EXPERIMENT 13

Chemical Separations by Paper Chromatography

INTRODUCTION

The word *chromatography* is derived from the Greek *chroma*, meaning color. Chromatography is any process in which a solvent is used to carry the components of a mixture through a solid, porous adsorbing material until the components are separated from each other. In most early experiments, the separated components were identified by their colors, and the procedure became known as chromatography. The name given to a particular kind of chromatography depends upon the manner in which the experiment is conducted. Thus, we have column, thin-layer, paper, and gas chromatography, all in very common use. Chromatography in its many possible variations is perhaps the best method for resolving a mixture into pure substances, regardless of whether that mixture consists of gas, a volatile liquid, or a group of nonvolatile, relatively unstable, complex organic compounds.

The term *adsorption*, used in the first paragraph, is different from the term *absorption*. Absorption is a process in which a substance permeates throughout another, such as water into a sponge. However, adsorption is a process in which a substance collects on the surface of another. In chromatography a mixture is adsorbed onto a solid material, and then a solvent is placed in contact with the adsorbing solid. The adsorbing solid used in chromatography can be a strip of filter paper, a thin layer of silica gel on a piece of glass, some finely divided charcoal packed loosely in a glass tube, or even some microscopic glass beads coated very thinly with a suitable adsorbing substance contained in a glass tube. As the solvent passes through the stationary adsorbing solid, the various components of the mixture are attracted both to the moving solvent and to the stationary solid. The rate at which a component will travel along with the moving solvent through the stationary adsorbing solid depends on its relative attractions to the solvent and to the solid. Because each component of the mixture attracts differently to the solvent and to the solid, the net effect is that as the solvent passes slowly through the stationary adsorbing solid, the components separate from each other.

In this experiment we will use paper chromatography to separate a mixture of metallic ions in solution. A spot of sample solution is applied near one end of a strip of paper, and the solvent is allowed to evaporate. The strip is then placed in a closed container with the spotted end immersed in a shallow layer of mixed water and organic solvents. The sample spot should be located just above the surface of this layer. Because they are polar, water molecules saturated atmosphere of the container are quickly adsorbed (bound) by the polar cellulose molecules of the paper. Actually, filter paper normally contains a significant percentage of water. As a result, the paper serves as an adsorbent for a stationary water phase.

Capillary action causes solvent from the shallow layer to flow through the paper. This flow, commonly referred to as development of the chromatogram, carries along the various components of the sample, each attracted by the stationary cellulose-bound water and the mobile organic liquids. The rate at which a particular component moves is determined by its *distribution coefficient, D*:

\[
D = \frac{\text{solubility in mobile phase}}{\text{solubility in stationary phase}}
\]

With a mixture of two components, for example, the one with the larger distribution coefficient will generally move more rapidly. Therefore, components with different distribution coefficients separate and are soon located at different positions along the paper.
One common technique for locating a component on a developed chromatogram is to spray the paper with a reagent that produces a particular color reaction. As a result, a small spot appears and the distance from its center to the original position of the sample is measured. From the point of sample application, the ratio of the distance traveled by a component (C) to the distance traveled by the solvent front (S) is called the \textit{retention factor}, \( R_f \) for that component:

\[
R_f = \frac{C}{S} = \frac{\text{distance component moves}}{\text{distance solvent moves}}
\]

Although a particular component has a characteristic \( R_f \) value under a specified set of conditions, the value is affected by so many variables that it can only serve as a rough indication of identity. For this reason, it is customary to chromatograph, along with a sample, substances presumed to be identical with the substances of the sample. If \( R_f \) values for a known and a component are the same, a fairly conclusive identification is obtained.

Other common types of chromatography include thin-layer, column, and gas chromatography. In thin-layer chromatography (TLC), the adsorbing solid material is silica gel spread uniformly on a glass plate. A spot of sample is applied and a chromatogram developed as in the paper method. Components are located with color producing reagents and \( R_f \) values are measured. Compared with paper chromatography, the thin-layer method provides a sharper and more rapid separation although it is usually more expensive.

Column chromatography uses finely divided charcoal, alumina, or even some microscopic glass beads coated very thinly with an adsorbing substance, contained in a glass tube. A mixture is added to the top of the vertical column, and then solvent is continually poured through the column. As the different components pass through at different rates, they can be collected individually in separate flasks below.
One of the most widely used chromatographic techniques is gas chromatography (GC). Here, the components are separated as vapors. A liquid or solid mixture is injected into a heated glass tube at one end. The tube is packed with a finely divided solid, often coated with a high-boiling viscous liquid. An unreactive carrier gas, often helium, is passed through the tube. The components of the sample gradually separate as they vaporize into the helium or adsorb onto the packing.
1. Students will work individually for this experiment. Except for the laboratory handout, remove all books, purses, and such items from the laboratory bench top, and placed them in the storage area by the front door. For laboratory experiments you should be wearing closed-toe shoes. Tie back long hair, and do not wear long, dangling jewelry or clothes with loose and baggy sleeves. Open you lab locker. Put on your safety goggles, your lab coat, and gloves.

2. Obtain a spot plate from drawer 026 in the back of the lab room and obtain a small piece of filter paper from the cart. The instructor will bring you an unknown solution. Write down the number of your unknown (found on the unknown’s vial) in the Unknown section the Data Table. Do not remove the number label from the unknown vial.

3. Set the spot plate on a piece of paper. Write $\text{Co}^{2+}$, $\text{Cu}^{2+}$, $\text{Fe}^{3+}$, and $\text{Ag}^+$ respectively on the paper next to four of the wells of the spot plate. Place two or three drops of 0.1 M solutions of cobalt (II) nitrate, copper (II) nitrate, iron (III) nitrate, and silver nitrate into the four separate, labeled wells of the spot plate. The metallic cations are $\text{Co}^{2+}$, $\text{Cu}^{2+}$, $\text{Fe}^{3+}$, and $\text{Ag}^+$ respectively. Capillary tubes will be used as the applicators for each solution. Test the application procedure by dipping a capillary tube into the colored cobalt (II) ion solution and touching it momentarily to the small piece of filter paper. The liquid from the capillary tube should form a spot about equal to, but no larger than, 5 mm in diameter. Use a ruler to verify the size. Practice making spots until you can reproduce the spot size each time. Keep this capillary tube with the cobalt (II) ion solution. Use one capillary tube for each of the solutions in order to prevent contamination.

4. Set out a paper towel on your lab bench, then obtain a rectangular piece of filter paper about 19 cm wide and 11 cm high from the cart to use as your chromatogram. **Place your chromatogram on a paper towel so it does not pick up any contamination from the lab bench, and do not use pen on your chromatogram.** With the chromatogram in landscape orientation, draw a horizontal pencil line 1 cm from the bottom (the 19 cm edge) using a ruler. Starting 3.50 cm from the left edge of the paper, mark the line at 3.00 cm intervals until 5 markings have been made. If you have done this correctly, the fifth marking will be 3.50 cm from the right edge of the paper. Label the markings on the line as shown in the figure below, with the formulas of the ions to be studied and the unknown mixture.

![Diagram of chromatogram](image)
5. With a capillary tube, form a spot of the cobalt (II) ion solution on the intersection of the pencil lines on the chromatogram where you have Co²⁺ labeled. Keep this capillary tube with the cobalt (II) ion solution. Use a clean capillary tube for the next solution. Dip the capillary tube into another cation solution and put a spot on the intersection of the pencil lines on the chromatogram labeled for that cation. Keep that capillary tube with its corresponding solution. Using another clean capillary tube, repeat the procedure with another solution. Continue this approach until you have put a spot for each of the four cations, and for your unknown on the chromatogram.

6. Dry the filter paper by moving it back and forth over a heat gun, using its lowest setting, so the paper doesn’t burn. Heat the paper just enough to dry the spots. Apply the cation solutions once more to the same spots, and apply the unknown solution two or three times more to its spots, drying between applications. The unknown solution is less concentrated than the cation solutions, so this procedure will increase the amount of each ion in the unknown spots. Make sure that you dry the spots between applications, since otherwise they will get larger.

7. Obtain 15 mL of the eluting solution (a mixture of hydrochloric acid, ethanol and butanol), pour it into a 600-mL or 800-mL beaker, and cover the beaker with a watch glass or a large Erlenmeyer flask. Position the snorkel hood so that it is about 15 to 18 inches above the lab bench, and set the covered beaker containing the eluting solution under the snorkel hood.

8. Check to make sure that spots on the chromatogram are all dry. Place a 4 to 5 cm length of Scotch tape along the upper end of the left edge of the paper, vertically as shown below, so that about half of the tape is on the paper. Form the paper into a cylinder by attaching the tape to the other edge, in such a way that the edges are parallel but do not overlap. When you are finished, the pencil line at the bottom of the cylinder should form a circle, approximately anyway, and the two edges of the paper should not quite touch. Stand the cylinder up on the lab bench to check that such is the case and readjust the tape if necessary. The cylinder should be small enough so that when it is placed into the 600-mL beaker it does not touch the inside wall of the beaker. Do not tape the lower edges of the paper together.

9. Place the cylinder in the eluting solution in the 600-mL or 800-mL beaker, with the sample spots down near the liquid surface. The paper should not touch the wall of the beaker. Cover the beaker with the watch glass or large Erlenmeyer flask. The solvent will gradually rise by capillary action up the filter paper, carrying along the cations at different rates. After the process has gone on for a few minutes, you should be able to see colored spots on the paper, showing the positions of some of the cations. Allow the eluting solution to rise up the filter paper for about 75 minutes. Do not disturb the beaker for the entire 75 minutes.
10. During this time, dispose of your cation solutions and your unknown solution in the waste bottle in the fume hood, and dispose of your used capillary tubes in the waste bag in Fume Hood A. Rinse and dry the spot plate and the unknown vial, and return them to the back of the lab room.

11. After the 75 minutes, remove the cylinder from the beaker and take off the tape. Dry the chromatogram by moving it back and forth over a heat gun, using its lowest setting. Draw a horizontal pencil line along the solvent front. Several cations will be colorless, but record the colors of any cation spots you can see, and draw a horizontal pencil line through the middle of the visible spots to mark their locations. Your unknown may contain anywhere from one to four cations. Record the colors of each cation spot found in your unknown and draw a horizontal pencil line through the middle of each of the visible spots to mark their locations.

12. In Fume Hood B hang your chromatogram on the metal support rod. Use the spray bottle of staining solution to spray the staining solution onto the chromatogram. Dry the chromatogram by moving it back and forth over a heat gun, using its lowest setting. Record the colors of the cation spots and draw a horizontal pencil line through the middle of any new spots that have been revealed with the staining solution. If you have trouble seeing some spots after the staining and drying, try rinsing the chromatogram with deionized water.

13. Measure the distance from the straight line on which you applied the spots to the solvent front, which is the distance $S$ in the equation on page 128. Then measure the distance from the pencil line on which you applied the spots to the center of the spot made by each of the cations; this is distance $C$. Calculate the $R_f$ value for each of the cations. Repeat the procedure for each of the spots in the unknown mixture, and calculate the $R_f$ value for each cation in the unknown.

14. By matching the $R_f$ values and colors, predict what cations are in your unknown mixture. In your Data Table under “CATIONS PRESENT IN UNKNOWN SOLUTION” write the cations you believe are present in your unknown solution.

15. Dispose of your eluting solution in the waste bottle in Fume Hood A.

16. Dry your chromatogram completely, and then staple it to the back of your lab report.

17. Clean and wipe dry your laboratory work area and all apparatus. When you have completed your lab report have the instructor inspect your working area. Once your working area has been checked your lab report can then be turned in to the instructor.
**EXPERIMENT 13 LAB REPORT**

Name: ___________________________________________  Student Lab Score: ___________
Date/Lab Start Time: _______________________________  Lab Station Number: _______________

**DATA TABLE**

<table>
<thead>
<tr>
<th>CATION SOLUTIONS</th>
<th>Co$^{2+}$</th>
<th>Cu$^{2+}$</th>
<th>Fe$^{3+}$</th>
<th>Ag$^{+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color Dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color with Staining Agent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance Solvent Moved ($S$)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Distance Cation Moved ($C$)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>1-4 $R_f$</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UNKNOWN __________</th>
<th>SPOT 1</th>
<th>SPOT 2</th>
<th>SPOT 3</th>
<th>SPOT 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color Dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color with Staining Agent</td>
<td></td>
<td></td>
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<tr>
<td>Distance Solvent Moved ($S$)</td>
<td>.</td>
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</tr>
<tr>
<td>Distance Cation Moved ($C$)</td>
<td>.</td>
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<td>.</td>
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</tr>
<tr>
<td>5-8 $R_f$</td>
<td>.</td>
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</tr>
</tbody>
</table>

**CATIONS PRESENT IN UNKNOWN SOLUTION:**

**CALCULATIONS**

1. ______  2. ______  3. ______  4. ______
**QUESTIONS**

1. Assume the left rectangle is your chromatogram. Draw (1) the line near the bottom where the spots were originally applied using the capillary tubes, (2) all of the cation spots after the 75 minute eluting time, and (3) the line showing the position of the final solvent front.

Assume the right rectangle is your chromatogram. Draw (1) the line near the bottom where the spots were originally applied using the capillary tubes, (2) all of the cation spots if the eluting time was only 15 minutes, and (3) the line showing the position of the final solvent front.

2. If the experiment was stopped after 15 minutes instead of waiting the entire 75 minutes, would the calculated $R_f$ values be too high, too low, or the same?

_________________________________________________________________________________

3. Aside from just being more accurate, why do we allow the eluting solution to rise up the filter paper for 75 minutes, instead of allowing it to rise up the filter paper for only 15 minutes?

_________________________________________________________________________________

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_________________________________________________________________________________