Experiment 3: The Chromatography of Organic Compounds

INTRODUCTION

When performing an organic reaction, it is very common to observe the formation of other compounds in addition to your desired product; these are typically referred to as byproducts. Other times, a reaction will not go to completion; that is, you end up with some of the desired product along with unreacted starting material. In either case, the organic chemist is left with a mixture of compounds and must find some method to isolate and purify the desired product.

In earlier experiments, you saw how the technique of recrystallization could be used to purify solid compounds. You will soon see how the technique of distillation could be used to purify liquids. Column chromatography, a third method of purification, is typically used when significant amounts of more than one compound are present. Notably, column chromatography is the most general of the three purification techniques and can be used to purify solids, liquids, and even oils.

Thin-layer chromatography (TLC) generally has two purposes, both of which are used for analysis. First, by using standards, or known compounds, one can identify unknown compounds; this is similar to the use of standards in gas chromatography (GC). Second, TLC can be used to follow the progress of a reaction over time by monitoring the formation of products and the disappearance of starting materials. TLC analysis is quick and easy to perform and requires only very small amounts of material.

All chromatographic methods (including column chromatography, TLC, and GC) rely on the differing molecular properties of organic compounds in order to effect separation. The most commonly used factor is polarity, since this determines how strongly the organic compound interacts with the stationary phase. Less polar compounds tend to adhere less tightly and thus move more rapidly. In contrast, more polar compounds move through the stationary phase more slowly because they are more tightly bound to it. The polarity of the solvent also plays a role, and obtaining good separation may require the use of several different solvents or even mixtures of solvents.

DISCUSSION OF THE EXPERIMENT

In this experiment, a two-component mixture comprised of ferrocene and acetylferrocene will be separated. Column chromatography will be performed, using alumina as the adsorbent. Use of two different solvent systems, petroleum ether and a
mixture of petroleum ether with diethyl ether, allows the use of the differences in solvent polarities to easily separate the two components of the mixture.

Thin layer chromatography will be used for identification of the components in the mixture and for the determination of the purity of the separated components. Melting point analysis will also be performed on the separated components in order to identify them and assess their purity.

**REAGENT TABLE:**

<table>
<thead>
<tr>
<th>Substance</th>
<th>MW (g/mol)</th>
<th>MP (ºC)</th>
<th>BP (ºC)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ferrocene</td>
<td>186.04</td>
<td>172-174</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>acetylferrocene</td>
<td>228.07</td>
<td>85-86</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>petroleum ether</td>
<td>N/A</td>
<td>N/A</td>
<td>35-60</td>
<td>N/A</td>
</tr>
<tr>
<td>diethyl ether</td>
<td>74.12</td>
<td>-116</td>
<td>34.6</td>
<td>0.713</td>
</tr>
</tbody>
</table>

A. **Purification by Column Chromatography: Separation of Ferrocene Compounds**

1. **Assembly of the chromatography column:**

   Dry pack (addition of dry powdery packing material) and slurry (addition of the packing material, already wet with solvent) methods are commonly used for assembling columns for separation. Due to the small size of the column, we will be using a dry pack method where the packing material, alumina, is added to the column as a dry powder. Be careful not to breathe the alumina dust. While this method is simpler, it generally tends to allow more air bubbles to be trapped in the column thus preventing separations of some compounds. The slurry pack method requires the formation of slurry of alumina by adding solvent to the dry powder until it is all wet and mobile in the liquid. This requires more solvent use than is desirable and is generally more difficult to do for an inexperienced person. It does present a more uniform surface though, without air bubbles thus it generally gives a better separation. Because the two compounds being separated in this experiment have vastly different polarities and are very easy to separate, we will opt to use the dry-pack method for your column formations.

   a. Prepare the column using the large plastic buret provided by your instructor.
b. Remove the stopcock from the end of the column, place a small cotton ball in the tube and replace the stopcock. Do not use too large of a wad of cotton, as this will slow down the ability of the solvent to move through the column. Clamp the column vertically to a support stand.

c. You may use your short-stem glass funnel to assist you in the addition of solids to the column. Add about a 1 cm thick layer of sand to the top of the cotton in the column to create a flat surface to build the column on. As an approximation, 1 cm is approximately the width of your pinky finger.

d. Slowly add 9-11 g of dry alumina on top of the sand, tapping the sides of the column with your spatula to help remove air pockets as the alumina settles.

e. Once settling is complete, follow this with another addition of an approximately 1 cm thick layer of sand.

2. Preparation and loading of the sample:

a. Weigh out approximately 0.10 g of the mixture of compounds, using a small watch glass or weighing boat. Record the mass used in your notebook. Observe what the mixture looks like.

b. Using a short-stem glass funnel, add the dry mixture to the top of the upper sand layer of the column.

3. Elution of the components:

a. Obtain a small beaker to be used for waste solvent; that is, the solvent that elutes from the column but not containing either of the components. Place the beaker under the buret column to prevent solvent from carelessly dripping onto the countertop.

b. Obtain two clean dry sample vials. Before weighing these, label them with your name(s), section number/day/time (whichever is appropriate), and “Vial 1” or “Vial 2”. Then, record the mass of each labeled vial (without the cap!) in your laboratory notebook.

c. Take a clean beaker or Erlenmeyer flask to the solvent hood and, using the provided, color-coded graduated cylinder, obtain approximately 25 ml of petroleum ether and bring back to your workspace. Using a disposable pipet, slowly add ~2 mL of petroleum ether drop-wise down the inside of the column. Do not disturb the compound at the top of the column. If you add too much petroleum ether too quickly, the compound will dissolve upwards into the solvent and make the band for the compound much wider. While this is generally not a problem for this experiment, it would be for other separations, so try to prevent this from occurring and practice good technique.
d. Continue to slowly add petroleum ether slowly until you see a narrow band of color appear at the top and begin to move down the column. Once all yellow color has moved into the column, slowly and carefully fill the column with petroleum ether and view the band descending the column. Remember to record observations of what is happening in your column.

e. As the band descends, continue to add more petroleum ether. Any solvent that elutes clear and colorless from column should be collected in the waste beaker. Once the yellow band reaches the bottom of the column, collect this band in Vial 1. Should you see the formation of crystals on the tip of the pipette column, you should rinse the outside edge of the column with a small amount of the solvent. Be sure to let this drip into Vial 1 as well. Record what the contents of Vial look like.

f. After the first band has completely eluted, place the waste beaker under the column to catch any solvent that continues to elute without a component. Should you completely fill Vial 1 and still have traces of yellow band eluting (your sample band was either wide or crooked!), you should continue to elute using petroleum ether into the waste beaker until all yellow color is gone from the column. If this happens, record this as an observation, as part of your compound is now in the waste container.

g. When the first band has completely exited the column and the petroleum ether solvent level is even with the top of the alumina, repeat the elution process for the second component, using approximately 25 ml of a 1:1 mixture of petroleum ether and diethyl ether (pre-mixed solution can be found in hood). Record any observations of the second band that you see.

h. When the second band of color is ready to elute off the column, collect the solvent in Vial 2. Continue adding the 1:1 solvent mixture until the second band is completely eluted. Observe what the contents of Vial 2 look like. Any additional solvent should be collected in the waste beaker. See Part (B), below, for TLC analysis of the contents of your vials.

i. After completing Part B, carefully place your two open, uncapped vials in the hood in the designated tray for your section and allow the solvent to evaporate from Vials 1 and 2 between lab periods.

B. Analysis of Components by Thin-layer Chromatography –

You will receive two TLC plates initially from your instructor. Should you make an error you will receive up to two replacement TLC plates to repeat the process, for a maximum total of four TLC plates for each set of students. Work carefully! The first TLC plate will be used for purposes of identification of the components, by comparison to known compounds. The second will be used to check the purity of your separated compounds. You will also receive five micropipets (must be clean each time - why?), a pencil (pens
cannot be used as the components of ink will also develop in the solvents), and one of the developing chambers. Prepare the TLC plates as shown and described below.

Plate 1: Identification of the Components

a. Be sure not to touch the plates with your fingers - handle only by the edges or with tweezers. Place the first plate on the bench and using the pencil - about 1 cm or so from the bottom of the plate - mark three tiny X's or dots on the powder face of the plate, in a horizontal fashion. Try not to “dig” into the adsorbent too much. These marks will be the positions on the plate that you will “spot” or apply the compounds. Mark the first spot as “F”, the second as “AF” and the third as “M”, to indicate ferrocene, acetylferrocene and your mixture of components, respectively.

b. Obtain the three standard solutions of known compounds, Ferrocene and Acetylferrocene and a pre-made solution of the mixture of both from your instructor. Using a clean micropipet each time, spot the contents of the ferrocene sample in the first position and acetylferrocene in the second position and the mixture solution in the third position. You should be able to see the colored spots on the plate. If you cannot see a spot, re-spot in the exact same position. The ferrocene solution may be too light to see and may require a multiple spotting in order for you to be able to visualize it.

c. Develop this plate using 1:1 diethyl ether: petroleum ether solution. First, place the clean, dry chamber on the bench and add a shallow layer (~0.5 cm) of the 1:1 diethyl ether: petroleum ether solution to the chamber. Carefully slowly lower the TLC plate straight down into the bottom of the chamber, being careful not to touch the plate to the sides of the chamber. Be sure not to touch the plate with your fingers - handle only by the edges or with tweezers. Allow the solvent to move up the plate vertically until it is within half of an inch of the top edge of the plate. Remove by grabbing the plate with the tweezers AT THE LEVEL OF THE SOLVENT FRONT and quickly mark where the solvent front stopped with

Plate 1: Plate 2:

| X | X | X | x | x | x | F | AF | M | V1 | V2 |
your pencil. The spots should be clearly visible on the plate. If they are not, see your instructor.
d. Place this TLC plate down on the page of your lab notebook and trace around it, then carefully sketch in the details that you see, including spots (shapes, colors) while keeping the distances fairly accurate. Include on your drawing the height of the solvent front from when the plate was removed from the chamber and the point of origin for each spot.

Plate 2: Verifying the Purity of your Separated Components

a. Repeat the steps using the second TLC plate to verify the purity of your separated components. Using Vials 1 and 2 from the chromatography column, spot the second TLC plate with each colored band solution.
b. Elute this TLC plate using the same 1:1 petroleum ether: diethyl ether solution. Before running the next TLC plate, empty out the chamber (find the organic waste container for the chromatography lab) and refill again with 0.5 cm of fresh solvent. Plate 2 may be done during the process of the column, once the second compound begins to elute off the column. If your separated components are pure, each lane on the TLC plate should contain only a single spot after the plate has been developed.
c. Again, draw this TLC plate in your notebook, as before, by tracing and sketching in the details.

C. Clean Up

Dispose of any remaining solvent in your waste beaker in the organic waste container (non-halogenated waste). Remove the column from the clamps and place in container marked “Used Columns”. Using soap and water, and acetone if necessary, clean all glassware. Clean up bench as generally required.

D. Calculations – Rf values

Using each TLC plate (not your sketches of the plates), each pair of students should measure the distance the solvent moved, from the origin (where the x’s are located, where the spots were placed initially) to the solvent front (value Y). Then measure the distances each spot moved from the origin to the center of each spot, (values of X). Calculate the Rf values for the spots on both TLC plates, as X/Y (six spots should have a total of six calculations).
E. Mass and Melting Point Analysis of Components (Day Two)

a. In the next lab period, remove any caps that were placed on your vials and determine the mass of vial 1 and vial 2 (if required). Observe what the crystals look like in each vial.

b. Each lab partner will obtain a melting point on one of the compounds (you will need to use a spatula to scrape the materials loose from the sides of the vial). Place both melting point capillaries in the Digimelt and input the following parameters for the ballpark runs (and adjust accordingly for the slow runs):

Vial 1: **START:** 140°C, **RAMP:** 2°C/min **STOP:** 190°C

Vial 2: **START:** 50°C, **RAMP:** 2°C/min **STOP:** 100°C

F. Calculations – Percent Recovery

Based on the masses of isolated ferrocene and acetylferrocene, **calculate a percent recovery for each component**. Assume that the mixture that you originally loaded onto the column consisted of a 1:1 mixture (by mass) of ferrocene and acetylferrocene. Then, **add the masses** of isolated ferrocene and acetylferrocene, and **calculate an overall percent recovery for the sample**.