Separation of Mixtures by Paper Chromatography

Objectives

In this experiment we will effect a separation of a mixture of amino acids based on their differences in solubility.

Background

When determining the physical and chemical characteristics of an element or a compound, chemists must be certain the substance is pure. Most substances in nature, however, are mixtures, and so it is necessary for chemists to be able to separate a mixture into its various pure components.

*Some mixtures of solids can be separated by dissolving one or more components.
*Mixtures containing solid and liquid components can often be separated by simple decantation, filtration, or centrifugation.
*Volatile liquids can be separated by fractional distillation, taking advantage of the difference in boiling points of the component substances.
*Certain materials, such as iodine and naphthalene, pass directly from the solid state to the vapor state upon heating and can be purified by sublimation.
*Other mixtures can be separated by crystallization on the basis of the differences in solubility.

One of the most versatile and powerful separation techniques used by chemists is chromatography, of which there are several types. The experiment today will utilize paper chromatography.

Chromatography is an analytical method that is widely used for the separation, identification, and determination of the chemical components in complex mixtures, many of which could not otherwise be resolved. Chromatography was invented and named by the Russian botanist Mikhail Tswett shortly after the turn of the century. He employed the technique to separate various plant pigments, such as chlorophylls and xanthophylls, by passing solutions of them through glass columns packed with finely divided calcium carbonate. The separated species appeared as colored bands on the column, which accounts for the name he chose for the method (Greek chroma meaning "color" and graphein meaning "to write").

The applications of chromatography have grown explosively in the last four decades, owing not only to the development of several new techniques but also to the expanding need of scientists for better methods of separating complex mixtures. The tremendous impact of these methods on science is attested by the 1952 Nobel prize awarded to A.J.P. Martin and R.L.M. Synge for their discoveries in the field. Perhaps more impressive is the list of 12 Nobel prizes awarded between 1937 and 1972 that are based upon work in which chromatography played a vital role. (Skoog and West, 5th ed.)
A General Description of Chromatography

The term chromatography is difficult to define rigorously, owing to the variety of systems and techniques to which it has been applied. All of these methods, however, have in common the use of a stationary phase and a mobile phase. Components of a mixture are carried through the stationary phase by the flow of gaseous or liquid mobile phase, separations being based on differences in migration rates among the sample components. These differences in migration rates can be caused by variations in polarity, size, solubility, etc.

Classification of Chromatographic Methods

Chromatographic methods can be categorized in two ways. The first is based upon the physical means by which the stationary and mobile phases are brought into contact with each other. In column chromatography, the stationary phase is held in a narrow tube, and the mobile phase is forced through the tube under pressure or by gravity. Figure 1 shows an example of elution chromatography in which a liquid solvent (the mobile phase) carries the sample mixture over a solid support (the stationary phase) packed into a glass column. As the sample migrates down the column, the separation of components in the mixture occurs due to their interaction with the solid support. Since the rate of migration is dependent on how long the solute stays in the mobile phase, those that interact with the stationary phase take longer to migrate.

In gas chromatography, the sample is vaporized and then forced through a long narrow heated column using a "carrier" gas (the mobile phase). The inner walls are coated with a support medium (the stationary phase) capable of interacting with the components in the sample. As the sample travels through the column, those components that interact more strongly with the walls of the column are slowed down and elute later than those that do not interact with the column walls.

In planar chromatography, the stationary phase is supported on a flat plate or in the pores of a paper. In the case of paper chromatography, as we will see in this experiment, the mobile phase (a liquid solvent) moves through the stationary phase (the paper) by capillary action. In today's experiment, a piece of porous paper is positioned with one end resting in the solvent. A drop of solution containing the substance to be separated is placed on one edge of the paper and the solvent is allowed to pass over the drop. Those components in the sample mixture that interact least strongly with the paper and most strongly with the solvent are drawn farthest up the paper with the moving liquid. This technique is used often in biochemistry to separate the products of a reaction.
Amino Acid Separation

Proteins are complex molecules essential to the structure and functions of the cells in your body. These macromolecules are polymers, made by linking many simpler units called amino acids. Twenty distinct amino acids have been obtained from the hydrolysis of proteins. We will study four amino acids today; Glycine, Tyrosine, Leucine, and Aspartic acid. Besides these amino acids each student will receive an Unknown containing from two to four of these same amino acids.

Procedure

1. Be careful to avoid touching the filter paper with your hands. Amino acids in the oils and perspiration on your skin will contaminate the chromatogram.

2. Obtain a piece of filter paper measuring approximately 10 x 20 cm. This size paper fits nicely into an 800 mL beaker. If another size beaker is used, the width of the paper should be slightly less than the height of the beaker and the length short enough so that the cylinder formed by the paper will fit in the beaker without touching the walls. (See Figure 3.)

3. Mark the paper with a pencil as shown in Figure 2.

4. Use a capillary tube to apply a small drop of each solution (0.05 M amino acids in 1.5% HCl) no greater than 2-3 mm in diameter at their respective starting marks.

5. Staple the filter paper into a cylinder with two staples as shown in Figure 3. such that the edges of the paper do not touch each other.

6. In the hood, place solvent (2 parts isopropyl alcohol: 1 part 2% NH₄OH) to a depth of 0.5 cm in the beaker. Place the paper cylinder in the beaker so that it does not touch the side (see Figure 3.). DO NOT MOVE THE BEAKER AFTER THE PAPER IS ADDED.

7. Cover the beaker with aluminum foil and allow the chromatogram to develop until the solvent front is three-quarters the way up the paper (about 90 minutes).
8. Remove the paper, mark the solvent "front" with a pencil, and allow it to dry.

9. Remove the staples. Under the hood, spray the paper with ninhydrin solution (2% ninhydrin in ethyl alcohol). Place the paper in the oven at 80°C for about 3 minutes.

10. Measure the distance (in mm) from the origin to the center of each spot. Also record the color of each spot.

11. Calculate the $R_f$ (the retention factor) for each spot. (Figure 4.)

\[
R_f = \frac{\text{distance the sample moved (D)}}{\text{distance the solvent moved (L)}}
\]

12. Identify the amino acids in the unknown mixtures by visual comparison of spot colors and by the relationships of $R_f$ values. For example, the chromatogram below indicates the presence of Aspartic acid and Tyrosine in the Unknown mixture.
REPORT SHEET
Separation of Mixtures by Paper Chromatography

Unknown Number _____________

Average distance solvent front traveled (from the starting line) ________________mm

**Standards (Knowns)**

<table>
<thead>
<tr>
<th>Distance sample moved (mm)</th>
<th>Glycine</th>
<th>Tyrosine</th>
<th>Leucine</th>
<th>Aspartic Acid</th>
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<tbody>
<tr>
<td>Trial 1</td>
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<tr>
<td>Trial 2</td>
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<tr>
<td>Average R&lt;sub&gt;f&lt;/sub&gt;</td>
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<tr>
<td>Color</td>
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**Unknown (May be 2-4 components)**

<table>
<thead>
<tr>
<th>Distance sample moved (mm)</th>
<th>Component</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Average R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Amino Acid Present</th>
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PRELAB: Exp.14 Paper Chromatography

1. Consider the chromatogram below. G, T, L and A stand for Glycine, Tyrosine, Leucine and Aspartic Acid. U₁ is Unknown #1 and U₂ is Unknown #2

<table>
<thead>
<tr>
<th>solvent front</th>
<th>G</th>
<th>T</th>
<th>L</th>
<th>A</th>
<th>U₁</th>
<th>G</th>
<th>T</th>
<th>L</th>
<th>A</th>
<th>U₂</th>
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</table>

a) Compute the $R_f$ value for each spot on the chromatogram.

b) What amino acids are present in Sample U₁?

c) What Amino Acids are present in Sample U₂?

d) If the solvent had traveled twice as far, would the $R_f$ values have changed? Explain.