PAPER CHROMATOGRAPHY

With thanks to Dr. Bassam Shakashiri and http://scifun.chem.wisc.edu/

Introduction

Chromatography is an analytical technique that is used to separate and to identify components of a mixture based on the interactions between molecules. It has a wide range of applications in the real world since many substances are mixtures of chemical compounds. For example, it is used extensively in forensic chemistry, the application of chemical techniques to law and the profession popularized by TV shows such as NCIS and the CSI franchise, where the samples that are received in a crime laboratory are complex mixtures of chemical compounds and chromatography is often ideal for separating and identifying these components.

To perform a chromatographic separation you need two things: a stationary phase and a mobile phase (the mobile phase is also known as the eluant). As you might suspect, the stationary phase doesn't move and mobile phase moves over, or through, the stationary phase. The mixture that you want to separate is introduced in the mobile phase and as it moves over the stationary phase its molecules interact with both the stationary phase and molecules of the mobile phase. Some components of the mixture will interact more strongly with the stationary phase (essentially sticking to the stationary phase) and their progress over the stationary phase is slowed down. Other components interact less strongly with the stationary phase (or more strongly with the mobile phase) and pass quickly over the stationary phase. The net effect is a separation of the components in the mixture by their differential interactions with the mobile and stationary phases.

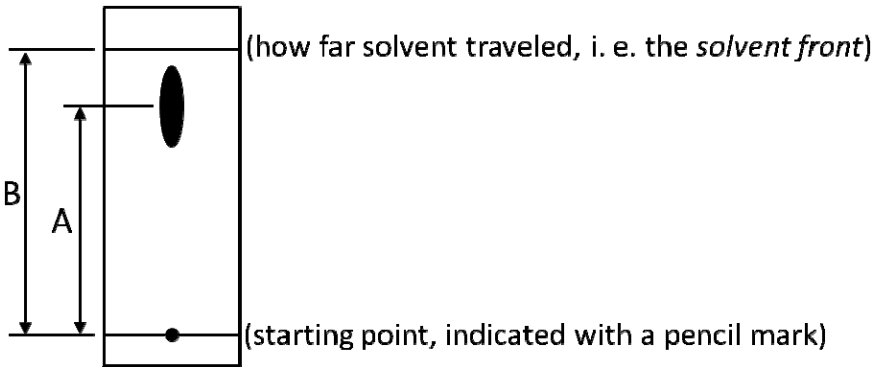
In paper chromatography, the chromatographic method you will be using today, the sample mixture is applied near the bottom edge of a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. While you might think the paper is the stationary phase, the actual picture is a little more complicated than that, but the general idea still applies. Paper is comprised of cellulose, which is a polymer of the simple sugar glucose, and as such is very polar due to the –OH groups present in glucose. Because of the many exposed –OH groups, cellulose interacts strongly with polar water molecules. This interaction is so strong that "dry" paper is approximate 22% water by weight (see Pavia, D. L.; Lampman, G. M. and Kriz, G. S. *Introduction to Organic Laboratory Techniques: A Contemporary Approach, 2nd Ed.*; Saunders College Publishing: New York, 1982, p. 584). It is this water adsorbed on the paper surface that is the stationary phase in paper chromatography. In general, the polarity of the mobile phase is adjusted by trial and error to affect the desired separation. Often for paper chromatography the mobile phase is a mixture of water and an alcohol. This mobile phase is fairly polar, but less polar than the stationary phase. Thus as the mixture moves up the paper by capillary action, the more polar components will travel up the paper more slowly than polar ones.

Performing a chromatographic experiment is basically a three-step process: 1) application of the sample, 2) "developing" the chromatogram by allowing the mobile phase to move up the paper, and 3) calculating *Rf* values and making conclusions.

In order to obtain a quantitative measure of the extent of movement of a component in a paper chromatography experiment, an "*Rf* value" (**R**etention **f**actor value) is calculated for each separated component in the developed chromatogram. The *Rf* value for a substance is

dependent on the polarity of the specific substance, so the *Rf* value can be used to roughly identify the substance. The *Rf* value is a number that is defined as:

$$Rf = \frac{\text{Distance traveled by component from application point}}{\text{Distance traveled by solvent from application point}} = \frac{A}{B}$$



The distance traveled by the spot is measured to the MIDDLE of the spot!

Experiment: the separation of colorants by paper chromatography

a. Obtain a strip of chromatography paper about 8 cm long. Along one of the ends, draw a horizontal line in pencil about 1.5 cm from the end of the strip. This will be your "baseline," the starting line where the samples will be spotted.

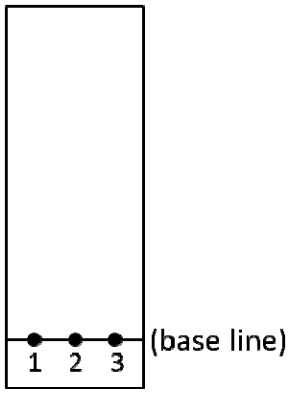
Hint: try to avoid excess handling of the paper in order to leave as little finger oil on the paper as possible. Finger oil will interfere with the solvent/solute interaction.

b. Make two dots on each paper with the pencil, equally spaced along the line, leaving about 1 cm between the dots and about 0.5 cm between each dot and the edge of the paper. Below the line, use the pencil to label each dot for the different colors of candy that you have.

c. Now make solutions of the color(s) in each candy. Take a piece of aluminum foil and lay it flat on a table. Place six small drops of water spaced evenly along the foil. Place one color of one kind of candy on each drop. Wait about three minutes for the color to from the candy to dissolve in the water. Remove and dispose of the candies, but do not dispose of the droplet solutions until the chromatography is finished.

d. Now "spot" the colors onto the filter paper. To do this, dampen the tip of one of the toothpicks in one of the colored solutions and lightly touch it to the corresponding labeled dot on your filter paper. Use a small droplet and a light touch, so that the dot of color stays small - less than 1/16 inch (2 mm) is best. Repeat using a different toothpick for each color.

e. After all the color spots on the filter paper have dried, go back and repeat the spotting process with the toothpicks to get more color on each spot. Do this three times, waiting for the spots to dry each time.



f. When the paper is dry, fold it in half longwise so that it stands up on its own, with the fold standing vertically and the dots on the bottom. Make a soft curving fold - do not crease the paper (creasing will change the way water travels up the paper), and make sure no dot lies on the fold.

g. To use as a developing solution, make 500 mL of a 1% (weight by volume) sodium chloride solution in your 600 mL beaker. Make certain all of the salt is dissolved in the water.

h. Now pour some of the salt solution into a 600 mL beaker to a depth of about ¼ inch (0.5 cm). The level of the solution should be low enough so that when you put the previously spotted chromatography paper in, the dots will initially be above the water level. Hold the filter paper with the dots at the bottom and gently set it in the beaker with the salt solution.

Hint: make sure the paper is not laid up lengthwise against the side of the beaker. This will cause fast solvent flow on the parts of the paper touching the beaker.

i. When the solvent has risen to within 1 cm of the top of the paper strip, remove the strip from the chamber and immediately mark the level to which the solvent has risen and very lightly (so you do not tear the paper) circle each spot on the strip with a pencil. Note that it is normal for the solvent front and the spots to continue to move slowly for several minutes after removing the strip from the beaker.

j. Measure the distance each spot traveled and calculate Rf values for each spot. Look at the picture on the front page for guidance in how to do this. Use the circles you drew around each spot with your pencil, and not the actual spot, since the spot will have moved after the paper was removed from solution.

k. Repeat the procedure for all the colors of the other type of candy.

l. Turn in your chromatogram(s) as part of your report.

SAFETY AND DISPOSAL

All solutions from this lab may be safely poured down the sink with running water. All solids may be disposed of in a trashcan.

DO NOT EAT THE CANDIES!