Title: Plant Pigment Analysis—Lab Class Set

**Introduction:**

When you look at a leaf, the green pigment chlorophyll is usually the only pigment that appears to be present. Actually, chlorophyll is only one of many types of pigments present in the thylakoid membranes of the leaf and one of several that are involved in gathering energy from the sun for photosynthesis. The slight molecular differences between pigments enable the pigments to absorb different wavelengths of light. These pigments absorb energy from the sun and use it to create ATP in light reactions of photosynthesis. Once removed from the leaf, the photosynthetic pigments can be separated from one another and identified using a process called chromatography.

Chromatography is a physical process in which several compounds are separated from a solution and from each other. In paper chromatography a solvent is absorbed by the paper. As the solvent moves up the paper, it carries with it the compounds that have been placed on the paper. Each compound will move a different distance up the paper.

The distance that each molecule travels is based on two factors: the attraction that the molecule has for the paper and the degree to which the molecule is soluble in the solvent.

Once the pigments have been separated, the isolated pigments can be identified by calculating the reference front (Rf):

Rf = distance the pigment/solute traveled(mm)

distance the solvent traveled(mm)

# Materials:

Assorted Leaves Coins

Large test tubes Cork/stopper

Chromatography paper Thumb tacks

Chromatography solvent Ruler

Scissors

# Procedure: Abbreviate this in your notebooks!

1. Obtain a large test tube to serve as a chromatography chamber.
2. Fill the chromatography chamber about 1/3 of the way full with solvent. Make sure the chamber is tightly stoppered due to the volatile nature of the solvent.
3. Cut a piece of chromatography paper so that the tip will just rest in the chromatography solution in the chamber. Cut the paper as shown below.

Use a pencil to draw a line about 2 cm from the bottom of the paper.

1. Use a quarter or nickel to rub and extract the pigments from the leaf cells of your selected plant variety. Roll the coin across a leaf placed on the pencil line that serves as the *pigment origin*. The leaf’s pigment is now transferred to the chromatography paper.
2. Use a thumb tack to attach the chromatography paper to the cork.
3. Place the chromatography paper into the chamber so that only the tip of the paper is immersed in the solvent. *Do not allow the pigment to be immersed in the solvent.*
4. Stopper the chamber and when the solvent is 1-2 cm from the top, remove the paper. *Immediately mark the location where the solvent stopped with pencil. This serves as the solvent front.*
5. With a pencil, mark the middle of each pigment band. Write the color of the pigment near the band as the colors fade over time. Record the color of each pigment band in Table 1. Sketch the chromatography paper in the Data Collection section.
6. Measure the distance that the solvent migrated from the pencil line in millimeters. Record the distance in Table 1.
7. Measure the distance that each pigment migrated from the pigment origin to the middle of the pigment band in millimeters. Record the distance in Table 1. Depending on the plant species, 1 to 5 bands should be observed.
8. Calculate the Rf values for each pigment band recorded. Record in Table 1.

Rf = distance the pigment/solute moved (mm)

distance the solvent moved (mm)

1. Identify the pigments by comparing the Rf values with known Rf values of the following pigments. Record identifications in Table 1.



**Data Collection: Copy these data tables and diagrams for TWO of the available plant materials**

Selected plant:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Chromatography Paper Sketch (Label the pigments to correlate with your chart below)

 *Solvent Front*

*Pigment Origin*

*TABLE 1: ISOLATION OF PIGMENTS*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Substance** | **Color** | **Distance (mm)** | **Rf** | **Identification** |
| Solvent |  -------------- |  | ---------------- | ----------------- |
| Pigment 1 |  |  |  |  |
| Pigment 2 |  |  |  |  |
| Pigment 3 |  |  |  |  |
| Pigment 4 |  |  |  |  |

Show one sample calculation of the Rf value:

# Analysis and Comprehension Questions:

1. Which pigment traveled the furthest? Explain why it traveled further than the others. Why is color alone not a good way to identify the pigment?
2. Would the Rf values be the same if a different solvent was used? Explain.

3. Why do leaves appear green even though there are other pigments present?

4. If you use the width of the band as an indication of the amount of pigment present, which pigments would you say are the most abundant in spinach leaves? The least abundant?

5. Many leaves change color in the autumn. How is it possible for this to happen? Base your answer on your new knowledge of pigments present in leaves. HINT: Chlorophyll a and chlorophyll b are easily broken down in the cooler autumn temperatures.

6. Using information you have learned about the absorption of wavelengths of light by pigments, explain why having many pigments in a leaf allows a plant to maximize the amount of light available for photosynthesis.

7. What are some possible sources of error in this laboratory exercise?