Chromatography Lab (Practical 4)

**Photosynthesis**, you might remember, is the process in which plants convert light energy from the sun to chemical food energy. Brightly colored **pigments** in leaves are essential to the first steps of light absorption, with **chlorophyll**being the most important pigment. There are two main types of chlorophyll: **chlorophyll A**, which is bluish-green, and **chlorophyll B**, which is yellowish-green. During most of the growing season, leaves contain more chlorophyll than any other pigment, making them appear green. In the fall, however, chlorophyll begins to break down, and the other pigments, which have been there all along, are finally revealed: we see pretty yellow and orange fall leaves. The yellow pigments in leaves are called **xanthophylls**, and the orange pigments are called **carotenoids**(carotenoids are easy to remember because they are orange like a carrot!).

If you want to see the hidden yellow and orange pigments in green leaves for yourself, you can use a simple but powerful technique called **chromatography,** which is the separation of a mixture by passing it through a medium (in our case, filter paper) through which different parts of the mixture will move at different rates. Chromatography works because different substances in a mixture have different degrees of solubility. **Solubility**refers to how much of a particular substance can dissolve in a particular solvent. For instance, you can dissolve lots of sugar in water, but not quite as much salt. In chromatography, the *least* soluble substances fall out of the filter paper column first, while the *most* soluble one travel the furthest up the paper. (source for background info: https://www.education.com/science-fair/article/find-color-pigments-hidden-green/)

Procedure:

1. Obtain about 10 cm worth of chromatography paper.
2. Using a coin, make a line of plant pigment on the chromatography paper, about 3 cm from the end. You can do this by rolling the coin over the leaf until it makes a straight mark on the paper.
3. Add a small amount of isopropyl alcohol (less than 3 cm depth) to the bottom of a test tube.
4. Place your chromatography paper in the tube so that it touches the alcohol, but make sure that the pigment from the leaf is not submerged.
5. Wait and observe as the isopropyl alcohol solvent moves up the chromatography paper. You will see the various pigments in the leaf stain begin to separate as the solvent moves.
6. Read about Rf values online and calculate the Rf value for each pigment observed.

Brief Lab report: Answer the following:

1. What are the Rf values of the pigments you observed? Explain how to calculate Rf values.
2. What did you learn about pigments in leaves?