Name _____

Paper Chromatography of Spinach Leaves
Background Information:
Paper chromatography is a process that uses special filter paper to separate and identify the different substances in a mixture. Chromatography means "to write with color." The substances in the mixture dissolve in the alcohol and move up the paper. The heavier substances move up the paper more slowly. The lighter substances move up the paper more quickly. So heavy and light substances get separated from one another on the paper. Plants contain chlorophyll, a green pigment, as well as carotenoids, pigments that range in color from red to orange to yellow.

Question: Do green leaves also contain other pigments?

Hypothesis: Record on the Analysis Sheet.

Materials:

Isopropyl alcohol	ruler	colored pencils
spinach leaf	scissors	beaker
chromatography paper	pencil	tape
paperclip		

Procedure:

1. Obtain a strip of chromatography paper.
2. Use a ruler to measure and draw a light pencil line 2-cm above the bottom of the paper strip.
3. Here is the tricky part! Place the edge of the spinach leaf over the pencil line and using the edge of a coin gently press on the spinach leaf to create a single green line over the pencil line. You want this line to be thin and concentrated with the pigment from the spinach leaf. Therefore, repeat this edging process carefully about 3-4 times. Be sure not to press too hard or you will poke a hole through the paper.
4. Tape the top of the paper strip to a pencil so that the end of the strip with the green line hangs down. The pencil should be able to sit across the top of the beaker with the bottom of the paper strip just touching the bottom of the beaker. Cut off any excess paper from the TOP of the strip if it is too long.
5. Remove the pencil/paper strip contraption from the beaker for the moment. Record observations in data table.
6. Carefully add isopropyl alcohol to the beaker until it reaches a depth of 1-cm in the beaker.
7. Lay the pencil across the top of the beaker with the paper strip extending into the alcohol. MAKE SURE THAT THE LEVEL OF THE ALCOHOL IS BELOW THE GREEN LINE ON YOUR PAPER STRIP! IF THE ALCOHOL IS GOING TO COVER THE GREEN LINE, POUR OUT SOME ALCOHOL BEFORE YOU GET THE GREEN LINE WET!
8. Observe as the alcohol gets absorbed and travels up the paper by capillary action. This may take up to 20 minutes. Do not touch your experiment during this time.

9. When the alcohol has absorbed to approximately 1-cm below the pencil, you may remove the pencil/paper strip from the beaker to dry on your counter. With a pencil, mark the distance the alcohol has traveled on the paper, as well as the distance each pigment has traveled.

10. Using colored pencils, draw your results in the data table.

11. Using a ruler and the following formula, measure the Rf values of each pigment. Since the fastest molecules will travel the greatest distance, or to the highest point along the strip, the relative distances can be measured, and the flow rate (migration) of the molecules (Rf) can be calculated by using the following formula:

$$Rf = \frac{\text{Distance pigment traveled}}{\text{Distance solvent traveled}}$$

Pre-lab Stuff:

Question: Do green leaves also contain other pigments?

Hypothesis: Propose your hypothesis knowing what you do about autumns in New England. Remember to use a measurable hypothesis.

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Data:

Filter Paper	Use colored pencils to draw your observations
Before Paper Chromatography	
After Paper Chromatography	

Distance Alcohol Traveled: _____ mm

Color of Pigment	Distance Traveled (mm)	R_f value

Analysis questions. Use your data to support all answers.

1. Did the leaf you test contain different pigments? Refer to your results to support your answer.

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2. Why did the separation of pigments in the spinach extract occur as it did? (i.e. How does paper chromatography work?)

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3. Why is it that light in the green portion of the area between 530 and 580 nm does not have any absorption?

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4. What would you conclude about the molecules of chlorophyll a, chlorophyll b, xanthophyll (a carotenoid), and carotene? Why do certain fronts travel further than others?

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5. Do a scientific inquiry about the functions of the 4 pigments you observed migrate along the chromatography paper. What are their specific functions and if photosynthetic pigments, what wavelengths of light do they absorb? _____

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6. What role does the solvent play in the migration of pigments? How do you think it does this (think about our properties of water lessons)?

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7. Based on what you have learned in this lab, explain why leaves tend to change color in autumn in New England given what you now know from this lab investigation.

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