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***Lab: Pigment Chromatography***

**Introduction:**

Visible light (or white light) is composed of many different colors or wavelengths. Plants use only a small portion of the visible light spectrum for photosynthesis. Their leaves contain pigments, which are molecules that absorb some wavelengths of light while reflecting other wavelengths. Photosynthetic pigments absorb these wavelengths of light and transfer this captured light energy to the reactions of photosynthesis and to the making of ATP. In this lab, you will use a technique called “chromatography” to separate the different pigments found in leaves.

Paper chromatography separates the pigments found in leaves based on the different chemical and physical properties of these pigments. When chromatography paper is placed in a liquid (solvent), the liquid will be absorbed by the paper and will migrate upward until the paper is saturated. If a material that is soluble in the liquid is placed on the paper, the material will dissolve as the liquid front moves through it. If the material placed on the paper is a mixture of substances, some of these substances are likely to be more soluble in the solvent than are others. A pigment that is more soluble in the solvent being used will move up the paper faster and to a greater distance than a pigment that is less soluble in the solvent being used. Molecular weight also plays a part in determining the separation of the components of a mixture. The lighter the molecular weight, the higher that substance will move up the paper, provided that it is also soluble in the solvent being used.

In this lab, R**f**  (Rate of flow) values will be calculated. The R**f** value represents the ratio of the distance the pigment traveled compared to the distance traveled by the solvent. Each plant pigment has a distinct R**f** value that can be used to identify it.

**Materials:** Mortar and Pestle Acetone

Fresh leaves Chromatography paper

Capillary tubing 25 x 200 test tube

#4 or #5 rubber stopper to fit test tubes Petroleum Ether

Ruler Calculator

Sand Test tube rack

**Safety Precautions:**

1. Safety goggles and laboratory aprons are required for this lab.

2. Both acetone and petroleum ether are flammable and are skin irritants. If there is a spill, notify the instructor.

3. Acetone and petroleum ether should only be used in well-ventilated areas.

**Procedure:**

1. Obtain several fresh leaves. Tear them into small pieces and place them in the mortar. Cover with a pinch of sand. Pour enough acetone into the mortar to just cover the leaves. Grind the mixture until all the color seems to have left the leaf, or until the acetone is dark green.

2. Obtain a piece of chromatography paper. Handle the paper by the edges since the smallest amount of oil from your skin will affect the results.

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3. Using a piece of capillary tubing, place a drop of chlorophyll extract on the paper about 1 cm from the bottom. Let the spot dry before applying another drop on the same spot. Repeat the procedure, drop by drop, until the spot is a dark green.

4. Place the paper in a large (25 x 200 mm) test tube. The bottom of the paper should be in solvent, but the green dot on the chromatography paper should be above the solvent. Place a rubber stopper in the test tube.

**DO NOT BREATHE THE PETROLEUM ETHER IN THE TEST TUBE.**

5. Allow the solvent to rise on the paper as far as it will go. This usually takes 20-30 minutes. Remove the paper from the tube and immediately replace the stopper. Using a pencil, quickly mark on the paper the level to which the solvent has risen.

6. Repeat the above procedure until three different leaves have been used.

7. In the space below, attach your three chromatograms with a staple or piece of tape. Label each of the bands of color with the name of the pigment. It may be helpful to turn your paper sideways as you staple the chromatograms to this page. Measure and record the distance moved by each pigment in the data tables below.

8. Measure the distance (in mm) traveled by the solvent Measure the distance (in mm) that each pigment traveled from the point of application to it highest point on the paper. Record your results in the data tables below. Calculate the R**f** value for each pigment.

Rate of Flow (R**f** value) = Distance moved by pigment (mm)

Distance moved by solvent (mm)

**Data Tables:**

|  |  |  |
| --- | --- | --- |
| **Pigments from Leaf #1** | **Distance Moved** | **Rf (Rate of flow) Value** |
| Solvent |  |  |
| Carotene (orange) |  |  |
| Xanthophyll (yellow) |  |  |
| Chlorophyll a (blue-green) |  |  |
| Chlorophyll b (yellow- green) |  |  |

|  |  |  |
| --- | --- | --- |
| **Pigments from Leaf #2** | **Distance Moved** | **Rf (Rate of flow) Value** |
| Solvent |  |  |
| Carotene (orange) |  |  |
| Xanthophyll (yellow) |  |  |
| Chlorophyll a (blue-green) |  |  |
| Chlorophyll b (yellow- green) |  |  |

|  |  |  |
| --- | --- | --- |
| **Pigments from Leaf #3** | **Distance Moved** | **Rf (Rate of flow) Value** |
| Solvent |  |  |
| Carotene (orange) |  |  |
| Xanthophyll (yellow) |  |  |
| Chlorophyll a (blue-green) |  |  |
| Chlorophyll b (yellow- green) |  |  |

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**Observations:**

1. What is another name for white light? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. What portion of the electromagnetic spectrum can be utilized by living organisms? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3. Was there any evidence that there was more than one pigment in the leaf **before** making the chromatogram? Explain. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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4. What is chromatography? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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5. Compare your chromatogram to another person in your class. Are the same pigments at about the same level on the chromatogram for the same leaf? Why should they be? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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6. Why did you have to make sure the spot you applied was above the level of the solvent when placing the chromatography paper in the test tube? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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7. Which pigment could you see in the leaf **before** it was ground up? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

8. What additional pigments showed up on the chromatogram? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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9. Why did you not see these other pigments in the whole leaf? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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10. Since only the chlorophylls are primarily used in photosynthesis, what do you suppose the other pigments are used for? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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11. Explain why the different pigments separated out where they did on the paper. Please give two reasons.

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12. Which of the pigments is most soluble in the solvent used? Which is the least soluble? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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13. If you had used a different solvent, would the pigments have appeared in the same order? Explain.

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14. Do you suppose that you have isolated every pigment from the leaf? Why or why not? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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15. Explain why leaves turn red and yellow in the fall. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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