Spinach Leaf Paper Chromatography Lab

Adapted from *Biology Junction Chromatography Lab*

Problem: How do you separate the different pigments in a plant?

Materials:

coffee filter paper strip (6 inches long and 1 inch wide)
large glass beaker or cup
Vinegar or water
toothpick
fresh spinach or other leaves

Introduction:

In this activity you will be experimenting with a technique called chromatography which will allow you to visually demonstrate that the pigment in leaves is a combination of several different colored pigments.

This technique is useful in that it can separate and identify the various components of mixtures, such as those contained in plant pigments. A pigment is a substance that absorbs light at specific wavelengths, chlorophyll is one of these pigments. Its green-yellow in color is due to the absorption of red, orange, blue, and violet wavelengths and the reflection of the green and yellow wavelengths. This occurs when white light (containing all of the light wavelengths, or the entire spectrum of colors) shines on the leaf surface, all of the wavelengths are absorbed except for the ones you see, which are green-yellow, those are the portions of the spectrum being reflected.

If the conditions are identical, the relative distance moved by a particular compound is the same from one mixture to another. This is why chromatography can be used to identify a compound. The actual identification requires a simple calculation as shown below:

> Rf = distance moved by compound from original spot divided by the distance moved by solvent from original spot

It is important to remember that several factors can influence the reliability of the Rf value, these include humidity, temperature, solvent, pigment extract preparation, and the amounts of the material present. Values are comparable only when the extracts are prepared in the same way and the chromatograms are prepared identically and developed together in the same container.

Procedure

1. Each lab group (or individual if not working in groups) will need 1 strip of filter paper, approximately 6 inches long and 1 inch wide, 1 chromatography development containers (500 ml beaker or large cup), 1 pencil to hang the filter paper from, vinegar.

2. Do the following with the fresh spinach leaves; tear leaf material and place in a glass container, cover with vinegar.

3. Place the solvent in the beaker or cup. Put a pencil across the top of the beaker. The pencil will be the mechanism for hanging the chromatography strips.

4. Make a pencil mark on the chromatography strip, in the center, about 1 inch from the tip of the paper. Using a tooth pick, apply the plant pigment to each filter paper strip. This is done by touching the tooth pick, which has been dipped in the pigment, to the pencil mark. Make an application, then wave the paper gently to dry it a little before the next application. Be patient, you will need 12 to 15 applications.

5. By now you should have a strip with spinach pigment. Suspend it in the chromatography development vessel by hanging it over the pencil. You may need to use tape to help secure it. The tip of the strip should just touch the solvent.

6. Wait 20 to 30 minutes for the chromatograms to develop. Remove the chromatograms. Mark with a pencil (NOT a pen) where the solvent stopped as it moved up the chromatogram. This is called the solvent front. Mark also where each pigment stopped moving up the chromatogram. Using the equation below, determine a reference number for each pigment on the chromatograms. Depending on which chromatogram you are viewing, you should see greens, yellow/yellow orange, and red. All measurements should be in mm. (Any material which did not move from the pencil dot is insoluble). Record your data in the table below:

Liquid color description (clear, yellow, red, green)	Distance from starting line where color stopped (measured in millimeters)	Rf value ** - see below the table for how to calculate	Pigment name

Rf = distance moved by compound from original spot divided by the distance moved by solvent from original spot

Note: each pigment has a special name,
green = chlorophyll a or b
yellow/yellow orange = carotene

red = anthocyanin
brown = xanthophyll

The reference numbers for the chlorophylls in this activity are:
0.28 = chlorophyll a, 0.18 = chlorophyll b (spinach). You need these numbers so that you can determine one chlorophyll from the other. Calculate reference fronts for all of your pigments.

See if your calculations come close to those above for chlorophyll a and b.

Conclusion Questions

1. What reference numbers (Rf) did you calculate for chlorophyll a and chlorophyll b?

2. With what you have discovered about pigments, what conclusions can you make regarding the changing color of leaves in autumn?

3. What adaptive purpose do different colored pigments serve for a plant?

4. Why do some pigments move farther up the chromatogram than others?

5. What are some possible sources of error in this lab?

Paper chromatography is a technique used to separate a mixture into its component molecules. The molecules migrate, or move up the paper, at different rates because of differences in solubility, molecular mass, and hydrogen bonding with the paper.