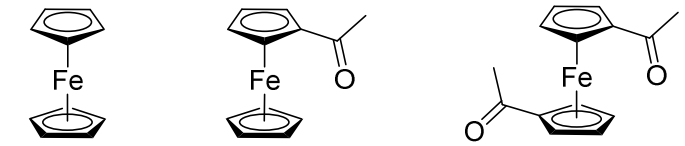
# Experiment 17

## Microscale Column Chromatography: Separation of Compounds

**Reading:** Handbook for Organic Chemistry Lab, section on Column Chromatography (Chapter 8).

This experiment involves purifying the product of another experiment, Electrophilic Aromatic Substitution, by column chromatography. Depending on which semester you are taking this course, you might have performed this experiment in the past, or you might be performing it in the future. If you have not performed it yet, you can use crude material from the recovery jar to perform today's experiment.

During the Electrophilic Aromatic Substitution experiment, acetyl groups were added to ferrocene. This created a mixture of unreacted starting material (ferrocene) as well as the desired product (monoacetylferrocene) and possibly some undesired side product (diacetylferrocene). These three compounds are shown in Figure 17-1. During today's experiment, the monoacetylferrocene will be purified by flash column chromatography, a commonly used method in modern organic chemistry research laboratories. Column chromatography works particularly well in this experiment because the three compounds have very different polarities and because they are highly colored. Ferrocene is a light orange-yellow, monoacetylferrocene is medium orange and diacetylferrocene is dark brown.




Ferrocene Monoacetylferrocene Diacetylferrocene

Figure 17-1: The three compounds which your crude sample might contain.

### Safety Precautions

Hexanes are flammable. Ferrocene and dichloromethane are moderate health hazards and contact should be minimized. Wear your gloves throughout this experiment.

### Procedure

You will perform this lab individually. Before you begin, clean two NMR tubes and let them dry so they are ready to use later.

If you have not yet performed the Electrophilic Aromatic Substitution experiment, or if you did not collect enough product, or if your crude product did not contain any monoacetylferrocene, you will have to take about 100 mg of crude product from the recovery jar. You can use this to preload some silica, perform TLC, and collect a crude NMR spectrum by following these directions:

- To pre-load your crude product onto silica, weigh out 50 mg of crude product from the recovery jar. Mix the crude product with 150 mg of silica gel and 1 mL of DCM. Allow the solvent to evaporate completely, stirring with your spatula until the silica is dry and free flowing – it should be orange or light brown now, instead of white. Leave this uncapped so it can finish drying while you perform the TLC and NMR.
- To perform a TLC, take about 10 mg of the crude product from the recovery jar and dissolve it in about 10 drops of DCM for TLC analysis. Spot it on a TLC plate, along with a ferrocene standard (you will have to make this up by dissolving about 10 mg pure ferrocene in 1 mL DCM). Use DCM as the eluting solvent. The spots should be visible, since they are colored, but if they

are not, a UV lamp will make them easier to see. You may or may not have spots for ferrocene and diacetylferrocene, depending on how far the reaction proceeded.

- Submit an NMR of the crude product in $CDCl_3$.

To separate your compounds, you will follow the directions for microscale flash chromatography given in the Handbook (see the assigned reading at the start of this experiment). However, unlike the procedure given in the Handbook, you do not need to collect small fractions in vials because you can visually keep track of your compounds as they move down the column.

Plug a Pasteur pipet with a small amount of cotton. Add dry silica to a depth of 5 cm. Tap the pipet to pack the silica. Pre-elute the column with hexanes; keep the column wet with hexanes as you prepare the product for loading.

Transfer the silica-product mixture to the column, then start eluting it with hexanes. Run a total of 10-15 mL of hexanes through the column, until the light orange ferrocene has eluted from the column. Collect and save the eluent in a small beaker or Erlenmeyer flask. If your TLC did not show a ferrocene spot, then you may not see a light orange band on the column. In this case, proceed on to the next step anyway.

Once the solvent eluting from the column has become colorless, change to a solvent mixture of 9:1 DCM-hexanes and continue eluting your column. Since monoacetylferrocene elutes very slowly with pure hexanes, the monoacetylferrocene should have stayed at the top of the column. In the new solvent system the monoacetylferrocene will begin to elute, and will move down the column as a dark-orange band. Again, collect this product in a small beaker, vial or Erlenmeyer flask. You may see a dark brown band remaining on the column; this band represents diacetylferrocene. You do not need to collect the diacetylferrocene.

Remove the solvent from the monoacetylferrocene sample by rotary evaporation. Run a TLC of both crude and purified products side-by-side and submit a sample of your purified product for NMR. If you don't have enough purified product in the bottom of your round-bottom flask to scrape it out easily, you can add 1-2 mL of $CDCl_3$ directly to the flask, allow it to dissolve the product, and then use this solution both for NMR and for spotting your TLC plate.

## Wastes

*Organic Waste:* DCM used to elute the TLC plates and all of the solvents used to elute the column.

*Solid Chemical Waste:* Used columns and TLC plates.

*Recovery Jars:* Place all recovered products in the proper recovery bottles provided.

## Lab Report

Your conclusions should include:

- Discussion of TLC plates.
- Based on your visual observations as the compounds moved down the column, how effectively did the column separate your compounds?
- Analysis of the NMR spectrum for the crude product, if you used a different sample than what you analyzed during the Electrophilic Aromatic Substitution experiment, or if you have not yet performed that experiment. Note that the chemical shift for pure ferrocene is 4.15 ppm (shown in study question 5 below), while the chemical shift for the unreacted ring of monoacetylferrocene is 4.20 ppm (shown in study question 6 below – peak **C**). If your peaks are more than 0.05 ppm from these locations, you might have to set your $CDCl_3$ reference peak to 7.26 ppm exactly. Based

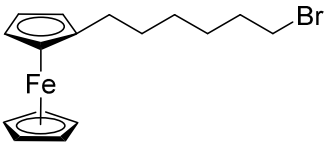
on these two peaks, calculate the relative amounts of ferrocene and monoacetylferrocene present in your crude product if possible. (You will likely not be able to calculate the relative amount of diacetylferrocene, since all of its peaks overlap with the peaks for monoacetylferrocene.) Do these results match your expectations based on the TLC results?

- Analysis of the NMR spectrum for the purified product. Compare it to the spectrum of the crude product. Based on NMR data, how effectively did the column separate your compounds?

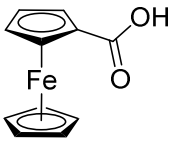
**Study Questions**

1) Consider the compounds ferrocene, acetylferrocene, and diacetylferrocene.
   a. Which is the most polar and which is the least polar?
   b. Which will have the lowest $R_f$ in TLC?
   c. Which will elute first in column chromatography?

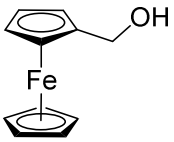
2) Shown below are some other ferrocene derivatives.



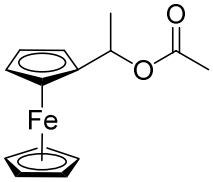

(6-Bromohexyl)ferrocene




Ferrocenecarboxylic acid



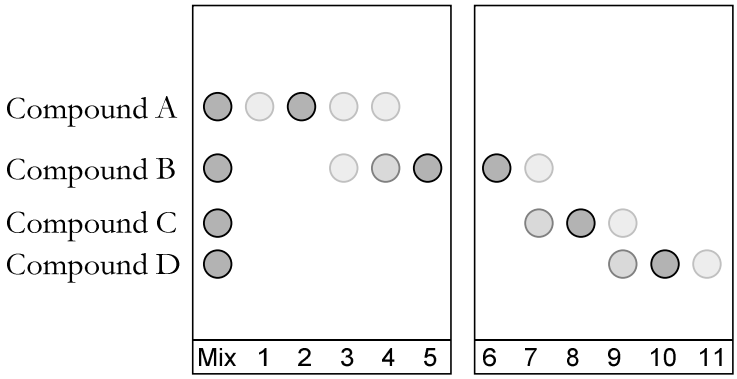

Ferrocenemethanol




(1-Acetoxyethyl)ferrocene

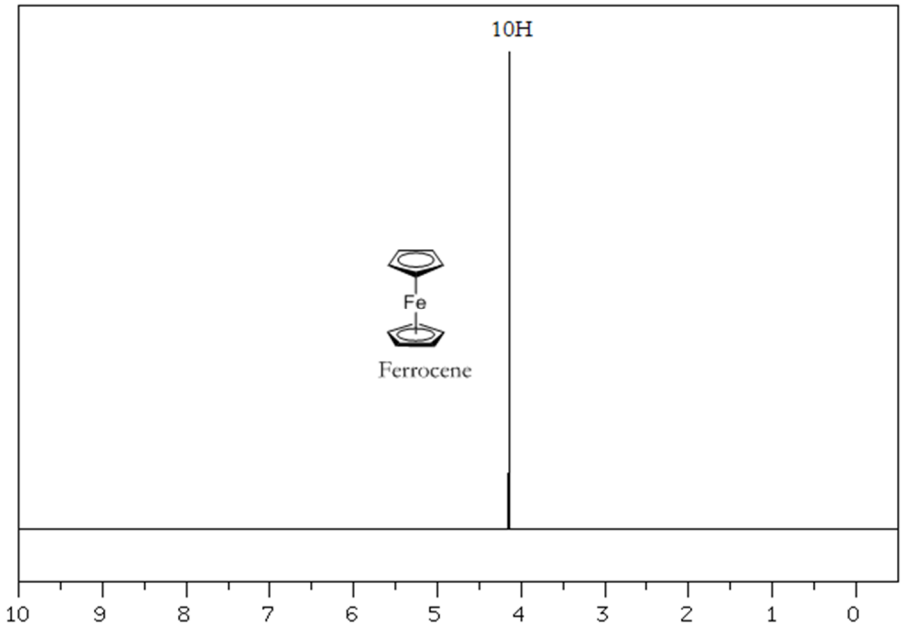
   a. Rank these compounds from most to least polar.
   b. Which will have the lowest $R_f$ in TLC?
   c. Which will elute first in column chromatography?

3) A chemist found that a mixture of four components (Compounds A–D) could be separated on a silica gel TLC plate using 10% diethyl ether in hexanes as the eluting solvent (see "original mixture" far left plate in the figure below). The mixture was then chromatographed on a silica gel column eluted with this same solvent mixture and 11 fractions of 15 mL each were collected. Thin-layer chromatographic analysis of the various fractions (1-11) under the conditions stated above gave the results shown in the figure below:

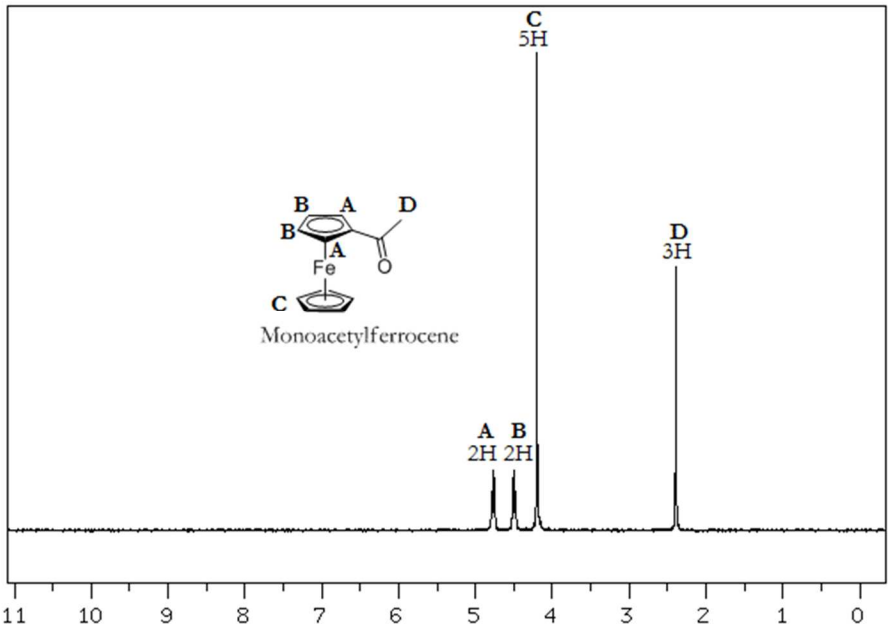



   a. According to these results which fractions should be combined to give pure samples of A, B, C, and D?
   b. Which fractions contain more than one component? Indicate for these 'mixed' fraction numbers what components of the original mixture are present.

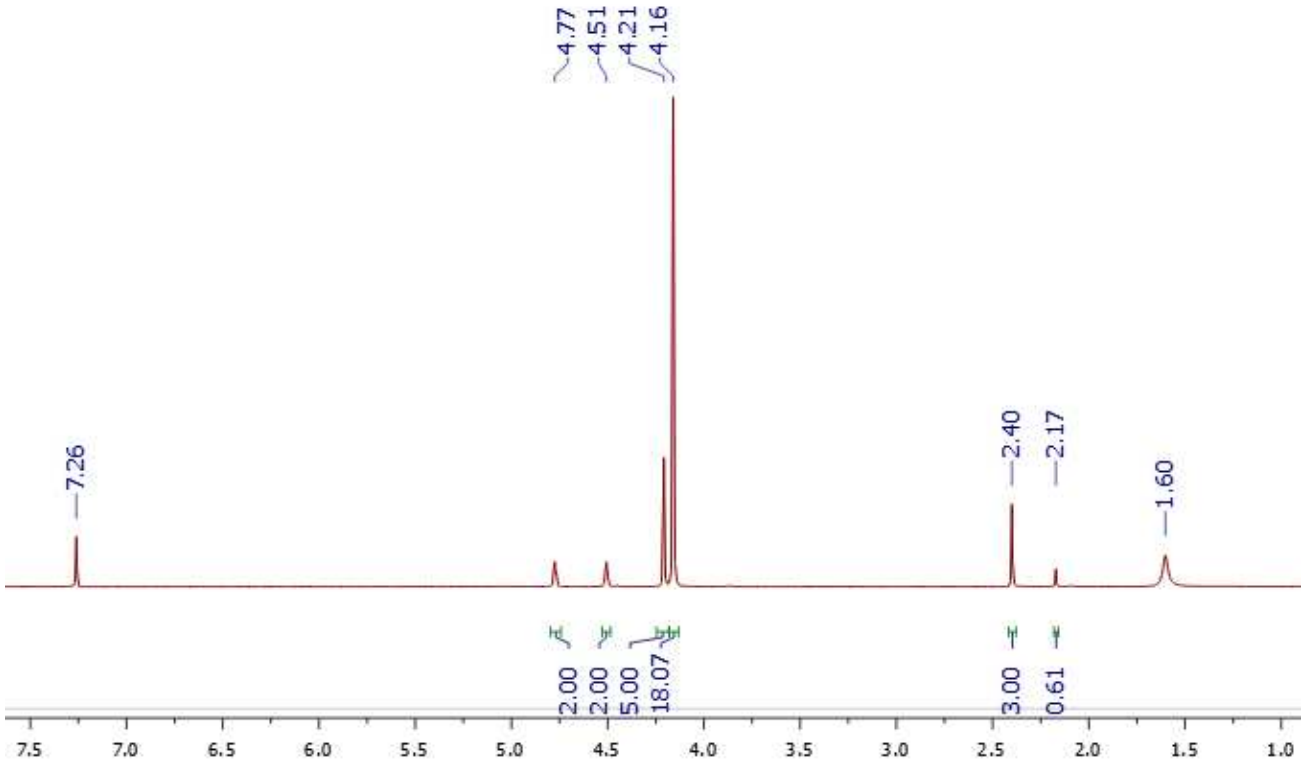
4) The NMR spectrum of ferrocene is shown below (taken from SDBS). Explain why the aromatic protons show a single band at 4.2 ppm.




5) The NMR spectrum of monoacetylferrocene is shown below (taken from SDBS). What would the NMR of diacetylferrocene look like?




6) A student performed this experiment and obtained the NMR spectrum shown below. What other impurities are present? What is the molar ratio of ferrocene to monoacetylferrocene in the crude product? What percent of the crude product is ferrocene vs. monoacetylferrocene?


4.77
4.51
4.21
4.16
7.26
2.40
2.17
1.60
2.00
2.00
5.00
18.07
3.00
0.61
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0