Paper Chromatography of Pigments in Chloroplasts

Objective
To use paper chromatography to observe the pigments in a solution of chloroplast (chlorophyll) extract.

Background Information
Chloroplasts contain several pigments. In terrestrial plants, chlorophylls a and b are usually found, along with carotenes and xanthophylls. In chromatography these pigments can be separated from one another based on their differing degrees of solubility in the chromatography solvent. A pigment that is very soluble will be moved higher on the chromatography paper.

Procedure
Part I: Leaf Pigment Preparation

1. Obtain a leaf from the desired plant and grind it using a mortar and pestle. A few grains of sand can be added to facilitate grinding. Add 2 mL of ethyl alcohol to extract the pigments from the leaf.
2. Continue grinding until you have several drops of dark green liquid. It might be necessary to add a little more alcohol.

Part II Chromatogram Preparation:

3. Obtain a strip of chromatography paper which is 1/6 of a standard strip. Cut one end of the strip to form a taper.
4. Using a PENCIL, draw a line across the paper approximately 2 cm from the tapered tip. This is your baseline.
5. Using a capillary tube, apply a small dot of the pigment extract at the center of the line. Let dry and repeat four or five times. You should have a small, very dark spot of pigment. If the spot is not dark, apply more extract.
6. Insert a paper clip hook into the bottom of a cork which will fit into a large test tube.
7. Pour the required amount of acetone into a large test tube so that the top of the solvent is above the tip of the paper but below the baseline. Attach a paper clip hook to the other, non-tapered end of the paper. When the cork is in place, the tip of the paper should be below the acetone. Hold your tube very still while proceeding.
8. Without moving or shaking the tube, attach the test tube to a clamp stand.
9. Allow the chromatogram to run until the solvent almost reaches the top of the paper strip.
10. Remove the paper and use a pencil to mark a line at the highest point the solvent reached. This is the solvent front.
11. Examine the chromatogram for the presence of different bands of color. Each color band is a different pigment. Chlorophyll a appears blue-green, chlorophyll b appears yellow-green, carotene appears bright yellow, and xanthophyll appears pale yellow-green. You may not see all of these bands.
12. Label your chromatogram and save it to attach to your lab report
13. Repeat the procedure using water in the test tube instead of acetone.

Discussion Questions (include these in your discussion)

1 What is the value of chromatography?
2 Which pigments are present in the smallest amounts in the leaf?
3 Which pigments are present in the greatest amount?
4 Many leaves change color in the fall. Explain. (Hint:
chlorophyll a and b are easily broken down in the cooler autumn temperatures.)
5 a) What is the role of chlorophyll a? b) What are the roles of carotene and xanthophyll?

(1) Which pigments were soluble in water?

(2) Which pigments were soluble in acetone?

(3) Which pigments were found in both red and green leaves?

(4) With what you have discovered about pigments, what conclusions can you make regarding the changing color of leaves in autumn?

Part 2 - Comparison of Pigment Amounts Due to Light

1. In part 2 of this activity, you will have the opportunity to compare pigment amounts. To do this, it is necessary to prepare plant pigment extracts with equal amounts of plant material in equal amounts of acetone. Use 3 grams of ivy leaf material in 10 ml acetone. Because ivy leaves are tough, they need to be ground in a mortar with clean sand and acetone. Two chromatograms will be made this time.

(1) Place 3 grams of full-sunlight ivy leaves in a mortar, sprinkle a little sand over the leaves and grind with a pestle while slowly adding the 10 mL acetone. Let the solution settle and remove any small particles.

(2) Repeat the above process with the same amount of shade-grown ivy leaf material.

(3) Write "light" on one strip of filter paper, and "shade" on the other.

(4) Use the same technique for placing extract on the paper strips as you used in part 1 of this activity.

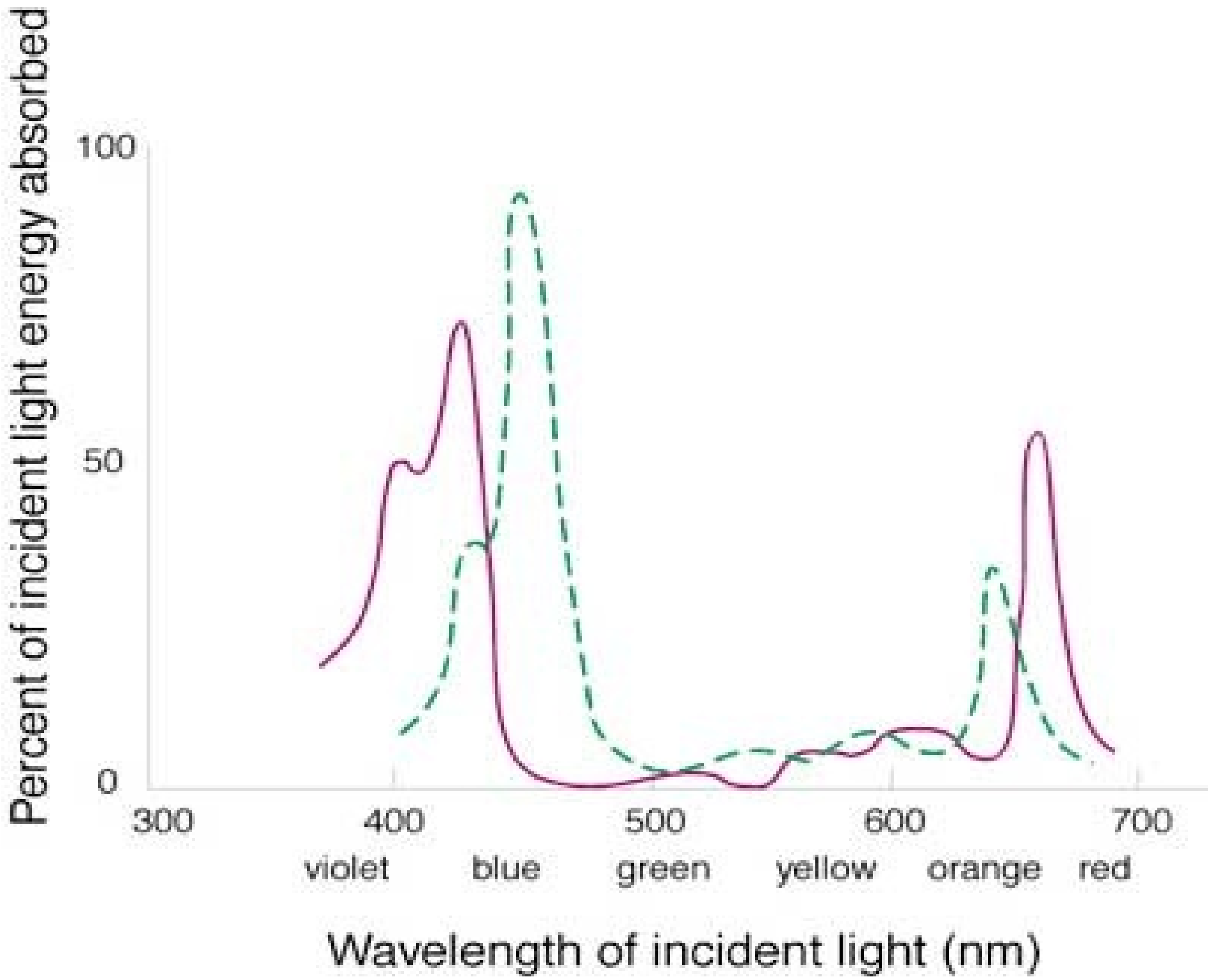
(5) Place both chromatograms in a developing tank with acetone and allow to develop for about 30 minutes.

Questions/conclusions:

1) Do the leaves grown in different amounts of light have the same kinds of chlorophyll? How can you determine this?

2) Do the leaves grown in light and grown in shade have the same amounts of each pigment? How can you determine this?

3) As you read the graph of the absorption spectrum for chlorophyll, which of the colors in the visible spectrum is seen the least when looking at reflected light from a green leaf?



4) What is the approximate wavelength of the color you identified in question 1?

5) What percent of light energy absorbed corresponds to the peak of the color you identified in question 1?

6) How much of the color you identified in question 1 is being reflected ?

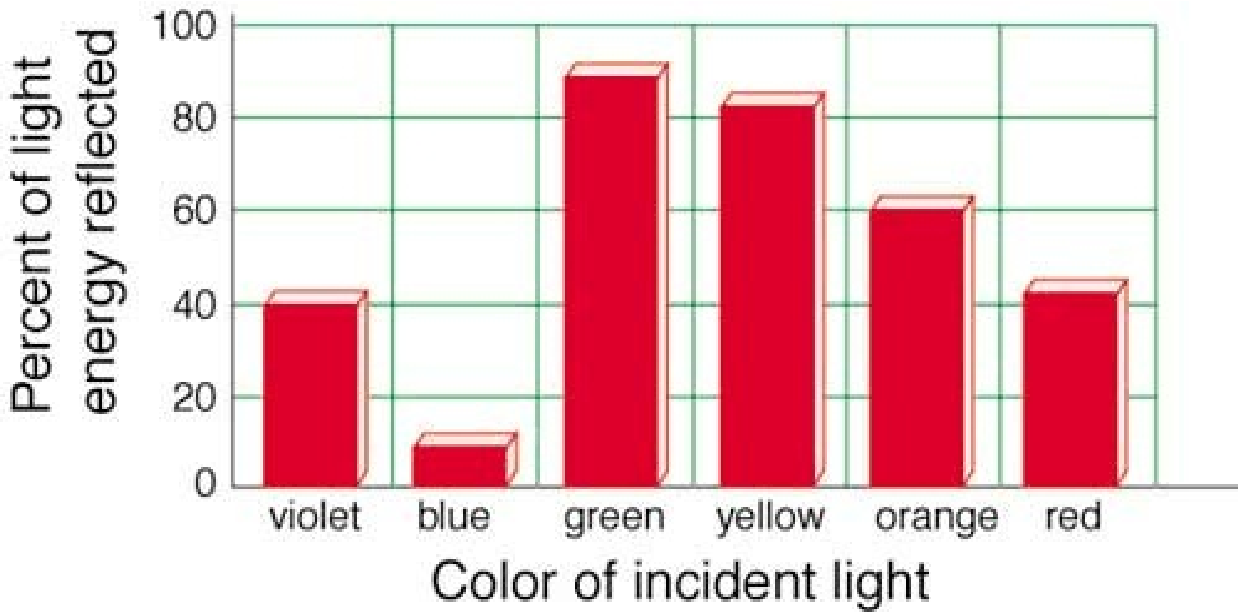
7) What percent of light energy absorbed by chlorophyll b does the orange spectrum peak represent?

8) Why are there no peaks in the range between 500 nm and 610 nm?

9) Are you able to see the light in the green-yellow portion of the spectrum? If so, how is this possible?

10) Arrange the colors in the absorption spectrum of chlorophyll a and b in order of their visibility. Place the most visible color first.

The bar graph in Figure 4 presents the opposite data compared to that presented in the previous graph. This graph shows the percentage of *reflection*, as opposed to *absorption.*



1) Referring to Figure 4, which color in this spectrum is most visible in the light reflected from a green leaf?

(2) What is the approximate percentage of the light energy reflected for the color you just named?

(3) What percentage of light energy absorbed by the color you just named does this represent?

(4) If everything above 50% of light energy reflected is visible to the human, is red light part of the mixture of colors seen in light reflected by chlorophyll?