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| **Chromatography of Plant Pigments** | NAME\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
|  | DATE\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |

**INTRODUCTION:**

**Chlorophyll** often hides the other pigments present in leaves. In Autumn, chlorophyll breaks down, allowing **xanthophyll** and **caroten**e, and newly made **anthocyanin**, to show their colors. The mix of pigments in a leaf may be separated into bands of color by the technique of **paper chromatography**. Chromatography involves the separation of mixtures into individual components. **Chromatography** means "color writing." With this technique the components of a mixture in a liquid medium are separated. The separation takes place by absorption and capillarity. The paper holds the 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.

**PRELAB PREPARATION:**

Gather leaves from several different plants. Autumn leaves from deciduous trees are especially interesting. Sort the leaves by kind (maple, etc.) and color. Review a diagram of a plant cell. Find the **grana(stacks of thylakoids)** and the **chloroplasts** of the cell.

**MATERIALS:**

Safety goggles
Chromatography solvent

Chromatography chambers - x3
Chromatography paper (or filter paper) about 1 cm x 15 cm - x3
Fresh spinach leaf

Two other leaf pieces of your choice.
Scissors and Ruler

Plastic Pipet

**PROCEDURE:**

**CAUTION:** Chromatography solvents are flammable and toxic. Have no open flames; maintain good ventilation; avoid inhaling fumes.

1. Cut a strip of filter paper or chromatography paper so that it just fits inside a 15-cm (or larger) test tube. Cut a point at one end. Draw a faint **pencil** line as shown in figure 1. Bend a paper clip and attach it to a cork stopper. Attach the paper strip so that it hangs inside the tube, as shown. The sides of the strip should not touch the glass.

2. Tear a spinach leaf into pieces about the size of a postage stamp. Put it on top of the pencil line and press the side of a coin down on the leaf. You should do this a couple of times and see pigment from the leaf transferred on to the paper.

4. Pour 2 **ml** chromatography solvent into the test tube. Fit the paper and cap the assembly inside. Adjust it so that the paper point just touches the solvent (but not the sides of the tube). The pigment dot must be above the level of the solvent. Watch the solvent rise up the paper, carrying and separating the pigments as it goes. **At the instant** the solvent reaches the top, remove the paper and let it dry. Observe the bands of pigment. The order, from the top, should be **carotenes** (orange), **xanthophylls** (yellow), **chlorophyll a** (yellow-green), **chlorophyll b** (blue-green), and **anthocyanin** (red). Identify and label the pigment bands on the dry strip. Write the species of leaf on the strip as well. Record the species, external color, and chromatogram pigments in the **DATA TABLE** of your report sheet.

5. Each pigment has an **Rf** value, the speed at which it moves over the paper compared with the speed of the solvent.

**Rf = Distance moved by the pigment / Distance moved by the solvent**

6. Measure the distance in **cm** from the starting point (pencil line) to the center of each pigment band. Then measure the entire distance traveled by the solvent. Remember, the starting point for the solvent is also the pencil line and the ending point for the solvent is the top edge of the paper. Do the required divisions and record your **Rf** values in the **DATA TABLE** of your report sheet.

7. Wash the chromatography chambers thoroughly.

8. Repeat steps **1** through **7** for each species.

**DATA TABLE:**

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| **Chromatography Data** |
| **Leaf Type (species)** | **External color** | **Chromatogram Pigments** |
| Colors from the Top | Pigment Names | Rf Values |
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Analysis Questions:

1. What purposes do the various pigments have in plant cells, be specific?
2. Summarize the process of photosynthesis (like what we did in class the other day).
3. How do the processes of photosynthesis and cellular respiration relate to each other?