Experiment-A2 - Paper Chromatography

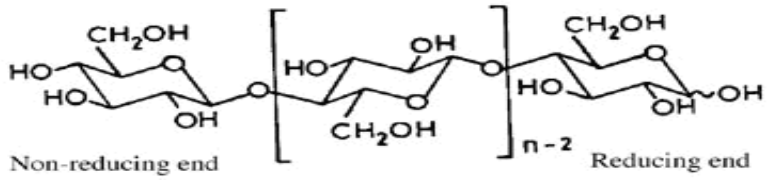
Purpose

In this experiment students will learn how to separate and identify components in an aqueous mixture of cobalt(II) chloride, copper(II) chloride, iron(III) chloride, and nickel(II) chloride, using paper chromatography.

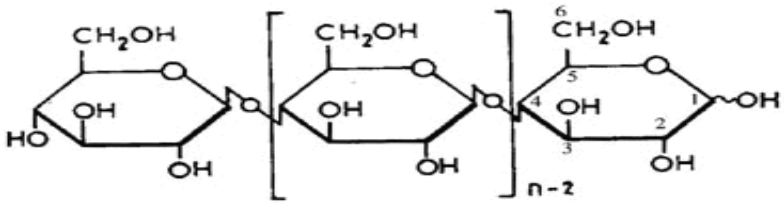
Introduction

Chromatography is *a technique that enables one to separate and identify components in a mixture*. Separation of components by chromatography is based on the different coefficients (K_d) that characterize the affinity of a given component between two *immiscible phases* – the *stationary phase* (S) and *mobile phases* (M). The *stationary phase* can be a *solid, gel, or liquid* that becomes immobilized on a solid support, while the *mobile phase* can be a *liquid* or a *gas* that flows through the stationary phase. A given component A that has a greater affinity for the mobile phase and a weaker affinity for the stationary phase will travel with the solvent at a faster rate than another component B that has a greater affinity for the stationary phase and a weaker affinity for the mobile phase. Thus, components A and B will be separated during chromatography.

In paper chromatography, the paper, which is made of cellulose, serves as the *stationary phase*. The cellulose structure (as shown in the diagram below) contains a large number of hydroxyl groups (–OH) that can form hydrogen bonds to water molecules. Therefore, the *stationary phase* is actually a layer of water hydrogen bonded to cellulose. If the solvent is water, the *mobile phase* is also water; but if it is a mixture of an organic solvent, such as acetone, and water, the mobile phase will contain a high proportion of the organic solvent. (Organic solvents are less polar than water and, therefore, they are less attracted to the cellulose of the paper.)



Sometimes shown as



Cellulose

In this experiment, you will use a solvent system that is a mixture of acetone 6 *M* hydrochloric acid. The cellulose structure of the paper absorbs the aqueous portion of the solvent, which becomes the *stationary phase*. While the rest of the solvent, which is composed of mainly acetone that makes up the *mobile phase*, moves up through the paper fibers by capillary action. A solute in the *mobile phase* moves along with the solvent during the chromatography development. As it moves, it undergoes many successive partitioning between the *mobile* and *stationary phases*. The relative fractions of time a given solute spends in the mobile

phase determines how fast it moves up on the paper during the development process. A solute that has a high affinity for the mobile phase and spends more time in the mobile phase will move up faster on the chromatographic paper. On the other hand, a solute that has a high affinity for the stationary phase will move up slower on the chromatographic paper. The different affinities of components in a solution mixture for the *mobile* and *stationary* phase allows their separation by paper of other chromatographic techniques. The solvents also determine how best the separation of two or more components in the mixture at the end of the chromatographic development. For a given solute, this relative affinity, which is a chromatographic property called the *retention factor* or R_f value, depends on temperature and the solvent used. At a given temperature and in a given solvent system, the R_f value of a solute will remain more or less constant. Therefore, by running a chromatography of a mixture containing unknown components with known samples of individual solutes, we can identify of each component in the mixture.

In the paper and thin-layer chromatography, a drop of solution containing a mixture of substances is applied near one end of a rectangular piece of paper, called the *point of application* (POA). This end of the paper is immersed in the developing solvent. Capillary action causes the solvent to rise up the chromatography paper. When the solvent reaches the spot where the mixture is applied, the components in the sample will begin to migrate upward with the solvent (mobile phase). Each component has characteristic affinities for the stationary phase (the cellulose paper) and for the solvent. These characteristic affinities determine the rate each component moves along with the solvent, which allows different components in the solution to be separated and identified.

A component's affinities for the stationary and mobile phases are entirely characteristic of that substance. Under a given set of conditions (including temperature and humidity), different substances have different characteristic affinities for the stationary and mobile phases. Therefore, each component in a mixture will travel up the paper at its own characteristic rate. If the paper is sufficiently large and enough time is allowed for the chromatogram to develop, all the components can be separated by the time the solvent has reached the top of the paper. A component's characteristic affinities for the stationary and mobile phases are expressed in term of its *retention factor*, or R_f value. This is calculated as the ratio of the distance traveled by the spot of a component to the distance traveled by the solvent,

$$R_f = \frac{\text{distance traveled by spot}}{\text{distance traveled by solvent}}$$

The R_f value of a substance is characteristic of that substance and should be a constant under a given experimental conditions, which include the type and thickness of the chromatography paper, the type of solvents, temperature, and humidity. Therefore, the separation and identification of components in a mixture using paper chromatography are normally accomplished by running the chromatography for the unknown sample together with the known substances.

At the end of the chromatography development, each component will appear as a separate spot. If the components are intensely colored, the spots will be visible. Weakly colored or colorless spots can be made visible by spraying them with substances that react with the components in the spots. The chromatography paper will now contain a vertical array of colored spots arranged according to their characteristic rates of ascent on the paper.

In this experiment, you will use paper chromatography to separate a mixture containing $CoCl_2$, $CuCl_2$, $FeCl_3$, and $NiCl_2$. The unknown mixture and samples containing individual substances will be chromatographed on the same paper. The mobile phase will be a mixture of acetone and dilute hydrochloric acid (HCl) solution. At the end of the experiment, you will place the chromatography paper over the vapor of concentrated ammonia. The NH_3 vapor will react with the cations, forming a distinctive colored complexes such as $Co(NH_3)_6^{2+}$, $Cu(NH_3)_4^{2+}$, $Fe(NH_3)_6^{2+}$, and $Ni(NH_3)_6^{2+}$, which make the spots easily

visible. You will determine the R_f value of each cation in the unknown mixture and in the pure sample, and subsequently identify the cations present in the unknown mixture.

Safety Precaution:

- Acetone and butanol are flammable liquid. No open flame is allowed during the experiment.
- Hydrochloric acid and ammonia can cause chemical burns. If you spill any of these chemical on you, wash the affected area thoroughly under running water for several minutes and report the incident to your instructor. If you spill hydrochloric acid or ammonia solution anywhere, please inform your instructor.
- Ammonia vapor has a strong choking odor. DO NOT inhale or breathe the vapor.
- DO NOT remove concentrated ammonia from the fume hood.

Equipment and Chemicals

A 400-mL beaker, a watch glass, two chromatography paper (already cut to size), acetone, 6 *M* HCl, aqueous solutions of $FeCl_3$, $CoCl_2$, $NiCl_2$, and $CuCl_2$, and an unknown mixture.

Procedure

1. Prepare a solvent mixture by mixing 10 ml of acetone and 3 mL of 6 M HCl in the 400-mL beaker. Cover the beaker with a watch glass or a piece saran wrap.
   (Note: Prepare the solvent first before working on the chromatography paper.)

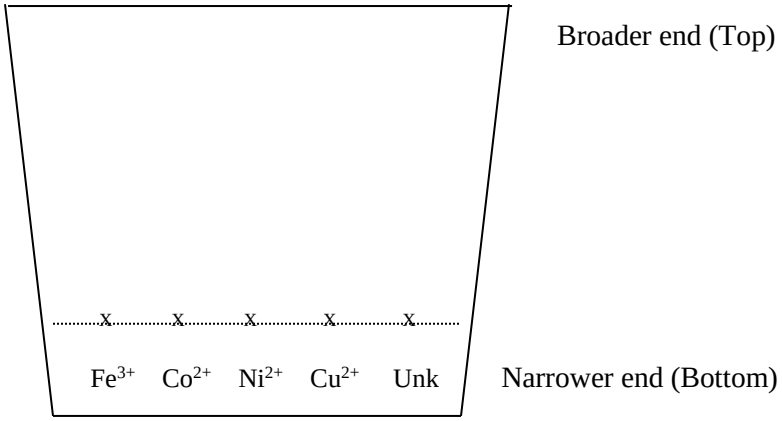
2. Obtain two (2) pieces of chromatography papers that have been cut to fit in a 400-mL beaker such as shown in the diagram below.

3. Make a pencil line about 1.5 cm from the narrower end of the paper (as shown in the diagram).

4. Use a pencil and mark five (5) spots, about 1 cm apart from each other on the pencil line. The spots at each end of the paper should also be about 1 cm from the side edge of the paper. Using a pencil, mark the spots with Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Unk(?) – indicate the letter ID for the unknown sample.

5. Obtain a solution-tray and put a drop of each known solution and two (2) different unknown mixtures in separate wells. Use a different capillary tube to apply each known cation ($FeCl_3$, $CoCl_2$, $NiCl_2$, or $CuCl_2$) and the unknown mixtures on appropriate spots on the paper. Apply only one unknown sample on each chromatography paper.

   The shape of chromatography paper used in this experiment.



[Note: When applying samples on the paper, you may need to apply each sample 3 – 4 times in order to saturate the spot with the cations, but make sure that these spots DO NOT touching each other. If they do, discard the paper and start over with a new piece of paper.]

6. Place both chromatography papers in the same developing tank (the 400-mL beaker) containing the solvent mixture prepared earlier with the narrow end of the paper in the solvent. Be sure that all spots of applied samples are above the solvent level and make sure that the papers are not touching one another. Cover each beaker with a watch glass and allow the chromatogram to develop for 40 – 45 minutes without disturbing or moving the beaker. (If you have any questions about your equipment set up, ask the instructor to come to your bench, but not carry the beaker to him/her. The sloshing of the solvent in the beaker can affect the development of the chromatogram.) Observe and record the color of each spot as it rises on the paper.

7. Allow the chromatograms to develop until the solvent front is about 1 - 1.5 cm from the top edge of the paper, which may take between 40 - 45 minutes. Keep an eye on the solvent front to make sure that it does not go over the top edge of the paper. By the end of the chromatography development, some of the spots may not be visible. It is important that you record the color of each spot in the solvent during the first 10 minutes of the developing time.

8. Remove the paper from the beaker and mark the *solvent front* with a pencil while it is still visible. Take the chromatograph paper to the fume hood and place it flat on top of the 250-mL beaker that contains some concentrated ammonia solution. Leave the paper in the NH_3 vapor for about 1 minute to allow the reaction of NH_3 with each cation. Record the color of each spot in the NH_3 vapor.

9. Circle each spot with a pen or a pencil. Measure the distance (in cm or mm) traveled by the solvent and by each cation. All measurements should be done from the point of application (poa) to the and the distance traveled by the solvent. Calculate the R_f values of each substance in the known sample and in the unknown mixture in each developing solvent.
   [Note: The unknown mixture contains more than one substance and will produce more than one spot. Measure the distance traveled by each spot and calculate its R_f value.]

10. Write the ID-letter of each unknown sample and identify the cations present in each unknown.

11. You will attach in this chromatogram to your lab report.

12. Dispose of the solvent into the chemical waste container labeled “Acetone-HCl Waste”. Dispose of all capillaries in the broken glass container and rinse off the solution tray.

Name:_____ Date: _____

Experiment-A2: Paper Chromatography

Pre-laboratory Assignment

1. Define the following terms:

   Chromatography, stationary phase, and mobile phase.

2. What is the composition of the mobile phase (the solvent) used in this experiment?

3. What components will be separated and analyzed in this chromatographic study?

4. Write the formula of each of the following substances used in this experiment:

   (a) Iron(III) chloride: _____; (b) Cobalt(II) chloride:_____;

   (c) Nickel(II) chloride:_____; (d) Copper(II) chloride:_____;

   (e) Acetone: _____; (f) Hydrochloric acid: _____.

5. A piece of chromatography paper is spotted with a solution containing $CoCl_2$ and $CuCl_2$. The chemical affinity of Co^{2+} for the stationary phase is less than that of Cu^{2+}, while the chemical affinity of Co^{2+} for the mobile phase is greater than that of Cu^{2+}. Which cation will travel farther at the completion of the chromatography experiment? Which cation will have the larger R_f value? Explain each answer.

6. Should a pen be used to draw lines or write symbols on the chromatography paper? Explain.

Name:_____ Date:_____

Experiment-A2: Paper Chromatography

Results (1)

Solvent: Acetone-HCl mixture

Substance	Color in solvent	Color in NH_3	Distances of spots	R_f Values
Fe^{3+}				
Co^{2+}				
Ni^{2+}				
Cu^{2+}				
Unknown: _____ _____				

Distance traveled by solvent:_____ cm

Cations present in unknown mixture:_____

Sample calculations of R_f:

Post-lab Questions

1. What cations were present in each unknown sample you analyzed?

2. (a) Which cation has the greatest affinity for the mobile phase and lowest affinity for the stationary phase?
   (b) Which cation has the highest affinity for the stationary phase and lowest affinity for the mobile phase?

3. Suppose that, under certain conditions, Co^{2+} and Cu^{2+} have R_f values of 0.80 and 0.65, respectively. If the distance travelled by the solvent is 6.0 cm, what are the distances traveled by Co^{2+} and Cu^{2+}, and how far apart are the two cations become separated at the end of chromatographic run?

4. Another method of making the spots visible is by spraying the chromatogram with sodium sulfide (Na_2S) solution. Sodium sulfide reacts with each of the cations to form precipitates, such as Fe_2S_3, CoS, NiS, and CuS, which are dark in color. Complete and balance each of the following equations, and appropriately label the products with "(s)" after the formula for the precipitates and with "(aq)" for those that are in solution.

   (a) $FeCl_3(aq) + Na_2S(aq) \rightarrow$

   (b) $CoCl_2(aq) + Na_2S(aq) \rightarrow$

   (c) $NiCl_2(aq) + Na_2S(aq) \rightarrow$

   (d) $CuCl_2(aq) + Na_2S(aq) \rightarrow$