

CH241 Experiment #1 (Weeks of September 10, 17, and 24, 2018)

Separation, Purification, and Recovery of Organic Compounds

Overview

In the first few weeks of this semester you will be learning a variety of laboratory 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 purify each of them. To accomplish this, you will use thin layer chromatography (TLC) to determine solvents appropriate to separate the components on a silica gel column, and then use this information the second week to run a chromatography column separation. In the third week, you will analyze the purity of the separated compounds by measuring their melting points and you will purify the compounds further by crystallizing each from an appropriate solvent or solvent pair. A brief discussion of most of the techniques is provided along with the experimental description below. However, you are responsible for reading more comprehensive treatments in the organic laboratory textbooks available in the Science Library.

Week 1

In week 1, you will receive a mixture of sand, 9H-fluorene, and 9-fluorenone. You will begin to separate the components using the technique of gravity filtration, and then you will analyze the mobility of your organic compounds on silica using thin layer chromatography (TLC). You will also test the miscibility of several solvents that you may choose to use for crystallization, which you will perform in week 3.

Separation of a Mixture Using Gravity Filtration

Obtain approximately 3.0 grams (weighed to the nearest 0.1 g) of one of the crude mixtures containing sand, 9H-fluorene, and 9-fluorenone. Place this into a 125 mL Erlenmeyer flask and add about 10 mL of dichloromethane, CH_2Cl_2 (which you may also hear called by an older name, methylene chloride). In a hood, add a magnetic stir bar to the flask and, while stirring the mixture, heat the flask on a stirring hot plate at a very low heat setting. Swirl the flask and note approximately what fraction of the solid has dissolved. Continue to add dichloromethane solvent a little at a time while watching to see if more of the remaining solid dissolves. Keep the solution stirring to avoid bumping. Try to add just enough solvent to completely dissolve the organic compounds (thus adding additional solvent does not change the amount of solid present). 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 the filter paper increases its surface area for faster filtering while allowing air to enter the flask to permit rapid pressure equalization. The technique of gravity filtration is commonly used to remove insoluble compounds (in this case, sand), from solutions where the solvent is near its boiling point (in other words, for solvents that have been heated or that are very volatile). Save a small amount of the filtrate for thin layer chromatography (just a few drops in a spot plate are sufficient), then transfer the rest to an appropriately sized round-bottomed flask, i.e. one that will be no more than half full, and evaporate the solvent from the filtrate on a rotary evaporator

(Rotovap). Transfer the residue from the round-bottomed flask into a storage vial labeled with the identity of the contents plus your name and lab day.

Separation of a Mixture Using Thin Layer Chromatography

The next step is to use thin layer chromatography (TLC) to find solvent conditions that will adequately separate the organic compounds in your filtrate on silica gel. To do so, you will spot your filtrate plus authentic samples of fluorene and fluorenone side-by-side on a TLC plate and elute the plate with an available solvent you think may separate them. TLC is a rapid technique, so if your “educated guess” proves incorrect, you can easily run plates using several different solvents in a short period of time.

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 TLC is reasonably straightforward. However, background reading on the theory and technique is important. You can find laboratory manuals in the reference section of the Science Library, in the organic laboratory, and also on the web. Since **Week 2** of this experiment will involve (flash) column chromatography, you may wish to read about both types at the same time to be prepared for that lab as well. Generally, every organic laboratory manual that you will find includes some treatment of both TLC and column chromatography.

In the lab you will find commercially prepared, precut TLC 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 four fractions can be spotted on one of these plates, perhaps more when you have mastered the technique. Every plate you run must include authentic samples of fluorene/fluorenone so that the R_f values of these compounds can be compared to the R_f values of the spots you find for your experimental compounds.

Identifying Miscible and Immiscible Mixtures

During the first week you will also run a 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, for instance, as shown 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

Week 2

In week 2, you will purify your mixture of 9H-fluorene and 9-fluorenone using column chromatography, isolate your organic solids by rotary evaporation, and use TLC to evaluate the success of your separation.

Separation of a Mixture Using Column Chromatography

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 larger (preparative) scale. In column chromatography, the sample is applied to the top of a column packed with a stationary phase (silica gel in your case) and the mobile phase, consisting of an 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). Read a treatment of column chromatography in one of the laboratory textbooks.

Weigh the mixture of fluorene and fluorenone you isolated in week 1. Use no more than 0.7 g dissolved in approximately 1 mL of dichloromethane for your column separation.

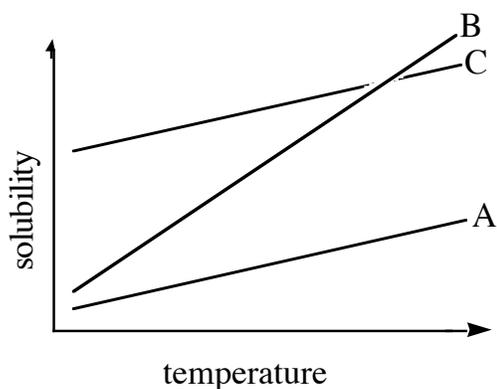
- Instructions for preparing your column, loading your sample, and operating the column equipment will be provided by your instructor.
- Elute the column with the appropriate solvent(s) and collect fractions using small test tubes. Analyze the fractions by TLC for the presence of each component. Combine your fractions containing only the same, pure component and evaporate the solvent using a rotary evaporator.
- Record the weights of your recovered compounds and save them in labeled storage vials.
- Dispose of the column as indicated by your instructor.

Week 3

In week 3, you will further purify your samples of 9H-fluorene and 9-fluorenone by crystallization and evaluate their purities by measuring their melting points.

Purification of a Compound by Crystallization

Crystallization is a purification process that effectively exchanges quantity for quality, since you cannot recover 100% of your compound from a crystallization experiment. However, it is important that you try to maximize your yield. In general, you choose a solvent in which your compound is quite soluble when that 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 or line for your compound (such as for solvent B in the graph below).



For example, since one gram of salicylic acid dissolves in 460 mL of room temperature water but only 15 mL of boiling water, 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 in reference books such as the *Merck Index* or the *CRC Handbook of Chemistry and Physics*, but it is often necessary to figure it out by trial and error in lab.

As stated above, it is not possible to recover all of your compound from a crystallization experiment. 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 amount of solvent for crystallization.

The crystallization process usually consists of the following steps:

- Dissolving the impure solid in a hot solvent (or mixture of hot solvents).
- Adding a decolorizing agent, such as activated carbon, to remove colored impurities. You need to do this **only** if there are such impurities.
- Gravity filtering to remove insoluble solid impurities. 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 to separate them from the "mother liquor" or filtrate.
- 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, and in some cases the identity, 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. For a mixture of two compounds (compounds A and B), 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 solution 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, $\Delta G = \Delta H - T\Delta S$. For an equilibrium process, such as melting, $\Delta G = 0$. Therefore $\Delta H = T_m\Delta S$ or $T_m = \Delta H/\Delta S$. If ΔH is considered a constant over the temperature range in question, then T_m is simply inversely proportional to ΔS , and ΔS for an impure sample is greater than ΔS 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 into 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. Record the melting point ranges of your samples.

Pre-laboratory Assignments (Prelabs)

Please write the prelab exercises in your notebook, and turn in the blue pages when you arrive in lab. Also make sure to read and review the appropriate material in preparation for both conducting the laboratory experiment and also the prelab quiz.

Prelab – Week 1 (Due in lab week of September 10, 2018)

In addition to the exercises below, make sure to carefully read through the week 1 procedure (above) and review the techniques of gravity filtration and thin layer chromatography.

The *Merck Index*, *CRC Handbook of Chemistry and Physics*, chemical catalogs, and other similar resources found on the reference shelves of the library, will be helpful in answering the following questions. You may also search online for the requested information. **Please be sure to cite your sources.**

1. Provide the structures (not just the molecular formulas), melting points, and solubility characteristics of 9H-fluorene and 9-fluorenone. *Note: structures for your pre-laboratory assignments can be drawn by hand in your notebook (but ChemDraw structures should be used for laboratory reports).*

2. Provide the structures, densities and boiling points of the solvents hexanes, ethyl acetate, water, dichloromethane, ethanol, toluene, diethyl ether and acetone.

Prelab – Week 2 (Due in lab week of September 17, 2018)

In addition to the exercises below, make sure to carefully read through the week 2 procedure (above) and review the technique of (flash) column chromatography.

1. Identify two different ways in which TLC can be helpful for performing column chromatography.

2. Which is more polar, fluorene or 9-fluorenone? Which would you expect to elute from the column first?

Prelab – Week 3 (Due in lab week of September 24, 2018)

In addition to the exercise below, make sure to carefully read through the week 3 procedure (above) and review the technique of crystallization (also called “recrystallization”) and use of a melting point apparatus to obtain an accurate melting point.

1. Clearly explain how you would test solvents to determine if they are appropriate for crystallization of your compounds.

What Should Be In Your Notebook?

1. An entry of the title, date, and page number in your table of contents.
2. An entry of the title, date, and partner’s name on the first page of your experiment (you should do this each week).
3. Masses and volumes of materials that you used in the experiment.
4. A sketch of the gravity filtration set-up followed by a brief description of the procedure you followed.
5. A sketch of the TLC plate that gave you the best separation, with size, shape, and R_f values for spots clearly recorded.
6. A table of the solvent miscibility tests with the boxes filled out.
7. A sketch of the column chromatography set-up with a brief description of the procedure you followed.

8. Yield of the two compounds recovered from your column.
9. A description of the crystallization procedure you followed.
10. Melting point ranges for your purified compounds.

What Should Be In Your Laboratory Report?

Use the **Experiment 1 Report Form** to write your lab report.

Remember to:

1. Submit an electronic copy to CH241Lab@colby.edu by the date your report is due.
2. Submit a hardcopy in lab on the due date (start of lab).