Bio I
Lab: Chromatography

Chlorophyll often hides the other pigments present in leaves. In Autumn, chlorophyll breaks down, allowing xanthophyll and carotene, 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.

Materials

chromatography solvent	test tube
chromatography paper	beaker
spinach	scissors
jade plant	stopper
shamrock	mortar and pestle
ruler	glass rod

Procedure

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

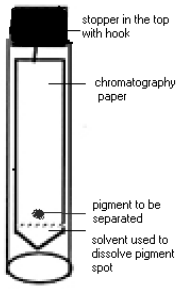
1. Cut a strip of filter paper or chromatography paper. Cut a point at one end. Draw a faint pencil line as shown below.

2. Tear one of the plant samples into pieces about the size of a postage stamp. Crush leaves with the pestle, using a circular motion, until the mixture is finely ground. The liquid in which the leaf pigments are now for paper chromatography dissolved is called the **pigment extract**.

3. Use a glass rod to touch a drop of the pigment extract to the center of the pencil line on the paper strip. Let it dry. Repeat as many as 20 times, to build up the pigment spot. **NOTE:** You must let the dot dry after each drop is added. The drying keeps the pigment dot from spreading out too much.

4. Pour **5 ml** chromatography solvent into the test tube.

5. Place the piece of chromatography paper inside of the provided test tube. The sides of the strip will slightly touch the edges of the test tube. Examine diagram below. Adjust the paper 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.



6. 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.