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Experiment 5
Superfund Lab: Isolating and Identifying Contaminates from a Decommissioned Chemical Plant

Chemistry 132
Spring 2013

Background

Mixtures of multiple compounds are common, both in the chemistry lab and in our everyday life. For example, virtually all food that is consumed is a mixture of some sort. And while mixtures are relatively easy to make in both the kitchen and the chemistry lab, a fair amount of work is required to isolate the individual ingredients of a mixture after it has been made. Several essential organic chemistry techniques are integral to the isolation process. In this lab, the techniques of ***extraction***, ***thin layer chromatography (TLC)*** and ***recrystallization*** will be used as tools for separating, identifying and purifying components of a chemical mixture.

Superfund Scenario

Your unknown sample mixture was isolated from the soil at the site of a decommissioned chemical plant. There is concern that the chemicals found in the soil could cause ecological harm if they contaminated the ground water and the surrounding environment. However, you don't know the identity of chemicals! They could be harmless and pose no threat, or could be toxic. Each mixture contains **two** of the following three classes of organic compounds: acidic, basic, and neutral. Your goal will be to use the differences in chemical and physical properties between each compound to isolate and identify the components in the mixture.

Four-Week Lab Overview (working ***individually***, but in parallel with a "buddy")

- **Week 1:** Separate the components of your unknown. The unknown will contain two of the following: an organic acid, an organic base, and a neutral organic compound. The two compounds will be separated by biphasic extraction with an organic solvent and a mineral acid or base.
- **Week 2:** Run TLC on the compounds to determine the purity and possible structure of each compound from a list of likely contaminates for the chemical site. Select recrystallization solvents based on the structure of the compounds and literature data.
- **Week 3:** Recrystallize components of your mixture. Run TLC of the crude extracted compounds versus the recrystallized compounds to assess the purity of each recrystallized compound and the effectiveness of the recrystallization process.
- **Week 4:** Take IR spectra and determine the melting point of the compounds present at the chemical plant site and identify of the compounds in your unknown mixture.

Week	Technique	Homework Assignment
1	Extraction	1. Assign acid/base/neutral to the list of possible chemical candidates 2. Extraction worksheet
2	TLC	1. Determine recrystallization solvents for each compound using recrystallization guidelines and literature searches 2. TLC worksheet
3	Recrystallization and TLC	1. Look up melting point and IR data for the compounds 2. Recrystallization worksheet
4	IR and Melting Point	Lab report: Results and Discussion section

Week 1: Separation and Extraction of Compounds using a Separatory Funnel

- Weigh the sample mixture and then dissolve it in ~30 mL of ethyl acetate. Place the mixture in a separatory funnel.

Extraction and Isolation of Compound 1

- Add ~10 mL of 2 M NaOH to the separatory funnel. Mix, extract, and separate the aqueous layer (see below for more details on extraction using a separatory funnel). Leave the organic layer in funnel.
- Repeat this step by adding an additional ~10 mL of 2 M NaOH to the organic layer that remains in the separatory funnel. Once both separations are completed successfully, combine the two aqueous solutions. (Leave the organic layer in the separatory funnel and use it for the *Extraction and Isolation of Compound 2* below.)
    - Which organic functional groups react with base?
    - Which of the molecules listed above contain those groups?
    - Why is Compound 1 able to be extracted from the organic solvent into the basic aqueous solution? Explain briefly.

- Place the basic aqueous solution in an ice bath. Calculate the amount of 6 M HCl needed to neutralize the basic solution. While stirring, **slowly** add the 6 M HCl solution to the chilled solution. Slowly add 6 M HCl until the pH of the aqueous layer is ~2. Use pH paper to determine the pH of the chilled aqueous solution. (Determine the pH by putting a drop of the aqueous solution onto the pH paper. Do not dip the pH paper into the aqueous solution.)

- How does the HCl react with the compound in the aqueous layer? What is the product of that reaction?
- Why is this desirable?
- Which of the possible components of your unknown mixture could have been extracted in this step?

- Recover the precipitate by filtering the chilled solution. Rinse the flask and the precipitate with a small amount of cold water. Allow the precipitate to air dry. Weigh and label the crude product. (If the product is still wet at the end of lab, let the product air dry and weigh it at the beginning of lab next week.)

Extraction and Isolation of Compound 2

- Add ~5 mL of 2 M HCl to the organic solution in the separatory funnel. Mix well and extract the aqueous layer. Leave the organic layer in the funnel.

- Add an additional ~5 mL of 2 M HCl to the organic solution in the separatory funnel. Mix well and extract the aqueous layer. Combine the two aqueous layers. (Leave the organic layer in the separatory funnel and use it for the *Isolation of Compound 3* below.)

  - Which organic functional groups react with acid?
  - Which of the molecules listed above contain those groups?
  - Why is Compound 2 able to be extracted from the organic solvent into the acidic aqueous solution? Explain briefly.

- Place the acidic solution in an ice bath. Calculate the amount of 6 M NaOH needed to neutralize the acidic solution. While stirring, **slowly** add the NaOH to the chilled solution. Slowly add NaOH until the pH of the aqueous layer is ~10. Again, use pH paper to determine the pH of the chilled aqueous solution.

  - How does the NaOH react with the compound in the aqueous layer? What is the product of that reaction?
  - Why is this desirable?
  - Which of the possible components of your unknown mixture could have been extracted in this step?

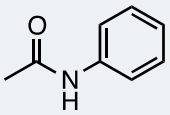
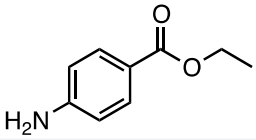
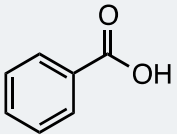
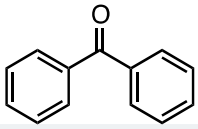
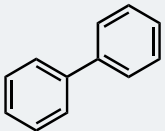
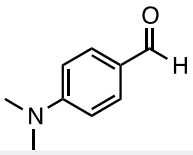
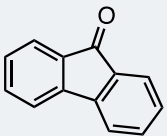
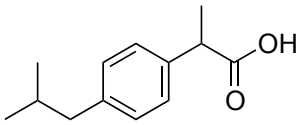
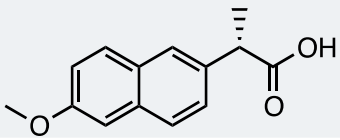
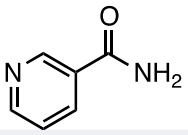
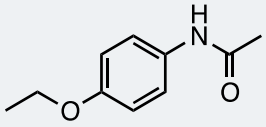
- Recover the precipitate by filtering the chilled solution. Rinse the flask and the precipitate with a small amount of cold water. Allow the precipitate to air dry. Weigh and label the crude

product. (If the product is still wet at the end of lab, let the product air dry and weigh it at the beginning of lab next week.)

Isolation of Compound 3

- Pour the organic layer into an Erlenmeyer flask or beaker, and dry using magnesium sulfate. Remove the drying agent by gravity filtration into an appropriately sized, ***pre-weighed*** flask. Evaporate the solvent in the organic layer, leaving your crude product in the flask. Weigh the crude product next week.
    - Is this an acidic, basic, or neutral compound? Explain briefly.
    - Which of the possible components of your unknown mixture could have been extracted in this step?

Assign acidic/basic/neutral to each of these chemicals.

Name	Structure	Information	Acid, Base or Neutral?
Acetanilide		Precursor in synthesis of penicillin and other pharmaceuticals	
Benzocaine		Local anesthetic	
Benzoic Acid		Common food preservative; common reagent in many organic syntheses	
Benzophenone		Photo-initiator in inks; Building block for organic molecules	
Biphenyl		Prevents growth of fungus and molds	
p-Dimethylamino benzaldehyde		Used to quantitatively detect hydrazine and urobilinogen	
Fluorenone		Used to make anti-malarial drugs	
Ibuprofen		Nonsteroidal anti-inflammatory drug; blood-thinner	
Naproxen		Nonsteroidal anti-inflammatory drug; Commonly used to reduce pain from arthritis, kidney stones, tendinitis, etc.	
Nicotinamide		Part of vitamin B group; used in acne medication	
Phenacetin		Once widely used analgesic	

Extraction Using a Separatory Funnel

Frequently in organic chemistry we deal with chemical mixtures. For instance, in many cases the initial product of a reaction is actually a mixture of the product and trace contaminants, including starting materials and byproducts. The question then arises: how do you isolate the product from the other components in the mixture? Extraction using a separatory funnel is one commonly used method.

Extraction using a separatory funnel relies on two key principles: **immiscible solvents** and **differential solubility and reactivity**. When we use a separatory funnel we will take advantage of differential solubility and reactivity in a **biphasic system**. In a biphasic system, the two solvents being used are immiscible and thus do not mix. Think of adding oil to water in the kitchen—they do not mix. In the lab we will use an organic solvent instead of oil, but we will still use an aqueous solution as the other immiscible solvent.

The two solvent layers are immiscible due to their different polarities. Water is a very polar solvent. Common polar aqueous solutions include acids (HCl, H_2SO_4), bases (NaOH, $NaHCO_3$) and salts (NaCl, KCl). Most organic solvents, in which organic compounds dissolve well, are much less polar than water, and are thus immiscible with water. Organic solvents commonly used in biphasic extractions include hexanes, ethyl acetate, and methylene chloride. More polar organic solvents like methanol, ethanol, and acetone are not suitable for biphasic extractions because they are miscible with water.

In the lab, the two solvents in a biphasic extraction can be easily separated using a separatory funnel (see above right). Think back to adding water to oil: two distinct layers form. In the separatory funnel the bottom layer can be drained out the bottom of the separatory funnel, leaving the top layer behind and effectively separating the two immiscible layers. The bottom layer will always be the more dense solution. Frequently this will be the aqueous solution, but it is always good to check the densities of the solvents you are using for the extraction. (In particular, halogenated solvents like dichloromethane have a higher density than most aqueous solutions, and therefore are likely to be the bottom layer in a separatory funnel extraction.)

We now have a system to separate two immiscible solvents. Let's relate that to the principles of differential solubility and reactivity. Different compounds that have different physical properties and different reactivity can separated based on those differences. For instance, one compound might be significantly more polar than another compound. The more polar compound can be extracted from the less polar compound due to its greater solubility in highly polar aqueous solvents. Another possibility is that one compound in a mixture readily reacts with a strong acid in an acid-base reaction to form a charged product with different solubility preferences. The other compound does not react, and now the two compounds can again be separated based on different solubilities.

The different acid-base reactivities allow for extraction and separation. Either way, it is important to know the molecular structure of the compounds involved, and to understand how that structure predicts its physical properties and its reactivity.

Procedure for Using a Separatory Funnel

1. Place the dissolved mixture of compounds in a separatory funnel. Typically, this mixture will be dissolved in an organic solvent. Before adding the solvent make sure the valve at the bottom of the separatory funnel (called a "stopcock") is closed. It is closed when the handle is parallel with the counter top. Place the funnel in a ring stand with an Erlenmeyer flask underneath the separatory funnel opening.
2. Add a second solvent that will extract one or more of the mixture components. Many times this second solvent is an aqueous acid or a base, and the compound(s) to be extracted can be protonated or deprotonated. The compounds that reacted are now more soluble in the aqueous layer.
3. Place the stopper in the top of the separatory funnel and mix the two solvents together by gently shaking or swirling the separatory funnel. Be sure to periodically release any gas or vapor that builds up inside the funnel by opening the valve end of the funnel. Make sure the tip of the funnel is not pointed at anyone or at the ground. Close the valve and resume mixing.
4. Once the two layers have been thoroughly mixed, place the separatory funnel in the ring stand, remove the stopper from the top of the separatory funnel, and allow the two layers to separate.
5. Determine which layer is organic and which is aqueous. This can be done before lab by comparing densities, but it can also be quickly determined in the lab. Add a small amount of water to the separatory funnel and closely watch to see which layer the newly added water joins.
6. Isolate and save the bottom layer. To isolate the bottom layer slowly turn the valve so that the bottom layer drains at a controlled pace from the separatory funnel into the Erlenmeyer flask. If a significant portion of the top layer accidentally drains into the Erlenmeyer flask, close the valve and empty the contents of the flask back into the separatory funnel and restart this step.
7. Repeat steps 2–6 to perform a second aqueous extraction if the lab protocol calls for it. If a repeat extraction is performed, extract the bottom layer into a separate flask and then combine the two layers once the second extraction has been successful. Often extractions will be performed twice with the same aqueous solution because the repetition allows for more effective extraction of the targeted compound.
8. Carefully label the extracted layer, and keep it until you have successfully recovered the product(s) at the end of the lab. Even if this solution is not used later in the lab, it is important to know the identity so you can properly dispose of it at the end of lab. Also, if a mistake has been made and you do not recover your product at the end of the lab, you can potentially recover your product from the labeled layers of the extraction process.

For a good YouTube video on extraction using a separatory funnel, check out:

http://youtu.be/vcwfhDhLiQU

Week 2: TLC of Crude Extracted Compounds

- As a result of the extraction process last week you should have separated two unknown compounds from the soil sample from your decommissioned chemical plant. After answering the questions during the lab you should also know whether each compound is acidic, basic or neutral.
- Of the possible compounds that could be found in your sample, you should have already labeled each as an acid, base, or neutral compound. If not, do this now.
- Dissolved samples of all of the candidates are in vials in the hoods.
- Run TLCs comparing all of the **acidic candidates** to the acidic compound you isolated last week. Or if appropriate, gather all of the basic candidates and run TLCs comparing all of the **basic candidates** to the basic compound you isolated last week.

  See the TLC technique procedure that follows for more details on how to run a TLC. Use the table below for guidelines on making the TLC solvents.

TLC Solvents	Ethyl Acetate	Hexanes	Glacial Acetic Acid
Acids	4 mL	6 mL	2 drops
Bases	4 mL	6 mL	none
Neutrals	4 mL	6 mL	2 drops

- Under the UV lamp, circle the spots for each compound, and then compare the R_f value of your unknown acidic compound with the R_f values of the acidic candidates.
  - What is the R_f of your acidic/basic compound? Of each of the known acidic/basic candidates?
  - What is the likely identity of your acidic/basic compound? Is there a second candidate? Explain briefly.
  - Rerun TLCs as necessary to confirm the identity of the compound.
- Repeat the TLC analysis for your neutral compound to determine the likely identity of the compound. Use the identified molecular structure to determine possible recrystallization solvents.
- Use the identity and structure of the your two candidate molecules to help you choose a recrystallization solvent. Use both the recrystallization chart and information you find on the web to aid in selecting a solvent.
- Discuss your TLC results and recrystallization solvent choices with your professor and lab assistants before leaving lab.

Thin-Layer Chromatography (TLC)

TLC is one of the most useful techniques in synthetic organic chemistry. It is a relatively inexpensive and quick procedure that can be used to quickly determine the identity of a compound, analyze the purity of a compound, determine conditions for purifying a reaction, and even used to 'watch' an organic reaction as it occurs to monitor its progress.

The TLC plate

We will be using pre-made plates to run our TLC experiments. These plates consist of a plastic support that is covered by a thin layer of an absorbent, porous material. This is referred to as the **stationary phase**. In our case, it will consist of silica gel, which is a fine white powder and is very polar compound. When handling TLC plates, be careful not to touch the side coated with silica gel with your bare hands (Look closely at both sides: one side is smooth plastic, and the other is covered with a fine white powder that is the silica gel). We will be working on the side with the silica gel!

How TLC Works

A small amount of the compound to be analyzed is dissolved in a good solvent, and the solution is then 'spotted' onto a TLC plate and allowed to dry. The plate is put into a glass beaker that contains about ¼ inch of solvent (the developing chamber). The solvent will slowly make its way up the plate; this solvent is referred to as the **mobile phase**.

When the solvent front reaches the compound that was spotted on the plate, if the compound is itself polar, it will bind more to the polar stationary phase, and thus not move very far. If it is non-polar, it will bind more to the less polar mobile phase and travel further up the TLC plate (or do something in between). Exactly how far the compound travels depends on the molecular properties of the compound, mostly the polarity. The distance traveled is reported as an R_f value for the particular mobile phase used (see below). As the polarity of each compound is unique, different compounds *may* have different R_f values! In this way you can compare your unknown to others; by spotting two or more solutions next to each other on the same TLC plate you will be able to determine if they are different or possibly the same compound!

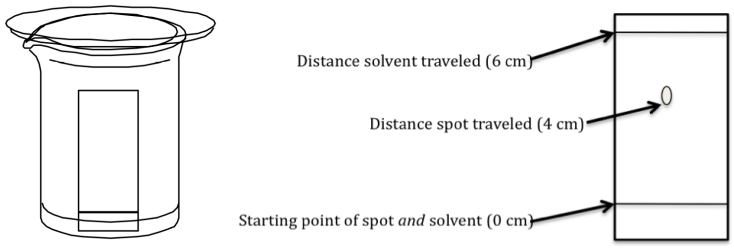


Figure. *Left*: Schematic of a TLC plate in a developing chamber (in this case, a beaker with a watch glass as a lid. *Right*: TLC place showing pencil markings of the staring point, the distance the compound traveled, and the distance the solvent traveled.

Visualizing the Spot

In most cases the compounds being testing do not absorb visible light, meaning they have no color and you cannot see them on the plate! We therefore require some other way to visualize how far up the plate the compound has moved. Many of the compounds we will be testing absorb UV light. The TLC plates we are using contain a small amount of dye that fluoresces when hit with UV-light (254 nm). You will notice that when you shine the UV light on your TLC plate it glows bright green. Your

compound will absorb the UV light at the spot it has traveled to, and you will see a dark spot on the plate. Carefully circle this spot so that you know where it is after the UV light is removed.

R_f Values

When comparing one TLC run to another (with the *same* eluting solvent) there are several variables that make it difficult to do a direct comparison (you can't just measure how far each spot has traveled). Therefore it is important that we normalized the distance traveled by each compound. This is done with a ratio called the R_f value, defined as:

$$R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}$$

In the case of the TLC plate in Figure 2, we can see that $R_f = 4/6 = 0.66$. This value can then be used to compare different TLC plates using the same solvent.

Thin-Layer Chromatography Procedure

1. With a **pencil**, draw a straight line on the powder side of your TLC plate 1–2 cm from the bottom. This is where you will 'spot' your plate with your sample.
2. Place a small amount of your unknown (just a few crystals) on a watch glass or in a vial, and dissolve it with about ten drops of solvent (one you have determined the compound to be soluble in).
3. Next take one of the capillary tubes (spotters) and touch one end of the tube to your dissolved unknown, allowing the liquid to be drawn up into the tube by capillary action.
4. Lightly touch your TLC plate with the spotter on the line that you drew. Carefully make a small identifying pencil mark under the spot and then allow it to dry.
5. In the solvent chamber (covered beaker or jar) stand a piece of filter paper on its side and add approximately 10 ml of your eluting solvent.
6. Carefully place your spotted TLC plate into the solvent and lean it against the side of the beaker. **Important:** make sure that the solvent level in the beaker is *below* the pencil line on your TLC plate!
7. Allow the solvent to make its way up the plate. Keep an eye on it and be sure to remove the TLC plate *before* the solvent reaches the top of the plate. Stopping about 1 cm from the top is usually a good idea (TLC plates are approximately 7.5 cm long to give an idea of how far the solvent should move). Immediately upon removal of the plate from the solvent chamber mark the level the solvent has traveled to with your pencil (be ready as the solvent will evaporate quickly!).
8. Mark your plate under the UV light, measure, and then calculate R_f values.

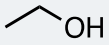
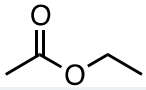
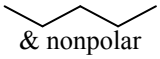
Before coming to lab, watch this YouTube video on TLC: http://youtu.be/EUn2skAAjHk

Week 3: Recrystallization of Crude Extracted Compounds

- Read below to find out more about recrystallization and for detailed instructions on doing a recrystallization.
- Heat the recrystallization solvent(s) for your acidic/basic compound to a *gentle* boiling. Place most of your crude acidic/basic compound in an Erlenmeyer flask, reserving a very small amount of the crude compound for further TLC analysis.
    - Based on the amount of crude material you have, what size Erlenmeyer flask would be appropriate for your recrystallization? *Use the best one!*
- Recrystallize your acidic/basic compound and collect the solid by vacuum filtration. After all of the recrystallization solvent has evaporated, weigh the crystals.
- Run a TLC comparing the crude acidic/basic compound, the recrystallized acidic/basic compound, and the pure candidate used for TLC identification last week. Use the same TLC solvent systems as you did last week.
    - Did the recrystallization process purify the acidic/basic compound? What evidence supports your analysis? Explain briefly.
    - How does the TLC of your recrystallized compound compare to the pure candidate?

Repeat the recrystallization process and TLC analysis for the neutral compound.

Recrystallization Solvent Table.

Name	Structure	bp (°C)*	Dielectric constant*	Details
Ethanol		78	24	polar, organic solvent can require drop-wise addition of water for effective recrystallization
Ethyl acetate		77	6	moderately polar organic solvent less polar than ethanol
Methanol	CH_3OH	65	33	polar, organic solvent more polar than ethanol can require drop wise addition of water for effective recrystallization
Petroleum ether (Ligroine)	 & nonpolar hydrocarbons	30–60	2	nonpolar, organic solvent mixture of nonpolar molecules
Water	H_2O	100	79	very polar, nonorganic solvent most polar solvent on the chart

*Boiling point and dielectric constant data taken from the 72nd Ed. of the *CRC Handbook of Chemistry and Physics*

Purification by Recrystallization

A very common and useful way to purify many solid compounds is by recrystallization. This technique involves dissolving an impure solid in a small amount of an appropriate solvent at elevated temperature, and then recovering pure crystals from the cooled solution. Finding the best solvents and conditions for recrystallization can be a delicate art; the beautiful crystals that can be obtained can be art themselves!

One method of recrystallization takes advantage of the fact that most solids are more soluble in hot solvent than in cold solvent. The key is to find a solvent that completely dissolves the compound at high temperature, yet at cool temperatures the compound is almost completely insoluble. The change in solubility with change in temperature is very important. The upper temperature limit is usually the boiling point of the solvent, and the lower is the solvent's melting point, or whatever cooling method is present (ice bath, freezer, dry ice bath, etc.). Choosing an appropriate solvent is the first step in recrystallization. Occasionally a single solvent cannot be found which is suitable, and in these cases two or more solvents can be used together in a 'mixed solvent recrystallization.'

Once a solvent has been selected the impure compound is added to ***as small an amount of solvent as possible*** so that when heated the solid is completely dissolved. The solution is then allowed to cool slowly, usually first to room temperature, and then on an ice bath or in a freezer. The slower the cooling occurs, the better! The hot solution is often insulated with cotton and foil to promote slower cooling. The point is to very gradually have crystals grow out of solution as the compound's solubility changes as the temperature drops. This process can form well-ordered crystals of the compound, and impurities originally in the solid remain dissolved in the supernatant. If the recrystallization happens too quickly, many small crystal grow rather than fewer large crystals. In this case, impurities can get trapped within the rapidly formed crystals of the desired compound, and the process will need to be repeated. Using a minimal amount of solvent allows for the recovery the maximum amount of crystals.

Occasionally the solid will not crystallize in the solution, even as it reaches low temperature. This is either because a poor solvent was selected, there is too much solvent, or the solution has become super-saturated. In the case of the latter, the crystals simply need a nucleation point to form. This can be achieved by adding a 'seed' crystal, but in the common event that there are no pure crystals available (the point of recrystallization in the first place) scratching the side of the container, or sharply agitating the container, can initiate crystallization.

Once crystals have finished forming, the solution is vacuum filtered and washed with a ***small amount of cold solvent***. This ensures that the impurities, which have remained dissolved, are washed off of the crystals and the purest possible compound obtained. Why is it important that the washing solution be cold?

Recrystallization Procedure

1. Identify a good recrystallization solvent. This may be as simple as reading the lab, require searching the literature, or may involve a great deal of trial and error.
2. Once a good recrystallization solvent has been identified, heat a moderate amount of the solvent to boiling.
3. Meanwhile, place the solid to be recrystallized in an appropriately sized flask. Make sure that you weighed the unrecrystallized solid. While you wait for the solvent to warm, crush the solid as much as possible. Do not heat the solid at this point!
4. Next add a small amount of the hot solvent to the impure solid. Swirl the solution and watch for signs that the solid is dissolving. Heat and swirl the flask containing the solid and solvent to encourage the dissolution process. If the solid does not completely dissolve in the hot solvent add another small portion of solvent and continue to heat and swirl the mixture. Slowly add hot solvent until the solid completely dissolved. Be sure to use the minimum amount of solvent as this will increase the amount of pure crystals that can be recovered.
    a) When heating the solid/solvent mixture to dissolve the solid, watch the solvent level. If the solvent is rapidly boiling, the solvent will evaporate quickly and the solid will never have a chance to dissolve.
    b) If too much recrystallization solvent is added, simply boil off the excess solvent. Closely watch this process to make sure that that you do not boil off *all* of the solvent!
    c) Sometimes the impurities in a solid will not dissolve in the recrystallization solvent. This means that there will always be solid particles in the recrystallization mixture no matter how much solvent you add. In this case a hot filtration may be necessary. Always check with your instructor before performing a hot filtration! A hot filtration involves filtering a solution while the solvent is hot. The solid particles will be caught by the filter paper and the particle free solution will be collected in the filter flask below. The recrystallization process can then proceed with the particle free solution.
    d) One method of mixed-solvent recrystallization involves dissolving the solid in a minimal amount of a hot recrystallization solvent. Then, the second miscible solvent is added drop-wise, with swirling, to the dissolved solution. After the addition of multiple drops of the second solvent the solution will eventually turn cloudy and no more is added. The addition of a few drops of the original solvent removes the cloudiness from the solution, and then the recrystallization can continue as outlined below.
5. Once the solid is fully dissolved, remove the solution from the hot plate. Allow the flask to cool ***slowly*** to room temperature. Do not rush it! You can use this time to perform another recrystallization, clean glassware, write your analysis in your notebook, etc.
6. Sometimes crystals will be visible after the recrystallization solution has cooled to room temperature, and other times no crystals will be visible. Either way, after it has reach room temperature cool the solution further by placing it in an ice bath, refrigerator, or freezer.

7. The cold solution should now have crystals. If crystals are not present try scratching the flask with a glass stirring rod or sharply shaking the solution to aid in crystal nucleation. It is also possible that there is too much recrystallization solvent for crystals to form. (See 4b above.)
8. Recover the crystals by vacuum filtration. Rinse the crystals with cold recrystallization solvent. If a solvent mixture was used, then rinse with the solvent in which the compound is *least* soluble.
9. Allow the crystals to dry before weighing them and getting a melting point.

Before coming to lab, watch this YouTube video on recrystallization:
http://youtu.be/Q47hTa1KvN0