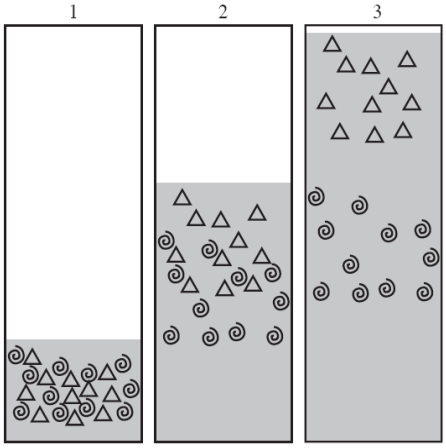
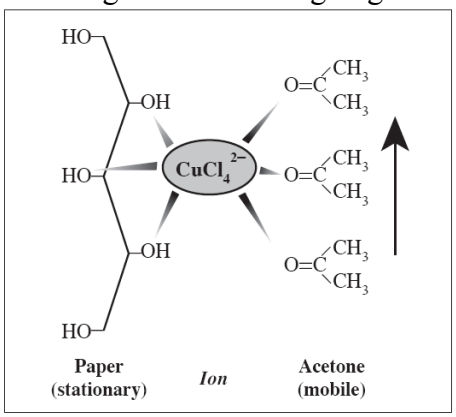
Paper Chromatography

INTRODUCTION

Separation techniques are of vital importance to chemists. Some of them are of ancient origin, such as distillation and fractional crystallization. Many others have been developed during modern times. Chromatography is a method of separating components one from another that takes advantage of the differing attractions molecules have for different substances. Here is a simple illustration showing two substances mixed together, one represented by a triangle, the other by a spiral, placed on a piece of absorbent paper. They are shown mixed in #1. As water rises (the grayed area), the triangles rise more rapidly than the spirals. In #2 and #3, the triangles ride with the water to near the top of the paper, while the spirals are only midway up.



Paper chromatography was first described in 1906 as a method for separating plant pigments. Its name is based on that use (khroma is the Greek word for color). All chromatography techniques use the same principle: The **stationary phase** and the **mobile phase** attract the various substances in the mixture to a different degree. In the first described use, a mixture of plant pigments was spotted on a piece of paper (the **stationary phase**), and water creeping up the paper by capillary action (the **mobile phase**) separated the pigments. Those pigments more attracted to paper did not move far from where they were placed. Those pigments more attracted to water rose with the moving front of water. Those pigments attracted to both the paper and water rose part-way. Today, you will separate and identify a mixture of metal ions using paper as the stationary phase, and a mixture of water, hydrochloric acid, and acetone as the mobile phase. Paper is primarily cellulose, which has numerous OH groups attached to a long molecule (a **polymer**). Hydrochloric acid provides Cl^- ions, which will stick to the metal ions to different degrees, depending on the metal. Acetone, $(CH_3)_2C{=}O$, has more or less attraction to the metal ions, depending on how strongly Cl^- attaches to the metal. The ions are also attracted to cellulose in varying degrees. Here is a stylized diagram of what is going on at a molecular level:

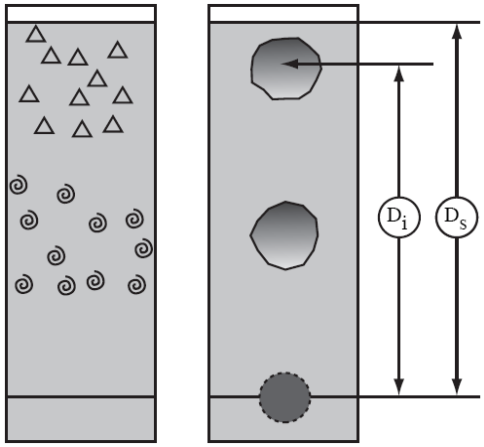


In this diagram, the acetone is shown moving upwards with the arrow. The $CuCl_4^{2-}$ is shown between a segment of cellulose and the acetone. To which of these is the $CuCl_4^{2-}$ more attracted? This will determine whether the copper ion stays in its original position on the paper (strongly attracted to the paper), moves up to the top of the sheet with the acetone (strongly attracted to the acetone), or rides partially up the sheet (attracted both to the paper and to the acetone).

The ions used in today's experiment differ in their attractions to paper and to acetone, so each will rise a different distance. By measuring how far the ion moves in relation to how far the solvent front moves, ions can be given an identifying value which is the ratio of those two measured numbers. This ratio is called the **retention factor**, R_f.

$$R_f = \frac{D_{ion}}{D_{solvent}}$$

For example, in the following illustration, the #3 arrangement of the triangles and spirals from page 1 is shown. Alongside is shown how this might look on a wet piece of paper. Where the original spot of the mixture was placed is shown on the bottom line. As this mixture moves up with the solvent, the bottom spot would disappear, and in its place would be the marks shown.



D_i is the distance travelled by the ion.
D_s is the distance travelled by the solvent.

The solvent front must be measured before it evaporates and is no longer visible. The spots caused by the separated substances are often invisible unless enhanced. Enhancing usually involves adding a reagent which brings out a colored compound, or viewing in light of an appropriate wavelength, such as ultraviolet, which sometimes causes fluorescence. Biologically important substances have a large array of tools used to identify where components of a mixture have migrated.

Although paper was the first stationary phase used, a great many other substances are used as this phase, depending on the nature of the mixture to be separated. Various silicates, aluminum oxide, waxes, and proteins are just a few. In place of water as the mobile phase, various liquid mixes, gases, or supercritical fluids are used. When gases are used, the stationary phase is contained in a tube, usually as a finely divided powder.

Name _____ **Date _____**

PRE-LAB QUESTIONS

MUST be completed before an experiment is started. The COPY pages will be collected as you enter the lab.

Please answer the following questions and show all work and units. Express all answers to the correct number of significant digits.

Q1. What does the technique of chromatography allow us to do?

Q2. In your own words, define mobile phase, stationary phase, retention time, and R_f value.

Q3. Explain in your own words the principles on which chromatography relies.

Q4. If the solvent front moved 111 mm and a component of a mixture moved 45 mm from the application point, what is the R_f value of the component? Show any required calculations.

Q5. Use the information below to predict the outcome of a paper chromatography separation of a mixture of three substances A, B, and C.
A has a greater R_f value than B, and B has a greater R_f value than C. Sketch the paper chromatogram below. Be sure to clearly label the spots.

Q6. If a substance has an R_f value of 1, is it strongly retained by the stationary phase or not retained by the stationary phase? Explain.

Q7. Does a large R_f value indicate that a substance is strongly retained by the stationary phase or weakly retained by the stationary phase? Explain

Q8. Does a long retention time indicate that a substance is strongly or weakly retained by the stationary phase?

Q9. Explain why methods of separating the components of mixtures rely on the differences in physical properties rather than chemical properties.

Q10. What does each spot on a PC chromatogram represent?

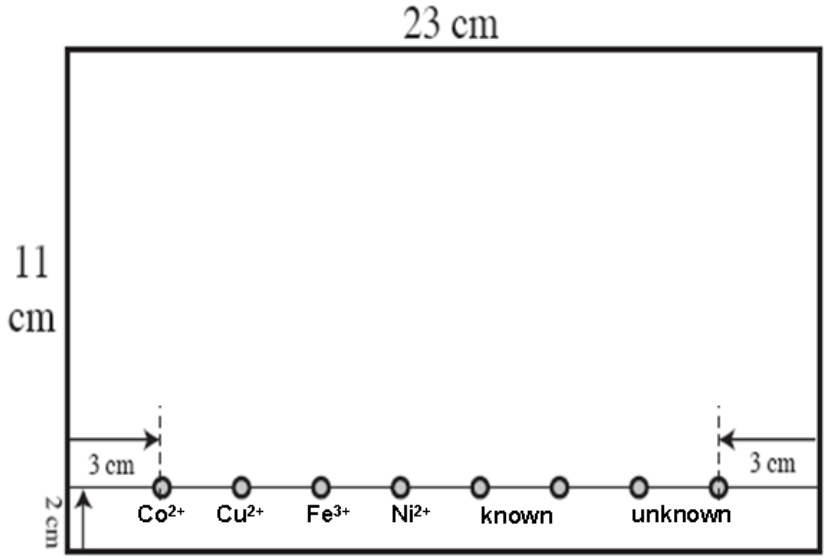
Q11. Explain how the components of a mixture are identified in chromatography?

Q12. Is it necessary that compounds be colored to be separated by chromatography?

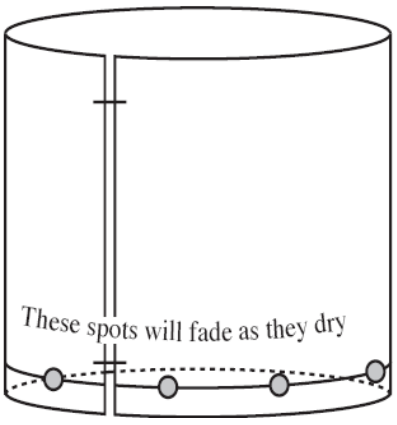
EXPERIMENTAL PROCEDURE

Safety: Wear your Goggles and lab Apron at all time.

1) Obtain a piece of precut filter paper about 23 cm long and 11 cm wide. Along the 19-cm edge, draw a *pencil* line along the long side 2 cm from the edge. Starting 3 cm from the end of that line, mark the line at 2-cm intervals. Label the identity of each spot with pencil as illustrated in the following figure.
2) Locate the aqueous solutions of $CoCl_2$, $CuCl_2$, $FeCl_3$, $NiCl_2$, the known solution containing all these four ionic compounds, and an unknown solution obtained from the instructor. Use capillary tube as the applicator for each solution.



3) Practice making spots with one of the solutions on a scrap piece of filter paper. The liquid from the applicator should form a spot no larger than 8 mm in diameter. Practice making spots until you can reproduce the spot size each time.
4) Then put a spot for each of the four solutions, the known mixture and the unknown mixture onto the assigned spots on the chromatogram paper.
5) Dry the spots under a heat lamp set at low setting. Do NOT let the paper char. Reapply the known and unknown solutions two or three more times to the same spots, as the ion concentrations in these mixtures are lower than the individual solutions. Make sure that you dry the spots between applications, but do NOT heat the paper more than necessary.
6) Use scotch tape or staples to hold the paper into a cylinder as shown:



7) There must be a small space between the ends of the paper. The solvent will move unevenly along the edges if they are touching. Squeeze the paper inward at the stapled side to make the assembly round rather than oblong.

8) Use graduated cylinder to measure about 15 mL of eluting solution from the supply of reagents. Pour it into a 600-mL beaker and quickly cover the beaker with a piece of plastic wrap. Place the sheet of chromatogram paper in the beaker, making sure it does not touch the sides. Quickly replace the plastic wrap, and do not move the beaker. The eluent, containing 3:1 acetone and HCl 6M, will gradually rise up the paper (by capillary action), carrying along the cations at different rates.

9) When the eluting solution has risen to about 2 cm of the top of the paper, remove the cylinder from the beaker, pull it apart from the staples. Immediately mark the position of the solvent front with a pencil while the paper is still moist. Then dry the paper under a heat lamp until it is quite dry.

<u>Caution</u>: Some jewelry may become discolored by the following procedure. Remove the jewelry to protect it.

10) To enhance the spots, take the paper to the fume hood. Place the paper on a piece of paper towel and spray it with the staining reagent. Spots should immediately form. Make sure the whole area of the solvent-soaked portion of the paper is wetted with the spray. Remove the paper from the fume hood. Dry the paper thoroughly under a heat lamp.

11) Measure the distance from the bottom line to the top solvent front line. Read the distance to the nearest millimeter. Record the distance the solvent traveled.

12) For each spot, make a line through the center of density of the spot. Measure and record the distance for each spot observed. Read the distance to the nearest millimeter. Calculate the R_f (retention factor) for

$$R_f = \frac{\text{distance travelled by ion}}{\text{distance travelled by solvent}}$$

each spot.

13) Note that in the mixture of all four ions, there should be four spots, spread along the solvent path. Calculate the R_f for each spot. Make a line in the center of each spot.

Look to see that the ions moved the same distance whether they were in a single ion solution or in the mixture of all four ions. That is, the cobalt sample will travel the same distance whether it is cobalt alone, cobalt mixed with other ions as in the known mixture.

This is the manner by which you will decide which ions are present in the *unknown solutions*. By visually comparing the location of the colored spots, list the contents of your unknown solution. Check to see if the R_f values for the spots in the unknown are consistent in their values whether the spot was for a solution of a single ion, or if the ion was in a mixture.

You will be graded on how reasonably you interpreted your results. Attach the chromatogram to your lab report.

To clean up, dispose of the eluting solution into the labeled container in the fume hood. Dispose of used capillaries in the broken glass container. Wash your hands before leaving the lab room.

Post-Lab Questions and Exercises

(All questions must be answered during the lab and submitted with your lab report at the end of the lab period).

Please answer the following questions and show all work and units. Express all answers to the correct number of significant digits.

Name _____ Date _____

Partner's Name _____

Substance Appearance after development	**Distance traveled**	**R_f Value**
Solvent	mm	
Co^{2+}	mm	
Cu^{2+}	mm	
Fe^{3+}	mm	
Ni^{2+}	mm	
Mixture Co^{2+} Cu^{2+} Fe^{3+} Ni^{2+}	_____ mm _____ mm _____ mm _____ mm	_____ _____ _____ _____
Unknown _____ Spot 1 Spot 2 Spot 3	_____ mm _____ mm _____ mm	R_f of spot and identity of ion _____ _____ _____ _____ _____ _____

Unknown number: _____

Post-Lab Questions and Exercises

(All questions must be answered during the lab and submitted with your lab report at the end of the lab period).

Please answer the following questions and show all work and units. Express all answers to the correct number of significant digits.

1) In the triangle/spiral diagram on page 1, which is more attracted to the paper, which to the water?

2) In the solution used in today's experiment, rank the ions for their attraction to the paper and to the acetone.

3) The solutions of ions were made using the following substances: $CoCl_2$, $CuCl_2$, $FeCl_3$, and $NiCl_2$. What is the formula mass for each of these substances?

4) Choose any ion that appears at least 3 times. What are the R_f values? How consistent are they?