**Chromatography using plant pigments-**Adapted from AP Biology lab text (edition D lab #4)

In this lab you will learn how to separate the photosynthetic pigments in leaves using paper chromatography. You will also learn how to calculate Rf values.

**INTRODUCTION:**

Chlorophyll often hides the other pigments present in leaves. In autumn the chlorophyll breaks down, allowing xanthophyll (yellow) and carotene (orange), chlorophyll a (yellow-green), chlorophyll b (blue-green), and newly made anthocyanin (reds, blues and purples), to show the fall colors. Different pigments allow a plant to use different wavelengths of sunlight.

The mix of pigments in a leaf may be separated into bands of color by using a technique called **chromatography**. **Chromatography** means "color writing". Chromatography separates mixtures into their individual color components. The separation occurs when the chromatography paper is placed barely touching a solvent. The pigment extract is wicked up and carries the various colors up the paper. The pigments will separate as they travel because they are attracted to the paper differently and dissolve in the solvent differently. The separated pigments will show up as colored streaks. The pattern of separated components on the paper is called a **chromatogram**.

Materials:
Chromatography paper 3 cm x 8 cm
Spinach
100 ml beaker
quarters
Pencil and tape
Chromatography solvent (9 petroleum ether:1 acetone)
Scissors and ruler

**PROCEDURE:**

Work in pairs.

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

**Procedure:**

1. Cut a 8 cm long piece of chromatography paper.
2. Make a small pencil line 1.5cm from the bottom. This is where you will apply the pigment mixture. **Touch the paper as little as possible because oils from your skin will interfere with the development of the chromatogram**.
3. Place a spinach leaf over the chromatography paper and roll the edge of a coin over the leaf, using a ruler as a guide, so that the pigments of the leaf are driven into the chromatography paper 1.5 cm from the bottom. This will produce a straight line of pigment that can be chromatographed. BE CAREFUL NOT TO PUSH/ROLL THE COIN SO HARD THAT YOU TEAR THE PAPER!!
4. Pour **10 ml** chromatography solvent into a beaker. Tape the chromatography paper to the middle of the pencil. Rest the pencil across the top of the beaker, adjusting it so that the paper just touches the solvent (but not the sides of the beaker). The pigment line must be above the level of the solvent. Watch the solvent rise up the paper, carrying and separating the pigments as it goes. Allow the paper to stay in the solvent for 5-7 minutes **or** until the solvent has risen almost to the top of the paper (**you do not want the solvent reaching the top of the paper**).

5. Remove the paper and using a pencil mark the location of the solvent mark before it disappears. Examine the paper for bands of color. Circle them before they disappear and draw a diagram of them

for your data. You should do this as soon as you remove the strips from the beaker because some of the fainter bands may fade quickly, especially under fluorescent lights. Compare your chromatograms with those of other members of the class, especially if any of your strips are faint or blurry.

6. Using the information in Table 1, identify the kind of pigment responsible for each band. Label the bands you have sketched.
7. Calculate your Rf values for each band using the following formula and put them into the attached table:

   Rf = distance pigment migrated (mm) / distance solvent front migrated (mm)
   **NOTE: for both distances start at the lower pencil line, where the pigments were applied.**

**Table 1. Characteristics of photosynthetic pigments.**

| Pigment | Color | Position on strip | Expected Rf | Actual Rf |
|---|---|---|---|---|
| Carotenoids: | | | | |
| carotene | usually very thin yellow | very high | .95-.99 | |
| xanthophylls | yellow | moderate to low | .4 | |
| Chlorophylls: | | | | |
| chlorophyll a | bright to bluish green | moderate to low | .20-.25 | |
| chlorophyll b | yellow to olive green | slightly lower than chlorophyll a | .1-.15 | |

* Note: A high position (nearer the top of the chromatogram) means the pigment adsorbs weakly to the chromatography paper, while a low position (nearer the origin) means it adsorbs strongly.

**Questions**

1. Calculate the Rf values for each of the bands using the equation above.
2. Make a table of the bands in order, including the color, the Rf value and your best attempt at identification it.
3. If most leaves appear green, why can't these other pigments normally be seen?
4. At what time of year might it be possible to see these other pigments in leaves? Why?
5. What are some possible advantages of having several different pigments present in leaves?
6. In what cell organelle will leaf pigments be found?

Conclusion:

Possible Sources of Error: