

Paper Chromatography: Separation of Transition Metal Cations

Objectives: Learn the analytical technique of paper chromatography, and use it to separate and identify components in a mixture of transition metal cations

Materials: Chromatography paper (cut into approx. 4" x 6" rectangles); eluting solvent (90% acetone, 10% 6 M HCl); developing solvent (conc. NH_3); 50-mL beaker; 600-mL beaker; 1000-mL beaker; capillary tubes (for spotting chromatographic paper); plastic wrap; ruler; pencil; solutions of transition metal cations (Fe^{3+} , Ni^{2+} , Co^{2+} , Cu^{2+}); unknown mixtures of transition metal salts

Safety: Hydrochloric acid (eluting solvent) is caustic and corrosive. Avoid contact with skin and clothes. Concentrated ammonia is caustic and an irritant, and should be kept in the fume hood at all times. Avoid contact with skin and inhalation of fumes. Wear safety goggles and appropriate lab attire in the lab at all times.

Waste Disposal: Follow disposal instructions provided by your instructor.

INTRODUCTION

Some of the materials we use every day, such as sugar, salt, and water, are pure substances, or substances that consist of only one kind of matter. But most materials we encounter are **mixtures**, containing more than one substance. To understand the chemical and physical properties of a mixture it is often necessary to separate it into its individual component parts and to identify them. One method for separating complex mixtures is **chromatography**.

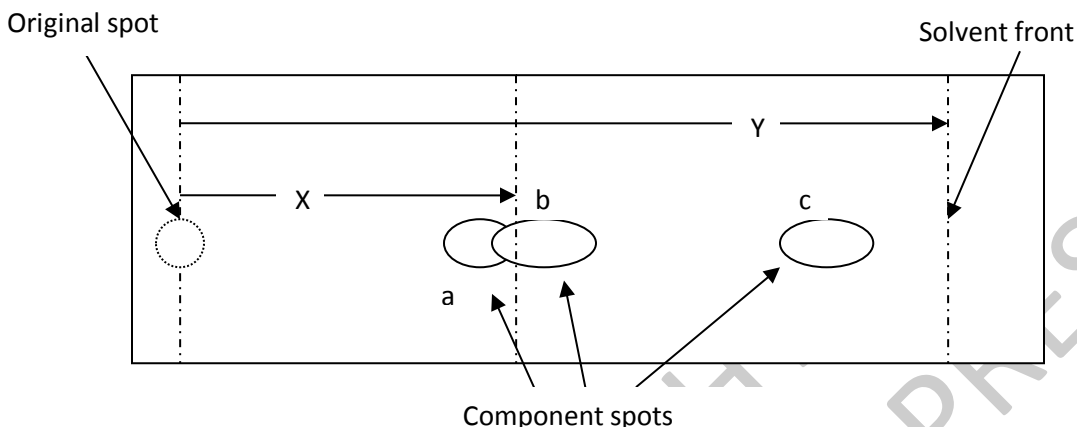
The name *chromatography* is derived from the original use of this method to separate plant pigments into colored bands (*chromos* = *color* in Greek). Most types of chromatography take advantage of differences in solubility, or the attraction between substances resulting from weak intermolecular forces. The technique used in this exercise is known as paper chromatography because the separating medium is porous paper.

In paper chromatography a small spot of a mixture is placed at one end of the paper. The paper is then placed vertically in a developing chamber containing a small amount of **eluting solvent** and the chamber is covered with a lid. The solvent travels up the paper by capillary action. As the solvent moves up the paper it dissolves the sample spot; those components that have a high affinity for the solvent (i.e., are more soluble) will travel up the paper more readily than components that are less soluble or that have a higher affinity for the cellulose in the paper. Over time, the components will separate as they move up the paper at different speeds.

How fast a component travels depends on the solvent, the type of paper, and the properties of the individual component. For a given system, the transport rate of a given component is fixed and can be characterized by the R_f value (or Retention factor), defined as:

$$R_f = \frac{\text{distance traveled by component spot}}{\text{distance traveled by solvent}} \quad (1)$$

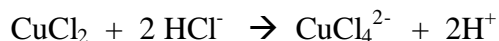
Each component spot in the sample moves independently and has a characteristic R_f value which can be used to identify the components. A sample chromatogram might look like this:



The R_f value for component (b) would be calculated as X/Y . Note that the R_f value will always be a fraction between 0 and 1 and is expressed as a decimal. Components that have a higher **affinity** for solvent (i.e., solubility or ability to interact with solvent) will travel a distance similar to the solvent and will have a R_f value closer to 1, such as component (c) in the diagram. Components with a low affinity (like component (a)) will not travel very far compared to the solvent and will have a R_f value closer to 0. Distances are usually recorded in centimeters (cm), although any units can be used. Since the units cancel in division, the R_f value is unitless.

A common variation on this technique is called thin layer chromatography (TLC). Instead of paper, a glass or plastic plate coated with a thin layer of silica gel is used. This technique is often used in biochemical assays, including identifying drug metabolites in urine samples. The identity of individual metabolites can be confirmed by comparison of R_f values with **standards**, or known substances. Since many components in a sample are not colored, the final chromatograms are often treated with chemicals that either fluoresce (or give off light) under ultraviolet light (i.e. “black light”), or that will stain the components so that they are visible.

In this lab you will use paper chromatography to separate and identify the individual components in a mixture of transition metal cations by comparison of R_f values with standard solutions. The separation will be performed on cellulose-based chromatography paper placed in a developing chamber containing the eluting solvent (HCl + acetone). The transition metal cations will form chloride complexes with the HCl:



The extent of the complex formation reaction can be described in terms of the stability constant. Those cations that form weak chloride complexes (i.e., small stability constants) will exhibit a higher affinity for the cellulose paper, while those that form strong complexes (larger stability constants) will exhibit a higher affinity for the acetone solvent and will be transported further up the paper. After some time, the chloride complexes should be visible at various locations on the paper as lightly colored spots. To enhance the visibility of the spots, the chromatogram can be placed in a beaker containing concentrated ammonia (NH₃). The NH₃ vapors react with the transition metal cations to form colored complexes that are more readily detected. The expected colors of the cation complexes formed with chloride and ammonia are provided in Table 1.

Table 1. Colors of Transition Metal Complexes

Cation:	Fe³⁺	Co²⁺	Ni²⁺	Cu²⁺
Ligand:				
Cl ⁻	Yellow-brown	Blue	Light green	Greenish blue
NH ₃	Red-brown	Pink	Light blue	Deep blue

Pre-Lab Questions

1. What is chromatography? What is the origin of the name?
2. What is the R_f value and how is it calculated?
3. How is the R_f value used to identify the components in a mixture?
4. What is the solvent used in this exercise?
5. Why must the spots on the paper not be allowed to touch the solvent in the bottom of the developing chamber (600-mL beaker)?

PROCEDURE

1. Obtain a piece of filter paper approximately 10 x 20 cm and several ink pens. Draw a line in pencil approximately 1 cm from the bottom end of the paper, as indicated by the dotted line in the diagram in Figure 1.
2. Obtain samples of the standard solutions of transition metal cations, and the cation mixture assigned by your instructor. Place a spot of each of the individual cation solutions on the penciled line on your paper, as shown in Figure 1. In addition, create a "Mixture" spot, on which you place a small drop of each of the four metal cation solutions. Finally, create a small spot containing the unknown solution. Write the identity of each spot (using either chemical symbols or numbers) below the spots (in pencil), as well as for your mixture and unknown spots. If you use numbers, record the numerical identity of each cation spot on your Data Sheet. Try to make the spots small enough so that they do not spread out too much during the separation. The resulting spots should be about this big: ●. Your finished paper should look like this:

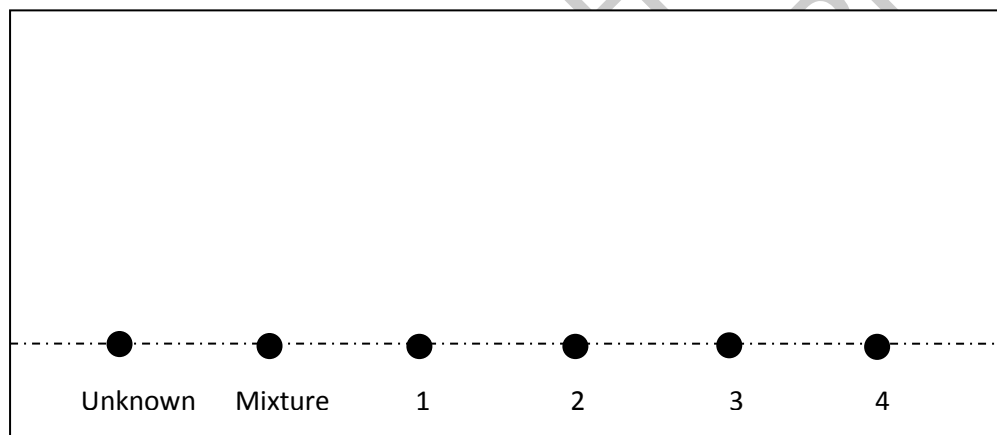


Figure 1. Filter paper with standard and unknown mixture spots before separation.

3. Carefully pour enough solvent to cover the bottom of the 600-mL beaker (10–15 mL). Carefully roll the chromatography paper into a cylinder and secure it at the top with a paper clip or staple. Place the spotted paper into the beaker (with the spotted end at the bottom) so that the sides of the paper do not touch the walls of the container. Be sure that the bottom edge of the paper is wetted, but that the spots are above the level of the solvent. Cover the beaker with the plastic wrap. Your paper chromatography chamber with the spotted paper should resemble the assembly in Figure 2.

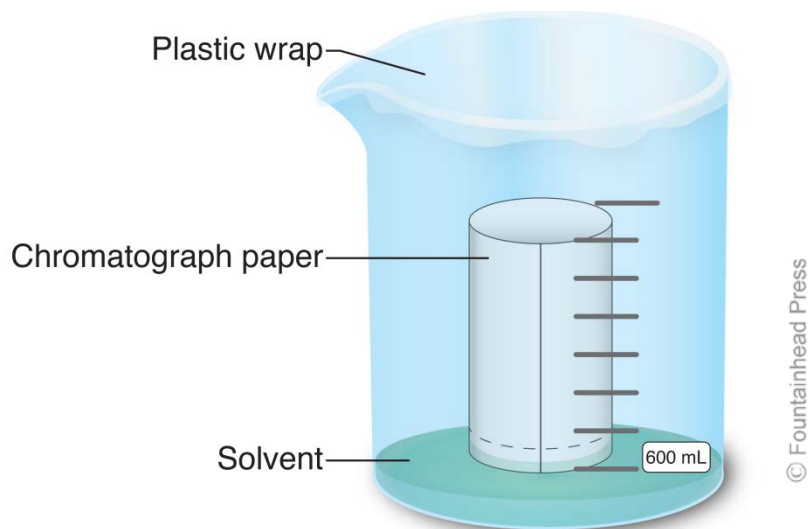


Figure 2. Chromatography developing chamber.

4. Check the paper occasionally to observe the progress of the solvent moving up the paper. When the solvent has moved to within $\frac{1}{2}$ inch of the top of the paper, or when it does not appear to be moving any further, the experiment can be stopped. **DO NOT ALLOW THE SOLVENT TO REACH THE TOP OF THE PAPER!** At this point your standard spots should have migrated up the paper to varying extents but may not be very visible, and the mixture and unknown spots should have separated into two or more smears, similar to those shown in Figure 3. **Remove the paper from the beaker and trace the solvent front with a pencil before the solvent evaporates.**

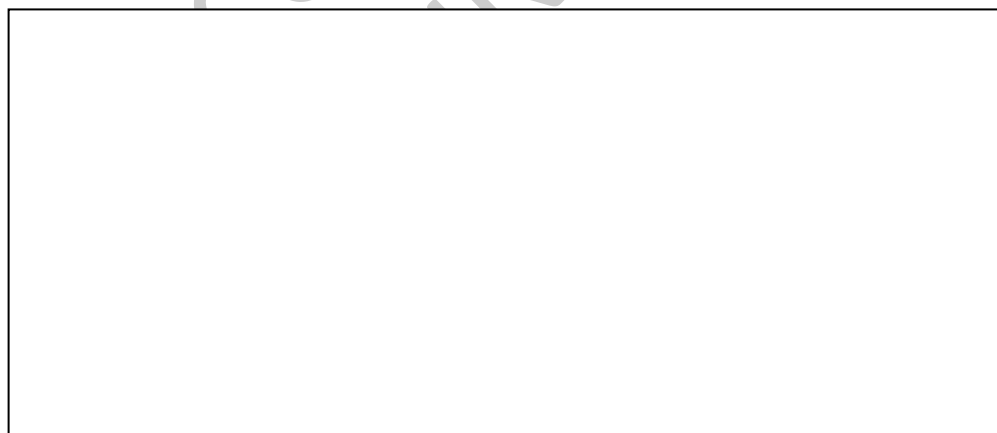


Figure 3. Typical chromatogram for sample and standard solutions.

5. Stand the paper up in an empty beaker and allow it to dry. To speed up the drying process, the paper can be placed in a drying oven, or can be gently heated with a heat gun or hair dryer (if available). When it is dry, place the paper in the ammonia chamber in the fume

hood. (Note: Other students may also have their paper rolls in the beaker, so be sure that you have some identifying information (name, drawer #, etc.) on your chromatogram. After a few minutes, the locations of the cation spots on your chromatogram should become visible as colored spots corresponding to the metal-ammonia complexes. When the colors of all spots are readily identifiable and consistent with the information in Table 1, remove your chromatogram from the ammonia chamber. Draw pencil lines around each component spot. Note the appearance for each of the standards, and the number of spots and their appearance for the unknown mixture and the standard mixture. Record this information on your Data Sheet.

6. Use a ruler to measure the distance of the solvent front and the distance traveled for the individual components in each of the standards and the mixture. Since the spots may be elongated, measure the distance to the center of the spot. Calculate the R_f values for each component spot and record these values on the Data Sheet.
7. Compare the colors and R_f values for the components in your standards with those in the mixture. Turn in your dry chromatograph with your Data Sheet. Be sure your name is on the chromatogram.

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Paper Chromatography Data Sheet

Observations: Describe the appearance of each component in your unknown and mixed ink samples. Calculate the R_f value for the individual components in each sample.

Distance traveled by solvent front: _____

A. Standard Cation Solutions:

Spot	ID (e.g., Fe^{3+} , Cu^{2+} , etc.)	Appearance	Distance traveled	R_f
#1				
#2				
#3				
#4				

B. Mixtures:

Spot	ID # (if applicable)	Appearance	Distance traveled	R_f	Component ID
Standard Mixture 1					
Unknown Cation Mixture					

Sample Calculations:

Post-Lab Questions

1. Were the R_f values for your cation spots in the standard mixture significantly different than for the R_f values for the individual cation standards? Explain why or why not.
2. Some of the spots will have R_f values close to 1, following the solvent front very closely. What does this tell you about the nature of these cation complexes?
3. The stability of transition metal complexes, quantified by the stability constant, would be expected to increase as $\text{Fe} < \text{Co} < \text{Ni} < \text{Cu}$. Is this order consistent with your experimental results? Explain.
4. In one experiment the solvent moved 3.0 inches while one of the components moved 5.2 cm. Calculate the R_f value for this component. (Watch your units and show your work.)