

Organic Chemistry I Laboratory

Recrystallization, Melting Point, and Chromatography: Separating and Identifying the Components of a Mixture¹

Experiment 2
Week 2

Background Reading

Zubrick, J. W. *The Organic Chem Lab Survival Manual*, 5th edition, Wiley & Sons, Inc., New York, 2001.

Melting Points: Pg 73-87.

Recrystallization Pg 89-102.

Chromatography, TLC & GC Pg 185-198; 213-220.

Background Scenario

This week's work is a continuation of last week's problem. At this point you should have separated the components of your pharmaceutical mixture and now your job is to identify the individual components.

Scientific Methodology

The problem you need to solve is to determine the identity of the individual components from your mixture. You will use several different techniques today and compare your experimental results to those of known standards. From this data you should be able to identify all of the components of your original mixture. Keep careful records of your observations and your interpretation of spectra so that you can succinctly describe them in your results and discussion.

Understanding the Techniques

Melting points²: The melting point of a substance can give an indication of its degree of purity and can also assist greatly in its identification. While not always strictly true, it is considered that a sharp melting point range (<2°C) between the first appearance of liquid within the sample to the disappearance of the last traces of solid constitutes good evidence for believing a substance to be pure. Rarely however, a mixture might give a sharp melting point if the components are present in the exact proportions to form a eutectic mixture.

Broad melting point ranges typically indicate that a substance is not pure. The exception is when pure substance decomposes on heating. When this happens, the decomposition products act as impurities in the sample and cause a broadening of the melting point range. A darkening of the sample color or evolution of a gas during heating is an indication that the sample is decomposing.

Melting Point Range: To explain why impure compounds have a broad melting point range, consider a hypothetical phase diagram of a mixture containing two components having different melting points. Component A of the mixture has a melting point of 90°C and component B melts at 110°C (Figure 1.).

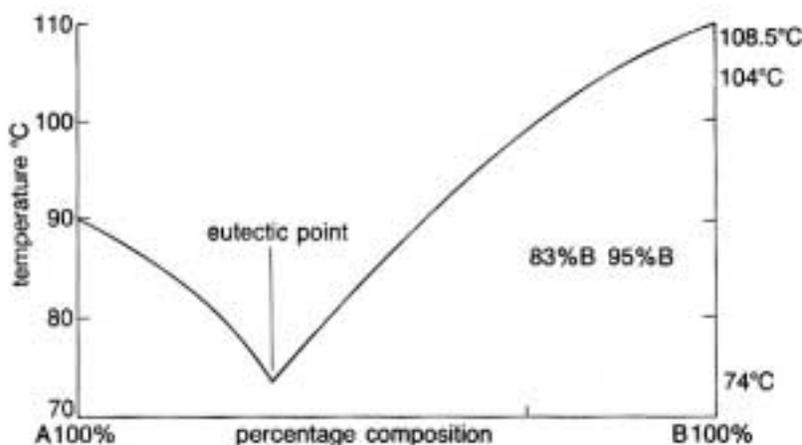


Figure 1. Simple Phase Diagram for a Two-Component Mixture

¹ Experiment is a modified version of an experiment found in: Lehman, J. W. *Operational Organic Chemistry: a problem-solving approach to the laboratory course*, 3rd ed., Prentice-Hall, Upper Saddle River, New Jersey, 1999.

² Harwood, L. M.; Moody, C. J. *Experimental Organic Chemistry, Principles and Practice*, Blackwell Scientific Publications, Cambridge, Massachusetts, 1989.

In the phase diagram, the mixture corresponding to 70% A and 30% B has the lowest melting point (74°C, the eutectic temperature). A mixture of A and B having exactly this composition would appear to have a sharp melting point at this reduced temperature. However, suppose that A is an impurity in B and present to the extent of 5%. Melting will first occur at 74°C and the liquid formed will have the eutectic composition (70% A and 30% B). In other words, the initially melted material will have a much higher concentration of A (70%) than the original mixture which contained 5% A. The mixture will continue to melt at 74°C until all of the impurity A is in the liquid phase. The total amount of material which will have melted at this point is:

$$A + B = 5\% + (30/70 \times 5)\% = 7.1\% \text{ of the total material}$$

This initial melting would probably not be visible to the naked eye, and at this stage the remaining solid, representing 92.9% of the original mixture, consists totally of B. As the temperature rises the remaining solid begins to melt, adding to the amount of B in the liquid until the first drop of liquid is observed--Let's assume this occurs when 30% of the mixture has melted. At this point the melt will contain 5 parts of A and 25 parts of B (approximately 17% A and 83% B). From the phase diagram in figure 1, it can be seen that the melting point of a mixture containing 83% B would be roughly 104°C and the observer would record this as the start of melting. As the temperature rises, B will continue to melt according to the phase diagram until the last crystal of B melts to give a liquid containing 95% B and 5% A (the original composition). This final temperature would be approximately 108.5°C. The recorded melting point range would thus be 104.0-108.5°C; broader and depressed compared with that of pure B.

Mixed Melting Points: The melting point of a pure substance can be used to provide information regarding possible structure by comparing the melting point obtained experimentally with those contained in data tables. Usually something is known about the substance which allows the field of possible candidates to be narrowed to a few prime candidates which have similar melting points. Identifying the unknown is then accomplished by taking a mixed melting point. A mixed melting point results from making a 50-50 mixture of your unknown with each of the possible candidate compounds identified from the data tables. If the two substances mixed are identical, then the substance will actually be a pure compound and the melting point will be sharp and unchanged from the melting point initial unknown. However, if the two compounds mixed are different, then the substance will be impure and result in a broad melting point range that is depressed compared to that of the original unknown. This technique is particularly handy when several possible candidate compounds have literature melting points within one or two degrees of the observed value for the unknown.

Crystallization and Recrystallization²: The simplest and most effective technique for the purification of solid organic compounds is crystallization. Crystalline compounds are easy to handle, their purity is easily assessed by melting points and solids are often easier to identify than liquids or oils. Crystals can be obtained in one of three ways: from the melted solid on cooling, by sublimation (solid to vapor to solid) or from a supersaturated solution. This last method is by far the most common in the organic laboratory.

A general plan for the purification of an organic compound by crystallization involves five stages: dissolution, filtration, crystallization, collection of the crystals and drying the crystals. The purity of the crystals can be determined and if necessary further purification by *recrystallization* can be carried out. First, let's consider how crystallization succeeds in purifying compounds.

The typical technique involves dissolving the impure solid in the minimum volume of hot solvent and quickly filtering to remove insoluble impurities. The resulting hot saturated solution of the compound, together with any soluble impurities, is set aside to cool slowly. During this cooling period, crystals of pure compound will separate from solution. The solution remaining after crystallization is known as the mother liquor. Why are the crystals pure? The process of crystallization is an equilibrium: molecules in solution are in equilibrium with those in the crystal lattice. Since a crystal lattice is highly ordered, other different molecules, such as impurities, will be excluded from the lattice and remain in solution. If you consider the developing crystal lattice as a jigsaw puzzle, then only molecules of the correct shape can fit into the lattice and build the solid. The impurity molecules have the wrong shape and can not add to the solid crystal. At the end of the crystallization process only molecules of the proper compound will be present in the solid crystal. In order for crystallization to be successful, the solution must be allowed to cool slowly, so that the crystals are formed slowly. This will allow the equilibrium process which excludes the impurities from the developing crystal to operate. If a solution is cooled rapidly, impurity molecules will be trapped or included in the rapidly growing crystal lattice. This rapid formation of solid material from solution is called *precipitation*, and is not the same as crystallization.

The ideal solvent for a crystallization (or recrystallization) should have a boiling point that is lower than the melting point of the compound to be crystallized. This will allow the solid crystals to be dried in an oven to remove the last traces of solvent. It is also important for the solid to be very soluble in hot solvent and insoluble in cold solvent. This will allow the crystals to slowly be forced out of solution as the solvent cools due to the decrease in the compound's solubility. Choosing a solvent for crystallization is not always easy. Organic chemists tend to follow

the rule that "like dissolves like." The crystallization of nonpolar compounds typically use nonpolar solvents such as hexane or petroleum ether (mixture of small molecular weight hydrocarbons). Compounds containing polar groups are best crystallized from polar -OH containing groups such as alcohols. Polar solvents are often preferred for other compounds because they tend to give better crystals. Sometimes a suitable solvent can not be found for a particular crystallization and a mixed solvent system must be used. A mixed solvent system is a pair of miscible solvents, chosen so that one of them (the good solvent) dissolves the compound readily and the other (the poor solvent) does not dissolve the compound. There are two methods for using mixed solvent systems. (1) Dissolve the solid in the minimum amount of hot good solvent and add the poor solvent dropwise until the solution becomes cloudy. Allow the cloudy solution to cool slowly to induce crystallization. (2) Suspend the solid in hot poor solvent and add the good solvent dropwise until the solid *just* dissolves. Set the solution aside to slowly cool and form crystals.

Chromatography: The term chromatography is derived from the fact that this technique was first used to separate pigments (Greek *chroma* = color, *graphein* = write), but the procedure, in its various modifications, is applicable to almost any chemical separation problem. The initial development of the methodology is ascribed to the Russian chemist Mikhail Tswett, who separated leaf extracts by percolation through a column packed with chalk. Since this pioneering work in the early 1900's, two Nobel Prizes have been awarded for work in the field of chromatography. The Swede, A. Tiselius, was awarded a Nobel Prize in 1948 in recognition of his contribution to work on electrophoresis and adsorption analysis and the Britons A. J. P. Martin and R. L. M. Syngé, were jointly awarded the Prize in 1952 for their work on partition chromatography.

All of the different chromatography techniques have one common theme. They all depend upon the differential distribution of the various components of a mixture between two phases-- the *mobile phase* and the *stationary phase*. The mobile phase may be either a liquid or a gas and the stationary phase either a solid or liquid.

Adsorption Chromatography: Organic chemists will frequently use *thin layer chromatography* (TLC) and *column chromatography* for the analysis and purification of organic compounds. Both of these methods are based on the principles of adsorption chromatography. The stationary phase or adsorbant for these methods is a porous solid capable of retaining both solvents and solutes. Many different materials fill these particular requirements but the two most used solid adsorbants are silica gel (SiO_2) and alumina (Al_2O_3) in highly purified and finely powdered form. Both of these adsorbants may be supplied with a fluorescent indicator (zinc sulfide) which aids in the visualization of certain compounds after TLC analysis. The activity of the silica or alumina is a measure of how tightly the adsorbant holds organic compounds. The activity is related to the moisture content in the silica or alumina. The more water that is bound to the adsorbant, the less tightly other organic compounds will adhere to the solid support. Typically, silica gel will contain 10-20% water when it is used for chromatography.

Elution Solvents: The chromatography column or TLC plate is first *loaded* with the mixture to be separated and then developed by allowing solvent to percolate through the solid adsorbant. The solvent moves either by the action of gravity (column chromatography) or capillary action (TLC). The eluting solvent will be able to displace the different components of the mixture to differing degrees. Both the solvent molecules and the different mixture molecules will be competing for the active sites on the solid adsorbant. As a result, some components of the mixture will spend more time in the moving solvent than stuck on the solid adsorbant. When a compound is not sticking to an active site on the solid support, they are moving with the bulk solvent through the silica gel or alumina. Compounds with different polarities and shapes will travel down the column or up a TLC plate at different rates. This results in a separation of the different components of a mixture. Column chromatography is used for preparative separation of mixtures. The solvent system is chosen so that the different mixture components will travel through the column at different rates and exit the column in different fractions. The polarity of the solvent will determine how fast the mixture components move through the column. The more polar the solvent the faster the organic components will move. This is because the solvent and compound molecules are competing for polar active sites on the solid adsorbant.

As the name suggests, TLC utilizes a thin coating of adsorbant on a rigid glass or plastic plate. The most useful size of TLC plate is about 2.5 x 7.0 cm which easily allows three samples to be spotted on the *baseline* (a line marked in lead pencil about 1 cm from the end of the plate) and has enough length to give good resolution in a short amount of time. TLC is used for analysis and the plate is developed by allowing the solvent to move the distance of the plate and then the plate is "visualized" either through chemical or fluorescent methods to determine how many components were in the original mixture. A two component mixture should result in two different spots on the developed TLC plate with different R_f values (see background reading). It is easy to overload TLC plates with sample. Figure 2 shows the results of sample overloading. When too much sample gets spotted onto the TLC plate, the spot not only is very large but also starts to streak. This streaking can easily obscure the presence of another spot with a very similar R_f value. It is important to realize that a single TLC

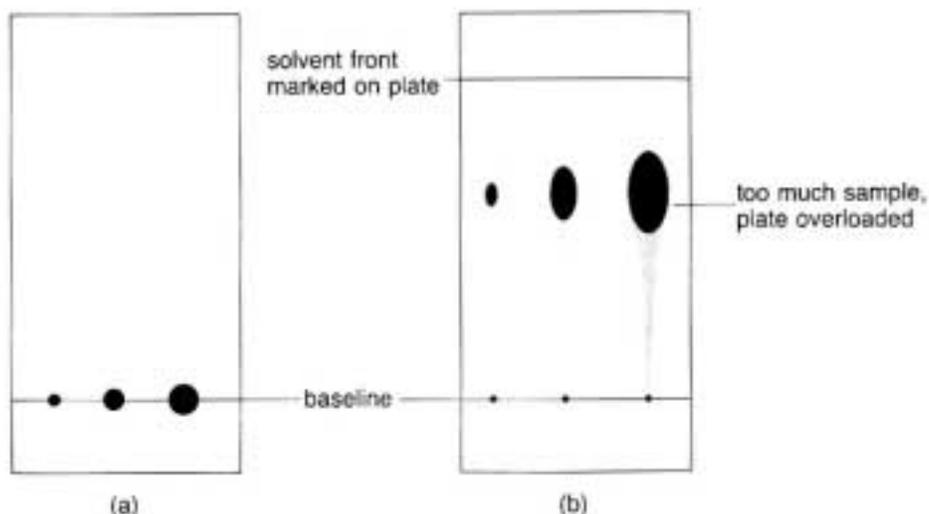
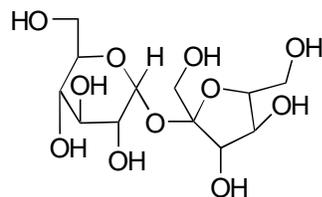


Figure 2. A TLC plate spotted with three different amounts of sample: (a) before development and (b) after development.

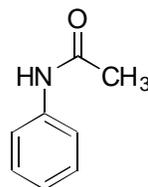
analysis is not enough evidence to identify an unknown compound. The use of co-spotting (see background reading) gives stronger evidence of compound identity. The appearance of one spot after TLC analysis also does not necessarily prove that your compound is pure. It is possible for two different compounds to have the same or very close R_f values in the same solvent system. This is why organic chemists typically do TLC analyses in several different solvent systems when they are trying to determine product purity.

Capillary Gas Chromatography: This technique uses an inert gas as the mobile phase instead of a liquid solvent. A temperature gradient is then used to get the compounds to move through the column. Like TLC, gas chromatography is typically an analytical technique used to determine the number and identity of compounds present in a mixture. Gas chromatography (GC) can also be coupled with other analytical techniques such as mass spectroscopy (MS) to aid in the identification of mixture components. The instrument we will use is a GC-MS but we will only be utilizing the GC during this experiment.

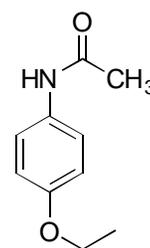
Structures and Properties



Sucrose



Acetanilide



Phenacetin

Physical Properties

	M.W.	mp	Solubility Cold H ₂ O	Solubility Hot H ₂ O
Acetanilide	135.2	114°C	0.54 g/100mL	5.0 g/100mL
Phenacetin	179.2	135°C	0.076 g/100mL	1.22 g/100mL
Sucrose	342.3	dec 160-186°C	200 g/100mL	500 g/100mL

Understanding the Experiment

In this experiment you will purify and identify the unknown component from your pharmaceutical mixture from last week's experiment. No separation is perfect. Traces of impurities will always remain in a substance that has

been separated from a mixture. Therefore, some kind of purification process is needed to remove them. Solids can be purified by such operations as recrystallization, chromatography and sublimation. Liquids are usually purified by distillation or chromatography. The solubility information given above (obtained from the *Merk Index*) indicates that both acetanilide and phenacetin are relatively soluble in boiling water but insoluble in cold water. This suggests that the unknown component can be purified by recrystallization. Based on the mass of unknown you recovered last week, you can estimate the volume of boiling water needed to dissolve that mass of either acetanilide or phenacetin. For example, the solubility of acetanilide in boiling water is 5.0 g per 100 mL water. If your unknown is acetanilide and you recovered 1.15 g of crude solid, the approximate volume of boiling water you will need to dissolve it is 23 mL.

$$1.15 \text{ g acetanilide (100 mL H}_2\text{O/5.0 g acetanilide)} = 23 \text{ mL water}$$

Phenacetin, which is much less soluble in boiling water, will require more water. You should begin the recrystallization using the smaller volume of water (do not add it all at once) and add more only if your compound does not dissolve in that amount of water. Save a small amount (~30-50 mg) for TLC and GC analysis to compare with the same analysis on the purified compound.

After a compound has been purified, it should be analyzed to establish its identity and degree of purity. We will analyze by melting point, TLC and GC analysis. Melting point and mixed-melting point analysis should establish whether you have isolated acetanilide or phenacetin. TLC and GC analysis will be compared to known standards to verify your identification.

Procedure:

Recrystallization: In a 50 mL beaker, recrystallize the unknown by adding just enough boiling water to dissolve the solid completely. Let the hot solution cool slowly to room temperature and then cool in an ice bath. If necessary, induce crystallization by scratching the side of the beaker. Collect the solid by vacuum filtration, washing the solid with a small amount of cold water. Dry the product to constant mass and weigh. Calculate the % recovery from the recrystallization.

Analysis: Grind a small amount of the purified product to a fine powder on a watchglass with a spatula or stirring rod. Divide the solid into three equal portions and prepare 1:1 mixtures by grinding one portion with a nearly equal amount of acetanilide and a second portion with a nearly equal amount of phenacetin. Measure the melting point ranges for (1) the purified unknown, (2) the mixture with acetanilide, and (3) the mixture with phenacetin. In each case record the temperature at which you see the first trace of liquid and the temperature at which the sample becomes completely liquid. You should carry out at least two measurements on each of the samples. Remember to also take a melting point of the aspirin which was isolated from the mixture.

Dissolve a small amount of each of your mixtures and your pure compound in dichloromethane and label the solutions. Use these solutions to spot a TLC plate. You should be able to spot all three compounds on one TLC plate. Draw a baseline in lead pencil about 1-1.5 cm from the bottom on the plate. Be sure that the line is straight. Carefully draw a hatch mark at the middle of the baseline. Place two more hatch marks midway between the middle of the plate and the edge. Label each of the marks to correspond with one of the three solutions that you have prepared. Spot your three solutions onto the TLC plate. Use a different micropipet to spot each of the solutions to avoid unwanted contamination. Develop the plate in the available developing solvent. Allow the solvent to move up the plate until it is 0.5 cm from the top edge. Remove the plate from the developing chamber and immediately mark with a pencil the distance that the solvent traveled by drawing a line across the plate at the appropriate place. Make sure that all of the developing solution has evaporated from the plate and then visualize under short-wave UV light. The plates are doped with a fluorescent indicator. The aromatic ring will be able to absorb this fluorescence and a dark spot will appear on the plate wherever the compound is present. Circle the dark spots with a pencil and calculate R_f values for each spot. If your TLC plate has been overloaded, try a second analysis.

For GC analysis, take an aliquot (~5 drops) of each solution you have made and add it to a GC-MS vial. Fill to the curve near the neck of the vial with dichloromethane solvent. Cap and invert once to mix the sample solution. Place sample vials into the autosampler on the instrument and set-up the experiment as shown during lab.

Discussion/Write-up

Your discussion should explain your results. Do not just write that one compound had a higher R_f value or a longer GC retention time than another. Instead, explain why these observations occurred. Apply the theory of the techniques used in lab to help explain the observed results. The reader should thoroughly understand your results after reading your discussion. Be sure to state the identity of the unknown component of your pharmaceutical mixture and briefly summarize your reasoning.

Questions:

1. (a) How much boiling water would be needed to dissolve 1.25 g of phenacetin? (b) How much phenacetin will remain dissolved after the solution is cooled to room temperature?
2. An unknown compound X is one of the four compounds listed in the following table. A mixture of X with benzoic acid begins to melt at 89°C, a mixture of X with phenyl succinate begins to melt at 120°C, and a mixture of X with *m*-aminophenol begins to melt at 102°C. Give the identity of X and explain your reasoning.

<u>Compound</u>	<u>mp, °C</u>
<i>o</i> -toluic acid	102
benzoic acid	121
phenyl succinate	121
<i>m</i> -aminophenol	122

3. Tell whether each of the following experimental errors will raise or lower the amount of product you recover, and why. (a) You failed to dry the product completely. (b) You used too much water to recrystallize your unknown. (c) In last week's experiment you did not extract all of the aspirin from the dichloromethane solution.
4. Tell whether each of the experimental errors in the previous question will affect the melting point of the unknown component. If it will, tell how it will affect the melting point and why.