

EXPERIMENT 4

COLUMN CHROMATOGRAPHY

A. PRELAB ASSIGNMENT

In your notebook, prepare a Table of Physical Constants for n-hexane, dichloromethane, ethyl acetate, ferrocene, and acetyl ferrocene.

B. THEORETICAL BACKGROUND

In the TLC experiment, you gained some experience with chromatography. As you may have noticed, TLC is a rapid, easy method for separating and identifying mixtures of compounds. However, it is limited to small samples (only a few milligrams). To handle larger amounts of material, more adsorbant is needed. One common approach to larger scale separations is column chromatography, where the adsorbant (usually silica gel or alumina) is packed into a tube, and a continuous flow of solvent (eluant) is passed through the adsorbant. As with TLC, the different compounds in the sample are carried along, going back and forth between solvent and adsorbant. The relative rates of these movements are dependent upon the relative strengths of the attraction of these compounds to the adsorbant and upon their solubilities in the eluant. With a polar adsorbant, the more polar compounds in the sample will be held more tightly to the stationary phase and move more slowly down the column. Therefore, when the same adsorbant and solvent system are used in both, TLC and column chromatography will yield the same order of elution for a given series of compounds. So, TLC is a valuable tool for rapidly devising appropriate solvent systems for column chromatographic separations.

Unlike TLC, column chromatography is not limited to only one solvent system for a given separation. Since the solvent is continuously supplied at the top of the column, it is possible to change the eluting solvent at any point in the separation. This advantage allows us to separate complex mixtures containing compounds of widely varying polarity. For separations of such

samples, the column is prepared in a relatively non-polar solvent. As the less polar components are eluted from the column, the polarity of the solvent can be progressively increased, thus allowing selective removal of compounds in order of increasing polarity.

In addition to this versatility for separating complex mixtures, column chromatography has the advantage that it can be used for preparative scale separations and purification, while TLC is usually limited to analytical procedures. The amount of material to be separated can be varied from milligrams to kilograms by increasing the size of the column and the amount of adsorbant. For convenience and economy, the separation of a mixture of ferrocene and acetyl ferrocene in our experiment will be done on a semi-micro scale.

C. EXPERIMENTAL PROCEDURE

1. Sample Preparation

On an analytical balance, weigh about 0.1500 g (note the exact amount to four decimal places) of starting material, a mixture of ferrocene and acetyl ferrocene, in a 20 mL beaker. Dissolve the sample in 1 mL of CH_2Cl_2 . Put a spot of the solution on a silica gel TLC plate, close to the left edge (about about 0.5 cm from the edge). This is spot A. Put the plate away in a safe place. More spots will be added to it later. Set aside. It will be used in step **2 g** below. Add about 0.5 g alumina to the dissolved sample in CH_2Cl_2 .

2. Column Preparation

a. Obtain about 80 ml of hexane in a clean and dry 125 mL flask. Also in clean, dry flasks, obtain about 20-25 mL of each of the two other solvents to be used for the elution of the column (10:1 hexane:ethyl acetate, and 1:1 hexane-ethyl acetate). Keep all these solvents covered. Clean and dry the column with a little acetone if needed. (Do this before preparing the sample.) Place a small wad of glass wool at the bottom of the column. Add enough sand to cover the glass wool completely. Place a clean, dry beaker under the stopcock tip.

- b.** Fill about 2/3 of the column with hexane. Measure 13 mL of basic alumina, activity I, in a dry 25 mL graduate cylinder. Alumina rapidly absorbs moisture from the air and this reduces its separating power, so **alumina should be measured just before adding it to the column.**
- c.** Pour the measured alumina into the column using a funnel. Tap the column gently as you pour the alumina.
- d.** When all of the alumina has been added, open the stopcock and allow the solvent to drain until its level is about 1 cm above the top of the alumina. Use the excess solvent in the flask to wash down any alumina adhering to the inside column walls.
- e.** Add a little bit of sand to form a thin layer above the alumina. Adjust the solvent level so that it is just above the level of the sand and let the column walls dry for a couple of minutes.
- f.** Use a funnel to transfer the sample prepared in step 1 to the column. Rinse the beaker with a **few drops** of hexane, and add this to the column. Allow the sample to run onto the column. Add a small amount of sand to form a thin layer above the sample.
- g.** Add small portions (about 1 mL) of hexane and open the stopcock, to allow the sample to run onto the column. Continue to add small portions of hexane until the solvent above the sand has no color.

3. Chromatography Procedure

- a.** Label three 100 mL beakers as **1**, **2**, and **3**. These beakers will be used to collect the components (fractions) of the mixture as they elute from the column.

After the sample has been loaded onto the column, begin eluting as described below.

- b.** Fill the top of the column with hexane. Open the stopcock and allow the column to run continuously. **DO NOT CLOSE THE STOPCOCK and DO NOT ALLOW THE LEVEL OF ELUTING SOLVENT TO FALL BELOW THE SAND AT THE TOP OF THE COLUMN.** (Add solvent as needed.) Collect the initial colorless eluant in a waste beaker. When the first colored band is about

1 cm from the bottom of the column, start collecting the fraction in the beaker labeled **1**, and continue eluting with hexane until all of the first colored band has been eluted from the column.

c. Change the eluting solvent to 10:1 hexane-ethyl acetate and collect the intermediate fraction in the beaker labeled **2**, until the second colored band is about 1 cm from the bottom of the column,

d. Change the eluting solvent to 1:1 hexane-ethyl acetate when the second colored band is about 1 cm from the bottom of the column, and change the collecting flask to the third beaker (labeled **3**). Continue to collect this fraction until the glass wool at the bottom of the column is white.

e. Rinse any crystals that adhere to the tip of the column into the beaker, using a plastic pipette to squirt some solvent onto the tip of the column.

4. Isolation and Identification of Products

a. Transfer fraction 1 to a pre-weighed round bottom flask (RBF). Rinse the beaker with small amounts (2 x 2 mL) of hexane. Transfer fraction 3 to another pre-weighed RBF, and rinse as above. Evaporate the solvent from both fractions using the RotoVap.

b. Weigh the two RBF's containing the solids from fractions 1 and 3. Find the weight of solid recovered from each fraction.

c. Dissolve the solid in each RBF in 2mL of CH_2Cl_2 . Spot each of these solutions on your TLC plate.

d. Spot standard ferrocene and acetyl ferrocene solutions on the same TLC plate.

e. Develop the plate with 30:1 toluene-absolute ethanol as the eluting solvent.

f. Draw (to scale) a replica of the TLC plate in your notebook. Calculate R_f values for all spots and record in tabular form in your notebook. Identify each spot.

Clean Up: At the end of the period, clean the chromatography column in the hood as demonstrated by an instructor. Empty the contents of the RBF's into the Halogenated Waste container.

D. CALCULATIONS

Calculate the total % recovery.

Total % recovery = % ferrocene + % acetyl ferrocene

Sample calculation:

% Ferrocene = (weight of ferrocene / weight of starting mixture)

E. QUESTIONS

1. The R_f values of three compounds on TLC plates developed with 10:1 hexane:acetone are (A) 0.33, (B) 0.91, and (C) 0.54.

a. What would be the order of elution from an alumina column which is eluted with 10:1 hexane:acetone (i.e., which would elute first, second and third)?

b. Explain your reasoning.

c. What difference if any would be seen if the compounds were eluted from the column with 7:1 hexane:acetone? Explain.

2. With reference to your experiment,

a. Which of your fractions contained ferrocene? Which contained acetyl ferrocene? Explain, using your data.

b. Did you isolate pure samples of ferrocene and acetyl ferrocene? What is your evidence?

c. Which of the two compounds is more polar? Explain. (Give evidence from this experiment and from the structures.)

F. DISCUSSION AND CONCLUSIONS

NOTES