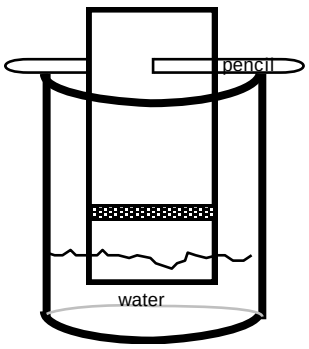
Chromatography Lab: Separation of Mixtures and Compounds; SC1b

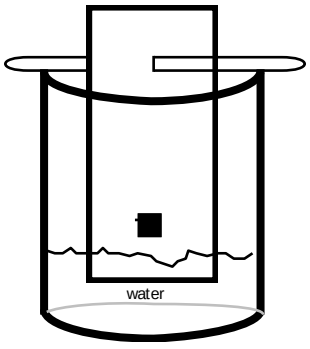
Background: Almost all substances we come into contact with on a daily basis are impure; that is, they are mixtures. Similarly, compounds synthesized in the chemical laboratory are rarely produced pure. As a result, a major focus of research in chemistry is designing methods of separating and identifying components of mixtures. The theory behind chromatography is to allow a mixture of different chemicals to be distributed or partitioned between a stationary phase and a mobile phase (eluent or solvent). The mobile phase may be a liquid or a gas; the stationary phase is typically a solid. As the mobile phase flows over the stationary phase, the components in the mixture are carried along. The more soluble a component is in the mobile phase the faster it will be transported along the stationary phase. Adsorption refers to the ability of a substance to 'stick' (or be adsorbed) to a surface. The more strongly a component is adsorbed to the stationary phase, the slower it will be transported by the mobile phase. As the mixture moves over the stationary phase, the components in the mixture move further and further apart into discrete zones. The purpose of this lab is to utilize paper chromatography to determine whether the colors of marker dyes coloring are due to a single compound or a mixture of several other colors.

Procedure: Remember to always "read before you proceed" and this includes the background information.

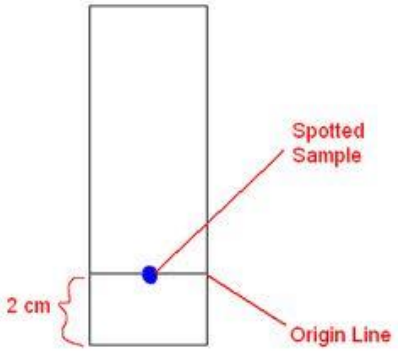
1. Obtain two small beakers and add about 10 mL of water (solvent), but note that you need the solvent line to be about 0.5 cm below your origin line on the chromatography paper (see figures below). So determine which size beaker would work best based on this information. Plan everything out first.
2. Next, carefully cut two strips of chromatography paper so that you have two set-ups representative of the figures below. It will work best to use either tape or a binder clip to attach the paper to the pencil. The length of the paper will depend on the size of beaker that you have chosen.
3. With a pencil and ruler make a straight line across the chromatography paper 2.0 cm above the end. This is the origin line. With a dark colored marker, make a thick line or a single dot along the origin line. With another color of your choice, make a thick line or a single dot on the center of the origin line of the second set-up (see figures).
4. Use either a sharp pencil or a pencil and some small pieces of tape to construct your set-ups as shown in the figures. Make sure the origin line is at least 0.5 cm above the solvent. Measure and plan first.
5. Make a prediction for each set-up in the form of an "If, then" statement. Record this for question 1.
6. Start the separation by placing the paper into the solvent and also make note of the starting time.
7. The separation is complete when the solvent is within 1.0 cm of the top. While you are waiting, you need to read the information on the back of this lab. Answer questions 1-3 before moving to step 8.
8. After your initial two chromatography runs are completed, design and conduct a new experiment using paper chromatography. Carefully, consider which variables you could manipulate before actually conducting the new experiment.



Thick Line Set-up



Dot Set-up



Lab Questions:

1. Make a prediction for each set-up in the form of an "If, then" statement. Be sure to justify your choice of color and marker type (e.g., permanent, Expo, Vis-à-vis, etc.)

2. Did you get the results that you predicted? Explain.

3. Compare and contrast your chromatogram with at least two other groups.

4. Describe your newly designed experiment from step 8. What was your hypothesis and what were the results?

5. What are the two major phases of chromatography? Describe each phase.

6. Explain in your own words why samples can be separated into their components by chromatography.

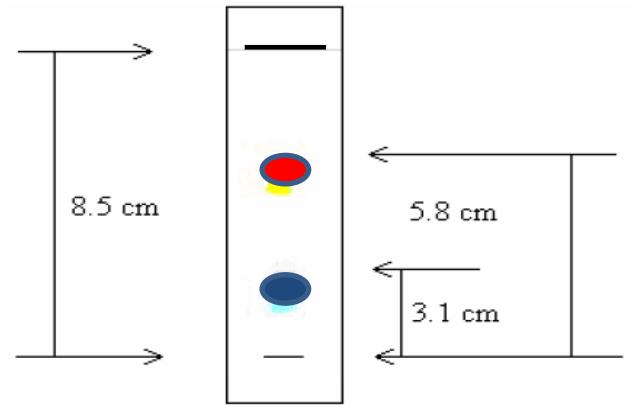
7. Calculate the retention factors (R_f) for both of your samples using the methods described below and in figure 1. You will need to show all of your work and use the correct significant figures.

8. If two components have the same R_f value in a chromatogram, are they necessarily identical in structure? Please justify your response.

9. Use the internet to find at least three other separation techniques that are commonly used in chemistry.

Retention Factors (R_f): The affinity of a substance for the stationary and mobile phases is characteristic of that substance. Different substances will have different competitive affinities. Since each component of a mixture will have its own characteristic affinities, each component will travel up the paper at its own characteristic rate. If the paper is sufficiently large, all the components can be separated by the time the solvent front has reached the top of the paper and each component will appear as a separate spot. The chromatographic paper will now contain a vertical array of colored spots arranged according to their characteristic rates of ascent. It is possible to describe the position of spots (so the substances that have separated) in terms of their retention factor, the R_f value (Figure 1). The retention factor is defined as: R_f = distance traveled by spot / distance traveled by solvent.

Figure 1: Example for calculating the R_f value



$$R_f \text{ (yellow)} = \frac{5.8\text{ cm}}{8.5\text{ cm}} = 0.72$$

$$R_f \text{ (cyan)} = \frac{3.1\text{ cm}}{8.5\text{ cm}} = 0.36$$