

CH 241 EXPERIMENT #1

WEEKS OF SEPTEMBER 10, 17, AND 24, 2001

SEPARATION AND RECOVERY OF ORGANIC COMPOUNDS, THIN LAYER CHROMATOGRAPHY, COLUMN CHROMATOGRAPHY, CRYSTALLIZATION AND MELTING POINTS

Overview

In the first few weeks of this semester you will be learning a variety of techniques that are routinely used by organic chemists. In the first week, you will separate two organic compounds from a mixture that also contains sand. Once you have recovered the organic compounds, you will need to separate them from each other, and to purify each of them. To accomplish this, you will use thin layer chromatography (tlc) to determine solvents appropriate to separate the components, and then use this information the second week to run a column separation. The third week you will check the purity of the separated compounds by taking melting points and then purify the compounds further by crystallizing each from an appropriate solvent or solvent pair. A brief discussion of most of the techniques is provided, but you are responsible for reading more comprehensive treatments in the laboratory textbooks available in the Science Library.

Week 1

Background and Procedure

Obtain approximately 3.0 grams (weighed to the nearest 0.1g) of one of the crude mixtures of sand, 9-fluorene and 9-fluorenone. Place this into a 125 mL Erlenmeyer flask and add about 10 mL of dichloromethane, CH_2Cl_2 , (an older name, still in use, is methylene chloride). Add a magnetic stir bar and heat on a stirrer-hot plate, set very low, under your hood. Swirl the flask and note that some of the organic material dissolves. Add dichloromethane, a little at a time, and observe if more solid dissolves. Keep the solution stirring to avoid bumping. Add just enough solvent to completely dissolve the organic compounds. The amount of solvent used is not terribly critical at this point because you will be evaporating it after you remove the sand. Filter the solution by gravity, using a fluted filter paper. Fluting filter paper increases the surface area of the paper and allows air to enter the flask to permit rapid pressure equalization. Gravity filtration is used to remove insoluble impurities, in this case, the sand. Save a small amount of the filtrate for thin layer chromatography, transfer the rest to an appropriately sized round-bottomed flask, i.e. one that will be no more than half full, and evaporate the filtrate on the rotary evaporator (Rotovap). Transfer the residue from the flask to a storage vial labeled with your name, lab day, and identity of the contents.

The next step is to spot your filtrate and authentic samples of fluorene and fluorenone side-by-side on a tlc plate and elute the plate with an available solvent you think should separate them. Thin layer chromatography is a very rapid technique, so if your “educated guess” proves incorrect, you can easily run plates using several different solvents in a short period of time. The following discussion of thin layer chromatography should help to familiarize you with the technique. Chromatography is defined as the

separation of a mixture of two or more different compounds or ions by distribution between two phases, one of which is stationary and one of which is moving. The experimental procedure for thin layer chromatography is straightforward and easy; however, background reading on the theory and technique is important. Most of the laboratory manuals in room 142 of the Science Library have sections dealing with chromatography. Since **Week 2** of this experiment will involve column chromatography, you may wish to read about both types in preparation for that lab as well. There is an especially nice treatment in Introduction to Organic Laboratory Techniques by Pavia, Lampman and Kriz, but every laboratory manual will include some treatment of both thin layer and column chromatography. In the lab you will find commercially prepared, precut, thin-layer plates of Silica Gel G. Be sure to handle them only by the edges or you may find that you chromatograph your fingerprints. Very fine glass capillary tubing will be available for spotting your compounds. Up to five fractions can be spotted on a single plate, perhaps more when you have mastered the technique. Every plate you run must include authentic samples of fluorene and fluorenone so that the R_f values of these compounds can be compared to the R_f values of the spots you find using your experimental compounds.

During the first week you will also run a very short experiment to familiarize yourself with solvents that are either miscible or immiscible in each other. Add about 1 ml of water to a small test tube with a Pasteur pipette. Then add 1 ml of an organic solvent (hexanes, dichloromethane, ethanol, or acetone) and mix. Let the test tube sit for a moment and note how many layers are present. If there is a single layer, the two liquids are miscible, but if there are two layers, then the liquids are immiscible in each other. Repeat the test using water and the remaining three organic solvents. For immiscible solvent pairs, identify the aqueous and organic layers. Record your observations in your notebook in table form, as indicated below.

| Solvent Pairs | √ if miscible. If not, go to next column | Immiscible Solvent Pair | |
|---------------------------|--|-------------------------|---------------|
| | | Aqueous Layer | Organic Layer |
| Water/Hexanes | | Top or Bottom | Top or Bottom |
| Water/Acetone | | Top or Bottom | Top or Bottom |
| Water/Ethanol | | Top or Bottom | Top or Bottom |
| Water/ Dichloromethane | | Top or Bottom | Top or Bottom |

Prelab Assignment

Because this is the first week of classes, no prelab will be due on Friday. However, there is an assignment to be completed in your laboratory notebook before you come to lab. This assignment will be checked by your instructor in the lab. The *Merck Index* and the *CRC Handbook of Chemistry and Physics*, found on the reference shelves of the library, will be helpful in answering the first two questions.

1. Look up the structures (not just the molecular formulas), melting points and solubility characteristics of 9H-fluorene and 9-fluorenone.
2. Look up the density and boiling point of dichloromethane.
3. From the laboratory textbooks in room 142 of the Science Library, read a treatment of thin layer chromatography. Write an outline of tlc procedures, including spotting, eluting, visualization of the plates and calculation of R_f values. Thin layer plates will be available for you, so a cursory reading of plate preparation is all that is necessary.
4. As will be the case for every lab period, write an outline of the procedure you will follow in **Week 1**. Remember that you should be able to successfully complete the experiment using only that outline, without referring to the laboratory handout.

Week 2

Background and Procedure

You are already familiar with the theory and practice of thin layer chromatography. A natural extension of that technique is column chromatography, which is useful for purifying compounds on a preparative scale. In column chromatography, the stationary phase is packed in a column and the sample is applied at the top of that column. The mobile phase, consisting of the appropriate solvent system, is then introduced to elute the sample. The different compounds in the sample travel down the column at rates that are dictated by their relative polarities. Fractions of the eluent are collected and analyzed for the presence of the desired compound(s).

Weigh the mixture of fluorene and fluorenone isolated in **Week 1**. Use no more than 0.7 gm for your column separation. Pack a chromatography column using 12.5 gm silica gel per 0.5 gm sample. Your crude sample should be dissolved in less than one mL of dichloromethane just before it is applied to the column.

- Pack the glass column provided to you with a slurry of silica gel, 60-200 mesh, in the solvent you have chosen to begin the separation. The columns in the lab have polyethylene discs in the bottom to keep the adsorbent in place, so a layer of sand in the bottom is unnecessary. Add a thin layer of sand, 3 mm, at the top. Make sure that the solvent level in the column does not drop below the level of the sand. Do not let the column go dry during the packing procedure.
- Drain the solvent from the column until the liquid is just level with the top of the sand layer. Introduce the solution of your crude sample, with a pipette, at the top of the column. Drain the sample onto the column by opening the stopcock briefly.
- Add small increments of solvent, draining almost to the sand layer, until the sample has been completely washed onto the column, then add a larger volume of

solvent and elute the column, collecting fractions using small test tubes. Analyze the fractions by tlc for the presence of the each component. Combine fractions containing each component and evaporate the solvent using a rotary evaporator.

- Record the weights of the recovered compounds and save them in labeled storage vials.
- Dispose of the column contents as indicated by your instructor.

Prelab Assignment

1. Back to room 142 of the Science Library! Read a treatment of column chromatography in one of the laboratory textbooks. Write an outline for the procedure, paying special attention to sections dealing with the packing of the column (we will be using the slurry method), application of the sample, elution of the column, and solvent polarities. We can help you to set up the experiment, but we assume you will be prepared when you come into lab. As you have heard before, all forms of chromatography deal with the separation of a mixture of two or more different compounds or ions by distribution between two phases, one of which is stationary and one of which is moving, so conceptually, you already know the theory.
2. Although you won't be using all of the compounds listed below in this experiment, it is important that you become familiar with the relative polarities of common organic solvents if you are going to perform column (and thin layer) chromatography experiments. Supply the structures of the following solvents, and rearrange them in order of increasing polarity: hexane, ethyl acetate, water, dichloromethane, ethanol, toluene, diethyl ether and acetone. Some are close in polarity, so we will give you a little leeway in your answers.
3. Which is more polar, fluorene or fluorenone? Which would you expect to elute from the column first?

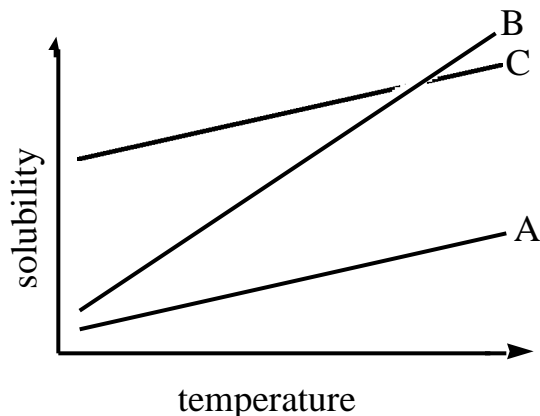
Week 3

Background and Procedure

This week you will be crystallizing your samples and taking melting points.

Crystallization

Crystallization is a purification process. In essence, you exchange quantity for quality, since you can never recover 100% of your compound. However, it is important that you try to maximize your yield. In general, you will choose a solvent in which your compound is quite soluble when the solvent is hot, and very much less soluble when that solvent is cold. In other words, you want a solvent that has a steep solubility vs temperature curve for your compound (solvent B in the graph below).



For example, one gram of salicylic acid dissolves in 460 mL of room temperature water or 15 mL of boiling water, making water an excellent solvent for crystallization of this compound. If you don't know what solvent is appropriate for crystallization of your compound, it is sometimes possible to find this information using the *Merck Index* or the *CRC Handbook of Chemistry and Physics*, but often it is necessary to go to the lab and figure it out by trial and error.

As stated above, it is not possible to recover all of your compound following a crystallization. This is because the compound will be at least slightly soluble, even in very cold solvent. Therefore, to maximize your yield, it is necessary to use a minimum of solvent for crystallization. Working question 1 in your prelab will illustrate these concepts.

The crystallization process usually consists of the following steps:

- Dissolving the impure solid in a hot solvent (or mixture of solvents).
- Adding a decolorizing agent, such as activated carbon, to remove colored impurities. You need to do this only if there are such impurities.
- Filtering to remove insoluble solid material. This is unnecessary if there are no undissolved solids.
- Cooling the hot solution slowly and undisturbed, to room temperature or below, allowing the pure solid to crystallize. Sometimes, it may be necessary to scratch the flask with a glass rod, or put in a "seed" crystal, to induce crystallization.
- Filtering the crystals by vacuum to separate them from the "mother liquor".
- Washing the crystals with the appropriate cold solvent to remove the mother liquor.
- Drying the crystals.

For this procedure to work, the impurities must either be insoluble in the chosen solvent so that they can be filtered away, or completely soluble in the solvent so that they remain in solution throughout the procedure.

Melting Points

The melting point of a pure crystalline compound is generally sharp and characteristic of that compound. When you take a melting point, it is important to note the range of the temperature when the sample first begins to melt (first drop of liquid seen) to the temperature at which it has fully melted. This range is very narrow for pure compounds but broadens when impurities are present. Impurities also lower the temperature at which melting begins to occur. Thus, a melting point can serve as a useful criterion to evaluate the purity of solid substances.

The following explanations of melting point behavior should be familiar to you from your work in general chemistry. A sample melts when the vapor pressure of the liquid phase and the solid phase are equal. When a mixture of A and B occurs, the component with the lower melting point begins to melt, let's say A, and B then starts to dissolve in the liquid A. The vapor pressure of the liquid AB is lower than the vapor pressure of pure A and B, which means that the temperature at which the pressures are equal between the two phases is lower. The second explanation relies on the Gibbs free energy equation,

$G = H - T S$. For an equilibrium process, such as melting, you know that $G = 0$. Therefore $H = T_m S$ or

$T_m = H / S$. If H is considered a constant over the temperature range in question, then T_m is simply inversely proportional to S , and S for an impure sample is greater than S for a pure sample. This makes T_m for the impure sample lower than T_m for the pure crystal.

Procedure

Save samples of your crude fluorene and fluorenone in melting point capillaries. Using the commercial samples of fluorene and fluorenone available in the lab, find an appropriate solvent or solvent pair for crystallization of each compound. Be sure to keep a complete record of the solvents tried, including those that did not work, if there are any. A table would be the most efficient way to present your experimental results. After you have determined the appropriate solvent, crystallize your own samples of fluorene and fluorenone. Collect and wash your crystals, and allow them to dry before taking their melting points.

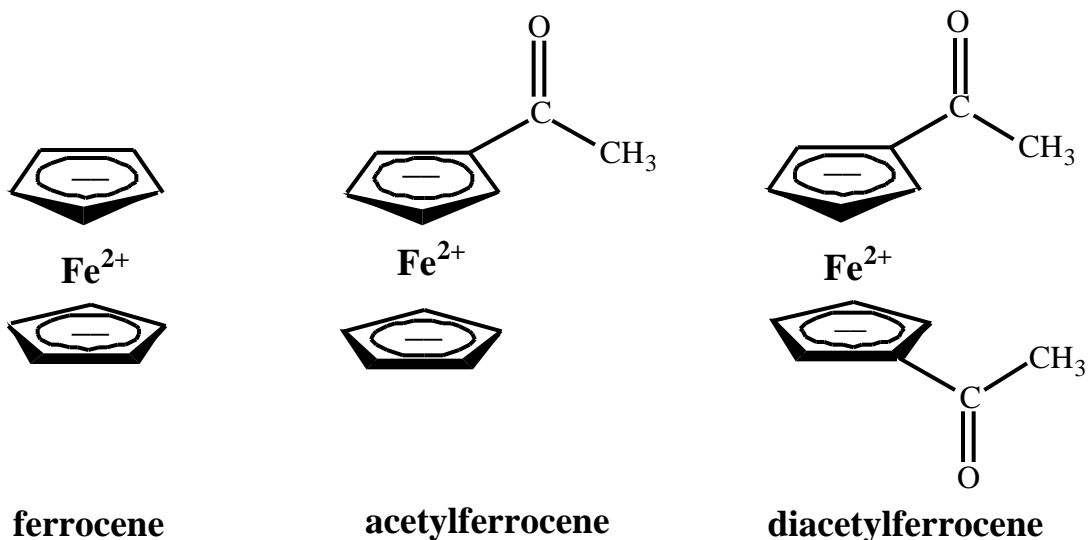
To take a melting point, place a small amount of finely powdered sample on a watch glass; use a spatula to grind the sample on the glass if necessary. Tap the open end of a capillary tube into the sample. Invert the capillary and, with the sealed end down, tap the tube on the desk to transfer the solid to the bottom of the tube. If that does not work, let the tube drop down a long glass tube. The solid will be carried to the bottom of the capillary as it bounces up and down inside the larger glass tube. Wipe the outside of the capillary and insert it into the melt-temp apparatus. You may insert up to three capillary tubes in the apparatus. You can therefore determine the melting points of your crude and crystallized samples, as well as an authentic sample, in a single run. Be careful to heat the samples slowly, especially near the expected melting point range, so that an accurate reading may be obtained. There is a chart in the lab that will help you determine where to set the melt-temp apparatus so that you are heating very slowly as you pass through the melting point of your sample. Record the melting ranges of your samples.

Prelab Assignment

1. You have 10 grams of a compound. Eight grams is soluble in 100 mL of boiling solvent and 3 grams is soluble in 100 mL of cold solvent. What is the minimum amount of solvent you will need to dissolve your compound? What is the maximum yield of crystals you would expect? Show your calculations.
2. Provide a detailed procedure for testing solvents to determine if they are appropriate for crystallization of your compounds. Use 20 mg of compound and one mL of solvent in small test tubes. Explain exactly what you would do. Thoroughly understanding this procedure should save you a lot of time in lab.
3. List two solvents that might be appropriate for the crystallization of fluorene and two for fluorenone. On what did you base your choices?

Report

1. By comparing the melting points and post-column yields of your fluorene and fluorenone with the melting points and yields of your crystallized products, briefly discuss the efficiency of your crystallization process.
2. You have an unknown that has a melting point of 130-133°C. You have either decanedioic acid (mp 133°C) or cinnamic acid (mp 133°C). Pure samples of both decanedioic acid and cinnamic acid are available in the lab. Design a melting point experiment by which you could determine the identity of your compound. Explain the concept you used to design the experiment.
3. So far you have used thin layer chromatography to determine appropriate solvents for a subsequent column separation and to check which column fractions contained your fluorene and fluorenone. However, tlc is much more versatile than that. Consider the following: You want to prepare acetylferrocene from ferrocene. This procedure should take about one hour. However, if you let the reaction go too long, diacetylferrocene will begin to form. You have authentic samples of ferrocene, acetylferrocene and diacetylferrocene (all colored compounds) available in the lab. Devise a way to monitor the reaction so you will maximize your yield of acetylferrocene.



4. Remember that your weekly lab sheets will be attached to the your report and be included in the grading. We will select areas to check for cogent procedures, complete observations and adherence to the notebook expectations as outlined at the beginning of the semester.