Chapter 8 **Photosynthesis** **Name ________________**

Lab Analysis of Plant Pigments

Background

The leaves of most plants contain many different pigments, or light-absorbing compounds. Chlorophyll, the main pigment of green plants, is needed for photosynthesis. The primary purpose of chlorophyll is to capture light energy, which is converted to chemical energy during photosynthesis. Chlorophyll is the most abundant and important photosynthetic plant pigment and exists in several forms, including *chlorophyll a* and *chlorophyll b.* Both chlorophylls absorb blue and red light and reflect green light.

Chlorophyll often hides the other pigments present in leaves. In autumn, chlorophyll breaks down, allowing *xanthophyll*, which reflects yellow light, and *carotene*, which reflects orange light, to show their colors. Other pigments may also be present in leaves.

The individual pigments in a mixture of pigments from a leaf may be separated by the technique of paper chromatography. *Chromatography* means color writing. The separation takes place by *absorption* and *capillarity.* The paper holds substances by absorption. Capillarity pulls the substances up the paper at different rates. Pigments are separated on the paper and show up as colored streaks. The pattern of separated components on the paper is called a *chromatogram*.

The relative rate of migration, the R_f value, for each pigment can be determined from the chromatogram. The R_f value is the ratio of the distance a pigment moved on the chromatogram to the distance the solvent front moved.

Materials (per group of two)

Spinach leaf	Solvent mixture (9 parts petroleum ether and 1 part acetone)
Paper towel	Pencil
Test tube (25 mm x 150 mm	Ruler
Cork or Rubber stopper with paper clip	Glass marking pencil
Test tube rack	Scissors
Transparent tape	Chromatography paper (20 mm x 150 mm)
Pasteur pipette	Coin

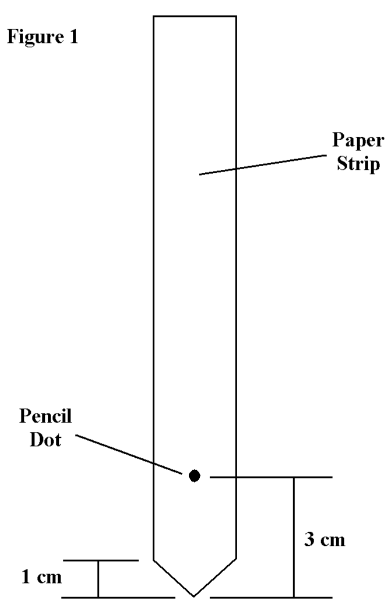
Procedure

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. See Figure 1. Then make a small pencil dot 3 cm from the tip of the point. Be careful not to bend the paper.

2. Attach the straight end of the paper strip to the paper clip located on the bottom of the stopper. Adjust the length of the strip so that the pointed end just reaches, but does **not** touch, the bottom of the test tube when the stopper is in place. See Figure 2 on the next page. Make sure that the pointed tip hangs free and is not bent. Also be sure that the strip does **not** touch the sides of the test tube at any point. This step is called the “dry fit”.

3. Carefully lay the stopper with the attached paper strip on a paper towel. Keep the strip clean and unbent.

Figure 1



4. Measure 1.5 cm from the round end of the test tube. Mark this distance on the test tube with a glass-marking pencil. See Figure 2. Then set the test tube in the test tube rack.

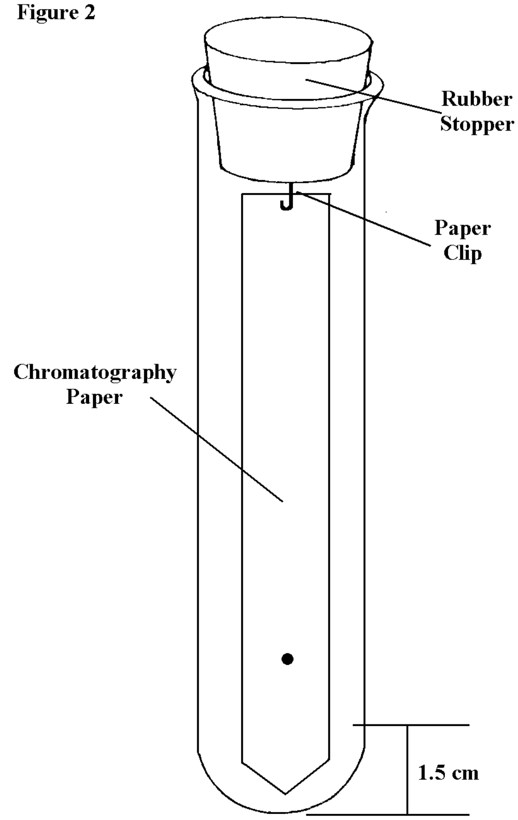
5. Use the "coin rolling technique" to apply a grass stain on the chromatography paper. Your teacher will explain the correct method for doing this process.

6. With the same care and without making the stain much larger, add another layer of grass stain directly on top of the first stain. Allow the grass stain to dry. Repeat step 5 at least four more times being sure to allow the stain to dry completely before adding another layer.

7. Examine the grass stain. If it looks very green, you have added enough pigment. If it still looks light green, repeat step 5 a few more times.

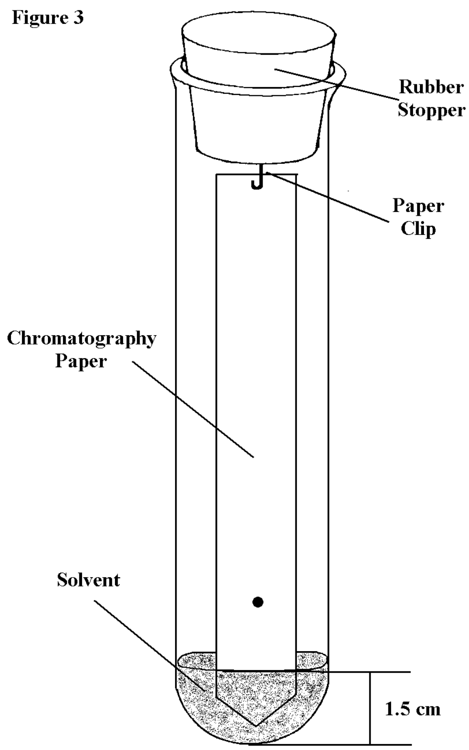
8. Obtain some chromatography solution from your teacher. Be careful not to breathe it in. Use a medicine dropper and add the solvent mixture to the level of the mark you made near the bottom of the test tube. Then place the test tube in the test tube rack so that it will stand upright.

Figure 2



9. Carefully lower the pointed end of the chromatography strip into the solvent mixture until the stopper is loosely in place. See Figure 3. **Check these points**: (1) the point of the paper strip is **not** bent; (2) the surface of the solvent does **not** reach the grass stain; (3) the edges of the strip do **not** touch the sides of the test tube; (4) the cork is **not** snugly in place; (5) the test tube is upright.

Figure 3



10. Do not disturb the test tube while the solvent is moving up the paper. Observe the progress of the solvent. As the solvent moves up through the paper, the paper will appear darker due to the moisture in it. As the chromatography fluid moves up the paper, the dissolved pigments will begin to move upward also. The leading line of the solvent is called the *solvent front*.

11. When the solvent front is about 2 cm from the top of the paper strip, remove the stopper and chromatography paper from the test tube.

12. Remove the paper strip from the stopper and immediately stopper the test tube again, to prevent the escape of fumes. Set the test tube back in the test tube rack. Hold the paper strip in the air until it dries completely.

13. With a pencil, immediately mark the line of the solvent front on the chromatogram. Also mark the top of each of the colored pigment bands, since the colors may fade. See Figure 4 in Observations.

Observations

1. How many colored bands are on the chromatogram?

2. Which plant pigment traveled the farthest?

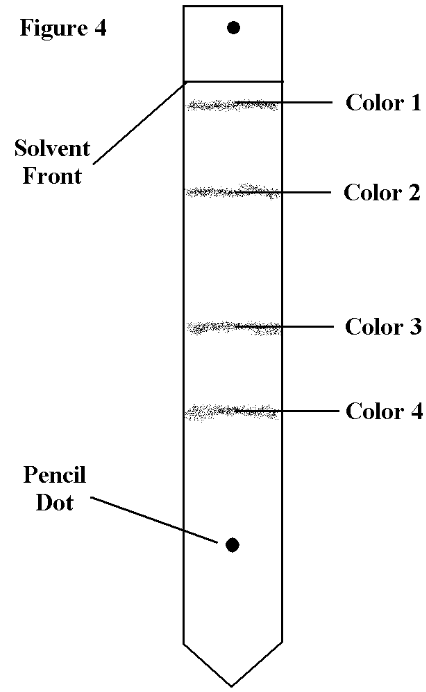
3. Did you see this pigment in the original spinach leaf?

4. Which pigment traveled the next farthest?

5. Did you see this pigment in the original spinach leaf?

6. Which color band seems to be the most abundant on the surface of the chromatogram?

Figure 4



7. The analysis of plant pigments by paper chromatography has been done many times. The four plant pigments that separate out when this solvent is used are given in Table 1. Compare your results with the experimental results shown in Table 1.

Table 1 Chromatography of Plant Pigments

Position of the Band	Color	Plant Pigment	R_f Value
Top	Yellow-orange	Carotene	.96
Second	Yellow	Xanthophyll	.72
Third	Blue-green	Chlorophyll *a*	.49
Bottom	Yellow-green	Chlorophyll *b*	.36

8. Tape your chromatogram to the bottom of this page in the space provided. Label each pigment band, the solvent front, and the beginning point of the grass stain.

Tape Chromatogram Here

9 The rate of' flow, the R_f value , can be calculated for each of the pigments. This number allows the comparison of experimental results. The R_f value is a fraction that indicates how far the pigment moved compared with how far the solvent moved. 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 the proper space in Table 2.

10. Measure the distance in cm that each pigment traveled, from the starting point in line with the pencil dot at the bottom of the strip to the top of each pigment band. Record these four numbers in the proper spaces in Table 2.

11. Study the formula for the R_f value shown below. Then calculate the R_f value for each pigment, rounding off the quotients to 2 decimal places. Record the R_f value for each pigment in Table 2.

$$R_f \text{ value} = \frac{\text{distance pigment traveled}}{\text{distance solvent traveled}}$$

Table 2 R_f Values of Plant Pigments

Pigment	Distance Solvent Traveled (cm)	Distance Pigment Traveled (cm)	Your R_f value
carotene	__________ cm	__________ cm	__________
xanthophyll	"	__________ cm	__________
chlorophyll *a*	"	__________ cm	__________
chlorophyll *b*	"	__________ cm	__________

12. Record your R_f value on the board. Then copy the class values to Table 3 below.

Table 3 Class Averages

R_f for Carotene	R_f for Xanthophyll	R_f for Chlorophyll a	R_f for Chlorophyll b
1. __________	1. __________	1. __________	1. __________
2. __________	2. __________	2. __________	2. __________
3. __________	3. __________	3. __________	3. __________
4. __________	4. __________	4. __________	4. __________
5. __________	5. __________	5. __________	5. __________
6. __________	6. __________	6. __________	6. __________
7. __________	7. __________	7. __________	7. __________
8. __________	8. __________	8. __________	8. __________
9. __________	9. __________	9. __________	9. __________
10. __________	10. __________	10. __________	10. __________
11. __________	11. __________	11. __________	11. __________
12. __________	12. __________	12. __________	12. __________
Total = __________	Total = __________	Total = __________	Total = __________
Mean = __________	Mean = __________	Mean = __________	Mean = __________

13. Find the mean value for each pigment's R_f value. How do they compare to the ideal R_f values on page 3?

Analysis and Conclusion

1. Why did you add more than one layer of grass stain to the chromatography paper?

2. Which pigment traveled the fastest on the paper and how do you know?

3. Why were the four plant pigments deposited on the chromatography paper at different distances from the starting point?

4. What is the relationship between the size of the pigment band and the amount of pigment in the spinach leaf?

5. What pigment gives leaves their green color?

6. What happens in the fall when leaves turn yellow, orange, or brown?

7. Which of the chlorophyll forms is more soluble in this solvent? How do you know?

8. Some leaves turn red or blue in the fall. Find out the name for the plant pigment responsible for these beautiful colors.