Name:_____

Date:_____ Period:_____

Biology 10

Paper Chromatography Lab

Introduction:

As we've discussed in class, plants use their specialized pigments to absorb light energy. This absorbed energy is used to power the reactions of photosynthesis. There are several pigments found in plants, including chlorophyll *a*, chlorophyll *b*, xanthophylls, and other carotenoids. Chlorophyll is the primary pigment, it absorbs light in the red and blue/violet spectrum. The xanthophylls and carotenoid pigments are used to absorb additional colors of light, and then pass that energy on to the chlorophylls.

In this paper chromatography lab, you will use an alcohol solvent to dissolve the various pigments from a leaf of spinach. A piece of filter paper is then placed in the pigment solution, which then absorbs the alcohol. As the alcohol migrates up the piece of filter paper, it carries the pigment molecules with it. The different pigments are carried up to different heights on the paper, based on the size of the pigment molecule. Smaller molecules are carried higher than larger molecules. This allows us to separate the different pigments found in a leaf in order to see what is in there. Chlorophyll *a* usually shows up as a bright green/blue green band. Chlorophyll *b* shows up as a yellow-green/olive green band. Carotene is generally orange/yellow, and xanthophylls are typically yellow.

Materials

Coffee filter
Spinach leaves
Beaker
Plastic wrap
Rubbing alcohol
Pencil

Pre-Lab Questions

1) What pigments are found in plant leaves? _____

_____

_____

2) What is the role of the different pigments in leaves? _____

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3) How does paper chromatography work? How are pigments separated? _____

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_____

4) What are some safety hazards associated with this lab? _____

Procedure:

1. Take 2-3 large leaves (or the equivalent with smaller leaves), tear them into tiny pieces, and place them into a 400 mL beaker.

2. Add enough alcohol to just cover the leaves.

3. Loosely cover the beaker with plastic wrap and set it into a shallow pan containing an inch or so of hot tap water.

4. Let the beaker sit in the hot water for at least 15 minutes. Replace the hot water as it cools and swirl the beakers from time to time.

5. The beakers are 'done' when the alcohol has picked up color from the leaves. The darker the color, the brighter the chromatogram will be.

6. Cut or tear a long strip of coffee filter paper for your beaker.

7. Place one strip of paper into the beaker, with one end in the alcohol and the other outside of the jar.

8. As the alcohol evaporates, it will pull the pigment up the paper, separating pigments according to size (largest will move the shortest distance).

9. After 30-40 minutes (or until the desired separation is obtained), remove the strips of paper and allow them to dry.

10. Can you identify which pigments are present?

Conclusion Questions:

1) Make a sketch of the filter paper below, labeling the pigment lines you found.

2) Why do the pigment molecules separate during chromatography?

3) List the pigments you found in order from the least-farthest moving to the most farthest moving.

4) Based on the distance traveled by the pigments, list them in order from the smallest molecules to the largest molecules.

5) Why do leaves change color in the autumn?

6) What adaptive purpose do different colored pigments serve in the leaf?

7) How do you think the results would have changed if the spinach leaves had been kept in the dark for 5 days before the experiment?