

Name _____

Hour _____

PAPER CHROMATOGRAPHY

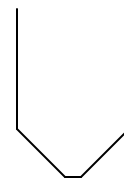
One of the main tasks of chemists and biologists is to unravel the complexities of chemical compounds and reduce them to their simple, individual components. Paper chromatography is an important technique used in the separation, isolation, and identification of chemical compounds. The term *chromatography* is derived from the Greek word *chromat*, which means color, and *graphon*, which means to write. Chromatography was originally used to separate pigments in leaves, berries, and natural dyes. Over the last 50 years, chromatography has been applied to the separation of a series of organic and biological compounds. Pigments are separated on the paper and show up as colored streaks. The pattern of separated components on the paper is called a chromatogram.

Paper chromatography is a useful technique for separating and identifying pigments and other molecules from cell extracts that contain a complex mixture of molecules. The solvent moves up the paper by capillary action, which occurs as a result of the attraction of solvent molecules to the paper and the attraction of solvent molecules to one another. As the solvent moves up the paper, it carries along any substances dissolved in it. The pigments are carried along at different rates because they are not equally soluble in the solvent and because they are attracted, to different degrees, to the fibers in the paper through the formation of intermolecular bonds, such as hydrogen bonds.

Beta carotene, the most abundant carotene in plants, is carried along near the solvent front because it is very soluble in the solvent being used and because it forms no hydrogen bonds with cellulose. Another pigment, xanthophylls, differs from carotene in that it contains oxygen. Xanthophyll is found further from the solvent front because it is less soluble in the solvent and has been slowed down by hydrogen bonding to the cellulose. Chlorophylls contain oxygen and nitrogen and are bound more tightly to the paper than are the other pigments.

PROCEDURE

- 2) Cut the end of the paper into a wide point.
- 3) With the pencil, draw a line about 2cm from the point.
- 4) Pour a small amount of solvent into your beaker. It should be ½ cm to 1 cm deep.



Keep the tube covered since the solvent is volatile

- 5) Practice rubbing a clean line of spinach on a scrap piece of paper. A thin, blunt object works best. Nickels are good.
- 6) Rub a clean, green line of spinach pigment onto the pencil line of new paper.
- 7) Place the spinach chromatography paper into the solvent. Make sure the level of the solvent does not surpass the pencil line. Do not allow the pigment to be in the solvent.
- 8) Cover the tube and let sit undisturbed for 15 minutes or until the solvent is about 1 cm from the top. Do not allow the solvent to run off the end of the paper.

9) After 15 minutes, remove the spinach paper and place it on a piece of paper towel.

Immediately mark the location of the solvent front before it evaporates.

10) Mark the **bottom** of each pigment band. Measure the distance each pigment migrated from the bottom of the pigment origin to the bottom of the separated pigment band. In TABLE 1 record the distance that each band, including the solvent front, moved. Depending on the species of plant used, you may be able to observe 4 or 5 pigment bands.

TABLE 1 – Distance traveled

<u>Band</u>	<u>Distance (mm)</u>	<u>Band Color</u>
Solvent front		
1		
2		
3		
4		
5		

ANALYSIS of RESULTS

The relationship of the distance moved by a pigment to the distance moved by the solvent is a constant called R_f . It can be calculated for each of the pigments using the following formula:

$$R_f = \frac{\text{migration distance of pigment}}{\text{migration distance of solvent}}$$

TABLE 2 – R_f values for pigments

_____	= R_f for Carotene (yellow to yellow orange) – top
_____	= R_f for Xanthophyll (yellow) – above the green
_____	= R_f for chlorophyll a (bright green to blue green)
_____	= R_f for chlorophyll b (yellow green to olive green) -bottom

ANALYSIS

- 1) Does spinach contain one or several pigments? What proof do you have? What are the roles of these pigments? Be as specific as possible.
- 2) What is the value of chromatography?
- 3) What factors are involved in the separation of the pigments?
- 4) Would you expect the R_f value of a pigment to be the same if a different solvent were used? Explain.