Lab 1: Separation of an Unknown Mixture

Reading

Zubrick: Chapters 10 (Drying Agents), 15 (Extraction and Washing), 19 (Clamps and Clamping), and 22 (Rotary Evaporator). Appendix 4 (about pre-lab and lab report), 6 (about rotovap) of this manual.

Summary

In this experiment, you will be given a mixture that contains two organic compounds. One will be an organic neutral compound and the other will be either a strong organic acid, a weak organic acid, or an organic base. You will separate the components in this lab using acid/base extractions; in the next lab you will purify them by recrystallization and distillation and finally identify them from a list of possible unknowns.

Introduction

Unlike on paper, where reactions do exactly what we want, in a real lab, reactions tend to give not only desired product(s) but also undesired by-products and unreacted starting materials. Thus, one of the most important techniques in organic chemistry is the separation of organic compounds from each other. A variety of methods are used for separations: chromatography, distillation, recrystallization, and extractions. In this lab we will learn the technique for a special kind of extraction—the acid/base solvent extraction.

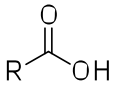
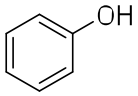
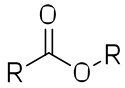
All extractions (acid/base and otherwise) depend on the fact that water and many organic solvents are not miscible (some exceptions: methanol, ethanol, acetone). This allows you to use an organic solvent and water in a separatory funnel ('sep funnel') to separate compounds based on their different solubilities in the aqueous and organic layers.

The strategy in an acid/base extraction is to transfer an organic molecule from a nonpolar solvent into an aqueous solution by converting it to a water-soluble salt. You probably remember from general chemistry that salts dissolve easily in water because they dissociate into charged ions which are attracted to and solvated by the highly polar water molecules. Most organic molecules, on the other hand, are not charged and thus do not share this attraction, and thus solubility in water. In general, we can divide organic compounds into four categories of acid-base behavior: strong acids, weak acids, bases, and neutral compounds. Keep in mind that in general chemistry you learned that strong acids and bases dissociate completely in aqueous solution – in organic chemistry we **also** use "strong" and "weak" as relative terms to compare the behavior of organic compounds. Always remember that *context is important* when using adjectives in chemistry!

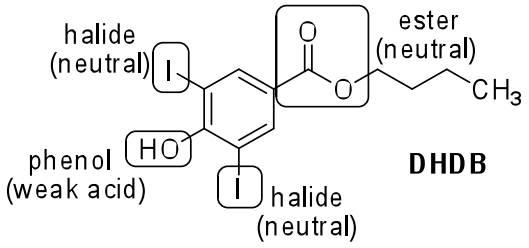
Perhaps the most important application of acid-base chemistry in medicine and biology is in the development of pharmaceutical agents. Potential drugs must be assessed with three questions in mind:

- Where will the drug be best absorbed? (e.g., skin, stomach, intestines)
- Will the drug be able to penetrate the blood-brain barrier?
- How stable is the drug under physiologic conditions?

We shall consider properties relevant to the first question in this lab, the second in lab 2, and the third in later chemistry courses. To this end, one must have a basic working understanding of functional groups and to which of the four categories of acid-base behavior they belong. Refer to the chart below for a partial listing of functional groups and their behavior under physiological conditions.

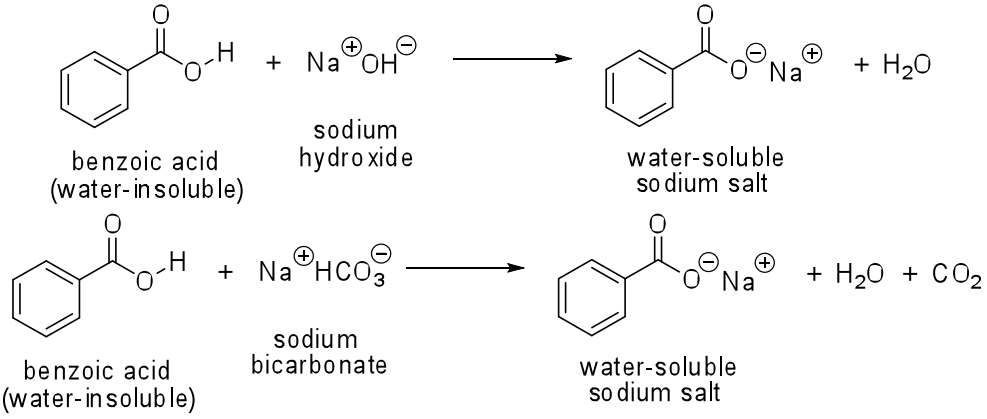
Functional Group	Example Structure	Acid/Base Behavior
Carboxylic Acid		Strong Acid
Phenol		Weak Acid
Amine	$R-NH_2$, $R-\overset{H}{N}-R$, NR_3	Base
Alcohol	$R-OH$	Neutral
Ether	$R-O-R$	Neutral
Ketone		Neutral
Ester		Neutral
Halide	$R-X$ ($X = F, Cl, Br, I$)	Neutral

Using this approach, we can identify the functional groups in the thyroid hormone analog DHDB, and then classify its overall acid/base behavior:

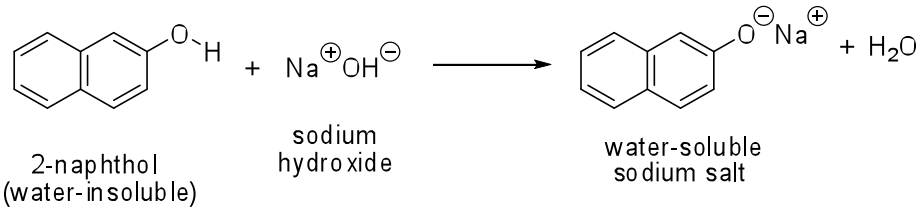


By the functional group assessment given in the chart, we would consider DHDB to be a weak acid overall. As simplistic as it may seem, the ability to assess a molecule's overall acid/base properties will be of inestimable value to you as the course progresses and the molecules we work with become more and more complex! Now let us turn our attention to how we will exploit acid/base chemistry to effect separation of organic molecules.

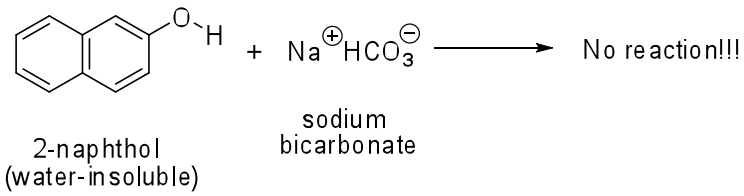
Strong organic acids (typically carboxylic acids) can be converted into water-soluble salts by allowing them to react with aqueous bases like sodium hydroxide (NaOH) and sodium bicarbonate ($NaHCO_3$, also known as baking soda). The salt formation reactions for an example molecule are shown below; note that in the case of reaction with sodium bicarbonate, not only is a water-soluble salt formed, but carbon dioxide gas (CO_2) will be evolved as well. Any basic compounds will remain dissolved and unchanged in the organic solvent layer.



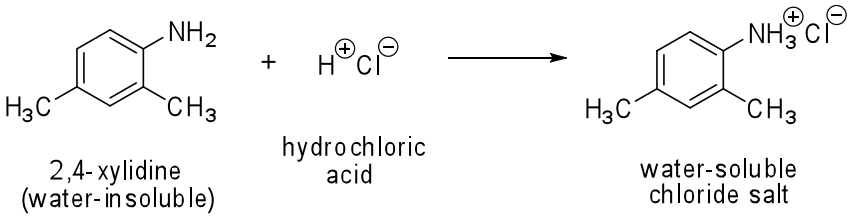
Weak organic acids (typically phenols) can be converted into water-soluble salts by allowing them to react with strong aqueous bases like NaOH. Since they are weaker acids, they will only react with stronger bases. While NaOH is an extremely strong base, $NaHCO_3$ is only a weak base, and weak acids will not form salts with it. This selective reactivity with strong bases over weak ones is often the best way to determine that an organic compound is a weak acid rather than a strong acid. Reactions for an example molecule are shown below. Any basic compounds will remain dissolved and unchanged in the organic solvent layer.



BUT


O
H
+
Na⊕HCO3⊖
No reaction!!!
sodium
bicarbonate
2-naphthol
(water-insoluble)

Organic bases (typically amines) can be converted into water-soluble salts by allowing them to react with aqueous acids like HCl. There is no straightforward way to classify organic bases into subgroups at this level, so reaction with HCl is enough to indicate the presence of such a compound. The reaction for an example molecule is shown below. Any acidic organic compounds will remain dissolved and unchanged in the organic solvent layer.



2,4-xylidine (water-insoluble) | hydrochloric acid | water-soluble chloride salt

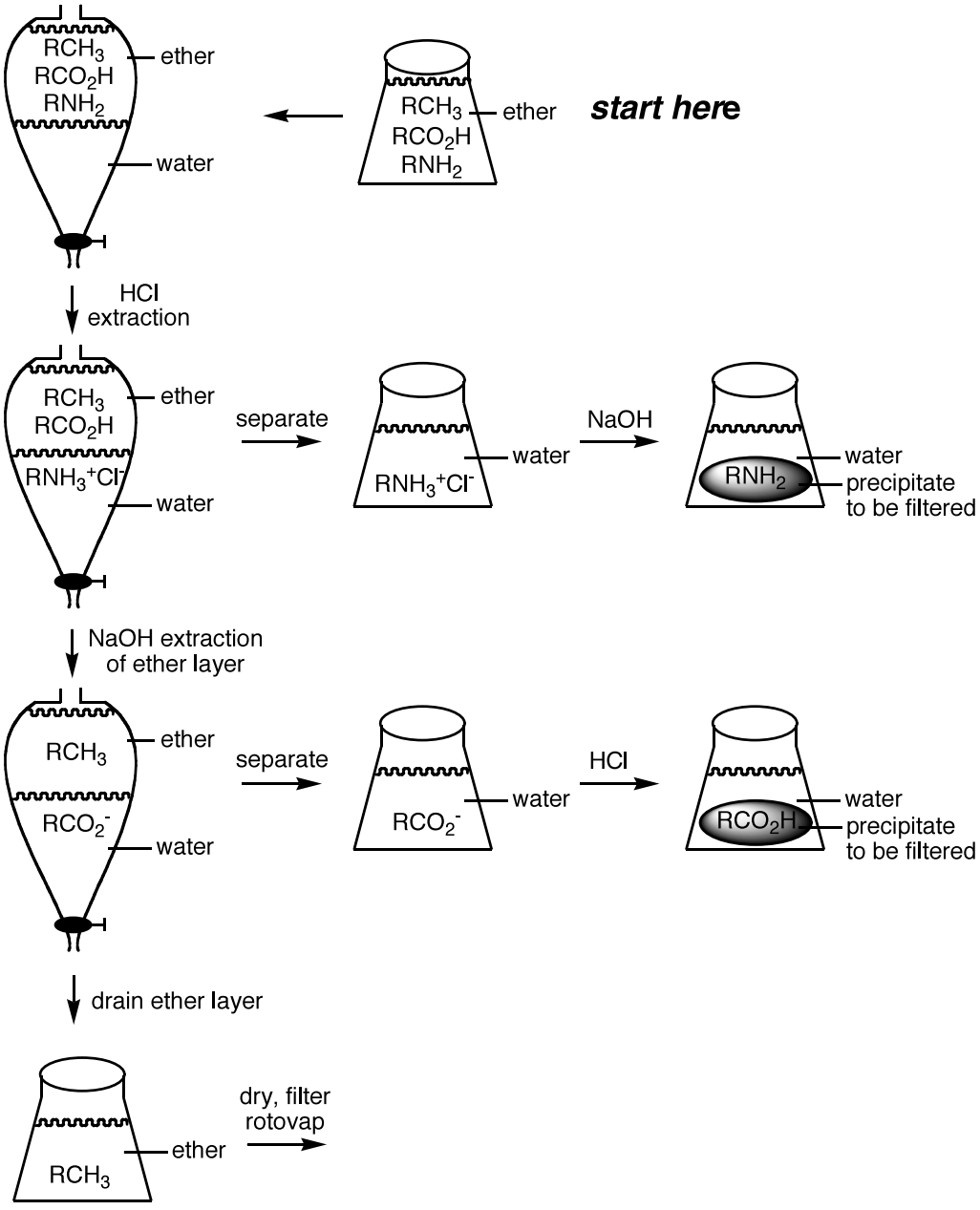
The acid or base added does not need to be an exact molarity as long as it is within a certain range – strong enough to deprotonate or protonate but not so strong that it is likely to decompose the compound. However, it is important to remember that if the base or acid added is too concentrated, there will not be enough water present to fully dissolve the resultant salt!

One caveat remains, however. In the event that a mixture contains both a strong organic acid and a weak organic acid, separation can ***only*** be effected by extraction **FIRST** with sodium bicarbonate, and **THEN** with sodium hydroxide. Extraction with sodium hydroxide first would result only in bringing ***both*** acidic organic compounds into the aqueous layer – and no separation!

In this lab you do not know what components are in your unknown. Therefore you will be conducting *microscale extractions* in order to determine which components you were given, and thus will be able to empirically decide on the best extraction sequence to separate and recover your unknowns. You will learn how to do this in the Procedure section below.

As an example, let's say your microscale extractions tell you that you have a mixture of an organic base, strong acid, and neutral. Shown here is a flow chart of what you would expect from a series of extractions of a ternary mixture. However, you will be receiving a binary mixture with only two organic compounds, and you should think through the various possibilities and develop your own flow chart as part of your pre-lab writeup. Remember that you will get only one precipitate since the organic neutral in your mixture is a liquid. The following notations are used in the flow chart:

RCH_3	Organic Neutral	RNH_2	Organic Base
RCO_2H	Strong Organic Acid	$RNH_3^+Cl^-$	Ammonium Salt
RCO_2^-	Carboxylate Salt		


RCH3
RCO2H
RNH2
ether
water
RCH3
RCO2H
RNH2
ether
start here
HCl
extraction
RCH3
RCO2H
RNH3+Cl-
ether
water
separate
RNH3+Cl-
water
NaOH
RNH2
water
precipitate
to be filtered
NaOH extraction
of ether layer
RCH3
RCO2-
ether
water
separate
RCO2-
water
HCl
RCO2H
water
precipitate
to be filtered
drain ether layer
RCH3
ether
dry, filter
rotovap

Safety Precautions

- All chemical work should be carried out in the hood.
- Wear the required personal protective equipment at all times – appropriate shoes, adequate leg coverage, lab coat, and goggles!
- Do not directly inhale any chemicals.
- Pressure can build up in the separatory funnel when mixing solvents. Be sure to vent frequently.
- This lab involves the use of concentrated solutions of strong acids (hydrochloric acid) and bases (sodium hydroxide). Use extra caution when handling strongly acidic or basic solutions such as hydrochloric acid (HCl) and sodium hydroxide (NaOH).

Procedure

Preparation and practice

Before you use a separatory funnel, make sure it does not leak. Test this by adding a little ether or water to the funnel and shaking upside-down and right-side up, looking for leaks. You may need a new glass stopper or teflon stopcock.

You may or may not have used a glass Pasteur pipet before, and this technique is crucial in organic chemistry lab, so your TF will demonstrate. Notice how the liquid squirts out all over the place if you tilt your hand to the side or if you hold onto the pipet too long (the heat from your hand vaporizes volatile solvents, builds up pressure, and squirts liquid out all over the place). Practice pipetting until you can reliably pipet 1.5 mL of ethanol back and forth between two graduated cylinders without spilling, squirting, or dripping. This may sound dull, but it's actually quite important. When it comes time to pipet something corrosive or the last drop of your final product, you'll know how (and your lab partner will stay safe and dry).

Microscale extractions

Record your unknown letter, and note the appearance and odor of the mixture. Remember that direct inhalation of organic vapors can be harmful, so be sure to *waft* the fragrance (if any) toward your nose. Your TF will demonstrate this if you are unsure. Gather three small (10 x 75) test tubes and clearly label them "HCl", "NaOH", and "$NaHCO_3$". Place these in a test tube rack or 100-mL beaker, and then use a Pasteur pipet to add a small amount (approximately half a mL) of your unknown mixture to each tube. If your mixture is not homogeneous, be sure to mix it up so that you include some of the undissolved solid in each sample. Now add approximately 1 mL of 1.5 M HCl to the tube labeled "HCl", mix well, and note any changes in the appearance of the mixture. If you see nothing, cover the mouth of the tube, and shake well to mix the reagents. You will likely see two layers (or oily bubbles) form since the organic neutral is water-insoluble, but this in itself indicates nothing. A reaction could be indicated by dissolution of solid matter, evolution of heat, or color changes. Ask your TF if you are unsure, but above all *take your time* and observe carefully – sometimes this can be tricky to see. You don't

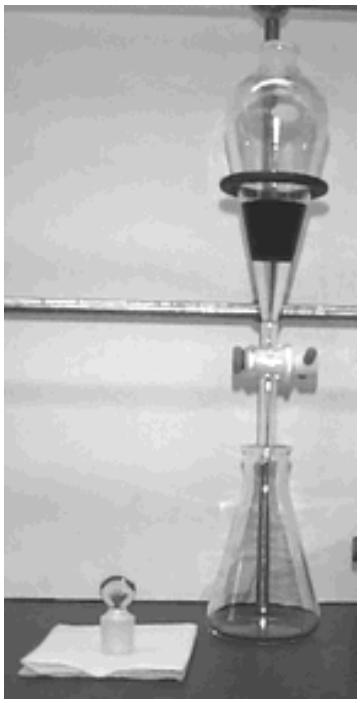
want to rush your observations and end up wasting lab time doing an ineffective extraction! Be sure to note any observations (or lack thereof) in your lab notebook.

Now do the same with the tube labeled "$NaHCO_3$" – add to the tube approximately 1 mL of 5% $NaHCO_3$ solution and observe. Typically the BEST indicator of reaction with sodium bicarbonate is the evolution of carbon dioxide gas – it will bubble and fizz! Don't confuse this bubbling with the oily bubbles that form anyway when you shake the tubes to mix. Try this with acetic acid (ask your TF) if you are unsure what to look for, and remember that reaction with a mixture will be less obvious than a reaction with a pure sample.

Lastly, to the tube labeled "NaOH" add approximately 1 mL of 1 M NaOH solution and observe; shake to mix well, and observe. Again, all observations should be recorded in your lab notebook. At this point you may or may not be confident in the classification of your unknowns, but you are not finished yet.

To the tube labeled "HCl", now neutralize the solution by adding approximately 1 mL of 3 M NaOH. Formation of a precipitate, cloudiness, or turbulence upon addition is evidence that you have an organic base; HCl forced the base into the acidic aqueous layer as a salt, but neutralization of the HCl with NaOH reverts the salt back to an organic base, which will be insoluble in water and precipitate out!

Likewise, to the tube labeled "$NaHCO_3$" now add approximately 1 mL of 6 M HCl (be careful, as bicarbonate solutions can react quite violently with the acid). Formation of a precipitate, cloudiness, or turbulence is evidence of a strong organic acid.



Finally, add approximately 1 mL of 6 M HCl to the tube labeled "NaOH" and observe. Formation of a precipitate, cloudiness, or turbulence is evidence of a weak organic acid. (Note: this step is unnecessary if you received positive results with both HCl *and* bicarbonate, as there are only three components in the mixture, and strong organic acids react with both bicarbonate and NaOH, making this test redundant in that case).

Identify whether your organic compound is a strong organic acid, a weak organic acid, or an organic base. Now, verify your results with your TF, and you are ready to begin your extraction!

<u>Extractions and separation</u>

Dissolve your mixture in approximately 35 mL of diethyl ether in an Erlenmeyer flask. Transfer this ether solution to a 125-mL separatory funnel. Whenever you transfer solutions of important reagents between different containers, you usually rinse the original container with a small amount of the appropriate solvent, and transfer that little bit to the new container. Rinse the Erlenmeyer with a few mL of ether, and pour the rinse into the sep funnel.

Remember that you have both aqueous and organic layers in your sep funnel so there is no need to use anhydrous ether. From here, follow the extraction technique below based on your results from the microscale extraction.

Extraction of the organic base

If your microscale extraction showed evidence of an organic base, you will need to extract the ether layer in the sep funnel twice with 25-mL portions of 1.5 M HCl, adding each extract to the same 125 mL Erlenmeyer flask labeled "HCl extract." Extracting twice means that you add the HCl, shake and vent the sep funnel, drain the HCl layer (which layer is this?), and repeat the process with the second aliquot of HCl. Place the combined aqueous HCl extracts in an ice/water bath (recall that an ice/water bath is a slush made from ice and water).

Extraction of the strong organic acid

If your microscale extraction showed evidence of a strong organic acid, you will need to extract the ether layer twice with 20-mL portions of 5% $NaHCO_3$. Be careful! You must vent the sep funnel regularly to avoid a buildup of pressure from the CO_2 gas being produced inside the funnel. Failure to vent could result in your unknown mixture ending up all over the hood, your lab partner, or your clothes! Collect the combined aqueous $NaHCO_3$ extracts in an Erlenmeyer flask labeled "$NaHCO_3$ extract", and cool it in an ice/water bath.

Extraction of the weak organic acid

If your microscale extraction showed evidence of a weak organic acid, you will need to extract the ether layer in the sep funnel twice with 25-mL portions of 1 M NaOH, adding each extract to the same 125 mL Erlenmeyer flask labeled "NaOH extract." Place the combined aqueous NaOH extracts in an Erlenmeyer flask labeled "NaOH extract," and cool it in an ice/water bath.

Separation of the organic neutral

Pour the remaining ether solution (which should now contain only the neutral component) through the top of the sep funnel into an Erlenmeyer flask labeled "neutral" (don't forget to rinse the sep funnel with a few mL of fresh ether and add this to the flask as well!) and add enough anhydrous magnesium sulfate to cover the bottom of the flask in a thin layer. The drying agent will absorb any unwanted water that might have crept into the solution during the extractions. Swirl the solution over the drying agent and observe. Hydrated $MgSO_4$ is clumpy while dry $MgSO_4$ is a fluffy white powder. Add a little more $MgSO_4$ at a time until fluffy white powder is observed (it will look a bit like a snow globe). Cover the mouth of the flask with aluminum foil (don't use parafilm!) and allow the mixture to sit over the drying agent for 10 minutes, swirling occasionally. Be sure to recap the drying agent as soon as you are done with it.

<u>Isolation of the unknowns</u>

Isolation of the organic base, if present

Make your HCl extract basic by slowly adding 3 M NaOH. Swirl the solution frequently. Remember that the neutralization reaction of acids and bases is exothermic, so the flask should still be in an ice bath. Check the pH with pH paper to make sure it's basic, adding more base if necessary (25 mL of 3 M NaOH should just neutralize 50 mL of 1.5 M HCl). You should see a precipitate when the extract has been made alkaline.

Isolation of the strong organic acid, if present

Make the $NaHCO_3$ extract acidic with 6 M HCl, following the procedure outlined above. You should see a precipitate when the extract has been acidified.

Isolation of the strong organic acid, if present

Make the NaOH extract acidic with 6 M HCl, following the procedure outlined above. You should see a precipitate when the extract has been acidified.

Obtaining the solid unknown

When a precipitate forms in your extract, collect it by vacuum (suction) filtration on a piece of filter paper in a Büchner funnel. The vacuum will pull the neutralized solution through, leaving your solid on the filter paper. Dry the crystals thoroughly and place in a **clearly labeled** scintillation vial. At the end of lab, give these vials to your TF to keep until the next lab session when you will purify them by recrystallization.

Isolation of the organic neutral

You must also isolate your crude organic neutral. Gravity-filter the dry neutral solution. Transfer this solution into an appropriately sized round-bottom flask for rotovapping, and rotovap off the ether. Do not heat the water bath when rotovapping off a low-boiling solvent like ether. It isn't necessary, and you may accidentally rotovap off your product! See Appendix 7 of this manual for instructions; your TF will help you get the hang of the rotovap.

After evaporating off the ether, transfer your compound to a **clearly labeled** scintillation vial, and give it to your TF to keep until the next lab session when you will purify the unknown liquid by distillation.

Cleanup

- The filtrates (liquids left over after filtration) as well as any unused acid or base solutions from the recrystallizations are likely to be quite acidic or basic. Dispose of these filtrates in either the acidic waste bucket or basic waste bucket. Never put any un-neutralized aqueous solutions into the aqueous waste container as this can lead to dangerous eruptions. **DO NOT** dump these solutions down the sink! **DO NOT** mix acids and bases!
- Dispose of test tubes, pipets, and glass vials in the glass waste containers. Remember to clean them first.
- Dispose of leftover solids and filter paper in the solid waste buckets.
- Dispose of paper towels and gloves in the trash.
- All acetone rinses must go into recyclable acetone waste.

- Empty the rotovap collection bulbs into nonhalogenated waste, and clean the rotovap bump traps.
- Aqueous (water) and ethanolic solutions of organic chemicals go in the aqueous waste bucket.
- Clean all glassware you obtained from the Glassware Storage room, and place it in the appropriate dirty glassware bins. A filter flask coated with organic slime is NOT clean enough to go in the cart; it must be rinsed with acetone first.
- Keep aspirator traps (for vacuum filtration) at your bench, SET UP and CLEANED OUT after use. Don't put them in the dirty glassware.
- Wipe off your benchtop with paper towels (water and acetone can be used if needed).
- Always wash your hands well before you leave the lab.

Pre-Lab Write-Up

- Summarize the procedure for doing a wash and an extraction for quick reference.
- Summarize the procedure for using the rotovap for quick reference.
- Draw a flowchart for determining whether a dissolved unknown solid is a strong organic acid, weak organic acid, or an organic base. Note that the flowchart provided in the manual does not account for weak versus strong organic acids!

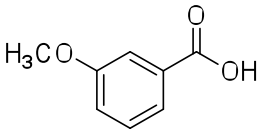
Lab Write-Up

Include the following in your Results and Conclusions Section:

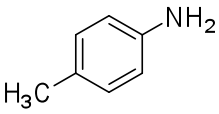
- Record the letter of your unknown sample.
- Clearly indicate all observations that led you to the extraction method you performed.
- For the acidic or basic components of the mixture, draw clear arrow-pushing mechanisms for each of the reactions that (a) extracted them into the aqueous layer and (b) forced them to precipitate out. You may use the example structures shown above (benzoic acid, 2-naphthol, and 2,4-xylidine) since you do not yet know the precise identities of your unknown compounds.

Possible Quiz Questions

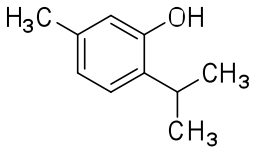
1) You have an ether solution that contains the following four compounds: m-anisic acid, p-toluidine, thymol, and indane (all pictured below). Propose (briefly) how these compounds could be separated using the techniques you will be using in today's lab. It might be easiest to simply list the steps you would take in order from start to finish, or draw a flowchart.



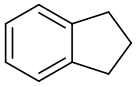
m-anisic acid



p-toluidine



thymol


indane

2) Sally Silly and Bobby Blunder are at it again! This time they were busy chatting about *The Jersey Shore* during the Head TF's safety orientation, and later in lab, Bobby carelessly splashed the ether layer from his extraction all over Sally's glove. Which of the following is the ***most appropriate*** course of action for Sally to take? Sally should:

a) wipe her glove on her jeans and hope for the best.
b) wipe up excess liquid with a paper towel, take off her gloves and wash her hands, and have Bobby notify the TF.
c) scream, panic, and have Bobby call 911 on his cell while the TF evacuates the lab.
d) scream and have Bobby hold her hair back while she uses the eyewash and continues screaming.
e) scream, remove her gloves and clothing, and have Bobby run for a fire extinguisher while she uses the safety shower and continues screaming.

3) Why should you vent the sep funnel when agitating the mixture? Why is it then necessary to remove the stopper when liquid is being drained from a sep funnel?

4) Would you predict water or ether to be the bottom phase in a sep funnel? Which is the bottom phase in a water/methylene chloride (d=1.325) system? Briefly explain your predictions.

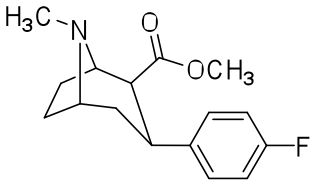
5) How would you dispose of a solution of indane (pictured above) in ethanol? What about indane in acetone? What about indane in ether?

6) What is the difference between washing and extracting?

7) Why do you want to use a drying agent to remove water before evaporating your solvent? (Hint: compare the boiling points of water and diethyl ether…)

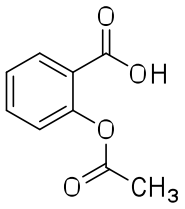
8) What does it mean for an organic compound to be "dry?"

9) Using the chart given in the introduction to this experiment, classify the dopamine transport blocker shown below (enigmatically named WIN35,428) as "strongly acidic", "weakly acidic", "basic", or "neutral" overall. You do not have to explain your choice.

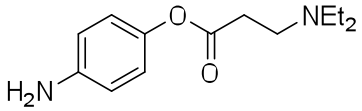


WIN35,428

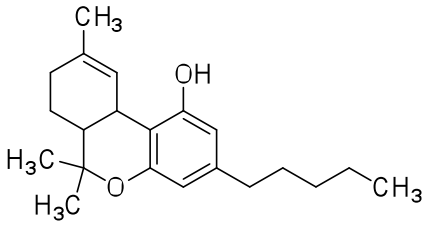
10) Since salts, being charged and polar, cannot be absorbed through highly nonpolar biological membranes, many drugs are selectively absorbed at particular sites in the body depending on whether they are present as salts or neutral organic molecules. Thus the body does its own form of "extraction" for ingested drugs by using the native pH of the stomach (highly acidic) and the intestines (basic to neutral). Where would you expect the following drugs to be uncharged (or of neutral/balanced charge) and thus preferentially absorbed?



aspirin



procaine



Δ^9-tetrahydrocannabinol ("THC")

11) List three possible safety risks specific to this experiment. Briefly describe precautions to address these risks.