the pigments play a role in? Describe the overall "goal" of this part of photosynthesis.
10. What is the other part of photosynthesis named and describe the overall "goal" of this part.

Part 2: Role of Light in Photosynthesis

Problem: Are light and chlorophyll necessary in photosynthesis?

Materials:

- Geranium plant kept in light
- Geranium plant kept in dark
- Alcohol
- Iodine
- 400 ml beaker
- 100 ml beaker
- tripod stand
- asbestos wire gauze
- burner

Procedure:

1. Place asbestos wire gauze on tripod stand and burner under it.
2. Fill a 400 ml beaker 3/4 full of water and place on stand. Light the burner and boil water.
3. Remove a leaf from the Geranium plant kept in the light and place it in the beaker of boiling water for 1 minute.
4. Fill a 100 ml beaker 3/4 full of alcohol and place the boiled leaf inside. Then place the beaker of alcohol inside the beaker of water. (This is a water bath.)
5. Boil the leaf in the alcohol for 3 to 5 minutes.
6. Removed the leaf and place it in the Petri dish. Cover the leaf with water and add 5 to 8 drops of iodine.
7. Record your results in the table below.
8. Repeat the procedure with a leaf from the Geranium plant kept in the dark

Data: Draw the leaves below using colored pencils.

Original Leaf	Stained Leaf from Plant Kept in Light	Stained Leaf from Plant Kept in Dark

Conclusion:

1. How much starch was stored in the dark-adapted leaf relative to the light-adapted leaf?
2. Write a conclusion about the relationship between chlorophyll and photosynthesis. Describe the relationship. Tell what supports your conclusion.
3. Write a conclusion about the relationship between light and photosynthesis. Describe the relationship. Tell what data supports your conclusion

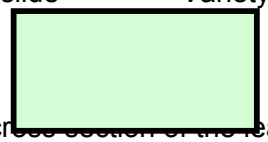
Part 3: Plant Leaf Morphology

Problem:

1. What parts of a leaf play a role in photosynthesis?
2. What adaptations do plants have that help them survive in an arid environment? Why is this important?

Materials:

Leaf cross section microscope slide Variety of live plants
Compound Microscope



Procedure:

1. Draw your observations of the cross section of the leaf under the microscope under low and medium power. On one of the diagrams label the following parts and the role of each part: cuticle, epidermis, mesophyll, stoma, gurad cells, vascular bundles
2. Walk around the school and draw three different plants, including a close up drawing of the leaf for each plant. Write down adaptations this plant exhibits in order to survive in an arid environment.

Data:

1. Microscope drawings
2. Plant drawings

Conclusion:

1. Overall, what did you learn from this section of the lab.