Chem 355
Lab Manual

Fall, 2005

Minnesota State University Moorhead
Department of Chemistry

Dr. Craig P. Jasperse
Phone: 477-2230
Email: jasperse@mnstate.edu
Website: www.mnstate.edu/jasperse

Table of Contents

Chem 355 Lab Manual **Fall, 2005**

CHEMISTRY 355 SYLLABUS
FALL 2005

Dr. Craig P. Jasperse
Office: 003 Science Lab (basement)
Telephone: 477-2230
e-mail: jasperse@mnstate.edu web: http://www.mnstate.edu/jasperse/

Office Hours: Mon 3-5
Tues-Wed-Fri 10-12
Fri 3-5

Required Text and Materials:
1. Safety Goggles

Date	Experiment
Aug 23-25	Checkin, Melting Points
Aug 30-Sept1	Molecular Structure
Sept 6-8	Solubility Tests; Crystallization of Phtalic Acid
Sept 13-15	Recrystallization of Acetanilide Using Mixed Solvent; Recrystallization of an Unknown
Sept 20-22	Simple and Fractional Distillation of an Ethanol-Water Mixture; Distillation of an Unknown Mixture
Sept 27-29	Liquid/liquid Extraction; Extraction of Acids and Bases
Oct 4-6	Nuclear Magnetic Resonance Spectroscopy; Determination of an Unknown by ^{13}C NMR
Oct 11-13	Nucleophilic Substitution of Alkyl Halides; Mechanistic Arrow Pushing
Oct 18-20	^{1}H NMR Determination of an Unknown; Infrared Spectroscopy
Oct 25-27	Thin-Layer Chromatography; Column Chromatography
Nov 1-3	Cholesterol Extraction from Gall Stones
Nov 8-10	Grignard Synthesis of Triphenylmethanol
Nov 15-17	Grignard Synthesis Continued
Nov 29-Dec 1	Sodium Borohydride Reduction of 2-Methylcyclohexanone. Use of 1H NMR to measure product ratios. Cleanup, Checkout

Grading Policy:

1) **Attendance**: Laboratory attendance is mandatory. In the event of an absence, you will receive zero points for that experiment, barring special grace. Doing an experiment during a different lab period will usually be OK if you make arrangements. NOTE: It will be routine that some data analysis will be required outside of the scheduled laboratory time. Often samples will need to dry for a while and be analyzed later.

2) **Individual Lab Scores**: Most experiment will require completion of a lab report and answers to some questions. Some of the grade will be based on quality of results, for example successful identification of an unknown, or high yield, or high product purity. Unless notified otherwise lab reports should be completed by the following lab period. For lab reports in which you are required to answer some questions, these will count into the lab report scores.

3) **Instructor's evaluation of your laboratory performance**. Laboratory preparation, performance, and understanding is not always easily quantified by lab reports alone, especially since you may sometimes work as partners. Part of the final grade will be influenced by the instructor's qualitative evaluation of your laboratory performance and understanding.

4) **Failure to Return Key** You will not receive a grade until you have turned in your lab key to the MSUM key office after the last lab. You will also be fined $25, and will be unable to register for subsequent courses?

Summary:

Individual Lab Score	14 x 10	140 points
Instructor's Evaluation of Your Techniques		40 points

Tentatively letter grades will be assigned as follows:

A	(≥91%)
B	(≥82%)
C	(≥73%)
D	(≥64%)

The instructor reserves the right to lower the requirement for a letter grade, but will not raise them.

Notes:

1. It is obvious that missing one lab and losing the associated 10 points is costly. The importance of attendance cannot be overemphasized.
2. **It is absolutely necessary that students have completed and passed the MSUM safety course/tests**. If you did not pass the MSU safety course/tests, it is absolutely vital that you take action to rectify the situation. A student who has not previously passed and who does not pass it **BY NOVEMBER 16** will not be allowed to continue in the organic laboratory.
3. Safety goggles must be worn at all times in the organic laboratory. A student who is caught without goggles on will be dismissed from lab and will receive a zero for that experiment. In other words, getting caught without safety goggles on is equivalent to dropping a letter grade! Don't let it happen to you!
4. Proper safety measures and disposal of chemical wastes will be important. If I catch you violating disposal policies, I reserve the right to dismiss you from laboratory immediately.
5. Clean up your hood before you leave! A messy hood and you are subject to losing points for the week.
6. The organic laboratory is **NOT APPROPRIATE IF YOU ARE PREGNANT**. The effect of chemicals on unborn children is not always fully documented, so being in the organic lab while you are pregnant represents an unknown risk. If pregnant, withdraw from Chem 355.
7. The doors will be locked all the time. You will always need your key-card (your MSUM ID card) in order to get in. The card will work **M-F, 7:30-5:30, in rooms 305, 307, and 316**. (These are the main lab, the NMR room, and the IR room). Students who were registered as of Monday, 8/15 should have functional cards. If yours isn't, see Jasperse and key office.
8. Your drawer key will need to be picked up at the key office (Owens 209). It should be ready within 48 hours after you check in.

Melting Range

Background Information The melting range of a pure solid organic is the temperature range at which the solid is in equilibrium with its liquid. As heat is added to a solid, the solid eventually changes to a liquid. This occurs as molecules acquire enough energy to overcome the intermolecular forces previously binding them together in an orderly crystalline lattice. Melting does not occur instantaneously, because molecules must absorb the energy and then physically break the binding forces. Typically the outside of a crystal will melt faster than the inside, because it takes time for heat to penetrate. (Imagine an ice cube melting from the outside in, and not doing so instantly…)

The melting range of a compound is one of the characteristic properties of a pure solid. The melting range is defined as the span of temperature from the point at which the crystals first begin to liquefy to the point at which the entire sample is liquid. Most pure organics melt over a narrow temperature range of 1-2ºC, if heated slowly enough. Impure samples will normally have melting ranges that are both larger (>1ºC) and begin lower.

Taking the melting range of a sample is useful for two reasons:

1. Identification of an unknown sample (compare it's observed melting range with that of known compounds)
2. Assessment of sample purity for a known substance. By comparing observed range for an actual sample to the known range for a pure sample, you can tell whether your actual sample is pure or contaminated (the range is depressed and broadened)

The presence of impurity has two effects on a substance's melting range:

1. Melting range depression (lower end of the range drops)
2. Melting range broadening (the range simply increases. Often the low end drops a lot, the high end less so or sometimes not much at all.) A melting range of 5º or more indicates that a compound is impure.

Why? The reason for this depression/broadening is that contaminants disrupt the consistency and organization of the crystal lattice at the molecular level. Contaminants don't "fit" correctly into what would be the normal pure lattice. **How does this manifest itself**?

1. The disruption weakens the lattice, so that the lattice can be broken down more easily; the weakened structure melts more easily at reduced temperature (depression).
2. Disruption of the lattice makes it non-uniform. At the molecular level, the molecules closest to the impurities melt fastest. Further away from the impurities, the crystal lattice is relatively undisturbed and therefore melts at or nearer the normal temperature.

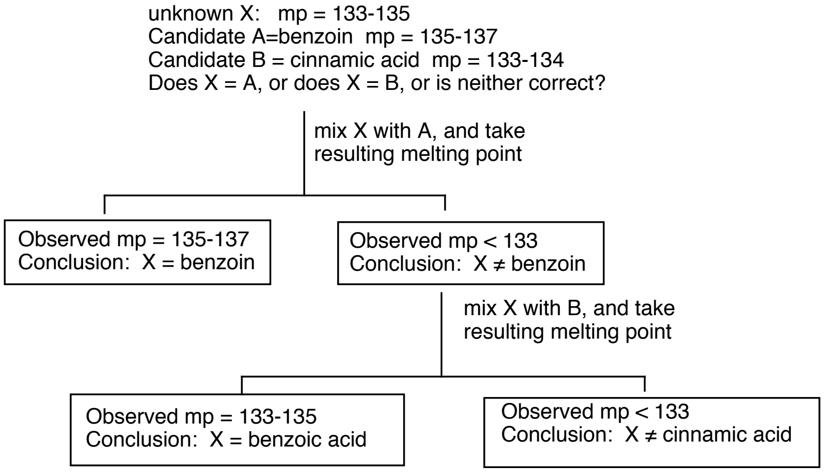
Miscellaneous notes on melting range depression/broadening:

1. Only "soluble" impurities, which are incorporated into the crystal structure at the molecular level, cause depression and broadening. An insoluble piece of metal or wood ionic salt crystal has negligible effect, because only a few organic molecules will be in contact and will be affected.
2. At the chemical level, it is impossible to "raise" the melting point of an already pure substance. It's melting point can be depressed by contamination, but not raised. Practical: If the melting point for an unknown sample is observed to be in between that of two candidates whose pure mp's are known, the unknown can't actually be equal to the lower-melting candidate. (Short of the rapid-heating effect, see later.) Most likely the unknown sample is an impure version of the higher melting candidate. For example: suppose an unknown sample X melts at 148-152º, and is thought to be either candidate A (known range is 141-142º) or B (known range is 161-162º). Sample X cannot be candidate A, but it can be an impure and thus depressed version of candidate B.

3. Often contaminated solids are purified by <u>recrystallization</u>. If the resulting melting range is unchanged, the original sample probably was pure to begin with. But if the resulting melting point gets higher, the original sample was obviously impure.
4. When crystals are isolated by filtration from a <u>solvent</u>, it is important to allow complete <u>drying/evaporation</u> of the solvent in order to get a good melting range. Residual solvent functions as a contaminant and will depress/broaden the melting range for a crystal.
5. When two chemicals are <u>mixed</u>, the resulting melting point is <u>**not**</u> the average of the two mp's. It is always depressed from the melting point of the major component in the mixture. This is true even if the impurity is higher melting (when pure) than the major component. For example, if a chemical that normally melts at 130° is contaminated by a small amount of material that when pure melts at 200°, the resulting mixture will not melt between 130° and 200°. Rather, the melting point of the major component will be depressed, and the observed melting range will begin lower than 130°.
6. Even when two chemicals with exactly the same melting point when pure are mixed, the resulting melting point is depressed.

<u>Mixed Melting Points</u>

That mixtures have depressed melting points, even when both components have comparable melting points when each is pure, provides a useful laboratory technique. Consider the following situation and flow chart. If an unknown candidate X melts at a temperature close to that of two potential candidates A and B, you can identify it by taking X+A mixed melting point, and X+B mixed melting point. If X is equal to either candidate, one of these mixed melting points will <u>not be depressed</u>. If the mixture with X+A is not depressed, X = A. if the mixture with X+B is not depressed, X = B. If both mixtures are depressed, then X ≠ A or B.



The Rate of Heating, and Some Practical Tips

It takes time for a crystal to absorb heat and to melt, from the outside in. Just as when you place an ice-cube into a liquid that is >0°, it doesn't melt instantly. To get maximal accuracy in taking a melting range, heating should proceed at only 1°/minute! This is the standard heating rate when publishing melting ranges in scientific journals. This is also inconveniently slow, especially if you don't know where your sample is likely to melt (as when examining an unknown).

- Q: What happens if I heat too fast? A: Your melting range will be too broad, but this time on the high end! If a sample should melt at 130-131°, but you are heating fast, it will still probable begin to melt at about 130°, but the full sample won't have time to absorb heat and finish melting by 131°. Instead, the heating device may have warmed well above 131° before the interior liquefies, so the observed range may appear to be 130-136°. Both the magnitude of the range and the high end of the range may be misleading.
- For doing routine samples, it is appropriate to be warming at 5 degrees per minute around the temperature at which melting occurs. This broadens the range somewhat, but not badly. And it keeps the melting point experiment from taking forever.
- Practical tip 1: If the approximate temperature at which your sample should melt is known, the sample can be quickly heated to within 10-15° of its melting point. Then the heating rate can be slowed to 2-4° per minute until the sample melts. For example, if you know your material should melt around 180°, but you are just trying to check the purity, you can heat rapidly until you are up to 165° or so, and only when you are getting close turn the heating rate down.
- Practical tip 2: If you have no clue where your sample will melt, it's common to heat rapidly to get a ballpark estimate of where melting will occur. 60°? 140°? 240°? If it turns out to be 240° and you heated only cautiously from the beginning, it will take a looooong time to get to the measurement. By heating rapidly, you can get an "orientation melting point" quickly, and then repeat with more care for a more precise reading. Often you don't even need to prepare a fresh sample, because after cooling the melted sample often recrystallizes.
- Practical tip 3: Heat transfer problems are minimized if the sample is ground finely. If the particles are too coarse, they do not pack as well, causing air pockets that slow heat transfer. Because the thermometer keeps heating while the sample is melting rather slowly, the high end of your range will be inflated.
- Practical tip 4: Loading too much sample makes it harder for the interior to get heated and melted. Because the thermometer keeps heating while the sample is melting rather slowly, the high end of your range will be inflated.

"Sagging"

Sometimes slight changes, such as shrinking and sagging, occur in the crystalline structure of the sample before melting occurs. The temperature at the bottom end of the melting range corresponds to the first appearance of **liquid** within the sample mixture; if the crystals are changing their appearance, but you don't yet see any actual liquid, you should not record this as the lower end of the melting range yet.

The Experiment: (Work alone or with One Partner)

Overview, if working with a partner: You will run three samples.

1. One will be either pure urea (mp = 132-133) or pure cinnamic acid (mp = 133-134). Whichever you run should be the opposite of what your partner runs. Share your observed results with your partner
2. The second will be mixture of the two, either 4:1 cinnamic acid:urea or 1:4 cinnamic acid:urea. Whichever mixture you run should be the opposite of the mixture that your partner runs. Share your observed results with your partner.
3. The third will be an unknown. (You and partner must run different unknowns. You do not need to share this result with your partner.)

If working alone: You will run five samples.

4. Run both pure urea (mp = 132-133) and pure cinnamic acid (mp = 133-134).
5. Run both the 4:1 cinnamic acid:urea and the 1:4 cinnamic acid:urea mixtures.
6. Run one unknown. (You and partner must run different unknowns.)

Goals:

- Learn how to run a melting point device and measure melting range
- By comparing results for the two mixtures, see how not all mixtures depress/broaden to the same extent.
- Identify your unknown from the list shown below.

Unknown Candidates

Acetanilide	112-115
Benzoic Acid	120-123
Cinnamic acid	133-134
Salicylic acid	158-160
Sulfanilamide	165-166
Succinic acid	184-185

Lab Report Requirements

No introduction or procedure write-up is required.

Fill in the data section on the report hand in, and answer the questions.

Melting Point Lab Report. Chem 355

Name:

Partner's Name (if you shared data with a partner:

Experimental Data	melting range
• My Known: (U or C or both)	
• My mixture: (4:1 C:U or 4:1 U:C or both)	
• Partner's mixture (4:1 C:U or 4:1 U:C)	
• My Unknown: (A, B, C, or D…)	
• Which compound is your unknown? (from the list on page 4)	

- Any doubts, discussion, or logic on your identification of unknown. (Not necessary, but if you have a tricky one or one that for whatever reason you get wrong, if your discussion shows some reasonable analysis or logic, it may help you get partial credit! ☺)

Discussion questions:

1. Compare the ranges observed with the two mixtures.
   a. Did they depress and broaden about the same, or different?

   b. What does that say about the degree of depression and broadening that occurs when mixtures are used? Do all impurities depress to the same degree, or by some predictable formula? Or do you think it's more of a case-by-case deal?

2. Strictly speaking, why is it incorrect to speak of a melting "point"?

3. How will your melting range be perturbed if you haven't completely dried your sample? (For example, after you've filtered crystals away from a solvent, and/or have washed the crystals with solvent…)

4. What's the advantage of a finely powdered sample over a coarser sample? How will your melting range be perturbed with coarse sample?

5. What's the advantage of putting in a relatively small amount of sample as opposed to putting in lots and lots of sample? How will your melting range be perturbed with huge sample?

6. Why is it desirable to heat the sample relatively slowly? How will your melting range be perturbed by heating too fast?

7. You have a sample that you are sure is Jaspersium, which has a list melting range of 145-146.
    - Suppose you run your sample and observe a melting range of 145-151. Is your sample impure, or did you heat too fast?
    - Suppose you run your sample and observe a melting range of 139-145. Is your sample impure, or did you heat too fast?

8. You have isolated an unknown compound that shows an observed melting range of 90-94. Which is it more likely to be, candidate X (list mp 97-98) or candidate Y (list mp 86-87). Why might your sample not have the same melting range as either of the known compounds, given that it must be one of them?

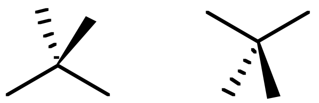
9. Three test tubes labeled A, B, and C contain substances with approximately the same melting points. How could you prove the test tubes contained three different chemical compounds?

MOLECULAR STRUCTURE

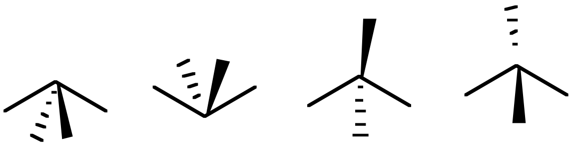
For each of the following molecules, make the models and then draw the models. Until the last page, use tetravalent atoms. Make double bonds by using two of the soft, flexible white bonds to make “banana double bonds”.
- For molecules involving lone-pairs, draw them with the lone pairs shown.

Guidelines for Drawing Models:
A. 3-D Perspective
1. Keep as many atoms as possible in a single plane (plane of the paper) by zig-zagging. Connections within the paper are drawn with straight lines.
2. Use wedges to indicate atoms that are in front of the plane.
3. Use hashes to indicate atoms behind the plane.

B. For any tetrahedral atom, only 2 attachments can be in the plane, 1 must be in front, and 1 behind.
-if the two in the plane are “down”, the hash/wedge should be up
-if the two in plane are “up”, the hash/wedge should be down.
-the hash/wedge should never point in same direction as the in-plane lines, or else the atom doesn’t looks tetrahedral
-for polyatomic molecules, it is strongly preferable to NOT have either of the in-plane atoms pointing straight up. Straight-up in-plane atoms do not lend themselves to extended 3-D structures.



Good! Look tetrahedral



Bad! These don' t look tetrahedral!

1. ALKANE. butane, $CH_3CH_2CH_2CH_3$
-take the chain and wiggle around all the single bonds.
-The most stable actual shape is the one with the carbons zig-zagged and co-planar.
-Notice the symmetry possible.

2. ALKANE. Pentane, $CH_3CH_2CH_2CH_2CH_3$

3. HALOALKANE. 2-bromobutane, $CH_3CHBrCH_2CH_3$
-notice that if the 4 carbons are co-planar zig-zagged,
the attached Br can't be in the same plane.
-try to compare with a partner 2 cases in which
Br is in front versus behind. Are they the same
molecule, or isomers?

4. ALKENE. Draw both: a) trans-2-butene, $CH_3CH=CHCH_3$
and b) cis-2-butene
(trans means the two CH_3 groups are on the opposite sides
of the double bond; cis means they are on same side)
-notice that not only the 2 double-bonded
C's but also the four atoms directly
attached are all co-planar.

5. ALKYNE. 2-butyne, CH_3CCCH_3
-draw Lewis structure first

6. WATER. H_2O
-DRAW at least 4 different orientations,
and specify the lone-pairs.
-try to have at least one picture in which
all of the atoms are in the plane of the paper.
-For building the model, visualize a
lone-pair by using a stick
without an atom at the end.
-draw in the lone pairs for this and all
following pictures. (For this assignment;
not normally required for class!)

7. ALCOHOL. Ethanol, CH_3CH_2OH

8. ETHER. Diethyl ether, $CH_3CH_2OCH_2CH_3$

9. FORMALDEHYDE. CH_2O.
-for 9-16, make sure you draw the Lewis
structure before you build models and draw
the 3-D picture. If you don't know the
connectivity, you have no chance!

10. ALDEHYDE. CH_3CH_2CHO.

11. KETONE. $CH_3CH_2C(O)CH_2CH_3$.

12. ACID. $CH_3CH_2CO_2H$.

13. ESTER. $CH_3CH_2CO_2CH_3$.

14. AMMONIA. NH_3

15. AMINE. $(CH_3CH_2)_2NH$

16. AMIDE CH_3CONH_2.
-FACT: contrary to what you would guess, the nitrogen is trigonal planar rather than tetrahedral. Draw accordingly!
-by remaining planar, the nitrogen does not hybridize all of its p-orbitals. One p orbital is left for orbital overlap/conjugation/resonance with the pi bond.
-because lone-pair is not hybridized, don't 3-D illustrate it
-the fact that amides are planar is crucial to biochemistry. The organization of proteins/peptides/enzymes is completely dependent on the planarity of amide nitrogens. Without it we would all be dead!

17. CYCLIC COMPOUNDS
A. Cyclopropane $(CH_2)_3$
-notice how hard this is, how the bonds "bend", etc.. Real cyclopropane experiences real "ring strain" based on the impossibility of achieving 109° bond angles.

B. Cyclohexane $(CH_2)_6$
-Don't bother to draw! Too tough! But do build the model.
1) notice that the 6 carbons do <u>not</u> easily remain coplanar. By puckering, ideal 109° bond angles can be achieved.
2) In the best model, 3 H's point straight down, 3 H's point straight up, and 6 H's essentially extend almost horizontally. The "horizontal" H's are called "equatorial" and the "vertical" H's are called "axial".
3) Try to put colored balls into the "axial" positions. Then try to manipulate the model so that the "axial" atoms become "equatorial", and the "equatorial" atoms become "axial".
-ask instructor to come over and give you cyclohexane spiel

18. Things that can't be completely drawn "3-D". 2-methylbutane, $CH_3CH_2CH(CH_3)_2$
-notice that not all 5 of the carbons can be coplanar. Structures like this can't be illustrated completely or easily. What you should do is simply draw "CH_3" as being out-of-plane, but don't try to illustrate the "3-D-ness" of that carbon. Ask instructor for confirmation.

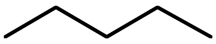
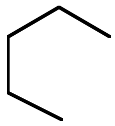
SAME OR DIFFERENT?

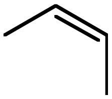
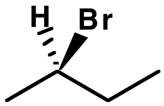
Rules:

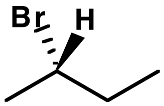
1. Structures which can be interchanged or made equivalent by rotations around single bonds are considered to be the same.
2. "Isomers" are things with the same formula that can't be made superimposable by simple rotations around single bonds.

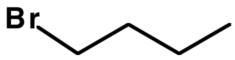
(not required, but for class will eventually need to be able to distinguish "structural isomers" from "stereoisomers")

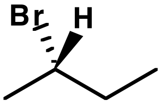
Classify the following pairs as "same" or "isomers"

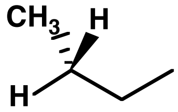



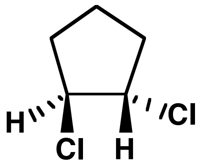



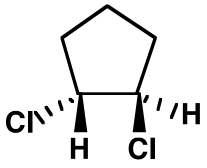


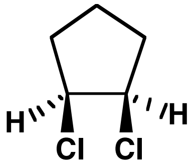


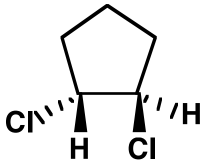


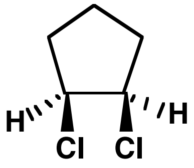



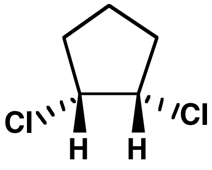






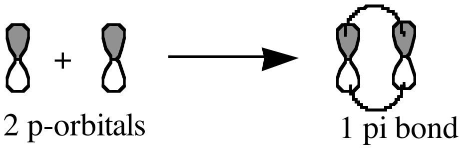






Orbitals and Pi-Bonds

The models you have built so far have used tetrahedral atoms and two flexible “banana bonds” for making double bonds. From a molecular orbital perspective, it is often more useful to consider a double bond as consisting of a straight sigma bond and a pi-bond made from the overlap of 2 parallel, non-hybridized p-orbitals.



Make a model of ethene, $CH_2=CH_2$, using trigonal atoms rather than tetrahedral atoms. The trigonal atoms all have holes in the middle. Poke sticks through the middle to represent p-orbitals.

1. Draw the molecule with the atoms in the plane of the paper (ignore the p-orbitals at first). Then draw in the p-orbitals as well.
-Notice: Are the p-orbitals in the plane of the paper? Do you like this picture? (It is expected to look yucky so that you appreciate why you normally draw double bonds as in pictures 2 or 3!)

2. Draw the molecule with the carbons and the p-orbitals in the plane of the paper. Then add the four hydrogens.
-notice: are the atoms in the plane of the paper?

3. Redraw the molecule with the atoms in the plane of the paper, but don’t draw the p-orbitals.

Comment: Ordinarily, picture 3 is considered the best when you aren’t emphasizing p-orbitals or the pi-bond.

However, if you are trying to illustrate p-orbitals and pi-bonds, picture 2 is considered easier than picture 1.

Chem 355 Jasperse RECRYSTALLIZATION

Background:

Impurities often contaminate organic compounds, whether they have been synthesized in the laboratory or isolated from natural sources. Recrystallization is the most important method for removing impurities from solid organic compounds. It is suitable for both small scale (<0.5 g) and large scale (>100g) work. **The basic recrystallization plan is to dissolve an impure solid in a hot solvent, then cool the solution so that the desired molecules recrystallize while the impurities remain in solution**. Subsequent filtration separates the solid crystal from the liquid solvent.

The dependence of solubility on temperature is key. Solubility of sample in solvent will always be higher at high temperature, but will decrease at low temperature. A solvent that can fully dissolve a solid while hot may thus become saturated as the temperature is reduced, resulting in crystal formation. Soluble impurities stay in solution because they are not concentrated enough to saturate the solution. However, even when the solvent is cold, at least some (if not all) of the desired compound will remain dissolved and will be lost during filtration.

The choice of solvent for a recrystallization is crucial. For a successful recrystallization **the dissolving power of the solvent must be "mediocre", neither too good nor too bad**. Why?

- If the solvent is too good, then even when the solvent is cold the sample will remain dissolved and you won't be able to harvest any crystals.
- If the solvent is too bad, then even when the solvent is hot it still won't be able to dissolve the sample, and the impurities won't be freed from the original sample.
- An effective solvent must be mediocre, good enough to dissolve the sample at high temperature (so that the impurities are freed), but weak enough so that at least some of your sample crystallizes out after cooling (so that you get at least some yield harvested.)

Necessary sources of mass loss: The yield for a recrystallization can never be 100%. Why not? Because while the chilled solvent is saturated and should release some crystals, at least some of your desired material will remain dissolved in the cold solvent and will be lost when the crystals and solvent are separated. **The primary necessary source of mass loss is to the solvent.** Obviously additional mass will be lost to physical handling, and some of the lost mass is simply the impurities that you wanted to lose. (But normally the mass of impurities is only a few percent or less.)

Unnecessary sources of mass loss: While losing mass to the solvent is inevitable, unnecessary losses to solvent are common and should be avoided. Some unnecessary losses to solvent result from the following:

- Using too much solvent. The more solvent that you use, the more sample will remain in the solvent even after cooling.
- Inadequate warming. If you don't warm your solvent to boiling temperature, you will not benefit from it's maximum dissolving ability, and will need to use extra solvent to make up for it. The unnecessary extra solvent will retain additional sample even after cooling.
- Inadequate cooling. If you don't cool below room temperature, for example, more sample will remain dissolved than if you cool to 0ºC.
- Excessive washing by solvent. Not only surface impurities but also some crystal will be lost when you wash your crystals. (There is usually some sort of happy medium required, because some rinsing is usually required).
- Choosing a poor solvent in the first place. If your solvent is too good, even after cooling it will still retain much or all of the sample.

Ideal: Use a Minimum of Hot Solvent so that you are at the Saturated/Dissolved Borderline at the Boiling Point. In order to maximize your purity, you'd like to use enough solvent to dissolve the crystals and keep the impurities in solution even after cooling. In order to maximize your yield, you'd like to minimize the amount of solvent used so that as little as possible sample remains in solution after cooling. The best way to accomplish both goals is to use enough solvent so that it can dissolve everything while hot, but to use no more than the minimum required so as to maximize yield after cooling. The minimum required is when your solution is just barely at the saturation point while boiling hot. Use just enough solvent so that the material is just barely soluble, or is just a little cloudy to show that it's just barely saturated.

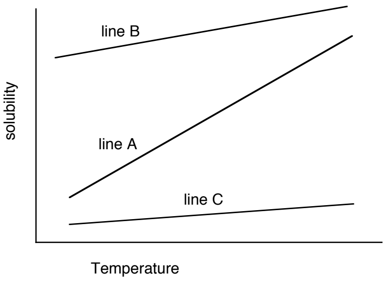
Three Ways To Achieve Just-Barely-Saturated/Just-Barely-Dissolved Borderline. In all cases, heat to boiling.

- Add more hot solvent (if solubility is too low)
- Boil solvent away (if solubility is too high)
- Add 'bad solvent' (if solubility is too high) that will reduce the solubility

Mixed Solvents Often it's difficult to find a single solvent that is appropriately "mediocre". Frequently it's easier to work with a solvent mixture, in which you use a good solvent and a bad solvent. In this process, the good solvent is able to dissolve the sample. As a solvent with poor dissolving properties is added, the overall dissolving power of the solvent gets worse and worse. Eventually, the saturation point should be reached. This is often the most convenient way to do recrystallizations. Use hot good solvent to get things dissolved, then add hot bad solvent until saturation is reached, then cool and filter.

- The most common "mixed solvent" combination involves an alcohol as the "good solvent" and water as the "bad solvent". This is effective because many organics are hydrophobic. By adding water, you can rapidly reduce their solubility.
- When mixed solvents are used, it is essential that both are cosoluble with each other. Trying to add water as the "bad solvent" to a hydrocarbon solvent like hexane or toluene fails, because the water simply forms a separate layer and doesn't actually do anything.

Choosing a Solvent As discussed previously, you don't want too good a solvent (line B) or too bad a solvent (line C). And you'd like a solvent with as sharp as possible a dependence of solubility on temperature (line A), so that it's pretty soluble hot but not very soluble cold. Trial and error experimentation is often required for finding a suitable solvent. Like-dissolves-like considerations can sometimes provide helpful guidance as to which solvents might be too good, or which might be hopelessly bad. For somewhat polar molecules containing oxygen or nitrogen atoms, ethanol/water mixed solvents are frequently a fine choice. The boiling point of the solvent is also worth considering. A relatively high boiling point is good because it allows a large temperature differential between boiling hot solvent and the chilled solvent. Diethyl ether, for example, boils at only 35ºC, so the solubility of a sample isn't likely to drop as much upon cooling from 35ºC to 0ºC as if water is used, where the hot/cold difference could be 100ºC. On the other hand, you don't want to use a solvent whose boiling point is so high that the sample will simply melt when the solvent is heated to boiling. It's also easier to dry the crystals if the solvent isn't too high boiling, so that it evaporates easily and won't depress the melting range.



Summary of the four criteria for selecting a recrystallizing solvent:

1. compound being purified must be insoluble in the solvent at room temperature
2. compound must be soluble in the boiling solvent
3. solvent's boiling point must be lower than the compound's melting point
4. an abundant quantity of crystals must be recoverable from the cool solvent

Crystallization Summary

1. **Choosing the Solvent.** "Like dissolves like." Some common solvents are water, methanol, ethanol, ligroin, and toluene. When you use a solvent pair, dissolve the solute in the better solvent and add the poorer solvent to the hot solution until saturation occurs. Some common solvent pairs are ethanol-water, diethyl ether-ligroin, toluene-ligroin, and t-butyl methyl ether-hexane.
2. **Dissolving the solute.** To the crushed or ground solute in an Erlenmeyer flask, add solvent, add a boiling stick, and heat the mixture to boiling. Add more solvent as necessary to obtain a hot, saturated solution. (Do not use a beaker, because the large mouth allows solvent evaporation to be too fast and uncontrolled.)
3. **Filtering suspended solids (if necessary).** If it is necessary to remove suspended solids, dilute the hot solution slightly to prevent crystallization from occurring during filtration. Filter the hot solution. Add solvent if crystallization begins in the funnel. Concentrate the filtrate to obtain a saturated solution.
4. **Crystallizing the solute.** Let the hot saturated solution cool spontaneously to room temperature. Do not disturb the solution. Slow cooling gives the best crystals. Cooling while clamped in the air, or while standing on a watch glass that is resting on your round-bottomed flask holder is a good way to do it. Put a watch glass or inverted beaker over the top of your flask so that solvent doesn't evaporate away while still hot. Then cool it in ice. If crystallization does not occur, scratch the insides of the container, add seed crystals, or for many solvents add ice chip(s).
5. **Collecting and washing the crystals.** Collect the crystals using a Hirsch funnel (<0.2 grams) or a Buchner funnel (>0.2 g), a filter flask, and aspirator suction. Place a filter paper on the surface, wet the filter paper with solvent, and apply suction to make sure the paper seals. Break the vacuum, add crystals and liquid, and apply vacuum. After solvent disappears, break vacuum, add cold wash solvent, apply vacuum, and repeat until crystals are clean and filtrate comes through clear. The wash solvent is normally either a small amount of an ice-cold portion of the main recrystallization solvent, or else a somewhat "worse" solvent (although it shouldn't normally be a totally "bad" solvent). For example, if 80% ethanol/water is used for a recrystallization, it would be common to wash with 60% ethanol/water to avoid dissolving much crystal.
6. **Drying the product**. Aspirate the sample for as long as is convenient. Press the product on the filter to remove solvent. Then remove it from the filter, squeeze it between sheets of filter paper to remove more solvent, and spread it on a watch glass to dry
7. **Analyzing the product.** Take a melting point of the final product. But since incomplete drying will contaminate the crystal and depress the melting point, it is normally best to wait for 15 hours or more before doing so.

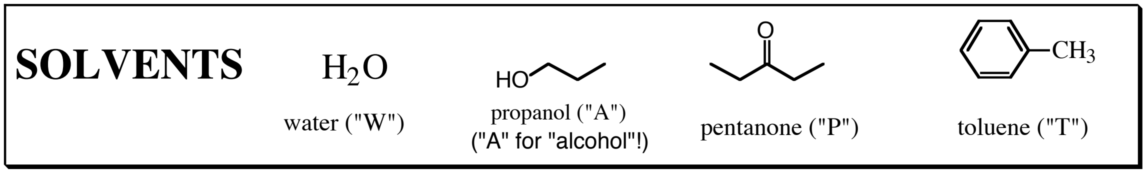
PART 1: Macroscale Recrystallization of Phthalic Acid from Water

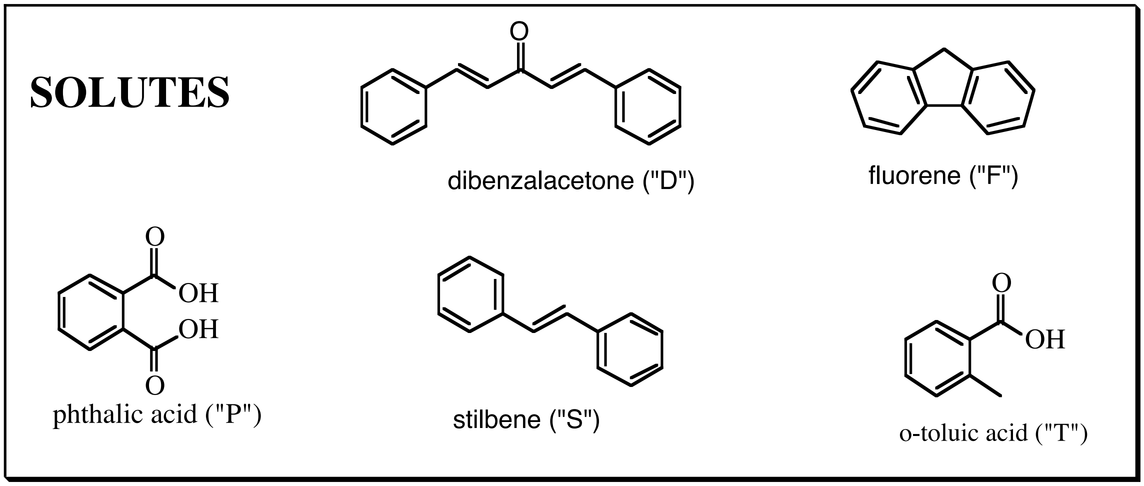
Weight out about 1 g of pthalic acid. (Record exact mass). Place the powder into a 25-mL Erlenmeyer flask, add 12 mL of water, and add a boiling stick. (The stick facilitates even boiling and prevents “bumping” explosions). Heat on a hot plate until the water begins to boil gently. (Avoid a hard boil. If much of your solvent boils away, the sample will either not dissolve in the first place or will not be able to remain dissolved). Once the solution has reached the boiling point and the sample has dissolved, remove it from the hot plate and move it onto a watch glass suspended on a round-bottomed flask holder (cork ring or rubber ring). (Two convenient ways to transfer a hot flask is to either hold it with tongs, or else to get a wet paper towel and transfer it by hand.) Remove the boiling stick and cover the top of the flask with an inverted 50-mL beaker. (This is to prevent further hot solvent from evaporating away.) The reason you don’t want to put your flask on the bench top is that heat transfer will be too fast and it will cool too quickly, resulting in inferior and less pure crystals. Allow the flask to cool until it is no longer hot to the touch. (10 minutes.) Then place it in an ice bath so that it gets as cold as possible and the smallest necessary amount of desired product stays in solution. (5 minutes).

Collect the crystals using a Buchner funnel. (See picture below, and instructions on page 3 of this handout). Make sure you have a filter flask (with an arm on the side for attaching the tubing), that you have a rubber adapter between the flask and the Buchner funnel, and that you put filter paper onto the funnel. With the Buchner funnel on top plus the tube, the flask will be top-heavy and will very easily tip over. To avoid this, you should clamp it to keep it secure (see picture). Attach the tubing to the aspirator, and turn on the water full blast to get maximum suction. Moisten the paper with solvent so that it makes a good seal. Once the paper is sealed, detach the tubing to break the seal, and then pour your crystals and solvent onto the paper. Use a spatula and perhaps additional cold water to try to get all of your crystals into the Buchner funnel. Reattach the tubing to suck the solvent through. Try to rinse the crystals with a little ice-cold water. Break the vacuum before you add the rinse solvent, and then reattach and pull the solvent through again. Maintain aspiration for five minutes (or longer, if you are doing something else).

If the crystals still seem very wet, press them with another piece of filter paper to squeeze out moisture. Once they are fairly dry, transfer them onto a watch glass and let it sit in your drawer to dry for at least 12 hours. (Otherwise they will still be damp and will have a depressed melting range). After allowing time to dry, come back, weigh the crystals, record your final mass and calculate your % yield, and take a melting range for the final product. (The range should fall somewhere in the 190-220 area. So set the melting apparatus high enough, maybe at 6 or so, so that it doesn’t take forever to warm up.)

PART II: Recrystallization Experiment 3.1





Overview: For each of 5 solutes, you will screen its solubility in each of the 4 different solvents shown above: water, propanol, 3-pentanone, and toluene. (5 x 4 = 20 tests/test tubes!) The big idea is that for each of the solutes, you should be able to decide which of the solvents would be suitable for carrying out a recrystallization. Repeat tests as needed.

Procedure: Add about 70 mL of hot tap water to a 150-mL beaker, and heat it on a hot plate to a gentle boil (just barely boiling). It will take a little while for the water to heat up, so start this before you'll actually need the hot water bath.

For each solute you will test the four solvents. It works best to try all four solvents for a given solute before beginning with the next solute.

For each solute: weight out about 0.16 g of the solute, and divide it into four roughly equal piles. Place these into four test tubes (about 40mg per tube). The easiest way to do this is to weigh it first, divide it on weighing paper, push the portions onto separate pieces of weighing paper, and then pour those portions into your test tubes. (Note: If the solid is "chunky", try to crush it before putting it into the test tubes to facilitate solubility.)

Then add 1 mL of each of the four solvents. (Be sure to label your test tubes adequately!) Stir with a wooden stick for 20-60 seconds. Record the results ("s" = soluble, "i" = insoluble, "ss" = slightly soluble.) For those that don't dissolve at room temperature, place the test tube into the hot water bath (2 minutes) and record the results again. If it still doesn't dissolve, add another 1mL of solvent to the hot solution, continue heating for another two minutes, and repeat your observation. (Sometimes more solvent will enable something to dissolve that wouldn't dissolve in a lesser amount of solvent.) (Note: There is no point in heating a test tube with something that dissolved already at low temp; things never "undissolve" at higher temperature!) Record all your observations.

- **Summary**: This is the standard process for finding which solvents are suitable for recrystallizing a particular solid.
- **What constitutes a suitable solvent**? If your results are "s" (soluble) even at room temperature, the solvent is unsuitable because it's too good. You'll never be able to harvest any crystals. If your results are "i" (insoluble) even at high temperature, the solvent is unsuitable because it's too bad. You'll never be able to free the impurities. The ideal solvent should be "i" (or perhaps "ss") at room temperature but then "s" at high temperature, so that you can both free the impurities (at high temperature) but also recover crystals (at low temperature).

RECRYSTALLIZATION REPORT Name:

Report requirements (Part 1):

1. Report your initial mass, your mass recovery, and your % yield.

2. Report your dry melting range.

3. Explain very briefly why dissolving and then reforming crystals can improve their purity.

4. Explain very briefly why recrystallization can never result in 100% mass recovery (even if you used perfectly pure material).

5. Explain very briefly why mass recovery is greater if you cool to 0˚C rather than merely room temperature.

6. Explain very briefly why mass recovery is reduced if you use an excess amount of boiling solvent.

7. Explain very briefly why washing product crystals with excessive amounts of solvent, especially warm solvent, can result in reduced mass recovery.

8. Given:
    - The solubility of **X** at 100ºC in water is 18.0 g/100 mL water.
    - The solubility of **X** at 0ºC in water is 3.6 g/100 mL water.

How many mL of boiling water would be required to dissolve 25g of **X**? If that solution was then cooled down to 0ºC, how many grams of **X** could then crystallize out? What would be the maximum yield recovery for **X?**

9. An ideal recrystallization solvent is able to fully dissolve a solute only when hot, but not when cold.

- Why is a solvent that can dissolve the solute even when it is cold useless for recrystallizations?

- Why is a solvent that can't dissolve the solute even when hot useless for recrystallizations?

Report requirements (Part 2):

Fill out the table below. Convenient standard abbreviations are “i” = insoluble, “ss” = slightly soluble, and “s” = soluble (completely). (Don’t worry about distinguishing “insoluble” from “slightly soluble”.

Beside the names for the five solutes, write down which (if any) of the solvents would be appropriate for recrystallization. (It’s possible that none of the solvents screened will look good for a particular substrate, and it’s also possible that several will.)

Recrystallization Solute/Solvent Sheet Chem 355

	WATER ("W")			PROPANOL ("A")			3-PENTANONE ("P")			TOLUENE ("T")		
Solute	RT	Hot	Extra Solvent	RT	Hot	Extra Solvent	RT	Hot	Extra Solvent	RT	Hot	Extra Solvent
"D" Dibenzalacetone												
"F" Fluorene												
"P" (Pthallic Acid)												
"S" Silbene												
"T" o-Toluic Acid												

Standard abbreviatons: "I" = insoluble, "ss" = slightly soluble, "s" = soluble

Which solvents (if any) would be appropriate for the recrystallization of the following substrates? There may be more Make sure that your answers are consistent with the observations in the table above.

"D"
Dibenzalacetone

"F"
Fluorene

"P"
(Pthallic Acid)

"S"
Silbene

"T"
o-Toluic Acid

Chem 355 Jasperse RECRYSTALLIZATION-Week 2

1. Mixed Solvent Recrystallization of Acetanilide
2. Mixed Solvent Recrystallization of Dibenzylacetone
3. Recrystallization of an Unknown

Background

Review: Recrystallization is an important technique for purifying organic solids. The contaminated solid is dissolved in a minimum of hot solvent, then cooled. The amount of solvent that is used should be just enough so that the solvent is just barely saturated or almost saturated when it is boiling hot. Upon cooling the solubility decreases, and crystal formation can occur. The new crystals are purer than the original because impurities are left in the solvent. Slow, gradual cooling is best for formation of pure crystals. Filtration then provides purified material. Some mass loss always occurs, because the solvent holds not only the impurities but also some of the desired material as well. Additional unnecessary mass loss can occur if you use too much solvent, don't heat your solution to boiling in the process of dissolving your sample, if you don't cool adequately, or if you wash your product crystals excessively. An appropriate solvent needs to have mediocre dissolving power: strong enough to dissolve the sample when boiling hot, but not able to dissolve too much of the sample when cold.

General Mixed Solvent Procedure, Concept: Often it's difficult to find a single solvent with appropriate dissolving power. Further, it's often difficult to decide exactly how much of the solvent is ideal. Frequently the use of mixed solvents is a practical and convenient solution.

In the usual mixed solvent recrystallization procedure, dissolve your sample in a sufficient quantity of your "better" solvent by heating it up to the boiling point. A 4 mL/1 g ratio is the default starting guess. Add more solvent if necessary, or boil some off if you judge it's obviously way more than needed. Then add "bad" solvent (usually hot water), until either you reach a visible "saturation" point (the "lucky" situation, where you can see crystals or slight cloudiness beginning to form) or until you have a 1:1 solvent ratio. Let cool so as to grow more crystals and harvest by filtration.

When mixed solvents are used, it is essential that they be co-soluble. Otherwise the bad solvent will simply create a second layer, but the sample can remain soluble in the original layer.

Ethanol/water combinations are commonly used because ethanol has good dissolving ability for many organics, but is also infinitely co-soluble with water. Addition of water can rapidly and dramatically reduce the solubility of many organics and thus induce crystallization. While organic mixtures are also frequently useful, the difference in character between two organics is rarely as dramatic as the difference between water and an organic solvent.

Difficult Crystallizations: Sometimes crystallization is slow or difficult. Crystallization must start on some nucleation center. (Crystals grow when molecules "fit" onto some preexisting surface.) Sometimes this will happen spontaneously, but sometimes it is difficult. The formation of "supersaturated" solutions, in which the solvent holds more sample than it could if equilibrium existed, are routine. Some common techniques for initiating crystallization include:

- Seeding the saturated solution with some of the desired sample that is already in solid form.
- Scratching the insides of your flask with a rough glass rod. (By scratching the surface of your glass, you can get a rough edge which may coincidentally serve as a crystallization surface.)
- Using old, scratched up flasks!
- Leaving a boiling stick, preferably broken off so it has rough shards sticking out at the end.
- Adding an ice chip. (The surface is often rather rough, and can serve as a nucleation site. In addition, because the surface is pure water, the solvent composition near the ice chip is not representative of the bulk solvent distribution. Being water-rich, solubility may be especially poor resulting in crystal initiation. The ice chip serves to provide a local area of extremely "bad solvent".)
- Wait a long time!
- Find a better solvent!

Crystallization Summary

1. **Choosing the Solvent.** “Like dissolves like.” Some common solvents are water, methanol, ethanol, ligroin, and toluene. When you use a solvent pair, dissolve the solute in the better solvent and add the poorer solvent to the hot solution until saturation occurs. Some common solvent pairs are ethanol-water, diethyl ether-ligroin, toluene-ligroin, and t-butyl methyl ether-hexane.
2. **Dissolving the solute.** To the crushed or ground solute in an Erlenmeyer flask, add solvent, add a boiling stick, and heat the mixture to boiling. Add more solvent as necessary to obtain a hot, saturated solution. (Do not use a beaker, because the large mouth allows solvent evaporation to be too fast and uncontrolled.)
3. **Filtering suspended solids (if necessary).** If it is necessary to remove suspended solids, dilute the hot solution slightly to prevent crystallization from occurring during filtration. Filter the hot solution. Add solvent if crystallization begins in the funnel. Concentrate the filtrate to obtain a saturated solution.
4. **Crystallizing the solute.** Let the hot saturated solution cool spontaneously to room temperature. Do not disturb the solution. Slow cooling gives the best crystals. Cooling while clamped in the air, or while standing on a watch glass that is resting on your round-bottomed flask holder is a good way to do it. Put a watch glass or inverted beaker over the top of your flask so that solvent doesn’t evaporate away while still hot. Then cool it in ice. If crystallization does not occur, scratch the insides of the container, add seed crystals, or for many solvents add ice chip(s).
5. **Collecting and washing the crystals.** Collect the crystals using a Hirsch funnel (<0.2 grams) or a Buchner funnel (>0.2 g), a filter flask, and aspirator suction. Place a filter paper on the surface, wet the filter paper with solvent, and apply suction to make sure the paper seals. Break the vacuum, add crystals and liquid, and apply vacuum. After solvent disappears, break vacuum, add cold wash solvent, apply vacuum, and repeat until crystals are clean and filtrate comes through clear. The wash solvent is normally either a small amount of an ice-cold portion of the main recrystallization solvent, or else a somewhat “worse” solvent (although it shouldn’t normally be a totally “bad” solvent). For example, if 80% ethanol/water is used for a recrystallization, it would be common to wash with 60% ethanol/water to avoid dissolving much crystal.
6. **Drying the product**. Aspirate the sample for as long as is convenient. Press the product on the filter to remove solvent. Then remove it from the filter, squeeze it between sheets of filter paper to remove more solvent, and spread it on a watch glass to dry
7. **Analyzing the product.** Take a melting point of the final product. But since incomplete drying will contaminate the crystal and depress the melting point, it is normally best to wait for 15 hours or more before doing so.

Part I: Mixed Solvent Recrystallization of Acetanilide

Purpose: Do mixed solvent recrystallization of a sometimes hard-to-crystalize substance using ethanol/water; practice tricks for inducing difficult crystallization.

Detailed Procedure: Add about 50-mL of hot water to a 150-mL beaker, and warm this on a hot plate. (Warm to a gentle boil or almost so.)

Weigh about 1 g (write down exactly) of acetanilide, and place it in a 25-mL Erlenmeyer flask. Add a boiling stick. Add 4 mL of ethanol and heat this to boiling by placing the flask into the hot water bath. If the material does not dissolve, even after heating, add additional ethanol until it does. Once you have gotten the material dissolved entirely, add the "bad" solvent (water) to the boiling solution. You can transfer hot water directly from your hot water bath by pipet. If you see some sign of "saturation" (formation of crystals; cloudiness that persists even after stirring; or formation of insoluble oil droplets which can give the solution a cloudy look that persists even after swirling), stop adding water. Assuming you never see visible indication of "saturation point", stop adding water after you have added an equal volume relative to the original "good solvent" (ethanol). (One full pipet holds about 2 mL of water.)

Let the solution cool slowly to room temp, and further cool on ice. If crystallization does not ensue, try standard crystallization tricks (scratch, add broken boiling stick, add ice crystals, seed, see instructor…). Once crystals have formed and are cold, filter using your Buchner funnel and the aspirator. Wash using some solvent mixture that is cold, and has a slightly higher water/ethanol ratio than what you used for your recrystallization. (So that you are less likely to dissolve away much of your product). Aspirate for as long as is convenient, so the sample is as dry as possible. Weigh the product (can do this day of lab). But let your sample further dry for at least a day prior to taking melting point and getting your final yield. Acetanilide mp: 113-115

Part II: Mixed Solvent Recrystallization of Dibenzalacetone

Purpose: Observe how dramatically a "bad solvent" (water) can induce saturation/crystallization.

Detailed Procedure: Weigh about 1 g (write down exactly) of dibenzalacetone and place it in a 25-mL Erlenmeyer flask. Add a stir bar and 4 mL of ethanol and heat to boiling by placing it in a hot water bath, as in Part I. Once you have the solution boiling and dissolved, add water slowly, dropwise, until the solution becomes and remains unclear. (Try to count your drops). At this point, your solution is saturated!

Let the solution cool slowly to room temp, and further cool on ice for at least 5 minutes. Once crystals have formed and are cold, filter using your Buchner funnel and the aspirator. Wash using some solvent mixture that is cold, and has a slightly higher water/ethanol ratio than what you used for your recrystallization. Get your yield, but let dry for at least a day prior to taking melting point. Dibenzalacetone mp: 110-111

Part III: Recrystallization and Identification of an Unknown.

Purpose: To identify a suitable recrystallization solvent (or mixed solvents) for an unknown, to do a recrystallization without a cookbook recipe, and to identify an unknown.

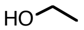
Choose one of the unknowns (make sure you write down your letter in your lab report!) Screen various solvents (as we did last week) to determine which might be suitable for recrystallizing your particular unknown. Weigh out about 0.16 g of the solute, and divide it into four roughly equal piles. Place these into four test tubes. Add 1 mL of each of the four solvents. Check the solubility at room temperature and at hot temperature. Solvents available: water, ethanol, pentanone, toluene.

After identifying a reasonable solvent, or one that could be used as the "good" solvent in a solvent pair (normally in combination with water), proceed to actually recrystallize about 1 g (write down exactly) of your unknown. Choose ethanol if possible as your solvent, because it "solvent pairs" so beautifully with water for inducing saturation and crystallization. Get your final yield, but let dry for at least a day prior to taking the melting point. If you have been successful, your melting point should be sufficiently accurate so that you can identify your unknown from the list of candidates on following page.

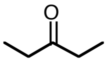
SOLVENTS

H_2O

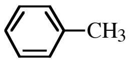
water ("W")



ethanol ("E")



pentanone ("P")

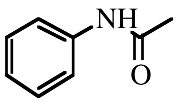


toluene ("T")

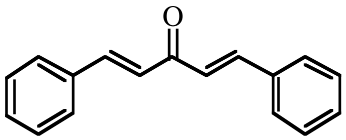
Recrystallization Unknown Candidates:

methyl 3-nitrobenzoate	78-80	cinnamic acid	133-134
1-naphthol	95-96	3-nitrobenzoic acid	140-142
1,4-di-t-butyl-2,5-dimethoxybenzene	104-105	4-nitroaniline	148-150
trans-Stilbene	121-122	triphenylmethanol	160-165

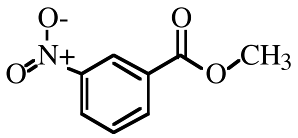
Recrystallization Unknown Candidates



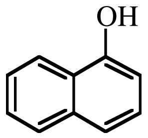
Acetanilide



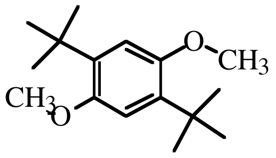
dibenzalacetone



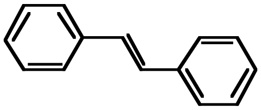
Methyl 3-Nitrobenzoate



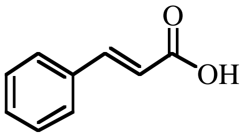
1-Naphthol



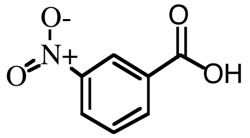
1,4-di-t-butyl-2,5-dimethoxybenzene



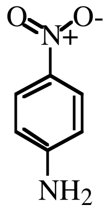
trans-stilbene



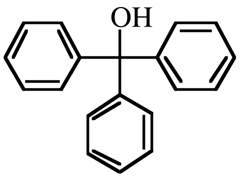
cinnamic acid



3-Nitrobenzoic acid



4-Nitroaniline



Triphenylmethanol

Name:

Recrystallization #2 Lab Report

Part 1: Acetanilide

Initial Mass: ________Final Mass:_________% Yield: ___________Melting Range: ____________

To the best of your knowledge, how much water did you add?

If you needed any "tricks" to induce crystallization, what did you try and what worked?

Any problems, difficulties, excuses, or interesting observations?

Part 2: Dibenzalacetone

Initial Mass: ________Final Mass:_________% Yield: ___________Melting Range: ____________

To the best of your knowledge, how much water did you add?

Any problems, difficulties, excuses, or interesting observations?

Part 3: Unknown

Initial Mass: ________Final Mass:_________% Yield: ___________Melting Range: ____________

Which unknown letter did you use? ______________

What was the chemical identity of your unknown? _________________________________
(See list of candidates on page 4)

Solvent Screening:

Water			Ethanol			Pentanone			Toluene		
RT	Hot	Extra Solvent	RT	Hot	Extra Solvent	RT	Hot	Extra Solvent	RT	Hot	Extra Solvent

What solvent or solvent mixture did you choose?

Approximately how much of each solvent did you use?

Any problems, difficulties, excuses, or interesting observations?

Chem 355 Jasperse DISTILLATION

Background Distillation is a widely used technique for purifying liquids. The basic distillation process involves heating a liquid such that liquid molecules vaporize. The vapors produced are subsequently passed through a water-cooled condenser. Upon cooling, the vapor returns to it's liquid phase. The liquid can then be collected.

The ability to separate mixtures of liquids depends on differences in volatility (the ability to vaporize). For separation to occur, the vapor that is condensed and collected must be more pure than the original liquid mix. Distillation can be used to remove a volatile solvent from a nonvolatile product; to separate a volatile product from nonvolatile impurities; or to separate two or more volatile products that have sufficiently different boiling points.

Vaporization and Boiling When a liquid is placed in a closed container, some of the molecules evaporate into any unoccupied space in the container. **Evaporation**, which occurs at temperatures below the boiling point of a compound, involves the transition from liquid to vapor of *only* those molecules at the liquid surface. Evaporation continues until an equilibrium is reached between molecules entering and leaving the liquid and vapor states. The pressure exerted by these gaseous molecules on the walls of the container is the **equilibrium vapor pressure**. The magnitude of this vapor pressure depends on the physical characteristics of the compound and increases as temperature increases. In an open container, equilibrium is never established, the vapor can simply leave, and the liquid eventually disappears. But whether in an open or closed situation, evaporation occurs only from the surface of the liquid.

If a liquid is heated to its boiling point, quite a different phenomenon occurs. The **boiling point** is the temperature at which the vapor pressure of a liquid is equal to the external pressure applied to the surface of the liquid. This external pressure is commonly atmospheric pressure. At the boiling point, bubbles of vapor are produced throughout the liquid, not just at the surface, and the vapor pressure inside the bubbles is sufficiently high to allow them to grow in size. The escape of these bubbles results in the characteristic chaotic motion of the liquid identified as **boiling**.

When a pure liquid boils, liquid is converted to vapor rapidly. Even if the heating rate increases, the temperature of the boiling liquid doesn't change, only the rate of vaporization. The energy supplied by heating is used by the liquid-vapor phas` e change.

Mixtures When a mixture of liquids is heated, the vapor pressure above the mixture equals the sum of the vapor pressures of the individual compounds. When their combined vapor pressures equal the external pressure, then boiling ensues just as for a pure liquid. However, the vapor above a mixture always has a different composition than the liquid mixture itself. The vapor above a liquid is always enriched in the more volatile component.

The table on the right shows the difference in composition between liquid and vapor for cyclohexane (bp 81ºC) and toluene (bp 111ºC) mixtures. Notice that an 85/15 liquid mix is 95/5 in the vapor, a 50/50 liquid mixture is 80/20 in the vapor, and a 32/68 mix is 54/46 in the vapor. In all cases, the vapor is significantly enriched in the lower boiling hexane.

The temperature column on the left gives the boiling points for the liquid mixtures. Notice that with pure hexane, the boiling point is 81ºC, that of pure hexane. With pure toluene, the boiling point is 111ºC, that of pure toluene. But for any mixture of the two, the boiling point is somewhere in between. In all cases, the boiling point of the

Temperature (ºC)	Cyclohexane/Toluene Ratio Liquid Phase	Cyclohexane/Toluene Ratio Vapor Phase
81	100/0	100/0
83	85/15	95/5
85	75/25	91/9
87	65/35	88/12
89	58/42	83/17
91	50/50	80/20
93	44/56	72/28
95	38/62	66/34
97	32/68	54/46
99	25/75	48/62
101	21/79	43/57
103	18/82	41/59
105	12/88	36/64
107	7/93	23/77
109	3/97	18/82
111	0/100	0/100

That the vapor is enriched in the more volatile component is key to separating mixtures by distillation. But note that the vapor is not pure; it is simply enriched. In a distillation, liquid is vaporized, then the vapor is condensed. So if you vaporize 50/50 cyclohexane/toluene and then condense the vapor, the condensate will still not be fully pure; it will be only 80% cyclohexane. For a single **simple distillation** to provide good separation, two liquids in a mixture should differ in boiling points by at least 40ºC. Simple distillation would enrich cyclohexane, but would not provide pure cyclohexane.

Fractional Distillation But consider what might happen if you did a series of vaporization-concentration cycles on a cyclohexane/toluene mixture. If you start with a 50/50 liquid mix, the vapor will be 80/20. If you condense some of that 80/20 vapor, the vapor above an 80/20 liquid mix would in turn be 93/7. If you condense that vapor, you will have 93/7 liquid. But the vapor above that liquid will in turn be >98/2 pure in cyclohexane. If you then condense that vapor, the resulting condensate will be quite pure in cyclohexane. Thus by doing four distillations, you could have relatively pure cyclohexane.

This kind of sequence of multiple distillations is involved in a process called "**fractional distillation**". A fractional distillation apparatus includes a column placed in between the boiling pot and the condenser. The fractionating column is filled with packing material with high surface area (typically glass beats or metal wire.) The vapors generated in the pot rise up the fractionating column and encounter cooler surfaces, upon which they condense. The condensed liquid is then reheated by rising hot vapors and revaporize. This process of condensation and revaporization may occur again and again as the vapors rise up the column. These composition changes are reflected by a *decrease* in boiling temperature as the mixture moves up the fractioning column. If the condensation-revaporization is repeated a sufficient number of times, the vapors of the more volatile compound reach the top of the column in a pure form. As these vapors move into the condenser, the compound condenses and is collected as a purified liquid.

Purification of the high-boiling component: As the more volatile component is being selectively removed, the residual liquid is increasingly enriched in the less volatile component. Thus, a separation of the two compounds is achieved.

However, as the more volatile compound is removed, and the composition of the residual liquid becomes enriched in the less volatile compound, the boiling temperature of the residual liquid also creeps up. If a cyclohexane/toluene mixture is originally 50/50, then the liquid boils at 91ºC. But as the cyclohexane is removed, the boiling temperature of the liquid gets higher and higher. (And it gets harder to purify the lower-boiling fraction.) After a while, all of the low-boiling material is removed. At this point, the only material that can climb all the way up the fractionating column is the low boiling component, and you can distill it over as well. By changing collectors, you can thus isolate both the more volatile and less volatile components in reasonable purity.

Technical Aspects

- The fractionating column must be positioned vertically so that condensed liquid can percolate down through the rising hot vapors. This percolation promotes equilibration/heat exchange between the liquid and vapor phases, a condition that allows the column to operate at maximum efficiency and provide an optimum separation.
- A crucial factor is the distillation rate. In order to get the maximum number of vaporization/condensation cycles and maximum purification, fractional distillation must be conducted slowly. A one drop per second rate is recommended for best results. Slow, gradual distillation essentially allows the best equilibration and heat transfer. If you heat too fast, vapors may not condense as quickly as desired, and may waste some of the column.
- Packing material is also crucial. High surface area packing material provides surface on which condensation can occur. The more easily vapor can condense, the more distillation cycles you get.

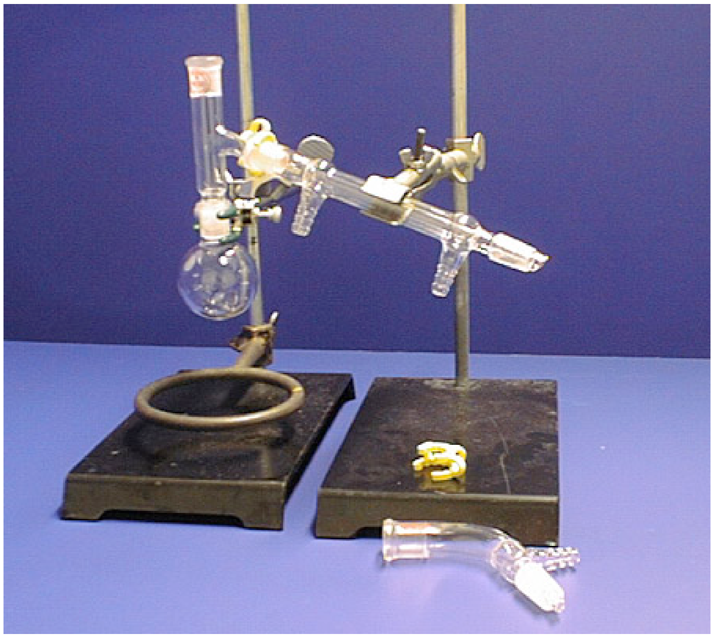
Miscellaneous

- At reduced pressure, liquids boil at lower temperatures. (The external pressure is less, so it's easier to build up enough vapor pressure to escape.) High-boiling liquids are often distilled under vacuum.
- Simple distillation is useful when there are large differences in boiling point (>40ºC.) Often organic solvents will be much more volatile than the target samples, so simple distillation is useful for rapid removal of the solvent. Simple distillation is faster than fractional. But fractional is much more powerful for more difficult separations.
- The temperature of the vapor is a direct reflection of it's composition.
- When the temperature of the vapor is changing, it's because the composition of the vapor is changing.
- The vapor temperature and composition of the vapor is almost constantly changing because the composition of the residual liquid is continuously getting depleted in the more volatile component.
- In a distillation curve, there will always be middle portion reflecting mixtures. For a typical purification, three separate collections would be made: the initial relatively horizontal portion (reflecting relatively pure volatile component); a middle portion which would be thrown away (reflecting mixtures not worth saving); and a subsequent relatively high-boiling horizontal portion (reflecting relatively pure less volatile component.)

Part I: Simple Distillation of Cyclohexane/Toluene

Setup:

1. Attach a large metal ring to one of the vertical rods on your rack
2. Rest your small heating mantle (the smaller of the two devices in your bottom cabinet with gray wells) on your metal ring, making sure that the plug-in cord for the mantle can reach one of the "Powermite" outlets. (Not a direct electrical outlet. Since the mantle has no power adjuster, if you plug it into the wall you will have full power and no control. The Powermite provides a dial that you can use to regulate the electricity going into your heating mantle and can thus regulate your heat.)
3. Securely clamp a 100-mL round-bottomed flask above this (find flask in your kit). (The neck of the flask has a "lip"; try to have your clamps below this, so that the "lip" has no chance of slipping through.)
4. Add a 3-way connecting tube (#10 in your kit map).
5. To the almost horizontal branch, attach a condensing tube (#12 in your kit map). Use a Keck clip to hold the joints snug.
6. Raise the entire array high enough and place a 100-mL graduated cylinder underneath the end of the condensing tube to collect the distillate. Make sure your 100-mL flask, on which the rest of the array rests, is very securely clamped! It should be able to hold everything up even without the support of the heating mantle. In turn, make sure that the heating mantle is also securely clamped.



7. Add an additional clamp to support the condensing tube, but do not clamp it tightly. (Joints might pull apart if you do.) (The picture doesn't have the graduated cylinder or the heating mantle in place, and has a smaller round-bottomed flask. But otherwise it shows how things should look at this point.)
8. Use rubber tubing to connect the condenser to a water tap (lower end of condenser) and to a drain (upper end of condenser). (Note: Be sure you connect to a water tap and not to an aspirator!)
9. Add 60 mL of 50/50 cyclohexane/toluene and 2-3 boiling chips to your flask. You can just drop in the chips, but you may wish to use your long-stemmed funnel to pour in the liquid.
10. To the open tube on top, add a straight adapter tube (#7 in your kit map) with a thermometer inserted.
11. Adjust the position of the thermometer so that the mercury is just below the branch point of your array.
12. Try to have a lab instructor check your setup to make sure everything is good!

Doing the Distillation

1. Turn the condenser water on, but do so only very gently. All you need is enough flow to keep the water circulating and keep the condenser cold. You do not need to turn it on full blast like when you use the aspirator.
2. Turn your Powermite setting to high to warm up the solution to the boiling point as fast as possible. Once you see boiling, you may wish to turn the power down a little, I'm not sure what setting is best. But since this is a simple distillation anyway, you may as well distill it over pretty quickly. You may wish to leave it at the highest setting even as you collect, if the solution isn't boiling out of control.
3. Record your thermometer temperatures (which reflect the composition of the vapor that is actually distilling over at any point in time) at 2 mL intervals. Since the 0-mL spot is meaningless, make your first reading after 1 mL, but from that point on record temperatures at 2, 4, 6, etc. mL. Continue the distillation until the temperature reaches and stays at 110ºC for a couple of milliliters, or until fewer than 5 mL of liquid remains in the pot.

4. Turn off the heater and lower the heating mantel away from the flask to allow cooling. Allow the flask to cool for a few minutes.

Part II: Fractional Distillation of Cyclohexane/Toluene

Setup

1. Again use 60-mL of 50/50 mL toluene, just like you did in your first distillation. Do this in the same 100-mL flask, after pouring out whatever residual liquid remained from the first experiment.
2. Have 2-3 boiling chips present.
3. Your setup for the fractional distillation will be very similar to what you did previously, except for the following changes:
    - You will insert a steel-wool packed distilling column (#13 in your kit map) in between your flask and your three-way connecting tube. (You do not need to clean out your condensing tube, you can use the exact same “top half” glassware from your previous setup, even if it is slightly contaminated by a little distillate.)
    - Because of the height of the fractionating column, you can probably rest your heating mantle on the bench top, or close to it, and still have enough height to fit your graduated cylinder to collect drops

Doing the Distillation

1. Proceed as above, with the following adjustments:
    a. Once some sample begins to distill, turn your power down significantly so that the drop rate is steady and not too fast. (Slow fractional distillation gives better separation). An ideal drop rate is one drop per second or less. It’s especially important that the solution climb through the packing relatively slowly at the beginning. I’m not sure, but perhaps try a power setting of around 3 on your Powermite to begin?
    b. This time record temperatures at 1-mL increments, again beginning at 1-mL.
    c. Continue the distillation until the temperature reaches and stays at around110ºC for a couple of milliliters, or until fewer than 5 mL of liquid remains in the pot.

Name:

Distillation Lab Report

1. Plot your temperature (y-axis) versus mL collected (x-axis). Plot both distillations on the same graph. Since little of the action takes place near room temperature, have the low end of your y-axis be 80ºC, with a high end of your y-axis 111ºC. You may prepare your graph on computer (Kaleidagraph works well…), but I will also accept hand-drawn graphs. Either staple your graph to this sheet or else generate your graph on the other side of this sheet for handing in your lab report.

2. Why is the vertical change in the fractional distillation so much sharper than in the simple distillation? (In other words, why does it jump from say 85ºC to 105ºC over such a smaller number of mL with fractional than with simple distillation? And why does it wait longer to creep up over, say, 90ºC?)

3. Give a brief discussion of how simple and fractional distillation differ. What is the difference? Why? When and why would you choose fractional distillation? Would there be any circumstances in which you'd choose simple distillation?

4. For the simple distillation, compare your vapor temperatures at 2 mL, 16 mL, and 30 mL. Why is the temperature different at these different times? Does the temperature also change like that in the fractional distillation?

5. Why is better separation of two liquids achieved by slow rather than fast distillation? (Particularly in the case of fractional distillation?)

6. Explain why a lot of packing material with a lot of surface area is helpful for an effective fractionating column? In our case, you had steel wool in your column. What would be worse if you didn't have the steel wool present?

7. What effect does doing a distillation under reduced pressure have? For a particular sample, will the boiling point be unchanged, go up, or go down if you try to distill it under a reduced pressure/vacuum type situation?

8. If you wanted to collect material that was relatively pure cyclohexane from your fractional distillation, which section would you save? (For example, the first 5 mL? First 10 mL?) If you wanted to collect material that was relatively pure toluene, which section would you save?

LIQUID/LIQUID SEPARATION: EXTRACTION OF ACIDS OR BASES FROM NEUTRAL ORGANICS

Background Extraction is one of humankind's oldest chemical operations. The preparation of a cup of coffee or tea involves the extraction of flavor and odor components from dried vegetable matter with hot water. Many other substances, flavors and spices and perfumes and medicines, have been extracted from plants for centuries (quinine, morphine, menthol…). Many undesirable drugs are also isolated by extraction (cocaine from coca leaves). Extraction, like recrystallization, is based on solubility factors.

The most common and simple separation in organic chemistry involves the separation of neutral organics from ionic compounds, whether the ionic compound is an inorganic salt (NaCl) or is an ionized version of the organic. The two most commonly ionized organic families are carboxylic acids, which are ionized by deprotonation to their carboxylate RCO_2- form, or basic amines, which are ionized by protonation to their ammonium RNH_3+ form.

Neutrals and ionics are easily separated because ionics are preferably soluble in water rather than in organic solvents, whereas neutral organics are preferably soluble in organic solvents rather than in water. The following three separations are thus common:

1. A neutral/ionic mixture is shaken with ether and water.
   - The neutral goes into the ether layer.
   - The ionic goes into the water layer.
2. A neutral/carboxylic acid mixture is shaken with ether and NaOH/water.
   - The neutral goes into the ether layer.
   - The carboxylic acid is deprotonated by NaOH to its carboxylate form (RCO_2-), which goes into the water layer.
3. A neutral/amine mixture is shaken with ether and HCl/water.
   - The neutral goes into the ether layer.
   - The basic amine is protonated by HCl to its ammonium form (RNH_3+), which goes into the water layer.

Once a chemical is separated from its original mixture, it must still be isolated from solvent.

1. Isolating a neutral from ether solvent:
   - **Dry** The ether will contain not only the neutral solute, but also some water. The water is absorbed by a chemical drying agent (usually sodium sulfate or magnesium sulfate or calcium chloride).
   - **Filter** The drying agent is then usually removed by filtration.
   - **Concentrate** The solvent is then removed by simple distillation, leaving the desired neutral as the residue. The simple distillation is usually done via a "rotary evaporator".

2. Isolating a neutral carboxylic acid from the NaOH/water layer:
   - **Acidify/Neutralize** HCl is added to acidify the water. In the process the carboxylate anion RCO_2- is protonated and converted back to its neutral form RCO_2H.
   - **Filter or Extract** Because the acid is now neutral, its solubility in water will be low. If it crystallizes, you can filter it. If it comes out of the water as an oil, you can extract it in ether!

3. Isolating a neutral amine from the HCl/water layer:
   - **Basify/Neutralize** NaOH is added to basify the water. In the process the ammonium cation RNH_3+ is deprotonated and converted back to its neutral form RNH_2.
   - **Filter or Extract** Because the amine is now neutral, its solubility in water will be low. If it crystallizes, you can filter it. If it comes out of the water as an oil, you can extract it in ether!

Partition Coefficients and Multiple Extractions

In the presence of two solvents (ether and water in our case), each specific chemical has a characteristic "partition coefficient", with the following formula:

Partition coefficient = solubility in ether/solubility in water.

The partition coefficient basically tells you what fraction of the material will partition into each solvent layer. If the value is 4:1, that means 80% will partition into the ether layer, and 20% will partition into the water layer.

- Ideally, the distribution will be either zero (all stays in water) or infinity (totally in ether).
- This is not often the case. Frequently some of the neutral organic material will be lost to the water layer, and sometimes some of the ionic material will go into the ether layer. In either case, either the yield and/or the purity will not be 100%.

To improve extraction efficiency, **often two (or more) extractions may be appropriate**.

- **Example 1**: **Suppose you are trying to get all of your organic material into the organic phase**, but the partition coefficient for your desired neutral organic is only 4:1. If you do one separation, you should have 80% of the material in the ether extract, and 20% of the material in the water phase. By extracting the water again with more ether, you should get 80% of the remaining 20% that was in the water, i.e. you should get another 16% out into the ether, and now only 4% of the neutral should remain in the water. A third ether extraction of the water should take out 80% of the remaining 4%, thus leaving less than 1% of your material left in the water layer. Combine all the ether extracts, dry/concentrate, and you should get 99% yield.
- **Example 2**: **Suppose you are trying to use aqueous base to extract a carboxylic acid from a neutral organic**, but only 90% of the acid goes into the NaOH/water and 10% stays in the ether. If you do only one separation, your neutral organic will still be contaminated by the residual 10% of acid. But if you do a second extraction with NaOH/water, 90% of that 10% will be extracted as well, and now only 1% of the acid will remain in the ether layer to contaminate your neutral.

The process of extracting from a particular phase, to either make sure you get all the target organic out (example 1) or to make sure you remove all of an undesired contaminant (example 2) is often referred to as "**washing**". In example 2, the carboxylic acid was "washed out" of the ether layer by a couple of NaOH/water "washes".

To determine how many "extractions" are required to achieve a target minimum of non-extracted material, use y^x, where "y" is the fraction that survives a particular extraction, and "x" is the number of extractions. (In example 1, $y^x = 0.20^3 = 0.008 = 0.8\%$.)

Choice of Organic Solvent

1. **Low Boiling Point** Since you normally have to distill off your solvent at the end, a low-boiling solvent that can be simply distilled away quickly and rapidly is very desirable.
2. **Good Dissolving Ability for Organics** Obviously you'd like your organic solvent to have much better dissolving ability for organics than does water. Sometimes the nature of the solute dictates which solvents are acceptable.
3. **Low Miscibility with Water** You'd like relatively water to dissolve into the organic phase, and vice versa.
4. **Higher or Lower Density Than Water, Depending on Extraction Purpose**
   Typically when multiple extractions/washes are used, it is desirable to have the "extraction solvent" be denser than the solvent that is "being washed".
   - **If you are going to "wash" the organic solvent several times with water** (example 2), it is technically convenient if the organic layer floats on the water layer. **This is true for ether**.
   - **If you are going to "wash" the water layer several times** to make sure you get all your organic material into the organic phase, it is more convenient to use a solvent that is more dense than water, so that the water will float on the organic solvent and you can pour the organic solvent out the bottom. This is not true for ether, so it isn't that convenient for multiple washes/extractions from water. **Dichloromethane, which is more dense than water, is frequently used** instead for doing multiple washes/extraction from a water layer.
5. **Safe, Cheap, Unreactive…**

LIQUID/LIQUID SEPARATION: EXTRACTION OF ACIDS OR BASES FROM NEUTRAL ORGANICS

Carboxylic acid unknown options (Part 1): benzoic acid (mp 123) or 2-chlorobenzoic acid (mp 141)
Amine unknown options (Part 2): 4-chloroaniline (mp 68-71) or ethyl 4-aminobenzoate (mp 90)
Neutral options (same choices for both Part 1 and Part 2): 1,4-dimethoxybenzene (mp 57), naphthalene (mp 82), dibenzalacetone (mp 110-111), or benzoin (137)

For flow chart, use "N" for neutral, "RCO_2H" for protonated carboxylic acid, "RCO_2-" for ionic carboxylate salt, "RNH_2" for neutral amine, and "RNH_3+" for ionic ammonium salt.

Part 1: Separation of a Neutral from a Carboxylic Acid.

Setup:

1. Attach your small metal ring to one of the vertical rods on your rack
2. Get your separatory funnel and a glass stopper out of your organic kit. Rest the separatory funnel into the ring.
3. Make sure the stopcock is closed!

Procedure:

Phase 1: Separating the two Chemicals into Two Liquid Phases. Extracting the Acid.

1. Weight out about 2 g (record exact weight) of a 50/50 mixture (by weight) of N/RCO_2H. (In other words, the mixture consists of 1 gram of neutral and one gram of acid). Pour the solid mix into your separatory funnel.
2. Add 20 mL of diethyl ether ("ether"). If the mixture doesn't dissolve, add enough ether to fully dissolve it.
3. Add 10 mL of 3M NaOH to the separatory funnel, stop it, shake vigorously, vent, and allow the layers to separate. (Purpose: to convert the neutral acid into carboxylate anion, which should then go into the aqueous layer rather than staying in the ether layer).
4. Label a 50-mL Erlenmeyer flask as "Flask 1" with your Sharpie pen, and a 150 mL beaker as "waste".
5. Get a couple of pieces of pH paper or litmus paper.
6. Drain off the aqueous layer into Flask 1. Pass a stick of pH paper into the draining stream to confirm that it is basic. (Be sure you have removed the stopper from your separatory funnel first.) (Note: it is better to have a little water stay in the ether layer than to have some of the ether layer go into the aqueous.)
7. Add an additional 5 mL of 3M NaOH to the separatory funnel, shake the mixture as before, let it settle, and again drain the aqueous layer into Flask 1.
8. Save Flask 1, the contents of which you will process a little later.

Phase 2: Isolating the Neutral Organic from the Ether Phase.

9. Add 15 mL of "brine" (saturated aqueous solution of sodium chloride) to the separatory funnel, shake the mixture thoroughly, allow the layers to separate, and drain off the aqueous layer into the "waste" beaker. The contents can be poured down the drain. (Purpose: the brine reduces the solubility of water in the ether, so the ether will be less wet.)
10. Carefully pour the ether layer into a 125-mL Erlenmeyer flask (labeled "Flask 2") from the top of the separatory funnel, taking care to minimize transfer of water droplets.
11. Rinse the separatory funnel with an additional 5-mL of ether, and add that rinse to Flask 2.
12. Add sodium sulfate to Flask 2 and swirl. The amount required depends on how much water is in the mixture. Typically one full scoopula of sodium sulfate should suffice, but frequently additional drying agent is required. If the solution is dry, the liquid should look very clear and not cloudy extract until it no longer clumps together. If all of the moisture has been absorbed, there should be at least some fine granular "non-clumpy" sodium sulfate granules left, and the solution should be clear. (Purpose: the sodium sulfate is intended to absorb any water that is in the ether solution.)
13. Pre-weigh a 50-mL round-bottomed flask, and then clamp it onto a vertical rod.
14. Take your long stem funnel and push a little glass wool into the neck. A little bit will suffice. A pipet is often helpful for pushing it in a little bit.

15. Pour the ether solution from Flask 2 through the glass-wool plugged funnel into the round-bottomed flask. The wool should be sufficient to filter off the solid sodium sulfate, and only allow the solution to get into the flask.
16. Rinse Flask 2 and the sodium sulfate pad with 10 mL of ether, and pour the rinse through the funnel into the round-bottomed flask.
    - At this point, there should be only ether and neutral in the flask. The acid should have been removed by the NaOH; the water should have been removed by the sodium sulfate; and the sodium sulfate should have been removed by the filtration.
17. Concentrate the ether solution in the round-bottomed flask by rotary evaporation in the lab next door. Be sure the aspirator power is on; that the top air valve is closed; and that you have an adapter for a good glass seal. Make sure that the spinner is also turned on. Get help the first time you use this!
    - Note: This is a standard simple distillation to remove the volatile ether while leaving the higher boiling, less volatile organic material behind. The vacuum further lowers the boiling point for the ether so that it comes off very quickly.
18. Once the sample has concentrated to dryness, weight the flask and calculate your mass yield.
19. Take a melting point, perhaps after waiting for 15 hours or more.
    - You have now completed isolation of sample from Flask 2.
    - Note: the melting points are likely to be somewhat depressed, because the products will have some impurities. The products could be further purified by recrystallization, but time does not permit!

<u>Phase 3: Isolating the Neutral Carboxylic Acid from the Aqueous Phase.</u>

20. Acidify the contents of Flask 1 by adding <u>concentrated</u> hydrochloric acid pipet-by-pipet, while testing with pH or litmus paper until the solution is decidedly acidic ($pH<4$). There is little harm in adding extra acid. (Be sure you use c<u>oncentrated</u> hydrochloric acid. Otherwise it will take too long to neutralize the water, and your yield will go down because of so much solvent.)
21. Cool flask 1 in ice, then filter (Buchner funnel), rinsing with a little cold water. Let the product dry, weigh it, and test its melting point. (Give it >15 hours of drying before taking melting point.)
    - Note: the melting points are likely to be somewhat depressed, because the products will have some impurities. The products could be further purified by recrystallization, but time does not permit!

<u>Part 2: Separation of a Neutral from a Basic Amine</u>.

(See introduction above Part 1 for list of unknown candidates.)

The procedure should be largely analogous to that used for extracting an acid, with one huge difference: now you want to extract a basic amine instead of an acid. (When you extracted the <u>acid</u>, you used dilute <u>basic water</u>; to extract the <u>basic amine</u> instead, should you again use dilute basic water, or will you want to use dilute <u>acidic water</u> instead?)

<u>Create a flow chart analogous to that used for the acid, and show it to instructor before going ahead</u>. Keys to consider: since an amine is basic rather than acidic, should you use HCl or NaOH to ionize it and make it water soluble in the first separation? And once you have ionized it, will you use HCl or NaOH to neutralize it and make it water insoluble? In others words, what changes in your flow chart and procedure result from the fact that you are extracting an amine rather than an acid?

Once you have established and checked your separation/purification plan, proceed to isolate the second neutral and the amine. Record their masses, and record their melting points.

Name:

Extraction/Acid-Base/Separatory Funnel Lab Report

1. Yields, Melting Ranges, and Identification:

Part 1:

Neutral: isolated yield (in grams) melting range: Identity:

Carboxylic Acid: isolated yield (in grams) melting range: Identity:

Part 2:

Neutral: isolated yield (in grams) melting range: Identity:

Amine: isolated yield (in grams) melting range: Identity:

2. Complete the flow chart for Part 1 (opposite side). Use “N” for the neutral, “RCO_2H” for the acid in it’s neutral form, and “RCO_2-“ for the carboxylate anion form.

3. Include an analogous flow chart for Part 2 (opposite side), with any adjustments required due to starting with a basic amine rather than an acid. Use “N” for the neutral, “RNH_2” for neutral amine, and “RNH_3+” for ionic ammonium salt.

4. Why is it necessary to remove the stopper from a separatory funnel when draining the liquid through the stopcock?

5. The pK_as of chemicals HX and HY are 5 and 7 respectively. The pK_a of carbonic acid H_2CO_3 is 6. If you made up an ether solution of chemicals HX and HY in a separatory funnel, and then added an aqueous solution of sodium bicarbonate $NaHCO_3$ to that separatory funnel, would both HX and HY stay in the ether layer? Or would either or both of them transfer into the aqueous layer? If one goes into the water layer, will it be in it’s neutral HX/HY form, or in it’s deprotonated anionic form?

HX: ether layer or water layer?

If in the water layer, in HX or X- form?

HY: ether layer or water layer?

If in water layer, in HY or Y- form?

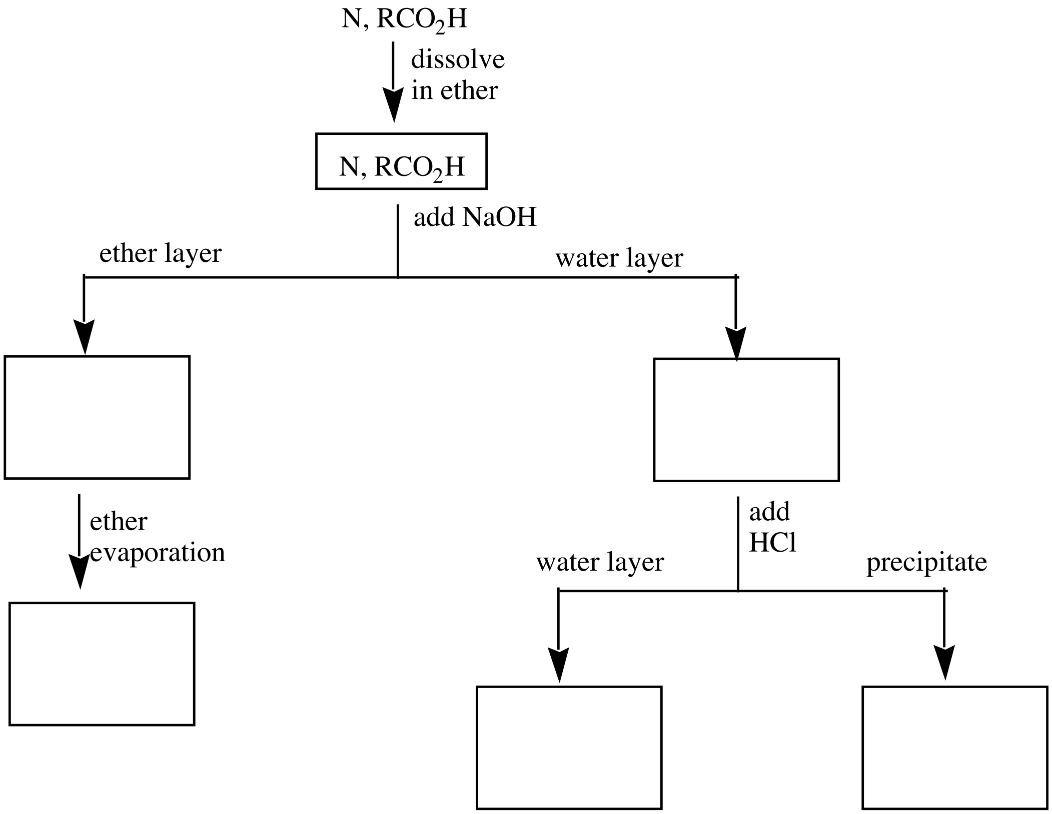
6. Suppose you have an organic sample **X** that is somewhat soluble in water, even though it is somewhat more soluble in dichlromethane or ether solvents. But if you do a single extraction, you get only 60% of your material to transfer from the water to the organic layer.

- How many “washes” would it take to extract over 90% of your organic material extracted from the water layer?

- In this case, would it be better to extract with dichloromethane or with ether?

7. Suppose you have an organic sample **X** that is contaminated with an impurity. When you wash with an aqueous phase, **X** stays exclusively in the organic phase, and most (about 80%) but not all of the impurity washes out into the water phase. How many aqueous extracts should you do so that less than 1% of your impurity remains in the organic layer? Will it be more convenient to use ether or dichloromethane as your organic solvent?

Flow Chart for the Separation of Neutral from Carboxylic Acid



Create Your Own Flow Chart for the Separation of a Neutral from a Basic Amine

Chem 355 Jasperse C-13 NMR

I. Introduction to Spectroscopy Spectroscopy involves gaining information from the absorption, emission, or reflection of light from a sample. There are many other examples of spectroscopy in our experience, but three familiar real-life examples include:

1. X-rays. Dense bone absorbs x-ray radiation.
2. Grocery store scanners. A monochromatic laser is either absorbed (black bar) or reflected (white bar). The simple black-or-white lines with their yes-or-no absorption-or-reflection response essentially produces a binary code, from which products and prices can be determined.
3. Stop lights. A lens is adjusted at timed intervals to enable emission of either green, red, or yellow light.

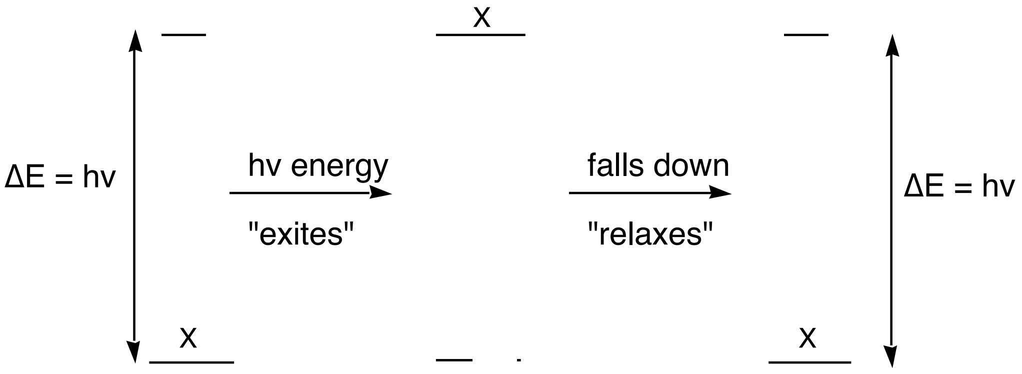
In organic chemistry, the most important type of spectroscopy is “NMR” (“**N**uclear **M**agnetic **R**esonance” spectroscopy). NMR spectroscopy is routinely used for chemical analysis, whether that be to identify the structure of an unknown, to assess the purity of a product, or to determine ratios of isomers. This week we will use carbon-13 NMR; in two weeks we will use hydrogen NMR. Both of these will be used later in the year, especially during second semester lab. During second semester lecture, we will revisit NMR and spend time and a test on interpretation of NMRs. Magnetic Resonance Imagine (“MRI”) is an important hospital application of NMR. (The name was changed from NMR to MRI because some patients were afraid of the word “nuclear”in NMR!)

II. General Aspects of Spectroscopy Physics The fundamental principles of chemical spectroscopy are illustrated below. Spectroscopy involves having quantized energy levels. You are familiar with the concept of quantized energy levels for electrons (1s, 2s, 2p, 3s, 3d etc.) and electron spins (spin up or spin down, but other things are also quantized (vibrational energies, rotational energies…).

Given that there is an exact energy gap between two quantized energy states, a photon of precise energy must be absorbed in order to excite a molecule from the ground state. When an excited state relaxes back to the ground state, that same photon is released. By measuring the exact frequencies of photons that are either absorbed or emitted, we can measure ΔE. The quantity of photons can tell us about how much material is absorbing or emitting.

The chemist must then be able to interpret what the frequencies of the photons mean in terms of chemical structure.

General Picture of Energetics and Spectroscopy



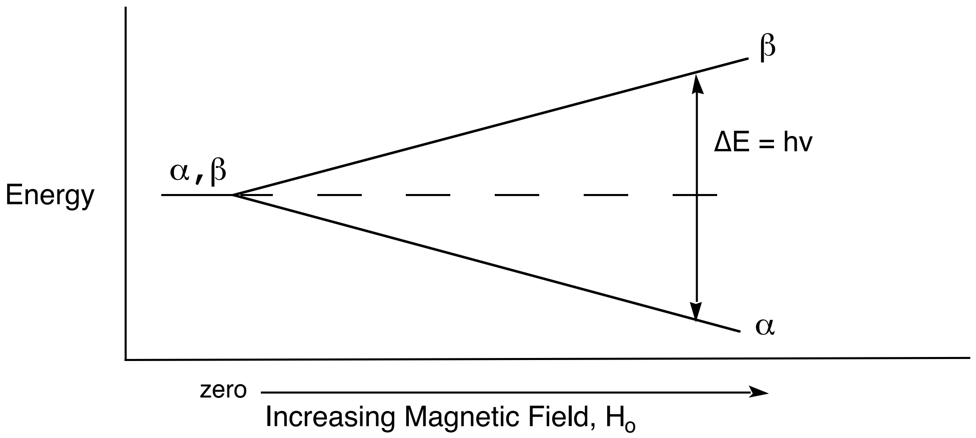
1. Quantized Energy Gaps
2. When a photon with exactly the right energy/frequency/wavelength is absorbed, a sample gets "excited" from its "ground state" to an "excited state"
3. When an exited state "relaxes" back to its ground state, the same ΔE is involved, and a photon with the same energy/frequency/wavelength is released

III. NMR Physics Certain nuclei (not all) have quantized "nuclear spins". Being charged objects that spin, a result is that they magnetic. (A circular flow of charge or electricity always produces a magnetic field, according to the "right hand rule" of electromagnetism.) Nuclei that have quantized spin states are referred to as "NMR active". Just as electrons have quantized spin states (spin up or spin down), NMR-active nuclei also have quantized spin states, spin up or spin down.

Some NMR-active nuclei: **H-1, C-13**, N-15, F-19, P-31, Si-29, Se-79, Sn-119
Some NMR-inactive nuclei: C-12, N-14, O-16

The list of NMR inactive nuclei is somewhat unfortunate for organic chemistry! We are largely interested in the chemistry of carbon and the 2nd row elements, but unfortunately the dominant isotopes for carbon, nitrogen, and oxygen are all NMR inactive! Fortunately at least carbon-13 is active. Although only 1% of carbons are C-13, that's still enough to give us useful information. Hydrogen is also NMR active, and can give us a lot of information (in two weeks…).

In the presence of an applied magnetic field, nuclear magnets can align with (spin down, α) or against (spin up, β) the field. The energy gap between these spin states is quantized, and depends on the strength of the magnetic field. (As with a bar magnet, the stronger the field, the greater the preference for the magnet to line up correctly…). To "excite" a nucleus from the more stable α state to the less stable β state, radiation with the correct photon frequency is required. When an excited nucleus relaxes back to the α state, a photon with that same frequency is emitted. Since magnetic field strength determines ΔE, and ΔE determines *v*, the magnetic field thus determines the frequency of the radiation absorbed or emitted.



1. Spin states are equal in energy in the absence of magnetic field
2. As the magnetic field increases, the difference between the two increases
3. Since field determines ΔE, and ΔE determines v, the magnetic field thus determines the frequency of the radiation absorbed or emitted when nuclei get exited or relax

When an external magnetic field is applied, will all nuclei have the same ΔE and the same photon frequency? No!

1. Different nuclei (H-1 versus C-13) have very different ΔE. Thus an MRI can easily identify whether a particular nuclei is or is not present.
2. In different chemical environments, the same nucleus will have different ΔE.

The second point is the key to 13C NMR. Although the external magnetic field (applied by the spectrometer) may be the same, different carbons in a molecule experience or "feel" difference magnetic fields. This is due to the magnetic fields produced by local electrons and by other nuclei (because moving electrons function as "electron magnets" and moving nuclei function as "nuclear magnets"). The magnetic influence of local electrons and nuclei can reinforce or partially counteract the external field, so that every different carbon "feels" a different H_{actual}.

$$H_{actual} = H_{applied} + H_{electrons} + H_{nuclei} \qquad H_{actual} \propto \Delta E \propto \nu$$

IV. The Actual Experiment

The actual steps in the experiment include:

1. Prepare the sample. For C-13, put in 5 drops of sample before diluting. For H-1, put in 2 drops of sample.
2. Insert the sample into the magnetic field.
3. "Lock" the magnet: (So that the magnetic field doesn't drift during the experiment)
4. "Shim" or "Tune" the magnetic field: Try to make it consistent from top to bottom, front to back, left to right. (This is very important for getting sharp lines).
5. Blast the sample with radiation to excite the nuclei. Rather than dialing through the different frequencies, a broad range of frequencies is applied so that all the carbon nuclei can get excited at the same time. After briefly blasting, the radiation is turned off.
6. Listen to the signals (actually in the radio frequency!) as the excited nuclei relax and release photons. (Many different signals with different frequencies are released simultaneously, each with it's own wave…)
7. Repeat the irradiate-then-listen sequence a number of times to build up the weak signal.
8. "Fourier Transform" (mathematical operation) to deconvolute the complex signal pattern resulting from the many overlapping frequencies. The Fourier Transform enables the computer to identify all the individual photon frequencies that summed up to give the total signal. An imperfect analogy would be to have every possible radio station broadcasting at the same time; then the Fourier Transform would essentially be able to identify and pick out each station one at a time and make sense of it.

Note: Many of these operations are best done by a computer. (The Fourier Transform especially!) Each of these steps also involves a number of software commands. So that you can acquire data and focus on chemical interpretation of the data, rather than being totally distracted by learning a lot of software commands, I have largely programmed the computer so that it can do most of this by itself. When it needs some input from the user, it will normally prompt you for input.

V. Interpreting C-13 NMR

While the physics of what happens is interesting, for the most part you the chemist will be engaged in interpreting the data that comes out at the end. This is true for the use of many instruments in science and health care. You need to learn some basic operational skills so that you can use the instrument safely and accurately. But being able to interpret the data is really what you need to be able to do at the end.

Summary of C-13 NMR Interpretation:

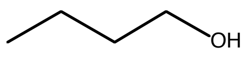
1. **Count how many lines** you have. **This will tell you how many types of carbons** you have. (Symmetry equivalent carbons can at times cause the number of lines to be less than the number of carbons in your structure.)
2. **Check diagnostic frequency windows** ("chemical shift windows") of the lines **to provide yes-or-no answers regarding the presence or absence of key functional groups** in your molecule.

1. Number of Lines and Number of Symmetry-Unique Carbons

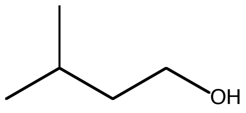
a. Each "unique"carbon gives a separate line.
  - This is due to having different electronic environments, and because spinning electrons create magnetic fields that counteract or reinforce the applied field.

b. Symmetry duplicates give the same line.
  - If due to molecular symmetry two carbons have exactly the same chemical environment, naturally they will absorb and emit exactly the same photon frequency, and give exactly the same line in the spectrum.

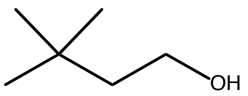
How Many Lines per Structure?
(Mark any symmetry duplicates)



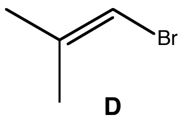
A



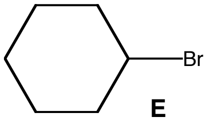
B



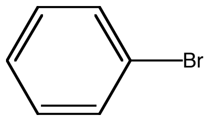
C



D



E



F

2. **<u>“Chemical Shifts” of the Lines</u>** (This reflects the energies or photon frequencies/wavelengths associated with the lines.)

220-160	C=O carbonyl carbons, sp^2 hybridized
160-100	C alkene or aromatic carbons, sp^2 hybridized
100-50	C-O oxygen-bearing carbons, single bonds only, sp^3 hybridized
50-0	C alkyl carbons, no oxygens attached, sp^3 hybridized

   a. Notice that sp^2 hybridized carbons come above 100, sp^3 hybridized come below
   b. Notice that oxygenated carbons come higher than non-oxygenated analogs. An sp^3-hybridized carbon with an oxygen comes higher than without, just as an sp^2-hybridized carbon comes higher with oxygen than without
   c. **<u>How do I process and use what I see from my Chemical Shifts?</u>**
      - **<u>Check each of the four zones. Each one gives you a yes or no answer about the presence of absence of the featured group</u>**.
      - Check 220-160. Do I have any carbonyl carbons or not? Easy yes or no question.
      - Check 160-100. Do I have any alkene/aromatic carbons? Yes or no! If I do, then how many? If I have two, I probably have an alkene! If I have four to six, I probably have a benzene!
      - Check 100-50. Do I have an oxygenated sp^3 carbon? Yes or no! Alcohols and esters will normally have one carbon in the 100-50 zone. Ethers will have two.
      - Check 50-0. I’ll almost always have some lines there! But how many should tell me how many types of non-oxygenated sp^3 carbons I have.

3. **<u>Signal Height/Size</u>** When we get to 1H-NMR, signal size will be very important. For 13C-NMR it isn’t that crucial. There is considerable variance in height. But there are two patterns that can be somewhat helpful.
   a. Carbons without any attached H’s are short. This is common for carbonyls (aldehydes are the only carbonyl carbons that have hydrogens attached) and for substituted carbons in a benzene ring.
   b. Symmetry duplication multiplies signal height (if you have two copies of a carbon, the line will probably be taller than normal!)

4. **<u>Subtracting the Solvent Lines: Don’t Count the Triplet at 77</u>** The sample is always diluted in a solvent. We will routinely use $CDCl_3$. When you use $CDCl_3$, it has carbons too! They give a 3-line “triplet” signal at 77, which is often kind of short (no H’s attached.) Ignore this signal! Don’t count it as three more unique carbons in your molecule! Don’t conclude that you have three oxygenated sp^3 carbons!

5. **<u>Subtracting the ReferenceLine: Don’t Count the Line at 0</u>** A reference chemical $[(CH_3)_4Si]$ is normally included that gets used to define where “zero” is. This zero marker is present all the time, but is not part of your actual molecule. Ignore this signal! Don’t count the zero marker as an additional sp^3 carbon!

6. **<u>How do I know what’s a real line, from a carbon in my compound from an impurity that I should ignore?</u>** No simple way! With experience you can often tell, but there is no automatic way to know. For today, <u>if in doubt ask the instructor</u>! The instructor will confirm which lines you should or shouldn’t consider in doing your analysis

Chem 355 C-13 NMR Lab (Jasperse): The Experiment

General: Work with a partner. Each pair should obtain spectra and identify at least **two** of the unknowns, whose identities will come from the list below. Each individual must have run one of the two samples. (If you don't have a partner, just run two yourself. All the better for you.)

Signing up for NMR Time: You can sign up on the web to reserve time in advance. Go to http://www.mnstate.edu/jasperse/ NMR Signup is the link on the far left, and there is a login and password in the pink column. When you get into the schedule, click "Add an Event" to reserve a spot during the current week. If you want to reserve a spot in the following week, click the "Next week" arrow in the upper right corner, and then "Add an Event" there. A typical student should be able to finish within 15 minutes. If two of you are doing both samples together, you may prefer to sign up for 45 minutes combined, in case either of you has any trouble.

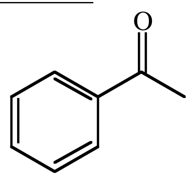
Preparing the sample

- Put in about 5 drops of sample, then add $CDCl_3$ solvent until it is about "three fingers" deep. (Typically about 1/3 full.) The volumes are not critical. Put a cap on the sample.
- Run the xmac "C13-notune".
- After you have analyzed your sample, pour the solution into the designated waste bottle, rinse your NMR tube with acetone, and return it to your drawer. Leave the tube open so that it can dry; put the cap on the closed end so that you don't lose it.

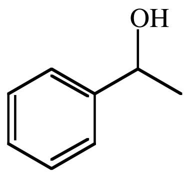
For each unknown, hand in the following:

1. For each of the 9 unknown candidates shown below, predict how many lines you'd get. Hand in this page.
2. Hand in a good, unexpanded NMR (label by hand)
   -to get one for your partner, simply type "plot" twice!!
3. Hand in at least one horizontal expansion (whether needed or not) for your sample.
4. Give a structure identification. (write the structure directly on the NMR plot)
5. Explain the reasoning for your identification. This doesn't need to be very complicated. "It's the only candidate that had 6 unique carbons and had a carbonyl group" would be sufficient, or "Had one carbonyl, one C-O carbon, and four carbons total. " Something that shows you can both count carbons and analyze the chemical shifts would suffice!

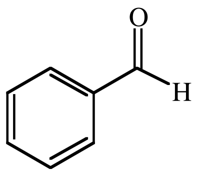
Unknown Candidates



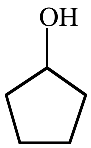
acetophenone



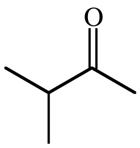
sec-phenethyl alcohol
(or DL-œ-Methyl-benzyl alcohol)



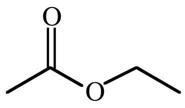
benzaldehyde



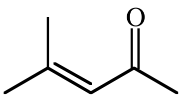
cyclopentanol



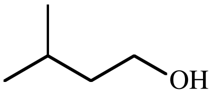
3-methyl-2-butanone



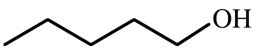
ethyl acetate
(ethyl ethanoate)



4-methyl-3-penten-2-one



isopentanol
(3-methyl-1-butanol)



1-pentanol

Running the Experiment, and What is the Instrument Actually Doing?

The overall experiment involves the following major steps:

1. Login to the Computer, Load the XWinNMR Program
2. Sample insertion
3. Calling up Standard Parameters. These include "acquisition parameters" (what experiment to run, how to run it at the instrumental level, how to tune the magnet if at all), how many "scans" to take, how to process the data once acquired, and how to plot the spectrum once the computer has it
4. Lock on Deuterium. A deuterium reference nucleus is required in the nucleus. The instrument determines the actual frequency of your photons by comparing them to the deuterium reference.
5. Tune the magnet. Inconsistencies in the magnetic field lead to line broadening. If not all nuclei experience the same magnetic field, their ΔE's will vary unnecessarily, and thus their photon frequencies, and thus the lines will be be wider. A well-tuned magnet is crucial to getting sharp spectra. The magnetic field tends to "drift" over time, and even sample to sample, so frequent tuning is necessary. Unfortunately the tuning often takes several minutes.
6. Acquire the raw data. The energy gap in NMR is in the radio frequency. To find which frequencies are absorbing/emitting, the instrument does not simply dial through all of the possible frequencies, the way that we do with a radio dialing through all the stations. Instead, the instrument irradiates all frequencies simultaneously, so as to simultaneously "excite" all the carbons at once. The irradiation then stops, after which the "excited" carbons "relax" to their ground state, and release their photons with specific frequencies. A detector "listens" to these signals. The signal is very weak, however. In order to build up signal, multiple "scans" are taken, each one consisting of an excite (irradiate)-relax (listen with detector) cycle. In today's experiment, 64 "scans" are taken and the raw signal is then averaged. This takes several minutes.
7. Process the raw data. Since each radio signal is a wave, the total signal "heard" by the detector consists of multiple radio waves superimposed on each other. Just as a human ear can recognize one "sound" amongst a multitude of sounds, so can the computer. By conducting a "Fourier Transform", the computer is able to deconvolute the complex raw signal and identify the specific frequencies involved. The spectrum is also phased so that the baseline looks level.
8. Plot

"lock" Locks the magnetic field on the deuterium signal of the $CDCl_3$. To watch this process, highlight "windows" on the top, and then click "lockdisplay" signal under the heading

"tune" The electronics that define the magnetic field are adjusted to make it homogeneous. Inhomogeneity in the magnetic field leads to line broadening and line shortening, which lead to diminished resolution and signal/noise.

"copy all" Copies all of the settings needed for the experiment to be executed, for the data to be processed, and for the spectrum to be plotted.

"rga" "Receiver Gain Automation". Optimized a signal amplifier.

"zg" "Zero go". The sample is actually scanned and the raw data is acquired.

"efp" "ExponentiaMultiplier-FourierTransform-Phase" The incomprehensible raw data is submitted to a complex mathematical treatment resulting in a meaningful, chemically sensible NMR spectrum!

"apk" "AutomaticPhase Korrection"(Bruker is a German company!) The spectrum is phased in order to give a flat baseline.

"abs" "Automatic Baseline Set" Sets a mark that enables automatic integration of proton spectra.

User's Guide to ^{1}H, ^{13}C NMR

- Note: the default mouse button is the left button. Always use the left one unless told otherwise.
- For help, see Dr. Jasperse, Hagen 411-I.

1. Login
    a. double click on **jasperse** icon (or type in "jasperse")
    (Research Users: You should use your boss's login.)
    b. Password "**chem355**" (need underline in between)
    c. double click on "**xwinnmr**"

2. Sample Insertion/Lock/Tune
    a. remove cap from spectrometer if needed, and then click **LIFT ON/OFF** key on upper lefthand corner of SCM keyboard (to right of computer) to lift lock sample
    b. place your sample in sample holder, adjust position using depth gage, and place in spectrometer [DO NOT PUT SAMPLE IN WITHOUT THE SAMPLE HOLDER! YOU WILL BREAK YOUR SAMPLE AND WRECK THE INSTRUMENT!]
    c. click **LIFT ON/OFF** key on SCM keyboard to lower sample
3. Acquiring the spectrum
    a. type "**xmac**"
        - a listing of suggestions will come up
        - at present, all of these assume $CDCl_3$ as solvent
        - instructors/researchers, these can be easily customized for you needs. See Jasperse
    b. select the experiment of interest, normally **ah1-tune** or **c13-tune** or **c13-notune**
        - Note: if you are going to run both ^{1}H and ^{13}C on the same sample, you don't need to tune twice. Run "ah1-tune" first for hydrogen, then "c13-notune"
    c. when asked for file name information, type your name into the name box and
    d. type "**chem355**" into the "user" box.
    e. click **SAVE**
    f. click **COPY ALL** when the box comes up
        - The computer will now do everything for you: read in the correct parameters, lock, tune if specified, adjust the receiver gain, acquire the spectrum, phase the spectrum, and store the phasing information for automatic integration.
    g. When "ns = 8 (or 128)" box pops up, hit return to accept default, or else enter something different for the number of scans
    h. wait patiently until either an "**xmac:finished**" or "**abs finished**" message appears
        - Hopefully this whole process will take less than 5 minutes for proton or less than 8 minutes for a carbon spectrum. If 8 minutes have passed and still incomplete, see Jasperse...
    i. click on the ⊼ icon (upper right corner of the icon group) to adjust the vertical scale of the viewed spectrum. (For example, if your baseline looks flat, this will fix it!)

4. Plot
    a. click **PLOT** icon
    b. hit return in response to any boxes that appear
    c. To do **horizontal expansions**, **manual integrations** or **vertical expansions**, see instructions on page 2.

5. Exiting:
    a. Replace your sample with the default sample, as described in part 3.
    b. Type "lock cdcl3"
    c. type "exit"
    d. Say OK if it asks you anything about closing things
    e. put cursor outside of any boxes into the blue area, then press the *right* mouse button, click **Logout** and click **Yes**.

1. Plotting **Horizontal Expansions**
   a. Make sure that the cursor is somewhere on the spectrum.
   b. Click the **left mouse button**. You will now get a doubled arrow.
   c. Move the doubled arrow to the left end of the area you want to expand and click the **center mouse button** do define the **left boundary**.
   d. Move the doubled arrow to the right end of the area you want to expand and again click the **center mouse button** to define the **right boundary**.
   e. click **PLOT** icon
   f. To get back to the full expansion, click the |◁▷| icon
   g. To get out of the "doubled arrow" mode, click the **left mouse** button

2. **Manual Integration**
   a. click **INTEGRATE**
   b. define the regions of interest (see horizontal expansion instructions above)
   c. click **RETURN** and save your integral regions
      - Sometimes you may wish to improve the “flatness” of the integral, or you may wish to assign calibration values of your own choosing. Do the following:
   d. put the arrow within the region of your integral, and click the **<u>left</u> mouse button**. The integral under consideration will then get a star by it.
   e. click **CALIBRATE** and respond accordingly
   f. adjust the **BIAS** in order to get the left side of the integral level
   g. then adjust the **SLOPE** to get the right side of the integral level

3. Reducing the noise in noisy, dilute ^{13}C spectra. "**Power Spectrum**" .
   [**<u>Do not use for 1H spectra!</u>**]
   a. After getting the normal spectrum, type "**ps**"
   b. click on the ⊼ icon (upper right corner of the icon group) to adjust the vertical scale of the viewed spectrum.
   c. click **PLOT** icon
   - Note: The "ps" command can make plots look prettier, by de-emphasizing noise. It does so by squaring all signals, however, so it will also de-emphasize small peaks that are real. In addition, by changing the relative sizes of peaks, it is incompatible with integration.

4. **Vertical Expansions**
   a. Type "**cy**" and increase or decrease the default value as you see fit. Doubling will double the printed heights, tripling will triple the printed heights, etc.
   - At default, cy=14, and is set so that the tallest peak in the spectrum will be 14cm tall. Thus, if you are wanting to expand a peak that is too tall, you need to multiply the cy as needed.

5. Manual Phasing
   a. click **PHASE**
   b. Click **BIGGEST**
   c. Click **PHO**, and keep finger held down
   d. -drag, to adjust phase of biggest, marked peak
   e. Click **PH1**, and keep finger held down
   f. -drag, to adjust phase of peaks distant from biggest
   g. click **RETURN**
   h. type "<u>abs</u>" if you want integrations to be automatically printed as a result

6. Printing Titles
   a. type “**setti**” (for “set title”)　　b. delete existing title and type in new one
   c. click **<u>save</u>**　　d. click **<u>quit</u>**　　e. type “**title**”　　f. choose **<u>yes</u>**

C13-NMR Interpretation

1. **Count how many lines** you have. **This will tell you how many types of carbons** you have. (Symmetry equivalent carbons can at times cause the number of lines to be less than the number of carbons in your structure.)
    a. Each “unique”carbon gives a separate line.
    b. Symmetry duplicates give the same line.
    c. If there are more carbons in your formula than there are lines in your spectrum, it means you have symmetry.

2. **Check diagnostic frequency windows** (“chemical shift windows”) of the lines **to provide yes-or-no answers regarding the presence or absence of key functional groups** in your molecule.

220-160	C=O carbonyl carbons, sp^2 hybridized
160-100	C alkene or aromatic carbons, sp^2 hybridized
100-50	C-O oxygen-bearing carbons, single bonds only, sp^3 hybridized
50-0	C alkyl carbons, no oxygens attached, sp^3 hybridized

3. **Check Splitting**. C13 NMR’s are often acquired as “decoupled” spectra, in which each carbon signal appears as a singlet. This is the way our laboratory C13 NMR’s come out. However, at the cost of extra time it is also possible to get “coupled” C13 NMR’s with splitting. These splitting values are very useful, and follow the N+1/N-1 rules (the number of lines is one greater than the number of attached H’s).

Quartert (q)	CH_3
Triplet (t)	CH_2
Doublet (d)	CH
Singlet (s)	C (no attached hydrogens).

4. **Signal Height/Size**
    a. Carbons without any attached H’s are short. This is common for carbonyls (aldehydes are the only carbonyl carbons that have hydrogens attached) and for substituted carbons in a benzene ring.
    b. Symmetry duplication multiplies signal height (if you have two copies of a carbon, the line will probably be taller than normal!)

5. **Aromatics, Symmetry, and C-13 Signals**. Most aromatics have symmetry, and both the number of aromatic lines and the splitting of the aromatic lines can be indicative of the substitution pattern on a benzene. Mono- and para-disubstituted benzenes have symmetry.

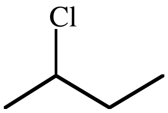
4 lines	s, d, d, d	Monosubstituted benzene. (Has symmetry).
4 lines	s, s, d, d	Para-disubstituted benzene. (Has symmetry).
6 lines	s, s, d, d, d, d	Ortho- or meta-disubstituted benzene. (Has no symmetry).

Chem 355-Jasperse
STRUCTURAL EFFECTS ON SUBSTITUTION REACTIONS

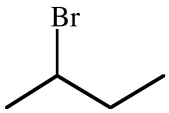
Structures:

1-chlorobutane
1

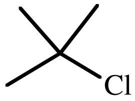
1-bromobutane
2



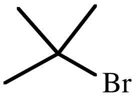
2-chlorobutane
3



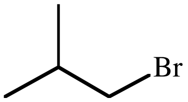
2-bromobutane
4



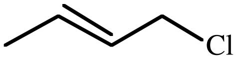
2-chloro-
2-methylpropane
5



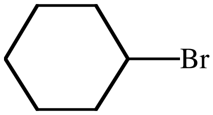
2-bromo-
2-methylpropane
6



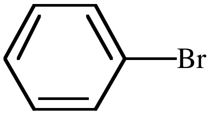
1-bromo-
2-methylpropane
7



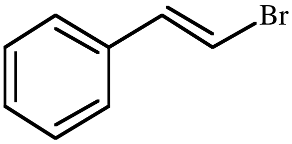
1-chloro-2-butene
8



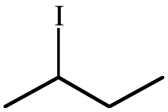
bromo-
cyclohexane
9



bromobenzene
10



ß-Bromostyrene
11



2-iodobutane
12

General Procedure: In each test, add 5 drops of haloalkane to a test-tube, then add 1 mL of solution (NaI/acetone for the S_N2 reactions, $AgNO_3$/ethanol for the S_N1 reactions), [stopper the tube in the case of **8**, which is smelly], mix by swirling vigorously (for NaI reactions, if you get a precipitate at first make sure you shake it/mix it initially; sometimes an initial false precipitate forms and persists that would dissolve if you swirl well), and watch for the formation of precipitate. For the NaI experiments, after 3 minutes warm test tubes in a 50° water bath if neither of them react; keep heating until at least one of them gives precipitate.

What is happening, and what are the precipitates? In the NaI experiments, substitution by iodide generates either insoluble NaCl or NaBr. In the second set of experiments insoluble AgCl or AgBr are reaction products as the halide is substituted by an ethoxy group. Thus, in both types of reaction the formation of precipitate gives a qualitative and visible measurement of relative reaction speed.

For the S_N2 reaction (Part 1), need samples 1, 2, 3, 4, 7, 8, 10, 11. For the S_N1 reaction (Part 2), need all samples except for 7.

Notes

1. Crotyl chloride **8** is a lachrymator (makes you cry). Do not spill it, and when you rinse it out do so in the hood!
2. You are using so many test tubes that you will need to wash them between sets of experiments. Make sure that they are washed very carefully, with water and then acetone, before reusing. If there is residual haloalkane in a tube, it can really mess up your results and give you false positives. If there is water in your test tubes, it will dissolve NaCl/NaBr salts and give you false negative data.
3. In part 1, the NaI/acetone should be added last. Otherwise you get false precipitate when relatively non-polar haloalkane can cause some of the NaI to precipitate. NaI precipitate should dissolve upon mixing/shaking.
4. In NaI reactions, often yellow color will develop. This means nothing. Iodide is air-oxidized to yellow iodine, but this has no pertinence to the experiment.
5. Silver nitrate spills give brown spots! Avoid spilling. A spot on your fingernail will last till your nail grows out! (And on your clothes, forever?).

Some Arrow-Pushing Guidelines

1. Arrows follow electron movement.

2. Some rules for the appearance of arrows
   - The arrow must begin from the electron source. There are two sources:
     a. An atom (which must have a lone pair to give)
     b. A bond pair (an old bond that breaks)
   - An arrow must always point directly to an atom, because when electrons move, they always go to some new atom.

3. Ignore any Spectator Atoms. Any metal atom is always a "spectator"
   - When you have a metal spectator atom, realize that the non-metal next to it must have negative charge

4. Draw all H's on any Atom Whose Bonding Changes

5. Draw all lone-pairs on any Atom whose bonding changes

6. **KEY ON BOND CHANGES**. Any two-electron bond that changes (either made or broken) must have an arrow to illustrate:
   - where it came from (new bond made) or
   - an arrow showing where it goes to (old bond broken)

7. **Watch for Formal Charges and Changes in Formal Charge**
   - If an atom's charge gets more positive ⇒ it's donating/losing an electron pair ⇒ arrow must emanate from that atom or one of it's associated bonds. There are two "more positive" transactions:
     - When an anion becomes neutral. In this case, an arrow will emanate from the atom. The atom has donated a lone pair which becomes a bond pair.
     - When a neutral atom becomes cationic. In this case, the atom will be losing a bond pair, so the arrow should emanate from the bond rather than from the atom.

   - If an atom's charge gets more negative ⇒ it's accepting an electron pair ⇒ an arrow must point to that atom. Ordinarily the arrow will have started from a bond and will point to the atom.

8. **When bonds change, but Formal Charge Doesn't Change, A "Substitution" is Involved**
   - Often an atom gives up an old bond and replaces it with a new bond. This is "substitution".
   - In this case, there will be an incoming arrow pointing directly at the atom (to illustrate formation of the new bond), and an outgoing arrow emanating from the old bond that breaks

Chem 355-Jasperse Name:

STRUCTURAL EFFECTS ON SUBSTITUTION REACTIONS

Part 1: The S_N2 Reaction (NaI/acetone)

Report your observations, based on quantity of precipitate formation per time. Do you get instant precipitation? Does it take minutes for much precipitate to build up? Do you need to heat in order to get much precipitate? After comparing, rank the relative reactivity of the competing substrates.

1. Leaving Group: Br vs Cl Run **1** vs **2**, and **3** vs **4**

2. Primary/Secondary/Tertiary: Run **2** vs **4**, **1** vs **3** (Also, make a prediction: Should **6** be fastest or slowest, compared to **2** and **4**?)

3. Double bonds part 1: Alkyl vs. Allylic: Run **1** vs **8**

4. Compare **2** vs **8**. This is an apples/oranges comparison; which is more important, the leaving group or the allylic double bond effect?

5. Double bonds part 2: Alkyl vs. Alkenyl (“vinyl”) or Aryl. Run **2** vs **11** vs. **10** (look for just one winner, neither of two losers should react at all).

6. Steric effects: Run **2** vs **7**
-notice that both are primary, so why should there be any difference between them?

7. Temperature. Did heating samples sometimes lead to reactions that didn’t go at room temperature?

Part 2: The S_N1 Reaction ($AgNO_3$/ethanol)

1. Leaving Group: I vs. Br vs Cl Run **12** vs **3** vs **4**; also run **5** vs **6**

2. Primary/Secondary/Tertiary: Run **1** vs **3** vs **5**; and run **2** vs **4** vs **6**

3. Double bonds part 1: Alkyl vs. Allylic: Run **1** vs **8**

4. Double bonds part 2: Alkyl vs. Alkenyl (“vinyl”)/Aryl. Run **9** vs **10**

Name:

STRUCTURAL EFFECTS ON SUBSTITUTION REACTIONS

1. When considering the leaving groups I, Br or Cl, what was the relative reactivity in S_N1 reactions? In S_N2 reactions (didn't actually use the iodide there)?

2. When considering primary versus secondary versus tertiary haloalkanes, what was the relative reactivity toward S_N1 reactions? Toward S_N2 reactions (we didn't actually run a tertiary there)?

3. What was the effect of the "allylic" double bond in **8** on S_N1 reactivity? On S_N2 reactivity?

4. What was the effect of the halide being <u>directly</u> attached to an aryl/alkenyl carbon (**10** and **11**) on the S_N2 reactivity? S_N1 reactivity?

5. Both **2** and **7** are primary bromides. Can you explain the difference in their S_N2 reactivity, if there was any?

6. What would be the mathematical effect of carrying out the sodium iodide-in-acetone reactions with the alkyl halides using an iodide solution half as concentrated?

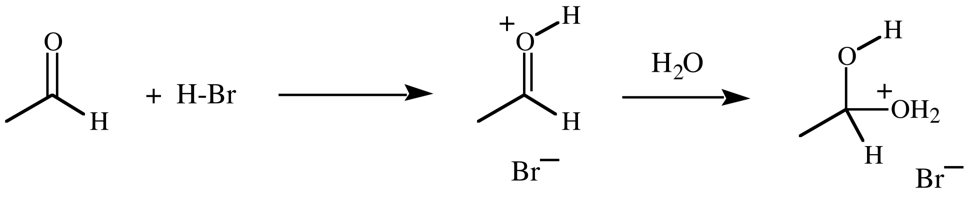
7. Thought question: The addition of KI is used to "catalyze" many S_N2 substitution reactions (R-Br to R-Z) when the nucleophile "Z" is relatively weak? [In these cases the reaction is slow in the absence of iodide and is much slower than the S_N2 reactions you observed with iodide as nucleophile in this experiment.] Why should iodide make the overall substitution go faster (considering that iodide can function as both a nucleophile and a leaving group)?

Name:

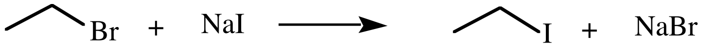
Arrow-Pushing Practice:

- Draw arrows for each of the steps in the following reactions.
- Include all formal charges, where present.
- Include all lone-pairs on atoms that react.
- Draw in all hydrogens on atoms that react. (It is not useful to draw in all H's on atoms that don't react.)
- Remember that arrows track the movement of electrons, so an arrow should go from the source of electrons and point directly to the atom that accepts them.

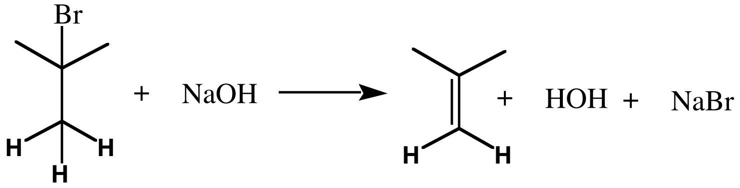
1. (Old Test)



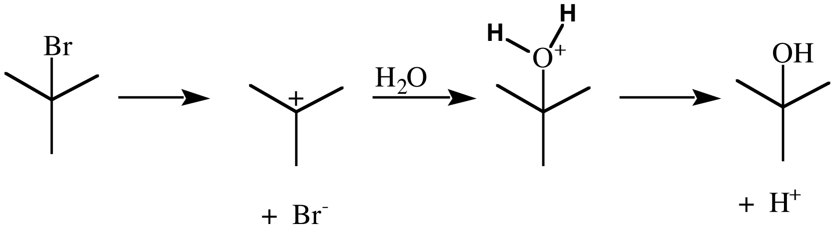
2. S_N2



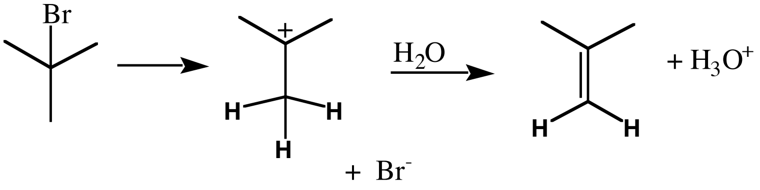
3. E2



4. S_N1



5. E1



Chem 355 Jasperse 1H-NMR

Introduction to 1H-NMR Spectroscopy Hydrogen NMR spectroscopy is considerably more complex than 13C-NMR. The interpretation is more difficult. However, the extra complexity provides extra information that is unavailable from carbon NMR. In interpreting carbon NMR, we basically focused on only two things, how many carbon lines were present, and where they were located (chemical shifts). Both of these are also central to hydrogen NMR, but two additional factors, "integration" and "splitting", are also useful.

The four facets of 1H NMR spectroscopy can be summarized as follows:

1. The number of signal sets ⇒ the number of symmetry-unique hydrogen types
2. The chemical shifts (frequency) of each signal set ⇒ the chemical environment/hybridization/functional groups
3. The integration of each signal set ⇒ how many hydrogen atoms cause a signal.
   - 3H ⇒ CH_3 group
   - 2H ⇒ CH_2 group
   - 1H ⇒ CH or OH group
4. The splitting of each signal set ⇒ information about what is connected to a given carbon
   - N lines ⇒ N-1 "neighbor" H's (when working from spectrum to structure)
   - N neighbors ⇒ N+1 lines (when you know what a structure is, and you're trying to predict what it's spectrum should look like)

Summary of Steps in 1H NMR Interpretation: (Not all will be needed to get the Answers Today)

3. **Count how many signal sets** you have. **This will tell you how many types of hydrogen-bearing carbons** you have. (Hydrogens attached to symmetry-equivalent carbons will give equivalent signals)
4. **Check diagnostic "chemical shift" windows** of the lines **to provide yes-or-no answers regarding the presence or absence of key functional groups** in your molecule.
5. Check the integration of each signal set.
   - 3H ⇒ CH_3 group 2H ⇒ CH_2 group 1H ⇒ CH or OH group
6. Check the splitting of each signal set.
   - For a signal set with N lines ⇒ N-1 hydrogens will be attached to carbons directly connected to the carbon of the signal set

I. Number of Signal Sets

1. Nonequivalent H's have different chemical environments and give different signals
2. Symmetry-equivalent H's have the same chemical environment and give the same signal
   - Thus the number of signal sets tells you how many different types of hydrogens are present
3. Ways for the number of signal sets to differ from the number of carbons:
   a. Symmetry duplication: two (or more) carbons give only one type of hydrogen and one signal set
   b. Hydrogen-free Carbons: No attached H, no H signal! (Carbonyl carbons rarely have H's…)
   c. OH Groups: OH as well as CH's give hydrogen signals
   d. CH_2 H's are NONEQUIVALENT in Two "Cis/Trans" Cases:
      - When there is a **chiral center** in the molecule. In 2-bromobutane, one of the CH_2 H's on C-3 is cis to the bromine, the other is trans. The cis and trans H's will give different signals.
      - In **Alkenes**. In propene ($CH_3CH{=}CH_2$), one of the CH_2 H's is cis to the methyl, the other is trans. They are in different environments and would give different signals.
4. On an **achiral** molecule (alkenes excepted), hydrogens on a given carbon will be equivalent.
   - all three H's on a CH_3 group will be equivalent
   - both H's on a CH_2 group will be equivalent.
5. Strategy Keys:
   a. If possible, determine how many signal sets you have in a spectrum. (Useful when working from spectrum to structure).
   b. For a particular structure, determine how many signal sets you should have. (Useful when matching unknown candidate structures with actual spectra, as in today's lab.)
   c. **End-Check: Check that the number of signal sets in your spectrum matches with the structure you believe you actually have! If not, structure needs correction!**

II. "Chemical Shifts" of the Signal Sets

9's (9.0-10.0)	**Aldehyde** sp^2 hybridized C-H's
7's (6.5-8.4)	**Aromatic** sp^2 hybridized C-H's
5's (4.8-6.8)	**Alkene** sp^2 hybridized C-H's
3's (2.8-4.5)	**Oxygenated** sp^3 hybridized C-H's (halogenated and nitrogenated alkyl C-H's will also come in this window, although no candidates for today's lab). Oxygenated sp^3–carbons are routinely present for the following functional groups that contain oxygen single bonds: • **alcohols**, • **ethers**, or • **esters**
2's (1.8-2.8)	**Allylic** sp^3 hybridized C-H's (sp^3 hybridized C-H's that has a double bond attached to the sp^3 hybridized C). Allylic signals routinely appear when one of the following double-bonded functional groups is present: • **carbonyls**, (ketones, esters, aldehydes, acids, amides) • **alkenes**, or • **aromatics**
1's (0.7-2.0)	sp^3 hybridized C-H's, with **no attached Functional Groups** • **Note:** Many molecules with non-functional alkyl portions will give a lot of signal in this area.
0-12 (anywhere!)	**Alcohol/Acid** O-H hydrogens (N-H hydrogens likewise) • **alcohols**, • **carboxylic acids**

How do I process and use what I see from my Chemical Shifts?

1. **Recognize OH's.**.
   - An OH can come anywhere, and can easily cause you to make a mistaken conclusion about a feature group. For example, if you have an OH and it comes in the 2's, and you conclude that you have an allylic C-H, that might send you down a bad blind alley. Or if you have an OH that appears in the 5's, you might falsely deduce that you have an alkene, etc.. Thus it is really helpful to recognize OH's when they appear so that they don't confuse you.
   - Two recognition factors for OH signals:
   1. They always **integrate for 1H**, never for 2H or 3H
   2. They normally **appear as singlets, normally somewhat broad**. C-H signals tend to be sharper, and any C-H signal that integrates for one will have significant splitting. The only way to have a 1H that doesn't split is for it to be an OH.
   3. If you have an OH signal, of course you will also have some C-H signals in the 3's area.

2. **Check each of the zones. Each one gives you a yes or no answer about the presence of absence of the featured group**.
   - o Do I have something in the 9's? If yes ⇒ aldehyde
   - o Do I have something in the 7's? (Other than a solvent singlet…)? If yes ⇒ aromatic
   - o Do I have something in the 5's? If yes ⇒ alkene
   - o Do I have something in the 3's? If yes ⇒ alcohol, ether, or ester
   - o Do I have something in the 2's? If yes ⇒ ketone, aromatic, or alkene
   - o Do I have something in the 1's? If yes ⇒ some nonfunctional alkyl carbons

3. **End-Check: Check that the functional groups indicated by your chemical shift information match with the structure you believe you actually have! If not, structure needs correction!**

Miscellaneous Chemical Shifts Notes

7. The regions are somewhat approximate, and have some spillover. But it's useful to basically talks about the "1's", "2's", "3's", etc. to discuss the major windows. Even though something might actually come at 4.2, it's still useful to refer to that as appearing in the "3's" group and make conclusions accordingly. I'll still refer to something as coming in the "1's" group even if it comes at 0.8.
8. Notice that sp^2 hybridized C-H's come above 5, sp^3 hybridized C-H's come below
9. Notice that oxygenated C-H's come higher than non-oxygenated analogs. An sp^3-hybridized C-H's with an attached oxygen comes higher than without (3's versus 1's), just as an sp^2-hybridized C-H's comes higher with an attached oxygen (10's) than without (5's, 7's)
10. The above windows are sufficient for this week's lab. In future, and for more complex molecules, there are more complex ways for a C-H to come in some of the above window. For example, an sp^3-hybridized C-H with two attached oxygens can come in the 5's, or an sp^3-hybridized C-H that is doubly allylic (for example, two attached carbonyls) can come in the 3's. But for beginning, none of our C-H's will be impacted by more than one attached functional group at a time.
11. OH's are real wildcards because they can come anywhere, and can easily get you confused.

III. Integration In C-13 NMR we didn't really use the heights or sizes of the signal in any quantitative way. However, the sizes of H-NMR signal sets are very useful and informative.

1. All hydrogens give an equal amount of signal
2. When there is symmetry duplication of a hydrogen, the resulting signal will be multiplied accordingly!
3. The key is not the signal height, but rather the signal **area**.
4. The signal **area** is measured by "integration lines". Make sure to differentiate integration marks, and what they mean, from signal lines themselves.
5. **Relative areas directly correlate ratios of H's**
6. These **must be simple whole-number ratios** (2:1, 3:1, 3:2, etc..)
   - Convert the "computer" ratios to simple whole-number ratios
   - Round off freely! The computer isn't normally very precise, easily 10% errors
7. Clean sets involving equivalent H's give clean, symmetric signal sets:
   a. 1H ⇒ CH or OH
   b. 2H ⇒ CH_2
   c. 3H ⇒ CH_3
   d. 6H ⇒ 2 equivalent CH_3 groups
8. Unsymmetrical messy sets involving overlapping signal sets: (these will routinely not look nice and symmetric…)
   a. 3H ⇒ OH overlapping a CH_2
   b. 4H ⇒ two overlapping but not exactly equivalent CH_2 groups; or a CH_3 overlapping an OH or CH
   c. 5H ⇒ common in the 7's, for 5 overlapping arene H's; also common in the 1's, when a CH_3 and CH_2 overlap
9. Unfortunately having signal sets overlap is all too common

How do I process and use what I see from my Integrations?

1. **Distinguish "Clean" Signal Sets from Overlapping Signal Sets**
   - o Clean ones look symmetric, overlapping sets do not

2. **For the Clean sets, the integration tells you what kind of group you have**
   a. 1H ⇒ CH or OH (methine or hydroxyl group)
   b. 2H ⇒ CH_2 (methylene group)
   c. 3H ⇒ CH_3 (methyl group)
   d. 6H ⇒ 2 equivalent CH_3 groups

3. **End-Check: Check that the "groups" your integration shows match with the structure you believe you actually have! If not, your structure needs to be corrected!**

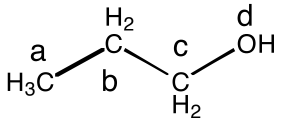
IV. Splitting In C-13 NMR all of our carbon lines came out as nice simple single lines. However, in H-NMR hydrogen signals are routinely split into multiple lines. The number of lines in a signal set tell us nothing about the C-H's themselves that cause the signal (whether it's a CH_3 or CH_2 group, whether it's an sp^3 or sp^2 carbon, whether it's allylic or oxygenated…). But the splitting tells us something else that is really useful: what kind of CH groups are attached to the group of interest! Splitting tells us nothing about the group itself, but it does provide great information about neighbor groups.

Rules of "Splitting"

- ☐ **N-1 Rule: N lines ⇒ N-1 neighbor H's** (H's directly attached to carbons attached to the C-H group causing the signal)
  - The N-1 Rule is useful when working from spectrum to actual structure
- ☐ **N+1 Rule: N neighbor H's ⇒ N+1 lines**
  - The N+1 Rule is useful when working from structure to actual spectrum

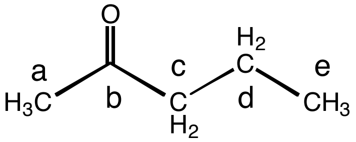
1. OH hydrogens don't participate in splitting (normally)
2. Only C-H hydrogens participate in splitting (normally)
3. For today's labs and for simple molecules, the N-1/N+1 rules are good. However, the rules actually are accurate only if the neighbor H's are equivalent. The rule can break down when some of the neighbor H's differ significantly from each other
4. Splitting from H's further distant than neighbor carbons sometimes occurs, but usually the amount of splitting is too small to worry about
5. Physics Origin: hydrogens are quantized little magnets. Having neighbor hydrogens is equivalent to having local magnets that can either reinforce the external field (spin up) or counteract the external magnetic field (spin down).

N+1 Rule (Given structure, how many lines a spectrum should give)



	a	b	c	d
Neighbors	2	3+2	2	0
Lines	3	6	3	1

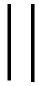
(Notice: OH doesn't split...)



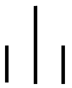
	a	b	c	d	e
Neighbors	0	-	2	2+3	2
Lines	1	-	3	6	3

N-1 Rule (Given spectrum, how many neighbors a structure should have)

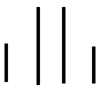
Lines 1 (s)inglet
Neighbors 0



Lines 2 (d)oublet
Neighbors 1



Lines 3 (t)riplet
Neighbors 2



Lines 4 (q)uartet
Neighbors 3

etc.

6. Splitting nicknames:
   - 1 line ⇒ singlet (s) 2 lines ⇒ doublet (d) 3 lines ⇒ triplet (t)
   - 4 lines ⇒ quartet (q) 5 lines ⇒ pentet (p) >5 lines ⇒ multiplet (m)

How do I process and use what I see from my Splitting?

1. For a given signal set, use integration to determine if you have a CH_3, CH_2, or CH group
2. Then use the number of lines in the signal set and the N-1 Rule to see how many hydrogens must be present on neighboring carbons
3. **End-Check: Check that the structure you believe you actually have would give the splitting you are actually seeing in your spectrum. If not, your structure needs to be corrected!**

V. Standard Summary Report There is a standard summary report format for H-NMR's which addresses chemical shift, integration, and splitting. Normally an interpretation/correlation with the actual structure is also included.

Ex: $CH_3OCH_2CH_2CH_2C(O)CH_3$ (I'll number the carbons from left to right…)

Standard report format (approximate chemical shift range, integration, splitting, and interpretation of which signal correlates to which group in the structure…)

3's, 3H, s (CH_3-1)
3's, 2H, t (CH_2-2)
1's, 2H, p (CH_2-3)
2's, 2H, t (CH_2-4)
2's, 3H, s (CH_3-6)

VI. Miscellaneous

1. **Subtracting the Solvent Lines: Don't Count the Singlet at 7.26** The sample is always diluted in a solvent. We will routinely use $CDCl_3$, specifically because it has no H's! However, it is not totally pure, and usually is contaminated by a small amount of $CHCl_3$, which gives a signal at 7.26. Ignore this signal!

2. **Subtracting the Reference Line: Don't Count the Line at 0** A reference chemical $[(CH_3)_4Si]$ is normally included that gets used to define where "zero" is. This zero marker is present all the time, but is not part of your actual molecule. Ignore this signal!

3. **Subtracting the Water Line:** Often a little moisture will be in the solution, probably because it gets into the $CDCl_3$ solvent bottle. This will often appear somewhere around 1.6, but it often drifts depending on hydrogen-bonding factors. Ignore this signal!

4. **Subtracting the Acetone Line?** Sometimes students will have washed their NMR tube with acetone, but not all the acetone will have had a chance to evaporate. If residual acetone is present, it will give a singlet at 2.15. Unfortunately this is about the same place where other methyl groups that are connected to carbonyls come. One hint that a 2.15 line is acetone and not actually part of your molecule is if it integrates funny, i.e. is either too big or too small to integrate correctly. One other hint is to ask the instructor!

5. **How do I know what's a real signal versus a signal arising from an impurity that I should ignore?** For today, if in doubt ask the instructor! The instructor will confirm which lines you should or shouldn't consider in doing your analysis. However, one useful recognition tip is if something integrates badly. Integrals are supposed to be nice whole-number ratios (1:1, 2:1, 3:2, etc.). So if something integrates at a 0.1:1 ratio compared to the next smallest signal set, it's likely just an impurity.

6. **Beware of Overlapping.** Overlapping is most routine in the benzene area (7's), and also in the alkyl area (1's), but happens elsewhere as well. OH signals also often overlap other signals.

VII. Review + Summary

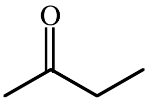
1. Count **how many signal sets** you have.
2. Check **"chemical shift" windows** of the lines to provide yes-or-no answers regarding the **presence or absence of key functional groups** in your molecule.
3. Check the **integration** of each signal set.
   - 3H ⇒ CH_3 group 2H ⇒ CH_2 group 1H ⇒ CH or OH group
4. Check the **splitting** of each signal set.
   - N lines ⇒ N-1 neighbor hydrogens

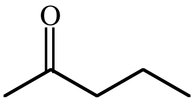
☐ Beware of misinterpreting overlapping signals
☐ Beware being confused by signal sets caused by solvents or impurities

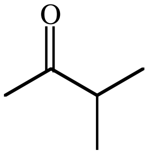
5. **End-Check: Check that the structure you believe you actually have would give the number of signal sets you have, the chemical shifts you have, the integrations you have, and the splittings that you have. If not, your structure needs to be corrected!**

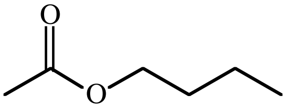
Chem 355 H-NMR Lab (Jasperse): The Experiment

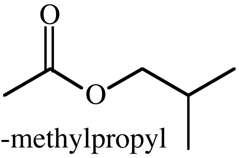
Unknown Candidates

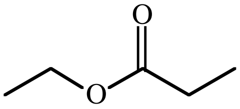

2-butanone

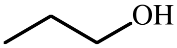

2-pentanone

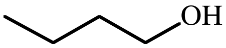

3-methyl-2-butanone

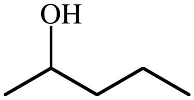

butyl ethanoate

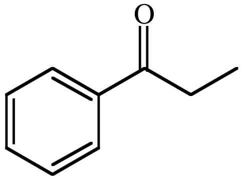

2-methylpropyl ethanoate

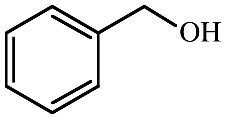

ethyl propanoate


1-propanol


1-butanol


2-pentanol

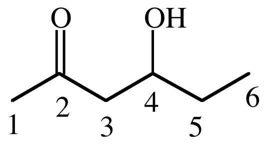

propiophenone


benzyl alcohol

Instructions for Lab Report

1. Each student (not partner pair) must run **two NMR spectra** (from unknowns labeled H1, H2, ...).
   - (See instructions below for sample preparation)
   - In some cases running horizontal expansions may make it easier for you to see splittings clearly. But horizontal expansions are not required on my account.
2. Identify both unknowns. Write the structures on your spectra.
3. For each unknown, next to the deduced structure provide a little summary with the format exemplified. Write the structure clearly, assign some arbitrary number system, and then summarize the observed spectroscopic information:

chemical shift (1's, 2's, or whatever), integration (1H, 2H, or whatever), splitting (s, d, t, or whatever) , and interpretation for each hydrogen type (CH_3-1, or whatever).



2's, 3H, singlet	CH_3-1
2's, 2H, doublet	CH_2-3
3's, 1H, multiplet	CH-4
1's, 2H, multiplet	CH_2-5
1's, 3H, triplet	CH_3-6
3's, 1H, singlet	OH

Sample preparation:

1. put in 2 drops of unknown, and dilute to the three-finger depth with $CDCl_3$.
2. Run the experiment "ah1-tune".
3. Do horizontal expansions if needed to see splittings clearly.

Summary of 1H-NMR Interpretation

I. Number of Signal Sets
II. "Chemical Shifts" of the Signal Sets

9's (9.0-10.0)	**Aldehyde** sp^2 hybridized C-H's
7's (6.5-8.4)	**Aromatic** sp^2 hybridized C-H's
5's (4.8-6.8)	**Alkene** sp^2 hybridized C-H's
3's (2.8-4.5)	**Oxygenated** or **Halogenated** sp^3 hybridized C-H's (halogenated and nitrogenated alkyl C-H's will also come in this window, although no candidates for today's lab). Oxygenated sp^3–carbons are routinely present for the following functional groups that contain oxygen single bonds: • **alcohols**, • **ethers**, or • **esters**
2's (1.8-2.8)	**Allylic** sp^3 hybridized C-H's (sp^3 hybridized C-H's that has a double bond attached to the sp^3 hybridized C). Allylic signals routinely appear when one of the following double-bonded functional groups is present: • **carbonyls**, (ketones, esters, aldehydes, acids, amides) • **alkenes**, or • **aromatics**
1's (0.7-2.0)	sp^3 hybridized C-H's, with **no attached Functional Groups** • **Note:** Many molecules with non-functional alkyl portions will give a lot of signal in this area.
0-12 (anywhere!)	**Alcohol/Acid** O-H hydrogens (N-H hydrogens likewise) • **alcohols**, • **carboxylic acids**

4. Recognize OH's..

Check each of the zones. Each one gives you a yes or no answer about the presence of absence of the featured group.

5. End-Check: Check that the functional groups indicated by your chemical shift information match with the structure you believe you actually have! If not, structure needs correction!
6. The regions are somewhat approximate, and have some spillover.
7. For multi-functional complex molecules, there are more complex ways for a C-H to come in some of the above window. For example, an sp^3-hybridized C-H with two attached oxygens can come in the 5's, or an sp^3-hybridized C-H that is doubly allylic can come in the 3's. In other words, the impact of functional groups is roughly additive.

III. Integration These **must be simple whole-number ratios** (2:1, 3:1, 3:2, etc..)

IV. Splitting

- **N-1 Rule: N lines ⇒ N-1 neighbor H's** (H's directly attached to carbons attached to the C-H group causing the signal)
  - The N-1 Rule is useful when working from spectrum to actual structure
- **N+1 Rule: N neighbor H's ⇒ N+1 lines**
  - The N+1 Rule is useful when working from structure to actual spectrum

Note: OH hydrogens don't participate in splitting (normally)

Chem 355 Jasperse **Chromatography**

BACKGROUND Chromatography is a powerful technique for the separation and purification of both solids and liquids on relatively small scale (ideally <10g). Chromatographic techniques are also invaluable as analytical techniques for analyzing tiny quantities of material (as little as 10^{-9} g). This is now our fourth purification technique. Advantages for chromatography are its power and generality (not limited to liquids or solids or the need for ionizability). A disadvantage is the limitation in scalability (has problems with hundreds of grams of material).

1. Recrystallization
2. Distillation
3. Liquid/Liquid Separation (Separatory Funnel Separation)
4. Chromatography

Every type of chromatography depends on the distribution of a substance between two phases, a **mobile phase** and a **stationary phase**. (In a river, the running water would be called the mobile phase and the riverbed the stationary phase…) In today's case, the mobile phase will be an organic solvent, the stationary phase a polar surface. A particular chemical will partition between being bound to the surface, where it doesn't move, and being dissolved in the solvent, such that it flows along. Thus different chemicals move at different speeds, depending on adsorption/solubility equilibrium. The more tightly the sample binds to the surface, the less it will move. Anything that impacts the sample's partition between binding to the stationary phase versus dissolving in the mobile phase will impact the sample's mobility.

Practical Summary: A sample "stuck" to a surface is "washed along" with a solvent. "Less sticky" things move faster!

Different types of chromatography use different binding principles for "sticking" to the stationary phase, and are useful in different contexts of science.

- Organic Chemistry (today): A polar surface binds polar organics
- Biochemistry
  - Size exclusion: surface pores can fit small molecules, exclude larger molecules
  - Charge: cationic surfaces bind anionic chemicals, anionic surfaces bind cationic chemicals
- Gas/Liquid Chromatography (Organic and Analytical Chemistry): the "stationary phase" is actually a nonvolatile liquid coating on the walls of a tube; the mobile phase is gas passing through the tube. Volatile chemicals are more likely to evaporate from the liquid phase and fly along in the gas phase. Less volatile chemicals are better retained in the liquid phase and thus move more slowly. Polarity can also be used to attract materials to the stationary phases.

ANALYTICAL TLC (THIN LAYER CHROMATOGRAPHY)

TLC chromatography uses glass or plastic plates coated with a thin layer of adsorbent as the stationary phase. Silica get (SiO_2•xH_2O) and alumina (Al_2O_3•xH_2O) are the most common solid adsorbents. Both are polar, hydrogen-bonding adsorbents, with lots of polar, hydrogen-bonding "sticky sites". Samples are applied to the surface, and then the organic "eluent" (solvent) is applied and runs up the plate. (The flow of the eluent results from capillary action.)

The mobility of a particular chemical depends on its partition between the mobile phase (the eluent) and the stationary phase (silica gel). The more tightly the sample binds to the silica (the "stickier" it is), the less it will move. The less well it binds, the more it will dissolve in the solvent and flow up the plate.

A typical ranking of polarity in terms of functional groups, all else being equal, is in the order shown. In practice, a more polar sample will bind to the stationary phase better, and as a result will be less mobile than a less polar, less sticky substrate.

- **Adding additional nonpolar hydrocarbon to a given molecule moves it in the non-polar direction**. For example, C_4H_9OH will be more polar than $C_7H_{15}OH$.
- **Polarity Pattern:**
  Carboxylic acids > alcohols > amines > ketones/aldehydes > esters > ethers > halocarbons > arenes > alkanes

Eluents have the same order of polarity. A good-binding polar eluent will compete for the sticky sites on the silica, and either "displace" the substrate from the surface or else prevent the solute from binding to the surface. The result is that the sample will have its adsorption/solubility partition moved away from the adsorbed side toward the dissolved side. The practical result is that the substrate will move to a greater degree.

Movement is quantified by "R_f" (**R**esponse$_{\underline{\text{f}}\text{actor}}$) values: distance the chemical travels divided by the distance that the eluent travels. For a given surface, substrate, and eluent, the R_f is characteristic and is useful for identification. For a series of substrates, their relative R_f's reflect their relative polarities.

Visualizing the samples is crucial, since most organics are colorless. UV (ultraviolet light) or a chemical colorizing dip will be used. (UV is ideal, but is only applicable to molecules with extensive systems of sp^2 atoms that are able to absorb UV light.)

Summary: For silica gel surfaces, which are polar, the following relationships are true:

1. When two substrates are run under identical conditions, **the more polar substrate will have the lower R_f; the less polar substrate will have the higher R_f**

2. When the same substrate is run under two different solvent conditions, **any substrate will have a higher R_f with the more polar eluent, and a lower R_f with the less polar eluent**.

COLUMN CHROMATOGRAPHY

While TLC is useful as an analytical tool, chromatography can also be used as a purification technique. But to separate larger than analytical quantities of chemical, a larger amount of solid surface material must also be used, and larger volumes of solvents must also be used.

The general idea is that if sufficient quantities of solvent are used, solutes will eventually "wash off" of the surface. The differential mobilities of different compounds allow them to come off at different speeds. Individual collection of the different fractions, followed by reconcentration, enables isolation of pure compounds from initial mixtures.

In practice solvent polarity "ramping" is commonly used. In this technique, a relatively nonpolar solvent is used first, which is only able to selectively wash off mobile, nonpolar substrates, while leaving polar substrates behind ("stuck" to the solid surface). Then a more polar solvent is applied which is able to selectively wash off the more polar substrate. In today's lab we will do a very abrupt increase in solvent polarity to make things go faster. But often this is done more gradually.

Pressure is frequently used to push solvent through more quickly and speed up the process. We will use modest pressure in today's experiment.

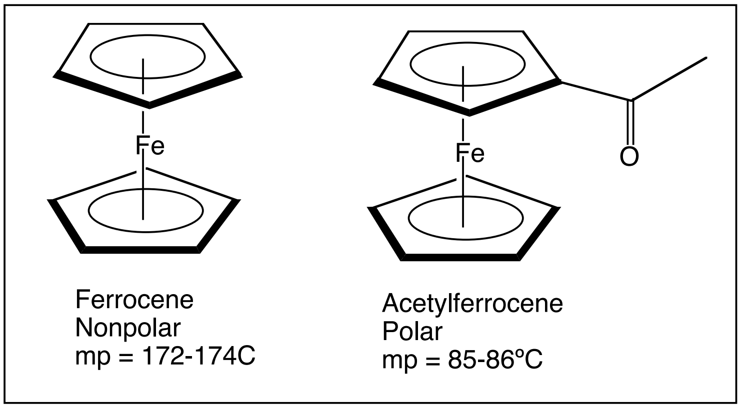
In today's experiment, the samples will be colored, so it will be relatively easy to see what is happening. Most ordinary organics are colorless. When colorless organics, a series of different solvent fractions are collected in test tubes or flasks. Then the same visualization techniques that are used for TLC are applied to determine which fractions actually have chemicals present.

In this experiment, a small-scale chromatographic separation of non-polar ferrocene (mp 172-174°) and relatively polar acetylferrocene (mp 85-86°) will be attempted. For the structures for these interesting iron-based chemicals, see p. 175. They have been chosen for the experiment specifically because, unlike most organics, they are colored. So their visible color will enable you to see the separation and chemical movement as it happens. The solid surface will be silica; the nonpolar solvent will be "petroleum ether" (a misnomer, it is a mixture of low-boiling alkane isomers); and the polar solvent will be 50/50 diethyl ether/petroleum ether.

Part I: COLUMN CHROMATOGRAPHY

Separation of nonpolar Ferrocene(mp 172-174°C) from polar Acetylferrocene (mp 85-86°C).

Overview



In this experiment, a small-scale chromatographic separation of non-polar ferrocene (mp 172-174°) and relatively polar acetylferrocene (mp 85-86°) will be attempted. These structurally interesting iron-containing molecules have been chosen for the experiment specifically because, unlike most organics, they are colored. So their visible color will enable you to see the separation and chemical movement as it happens. The solid surface will be silica; the nonpolar solvent will be "petroleum ether" (a misnomer, it is a mixture of low-boiling alkane isomers); and the polar solvent will be 50/50 diethyl ether/petroleum ether. A solution of a 50/50 mixture of ferrocene and acetylferrocene will be adsorbed onto some silica gel. Then the silica gel/sample powder will be layered on top of some clean silica. Nonpolar solvent will wash off the nonpolar ferrocene while leaving the polar acetylferrocene behind. A more polar solvent will then be used to wash off the acetylferrocene. Both wash solutions will then be concentrated to give the purified materials.

Preparing the sample:

1. Weigh out approximately 0.1g of 50/50 pre-mixed ferrocene/acetylferrocene material onto some weighing paper. (Record the exact weight of the mixture).

Preparing the column:

2. Plug the end of a 4-mL pipette (the "column") with glass wool. A long-nosed regular pipette serves as a good ramrod.
3. Weight out 1g of silica and add this to the pipet. (Use glassine weighing paper to try to make a funnel to funnel it in). Your column should be approximately half full.
4. Securely clamp the column, and tap it to try to level the surface of the silica.
5. Get 25 mL of petroleum ether.
6. Flush the column with petroleum ether three times to remove air bubbles and to saturate the column with petroleum ether.
    - While you can rely on gravity, you can save time by using a big blue pipet bulb to "push" the solvent through more quickly.
    - *Be very careful when doing this. In order to apply much pressure, you will need to push down kind of firmly. However, if you let the pipet "suck" back while it is pressed down on the column, you will suck all your column and solvent back up and mess everything up. So try to carefully remove the contact between the pressure bulb and the column before allowing a compressed bulb to re-expand. Keep the squeeze on until the bulb is clearly off the column!*
    - The petroleum ether used in these initial rinses can be recovered in your original petroleum ether flask and reused.
7. Once you have first added the solvent, *never allow the column to dry out*; this creates channels that will result in uneven bands and non-optimal separation. So avoid pushing the solvent much beyond the surface of the silica. The petroleum ether used in these initial rinses can be recovered in your original petroleum ether flask and reused.

Applying the sample to the column:

8. Take your dry sample (see above) and pour it (carefully!) into the column, again using the folded weighing paper. Again tap the surface to try to level the material.

<u>Eluting the Nonpolar Substrate</u>:

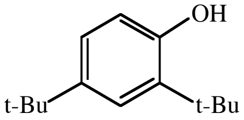
9. Select three large test tubes
10. Mark them as Tubes 1-3.
11. Pre-weight Tubes 1 and 3.
12. Use 15 mL of petroleum ether to wash the mobile, nonpolar substrate off from your column. Collect the yellow solution in Tube 1.
    - By the end of the 15 mL, the bottom of the column should be almost white, the drops coming off relatively colorless, and the orange polar material should remain at the top of the column.
13. However, to ensure that the nonpolar ferrocene is completely removed, use an additional 10 mL of petroleum ether to further wash the column. Collect these 10 mL in Tube 2. Since there should be little or no solute in this fraction, it can be thrown away after the experiment.
14. If the rotovap is free, transfer the solution from Tube 1 into a pre-weighed round-bottomed flask and use the rotovap to concentrate the solution. If the rotovap is too busy, you may prefer to concentrate your solution by simply placing Tube 1 into a hot-water bath and simply boil off the petroleum ether. The petroleum ether is quite volatile, so allowing it to stand in your drawer for a day or more will probably also allow completely dry material.
15. Determine your mass yield for your ferrocene.
16. Take a melting point for your ferrocene.

<u>Eluting the Polar Substrate</u>:

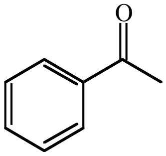
17. Following step 4 above, use 15 mL of the more polar 50/50 diethyl ether/petroleum ether solvent to wash the more polar acetylferrocene off of the column. Collect the solution in Tube 3. You should be able to see the colored band as it moves through the column.
18. To isolate the polar acetylferrocene, the solution in Tube 3 needs to be reconcentrated. You can again do this either by rotary evaporation (preweigh your flask!), by boiling Tube 3 in a hot water bath, or by allowing the solution to simply evaporate to dryness.
19. Determine your mass yield for your ferrocene.
20. Take a melting point for your ferrocene.

Part II: TLC (Thin Layer Chromatography)

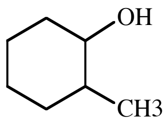
4 Substrates, Unknown Candidates



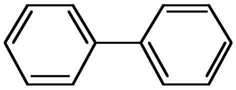
1
2,4-di-t-butylphenol



2
acetophenone



3
cis/trans mixture
of 2-methylcyclohexanol



4
Biphenyl

Goals

Use TLC mobility to observe the TLC behavior and rank the "TLC polarity" of the 4 reference substrates, from least polar to most polar. (Some may be essentially tied).

Determine how each substrate can be visualized. Which are UV active, and which appear only when using a visualizing "dip"?

Observe the dependence of TLC mobility on solvent polarity.

Calculate R_f values

Identify two unknowns by TLC.

Learn general analytical TLC techniques.

Procedure

Work with a partner on this experiment. Each of you will need at least 4 TLC plates (two for each eluent). Each of you will identify two unknowns; for one of your unknowns, use the same one as one of your partners. For the second, make it different from one your partner uses.

Preparing the Plates:

1. Prepare the two plates for your first eluent: Use a pencil to mark 4 spots.
    - Give at least a 1 cm margin from the bottom. **Otherwise the sample may subsequently submerge, get dissolved away, and not get drawn up the plate.**
    - Avoid placing spots within 0.8 cm of the sides.
    - Use a pencil to mark placement of your original spots.
2. On plate 1, write **1**, **X** (the letter for your first unknown), **Y** (the letter for your second unknown) and **2**.
3. On plate 2, write **3**, **X** (the letter for your first unknown), **Y** (the letter for your second unknown) and **4**. (Both unknowns should be on each plate).
4. Then use the capillary tubes to apply your chemical solutions onto your TLC plates.
    - Spots should be neither too heavy nor too light (strong enough to be able to visualize, but **light enough to avoid overlap and chemical "tailing"**).
    - Before running your plates, check your second plate by UV. Biphenyl (**4**) should be easily visible. If not, your spot sizes may be too small. [Note: some spots are not very UV active, and may not appear. The point here is that if you don't even see #4, which is strongly visible, something is wrong. But don't worry if some of your spots show little or nothing by UV.]

To run the TLC's:

1. Place 1 full pipette of eluting solvent into a 50-mL beaker, and put a watch-glass on top to prevent solvent evaporation.
    - The eluent choices are 2% ethyl acetate/hexane, 5% ethyl acetate/hexane, and 10% ethyl acetate/hexane.
    - One partner should run both the 2% and 5% solutions. The other partner should run both the 5% and 10% solutions. (I want both of you to run 5% just to see how much scatter there is or isn't between two different scientists.)
    - You don't want to put in so much eluent that your spot will get submerged, in which case it will get dissolved away and not get drawn up the plate.

- Ethyl acetate is an ester. The more ethyl acetate is present in the mixture, the more polar the eluent should be.

2. Then carefully put your TLC plate(s) into the eluent, and put the watch-glass back on top. The eluent will creep up the plate.
    - You can easily put both plates 1 and 2 in at the same time.
    - Avoid putting plates in crooked, or touching the walls.
    - You'd like the solvent line on the bottom to be level.
    - Note: the spots must not be submerged in solvent, or they will simply dissolve and not be drawn up the plate.
3. After the eluent has risen a significant distance, (it shouldn't actually hit the top), remove the plate(s) and immediately mark with pencil the top distance that the eluent went.
    - If it hits the top, your sample can keep creeping up the plate and you will get falsely high R_f's.
    - Note: It's important to keep the cover on your beaker. Otherwise evaporation competes with elution and you will get falsely high Rf's.

Eluent choice: You will test two different solvent mixtures, as will your partner. One of you should run both the 2% and 10% ethyl acetate/hexane solvents; the other should run both the 10% and 40% ethyl acetate/hexane solvents. Since each of you will test two solvents, and for each solvent you will need 2 plates, that means each of you will be running 4 plates total. (Or more; you may need to repeat some!)

Visualizing the spots:

1. Look at your plates under UV light, and circle with pencil the spots that you can see.
    - Spots may seem weaker than before; during the process the sample gets "stretched" out, so the signal intensity essentially gets diluted.
    - Not all samples will give UV spots.
2. After marking the UV active spots, take your plate to the "p-Anisaldehyde Dip" station. (Caution: 5% sulfuric acid!)
    - Dip your plate into the solution, using forceps
    - Try to let the excess liquid drain off
    - Then dry the plate with a heat gun.
3. Circle the new spots that appear, and record their color. (Some spots may differ in color, and the colors may have diagnostic value).

Calculate the R_f Values for All Your Spots: This will be the ratio of the distance traveled by your spot relative to the distance traveled by the solvent. While these can be evaluated with rulers, it's satisfactory to simply eyeball it.

- Do not measure relative to the bottom of the plate. Measure relative to where the spot began.

Name:

Chromatography Lab Report

Part 1: Column Chromatography

1. Part 1 Yields and Melting Ranges:

Nonpolar Ferrocene: isolated yield (in grams) melting range:

Polar Acetylferrocene: isolated yield (in grams) melting range:

Part 2: TLC

2. Fill out the Rf data in the chart below for the two columns that you ran yourself (either first two, or last two).
    - You are not required to fill in the "distance traveled" information, but many students like to write it down if they have a ruler or something. However, an eyeball estimate is satisfactory, given the qualitative nature of this experiment. Only one significant figure is probably appropriate (0.2, 0.3...)
    - Remember that the Rf value for a spot will be fall somewhere from $0.0 \leq Rf \leq 1.0$, and is the ratio of the distance traveled by the spot (middle of the spot) relative to the distance traveled by the solvent, relative to where the spot began.
    - Put a star above each of the two columns that you did yourself.
    - Copy Rf data from partner and fill in those two columns.
    - Note: There is a high likelihood that the Rf's that you and your partner got for the same chemicals in the same solvent will differ noticeably, due to experimental variance. But the relative ordering should be analogous.

	2% Ethyl Acetate/Hexane	5% Ethyl Acetate/Hexane	5% Ethyl Acetate/Hexane	10% Ethyl Acetate/Hexane
Substrate	distance traveled/Rf	distance traveled/Rf	distance traveled/Rf	distance traveled/Rf
2,4-di-t-Butylphenol **1**				
Acetophenone **2**				
2-Methylcyclohexanol **3** (this is a cis/trans mixture, and may perhaps separate into two spots)				
Biphenyl **4**				
First Unknown				
Second Unknown				

3. Rank the observed polarity of the 4 substrates **1-4**, from least polar to most polar. In case of a close race, your eyes will be best for seeing which is a little faster.

Most Polar: ________ > ________ > ________ > ________ Least Polar

4. Identify your two TLC Unknowns:

First Unknown: Letter ______ Identity_______________________________

Second Unknown: Letter ______ Identity_______________________________

5. Did increasing the polarity of your eluent increase or decrease your Rf's?

6. Given that the solid surface is polar, explain why polar substrates have lower Rf values than less polar ones.

7. Explain why increasing solvent polarity increases the Rf value for a given substrate.

8. What problem happens if the spot is placed so low on the plate and the eluent pool is so deep that the spot is actually submerged under the solvent?

9. What problem happens if the TLC beaker is left uncovered? Will the resulting Rf be fine, too high, or too low?

10. In what order, from top to bottom, would you expect to see the spots for naphthalene ($C_{10}H_8$), butyric acid ($CH_3CH_2CH_2CO_2H$), and phenyl acetate ($CH_3CO_2C_6H_5$)?

11. In what order, from top to bottom, would you expect to see the spots for the following:
    - acetic acid=CH_3CO_2H
    - butanal=$CH_3CH_2CH_2CHO$;
    - 2-octanone=$CH_3COCH_2CH_2CH_2CH_2CH_2CH_3$
    - decane=$C_{10}H_{22}$;
    - 1-butanol=$CH_3CH_2CH_2CH_2OH$

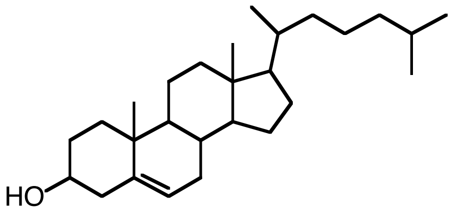
12. What will be the appearance of a TLC plate if a solvent of too low polarity is used for the development? (Will the spots be somewhere in the middle, way at the bottom, or way at the top of the plate?)

13. What will be the appearance of a TLC plate if a solvent of too high polarity is used for the development? (Will the spots be somewhere in the middle, way at the bottom, or way at the top of the plate?)

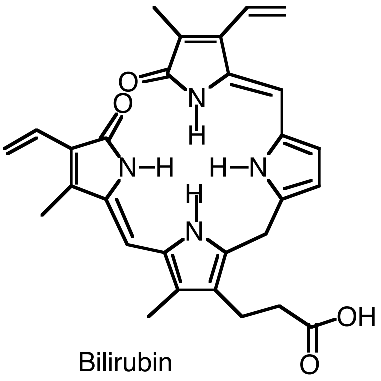
Chem 355 Jasperse

Cholesterol from Human Gallstones

In this experiment, cholesterol will be isolated from human gallstones. Cholesterol is an unsaturated alcohol containing 27 carbon atoms. It is a solid (mp = 148-149°C), and it is insoluble in water but soluble in a variety of hot organic solvents.



Cholesterol



Bilirubin

The gall bladder is attached to the undersurface of the liver just below the rib cage. It retains bile produced by the liver and feeds it into the upper part of the small intestine as needed for digestion. Bile consists primarily of bile acids, which are carboxylic acids closely resembling cholesterol and which aid in the digestion of fats by functioning as emulsifying agents. The gall bladder also harbors free cholesterol. If the concentration of cholesterol in the bile exceeds a certain critical level, it will come out of solution and agglomerate into particles that grow to form gallstones. An amateur geologist given a bottle of gallstones to identify once labeled them a "riverbed conglomerate" -- and indeed they do resemble stones in color, texture, and hardness. They come in a variety of shapes and colors and can be up to an inch in diameter.

As gallstones collect, they irritate the lining of the gall bladder, causing severe pain, nausea, and vomiting. The stones can block the bile duct and at times even lead to fatal complications. Formerly, the only remedy was major surgery. While surgery remains a frequent procedure, gallstones can sometimes be disintegrated in the gall bladder and the entire organ removed through a small incision in the navel. Consequently, it may soon be impossible to obtain whole human gallstones.

In the average human, approximately 200g of cholesterol is concentrated primarily in the spinal cord, brain, and nerve tissue. Insoluble in water and plasma, it is transported in the bloodstream bound to lipoproteins, which are proteins attached to lipids (fats). Recent research has divided these lipoproteins, when centrifuged, into two broad classes—high density (HDL) and low-density (LDL) lipoproteins. A relatively high concentration of HDL bound to cholesterol seems to cause no problems and in fact is beneficial, but a high ratio of LDL-cholesterol leads to the deposition of cholesterol both in the gall bladder (resulting in gallstones) and on the walls of the arteries (causing a plaque that cuts off blood flow and hastens hardening of the arteries or arteriosclerosis).

Mounting evidence points to unsaturated fats such as those found in vegetable oils as favoring the HDL-cholesterol bond, while LDL-cholesterol formation is speeded by saturated fats such as those found in animals. The HDL-cholesterol level goes down with smoking or eating large amounts of sugar. It goes up with regular exercise and with the consumption of moderate amounts of alcohol.

The average American woman at age 75 has a 50% chance of developing gallstones, while for a man of the same age the chance is only half as great. Gallstones and coronary heart disease are also much more common in overweight people. Almost 70% of the women in certain Native American tribes get gallstones before the age of 30, whereas only 10% of black women are afflicted. Swedes and Finns have gallstones more often than Americans; the problem is almost unknown among the Masai people of East Africa.

Experiment

Swirl 0.6 g of crushed gallstones in a 25-mL Erlenmeyer flask with about 6mL of 2-butanone. Add a boiling stick, and heat gently on a hot plate for five minutes until the solid has disintegrated and the cholesterol has dissolved (a brown residue, bilirubin, will not dissolve).

Filter the solution while hot to remove the bilirubin, using a Hirsch funnel and a small filter flask (50-, 75-, or 125-mL). The filtration is often difficult, because some of the bilirubin can get through. Keys to success: 1) Wet the filter paper with 2-butanone immediately before pouring the mixture, and 2) turn the vacuum on immediately before pouring the mixture, so that the filter paper stays in place. Why do these things? A) If you don't wet the filter paper it will often be knocked out of position and bilirubin will get underneath. B) If you don't have the vacuum running, the filter paper will again often lose its place and float up or get knocked out of position as soon as you pour the mixture on. C) If you wet the filter paper too much in advance of your filtration, the volatile butanone will simply evaporate and you'll be right back to the dry-filter paper problem situation A again!

Use some additional hot 2-butanone to rinse your Erlenmeyer and to rinse the Hirsch funnel. If some bilirubin did get through, you'll need to filter again (and again, etc.) until your liquid doesn't have any brown particles in it.

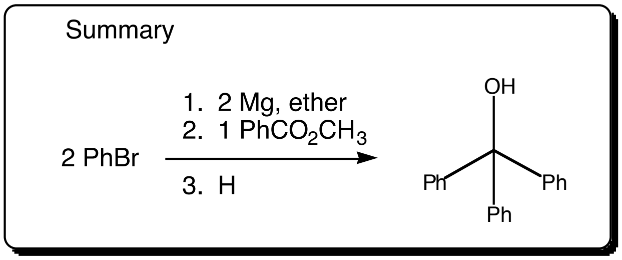
Rinse your Erlenmeyer and your Hirsch funnel with an additional 10 mL of methanol. Heat the combined cholesterol/butanone/methanol solution back to boiling, and reduce the total volume to ≤15 mL (best observed if you are working in a relatively small flask with volume markings on it). Add drops of hot water to the hot solution until you achieve saturation. As soon as you see product coming out of solution, don't add any more water. Cool the mixture and filter. Use some 80% methanol/20% water solution to rinse. (This is organic enough to help wash off impurities, but not a good enough solvent to dissolve away much of your yield.) Let dry for at least a day before taking final melting point and determining your final % yield.

Lab Report Requirements

- Write up your procedure, with full description of what you did and what you observed.
- Include you percent yield
- Include the melting point for your product cholesterol.

Questions

1. Given the structure of cholesterol, explain why it is more soluble in organic solvents than in water. Explain why the solubility of free cholesterol in the blood stream is so low.
2. Given that cholesterol has 27 carbon atoms, how many hydrogen atoms does it have?



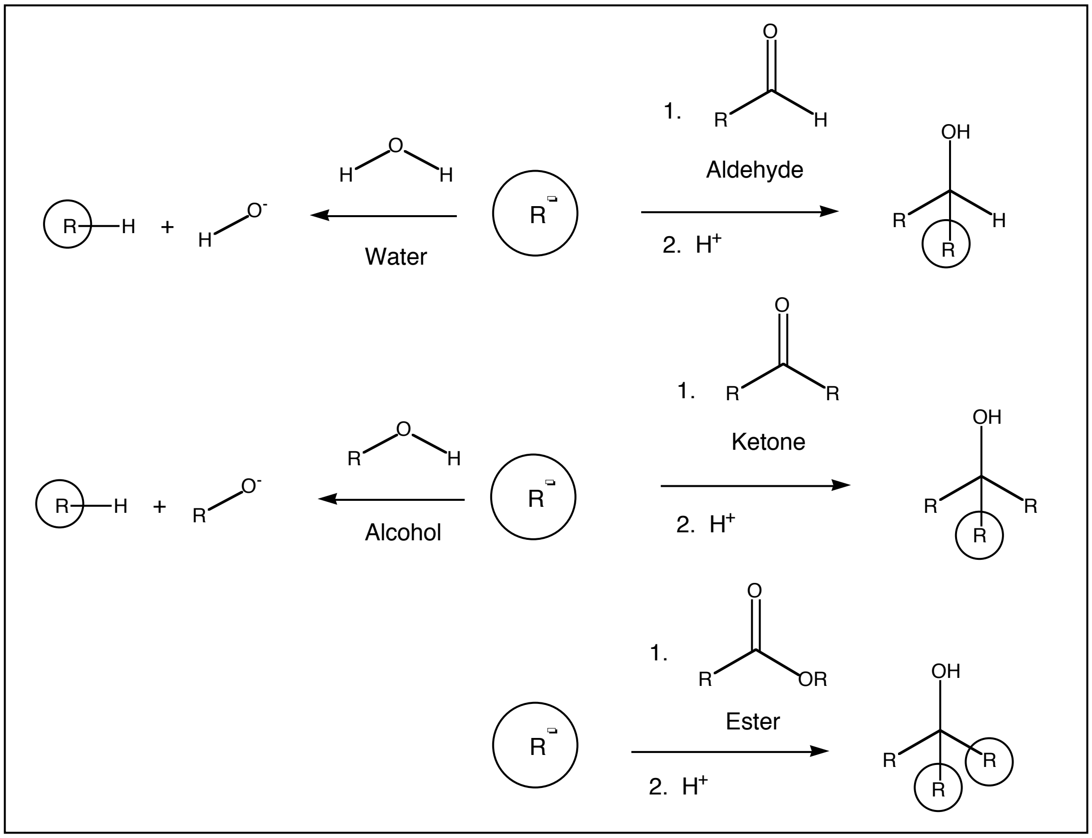
I. Background In 1912 Victor Grignard received the Nobel prize in chemistry for his work on the reaction that bears his name, a carbon-carbon bond-forming reaction by which almost any alcohol may be formed from appropriate alkyl halides and carbonyl compounds. The Grignard reagent RMgBr is easily formed by redox reaction of an alkyl halide with magnesium metal in anhydrous diethyl ether solvent.

$$\text{R-Br} + \text{Mg} \rightarrow \text{RMgBr}$$

The Grignard reagent **can be viewed as an ionic species consisting of carbanion R^-,** with a Mg^{2+} counterion and an additional Br^- counterion. **The carbanion R^- is very reactive, and functions both as an extremely strong base and an extremely strong nucleophile**.

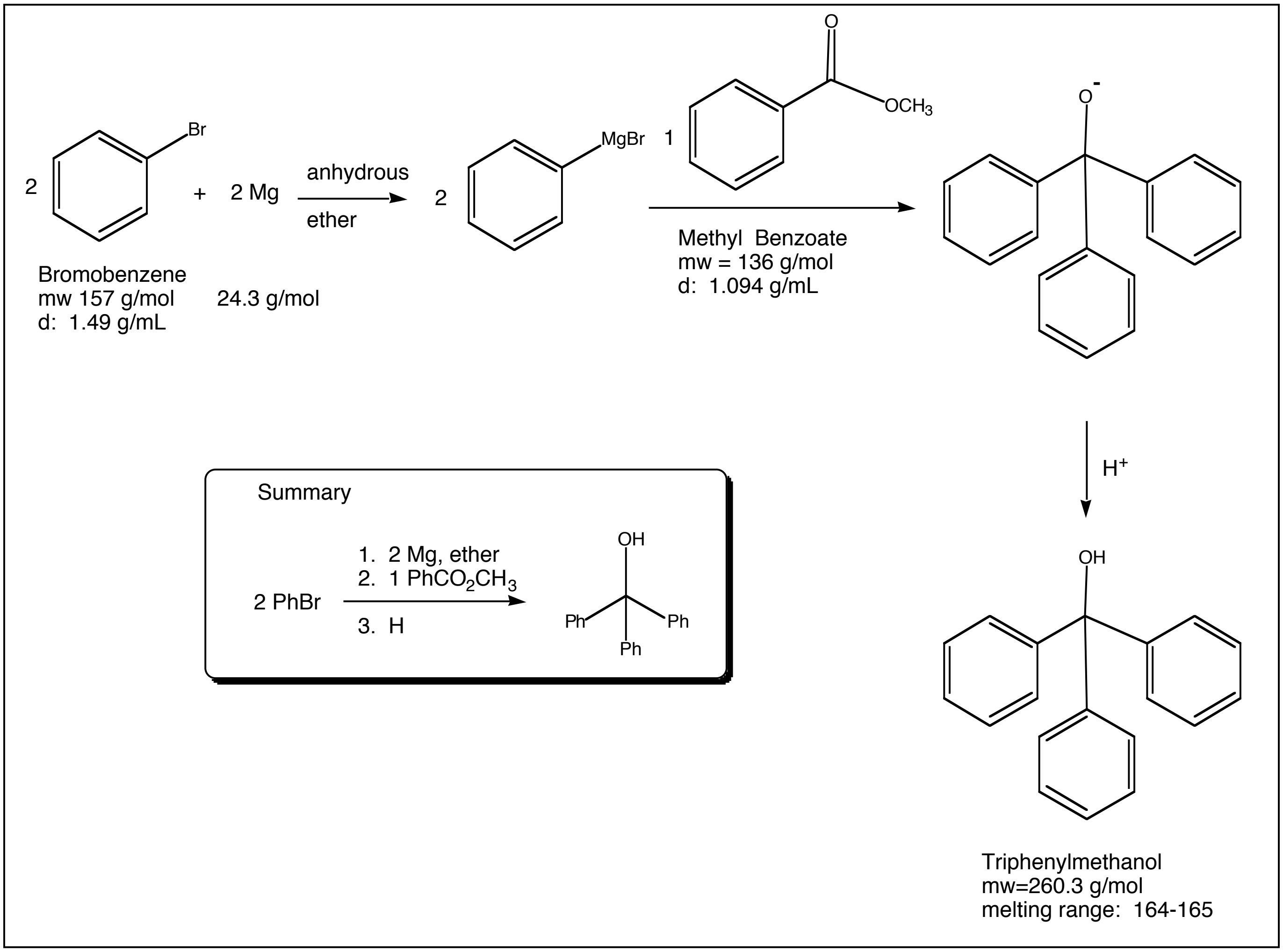
Some of its reactions are shown below.

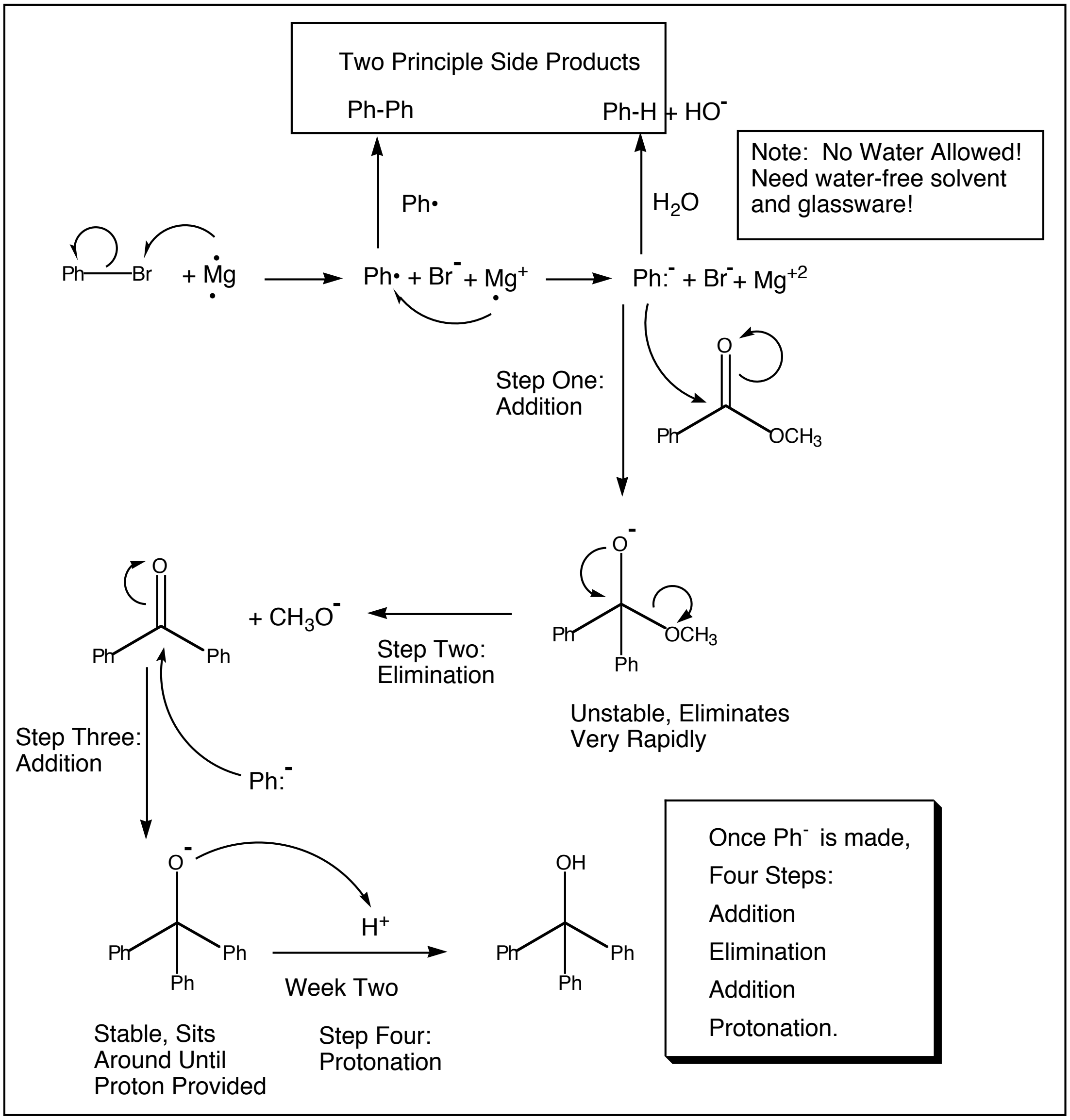
- **It reacts as a strong base with water or alcohols**.
  - Conversion from less stable R^- to more stable HO^- or RO^- is favorable.
- **It reacts as a strong nucleophile with carbonyl groups aldehydes, ketones, and esters.**
  - Conversion from less stable R^- to more stable RO^- is favorable, followed by protonation to give alcohols ROH.



II. Overview of Our Experiment

Our experiment is shown below. During week one we will generate the Grignard reagent (step one) and react it with the ester (step two). During the second week we will neutralize the alkoxide (step three), isolate the alcohol, purify the alcohol by recrystallization, and do product analysis.





The overall mechanism is illustrated above. The carbanion is generated by electron transfer from magnesium metal. The reactive carbanion then attacks electrophilic carbonyl to give an anionic intermediate (step one). This unstable intermediate rapidly eliminates a methoxide anion (step two). The resulting ketone is attacked again (step three). The resulting anion waits patiently until next laboratory period, at which time acid will be added to protonate the anion (step four).

Byproducts and Potential Problems There are two main byproducts and three problems.

1. **The first side product is biphenyl, Ph-Ph**, which is formed in competition with the Grignard reagent PhMgBr. Following initial electron transfer, the phenyl radical Ph• can either accept another electron leading to the desired carbanion, or combine with another phenyl radical to make biphenyl.
2. **The second side product is benzene** (Ph-H)**, resulting from protonation of the carbanion**. The carbanion is supremely basic, so if there is any water in the solvent or in the glassware, or if moist air is allowed to enter the reaction mixture, some of the carbanion will be protonated. **Great care is thus required to ensure "dry", water-free conditions.**
3. **The third problem is getting the magnesium to actually do the electron transfers**! Pure magnesium is an active metal, so active that any magnesium that has been exposed to air is inevitably coated with a film of magnesium oxide on its surface. This oxide film blocks the bromobenzene from actually contacting active magnesium, and thus prevents the requisite electron transfer. **For a Grignard reaction to work, it is necessary that fresh active magnesium be exposed**. Otherwise no electron transfer from magnesium to bromobenzene can take place, no carbanion can be formed, and no reaction proceeds. We will use two techniques, iodine activation and physical crushing, to activate our magnesium.

III. Procedure: Week One

Note: All equipment and reagents must be dry!

Phase 1: Preparing the Grignard Reagent

1. Dig out the following pieces of glassware:
    a. 250-mL round-bottomed flask
    b. "Claisen" two-branched connecting adapter (piece #9 in your kit)
    c. reflux condenser (piece #12 in your kit)
    d. separatory funnel with stopper
    e. thermometer adapter (piece #7 in your kit)
    f. drying tube packed with calcium chloride
    g. stick the drying tube into the rubber end of the thermometer adapter

2. Clamp the 250-mL round-bottomed flask to a vertical rod. If possible, use a clamp with grips that are either pure metal or else have non-flammable white coating rather than gray rubber coating. (Rubber clamps will melt and stink when subjected to Bunsen-burner flame!)
3. Light your Bunsen burner and pass the flame over the flask until there is no more steam visible on the surface of the glass.
4. As soon as the steam is gone from the flask, add the Claisen adapter to the flask and flame dry it as well
5. As soon as the steam is gone from both the flask and the adapter, add the reflux condenser to the flask, and flame dry as best you can.
6. While everything is still hot, attach the thermometer adapter with the drying tube into the top of the reflux condenser, add the separatory funnel with it's stopper on into the other arm of the Claisen adapter.
    - **At this point, the interior should be entirely closed from wet air getting in. The separatory funnel blocks out one side, and any air coming in through the column must pass through the drying tube.**
7. Weigh out about <u>2 grams of magnesium metal</u>. (Record weight)
8. When the glassware is cool enough to handle, add tubing to the condenser so that you can run a slow stream of tap water through the condenser. Reassemble the array as quickly as possible.
9. When the glassware is cool enough to handle, lift out the condenser and pour in the magnesium, then replace the condenser as soon as possible.
10. Pour <u>15 mL of ether</u> into the separatory funnel and put stopper back on.
11. Add one small chip of iodine (may ask the instructor to do this…) into the separatory funnel so that the color turns pink.
12. Drain the ether/iodine solution into the round-bottomed flask.
    - **<u>The iodine serves two functions.</u>**
        a. The first is as an **<u>indicator</u>**. The pink color will disappear when the magnesium is activated and is able to do redox chemistry with bromobenzene.
        b. The second is as an **<u>activator</u>**. Iodine is sometimes able to chemically "clean" the surface of the magnesium so that fresh, active magnesium is exposed so that it can do redox chemistry with bromobenzene. However, it doesn't always work!
    - **Make a mental picture of how much magnesium you have to begin with, so you can remember later on for comparison.**
13. Measure out <u>9.0 mL of bromobenzene</u> in a graduated cylinder. Record the volume as accurately as possible. Pour this into the separatory funnel and drain this into the round-bottomed flask.
14. If the redox chemistry of the Grignard reaction initiates, the iodine color will go away, the solution will begin to get hot, there will be some bubbling, and things may become slightly cloudy.
15. If there is no indication of reaction after two minutes, beg the instructor to come over to crush some magnesium.
16. The instructor will use a glass rod to try to crush some of the pieces of magnesium firmly against the bottom of the flask. This will expose fresh, active magnesium that should be able to initiate the redox chemistry and the formation of the Grignard reagent. Trying to crush very very hard magnesium pieces inside a glass flask is dangerous, though; it's easily possible to punch a hole in the glass! So if somebody is going to poke a hole in your flask, let it be the instructor so he can take the blame rather than doing it yourself!
17. Once the reaction has clearly initiated, get another <u>25-mL of ether</u>, and pour this into the reaction through the separatory funnel. (The dilution minimizes formation of the side-product biphenyl, but the more dilute it is at the beginning, the harder it is to initiate the reaction. Thus we don't want it too dilute until certain that initiation has succeeded.)

18. The reaction should be so exothermic that it will be self-boiling for some time. If the rate of boiling subsides, apply a heating mantel (connected to the Variac, not directly to the wall outlet) and apply heat to maintain a good rate of boiling.
19. Maintain boiling for one hour.
    - **Note: notice how the reflux condenser works. The bottom flask can be boiling hot (which facilitates maximum reaction rate), but the condenser enables you to liquify and recycle all of the boiling solvent.**
    - **Keep good procedural and observational notes of everything that you see and do!**

Phase 2: Things to do during the Grignard Hour…

Once the reaction is clearly going, prepare for Phase 3, in which you will add the methyl benzoate ester electrophile to the carbanion that you are making. And do the calculations that you will eventually need to include in your report.

1. Calculate what <u>volume</u> (in mL) it will take to add <u>5.0 grams of liquid methyl benzoate (density = 1.094 g/mL)</u>.
2. Calculate the number of **moles** used for magnesium, bromobenzene, and methyl benzoate.
3. Calculate the **<u>overall theoretical yield</u>** for your final product of next week, triphenylmethanol (mw = 260 g/mol).
    - To do this, you must **<u>first identify</u>** which if the three reactants (Mg, PhBr, or $PhCO_2CH_3$) is the **<u>limiting reactant</u>**
    - To do this, you must factor in the overall stoichiometry, which is not all 1:1:1:1. (Given your calculated moles of Mg, how many moles of Ph_3COH could you make? Given your calculated moles of PhBr, how many moles of Ph_3COH could you make? Given your calculated moles of $PhCO_2CH_3$, how many moles of Ph_3COH could you make?)
    - In calculating theoretical yield for a multistep reaction, theoretically every step will be perfect. (We know otherwise, but we're talking theoretical yield here…) Thus you don't need to calculate or measure quantities for any intermediates. <u>Your limiting reactant and theoretical yield should consider only original reactants and final product</u>, all things which are easily quantified.
4. After the Grignard solution has reacted for one hour, check to see how much magnesium is left. Any qualitative estimate of about how much is left? (None? 10%? 50%?)
    - What implications might this have on your possible yield? Is it necessary for all of your magnesium to have reacted completely in order to get 100% yield? Or could you get 100% yield even if some of your magnesium remains unreacted?

Phase 3: Reacting the Grignard Reagent with the Methyl Benzoate

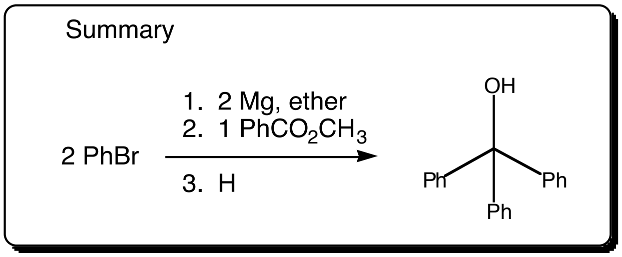
1. After the hour is up, let the reaction cool down (an ice-water bath might help).
2. Add <u>15 mL of ether</u> to your separatory funnel. (Stopcock closed).
3. Add <u>5.0 grams of methyl benzoate</u> to your separatory funnel by syringe. (Remember, you calculated this volume in Phase 2….)
4. Remove the cold bath (if you have one on), then drain the ester/ether solution into the round-bottomed flask, slowly so that the reaction doesn't overheat to much. Try to shake the solution around as much as possible (hard to do when it's clamped!) If things start to boil hard, reapply the cold bath.
    - Record your observations!
5. If everything is added without excessive boiling, try to shake everything up, and give it five minutes or so to continue reacting.
6. If the reaction is still hot, cool it with the ice bath.
7. Remove all the glassware from the top of the round-bottomed flask, and stuff in a rubber stopper.
    - Note: it is essential that the solution isn't hot when you do this. If it is, then when it cools it will create a vacuum and suck the stopper in…)
    - Note: it is essential that the vigorous exothermic reaction is done before you stopper the flask. Otherwise if stirring or further reaction generates enough heat, it will cause the ether to boil and blow the stopper off!
8. Stash the round-bottomed flask with the chemicals and the stopper into a secure spot in your drawer, and wait till next lab to finish!

<u>IV. Procedure: Week Two</u>

1. Record you observations for what your mixture looks like at this point.

2. Remove the stopper, and add about 25 grams of ice and 50mL of 2M sulfuric acid
    - The acid will react exothermically with both the anion and unreacted magnesium. The ice is there simply to absorb the heat.
3. Swirl well to promote hydrolysis and breakup of the solid clumps. Use a spatula to try to break up the chunks.
4. In the process, three things should happen:
    - The anion should be protonated, giving the neutral organic alcohol product. This should partition into the organic ether layer.
    - Magnsesium salts should be ionic, so they should partition into the aqueous layer.
    - Unreacted leftover magnesium metal will react with the acid to give molecular hydrogen. That's what causes the bubbling. (1 Mg + 2 $H^+ \rightarrow Mg^{2+} + H_2$ gas)
5. Pour the mixture into your separatory funnel. (The magnesium doesn't need to be totally dissolved…)
6. Pour an additional 10 mL of sulfurice acid and 10 mL of ether into your flask, swirl to try to dissolve up anything left on the walls, and pour into the seperatory funnel. (These need not be measured, just pour some in approximately.)
7. Drain off the bottom aqueous layer into a beaker.
8. Add another 20 mL of sulfuric acid into the separatory funnel, shake it up, and drain off the aqueous layer again. Pour the combined aquous layers into the aqueous waste bottle in the hood.
9. Drain the organic layer from the separatory funnel into an Erlenmeyer flask.
10. Add about 5 grams of sodium sulfate to "dry" the ether layer. Add additional scoops if the sodium sulfate is all clumped up (indicating that there may be too much water for the sodium sulfate to handle).
11. Plug your long-stem funnel with a little glass wool, then pour the solutio
12. Pour the ether solution through the glass-wool plugged funnel into a different Erlenmeyer flask. The wool should be sufficient to filter off the solid sodium sulfate, and only allow the solution to get into the flask. Rinse your original flask and the sodium sulfate with an additional portion of ether.
    - At this point, your solution should be free of water and of magnesium salts. Other than the ether solvent itself, you should have nothing but the desired product and organic contaminants.
13. Make a TLC plate with five pencil marks for five tracks ready:
    a. Authentic biphenyl b. Authentic methyl benzoate c. Crude mixture
    d. Purified mixture e. Post-crystallization solvent
14. Take a capillary droplet from your mixture, and put it on the "crude mixture" spot. Take droplets from the authentic biphenyl and methyl benzoate bottles in the hood and apply them as well. Save the plate until you've finished purifying the product, at which point you'll be able to apply your last spot.
15. Add 25 mL of "ligroin" solvent (all hydrocarbons, mostly hexanes, but not pure) to your ether solution. The product is more soluble in ether than in hydrocarbons, so you are essentially adding some "bad solvent" to facilitate a mixed solvent recrystallization.
16. Prepare a little distillation chimney, by connecting a hose to your long-stemmed funnel. Connect the other end of the hose to the aspirator outlet.
17. Add a boiling stick to your organic solution
18. Now heat your solution on a hot plate. Place the funnel over the top of your flask, and run the aspirator. This will cause the boiling ether to get sucked away.
19. Boil the solution down to 25-30 mL or so. (Crystals should start to form before this, depending on your yield. But if you stop boiling as soon as the first crystals form, you'll still have too much solvent and will get a low yield.)
20. Remove from heat, and let cool slowly to grow your crystals, first to room temperature and then to 0ºC.
21. Filter your crystals with Buchner funnel and aspirator.
22. Take a droplet from the solvent and put it on the tlc plate in the "post-crystallization solvent" spot
23. Take about 0.1 grams of your crystals (needn't be bone dry) and dissolve in 3 mL of ether. Then take a capillary and put a droplet of this purified material onto your tlc plate in the "purified" spot.
24. Run the tlc in designated solvent (2% ethyl acetate/hexane?), and analyze by UV and the "dip" solution.
    - Mark down the results, with the following questions in mind:
        - o Is biphenyl present in the crude mix? In the purified material?
        - o Is methyl benzoate present in the crude mix? In the purified material?
        - o Did recrystallization purify the material at all?
        - o Did crystallization get all of the product out of the solvent?
25. Take a melting range on your final product. (Should melt above 150º, so heat accordingly)
26. Get your final mass.

Lab Report Requirements and Format: The following layout is standard for a "synthesis reaction" report. Provide the parts and information in the sequence specified.



1. Title = Reaction Summary

For an organic reaction, there is no point in having a Worded Title: The chemical reaction is the best title summary of what you did!

2. Listing of all Chemicals Used

- This should include all chemicals used, including solvents.
- For each chemical, you should include the actual quantity used and measured. For example, with the methyl benzoate you measured a volume by syringe, rather than by weighing on a balance. So you should list the volume you actually used rather than just the weight.
- If a person was later to repeat your experiment, they should be able to look at this list and know all the chemicals they'd need to have on hand and in what quantities, in order to complete the experiment.
- In some cases, there may be considerable roundoff (you needn't keep precise record of the quantity of solvent that was used, for example, or of sodium sulfate drying agent…)
- For reactants that might possibly be limiting reactants and might possibly factor into calculation of the theoretical yield, however, you must include more than just the quantity of chemical used. You should also include a conversion from what you measured into the number of moles used.

3. Calculation of Theoretical Yield

- Specify which chemical is the limiting reactant
- Given moles of limiting reactant, calculate theoretical moles of product
- Given moles of product, calculate theoretical grams of product.
- Note: Why do this so early in report?
  - First, because it fits in near your mole calculations above.
  - Second, if calculated in advance, as with most research, you know which chemical is limiting and thus must be measured most carefully, but you also know which are in excess and thus need not be measured with equal precision.
  - Third, it's nice to know approximately how much material is expected, so you can ecognize whether your actual results are reasonable or way off.

4. Writeup of Actual Procedure.

- For this particular experiment, the "procedure" section will be by far the biggest portion of your report.
- This should be a concise but detailed description of things, including:
  - What you actually did (even if not recommended or not from recipe)
  - All observations should be included. These include all observed changes, such as:
    - Changes in **color**
    - Changes in **solubility** (formation of precipitate or cloudiness…)
    - Formation of **bubbles**
    - Changes in **temperature** (like, reaction became hot…)
  - Time and temperature details:
    - Whenever you heat something or cool something, the procedure should specify
    - Specify times. Whether you boiled for 5 minutes or 5 hours matters!
- Writing details: As a record of what actually happened, the report must be written in **past tense**, not **command tense**. (Rather than "Add this", should read "I added this", or "I dropped that…")
  - Use of personal pronouns is accepted in this class. (Teachers in other classes may have different requirements). But you are not obligated to avoid references to "I" or "we" in this class.

5. Product Analysis

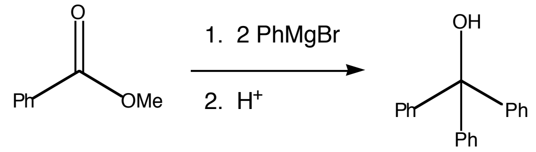
- Any NMR, mp, bp, TLC information. For this report, mp and TLC information must be included.
- Final yield and percent yield information.

6. Discussion/Summary. Need not be long, but any conclusions or excuses would go here…

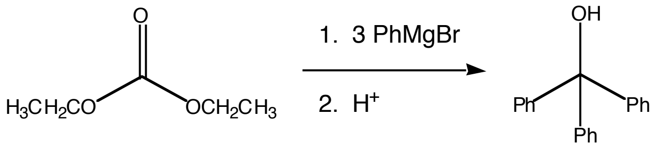
7. Answers to any assigned Questions

Assigned Questions, Grignard Lab

1. Draw a detailed, step-by-step mechanism for the reaction you actually did: (on attached sheet?)



2. Triphenylmethanol can also be prepared by the reaction of PhMgBr with diethylcarbonate $(CH_3CH_2O)_2C{=}O$, followed by H^+ workup. Draw a detailed, step-by-step mechanism for the following reaction: (on attached sheet?)



3. If the methyl benzoate you used had been wet (contained water), what byproduct would have formed?

4. If you hadn't bothered to flame-dry your glassware or used a drying tube, what byproduct would have formed?

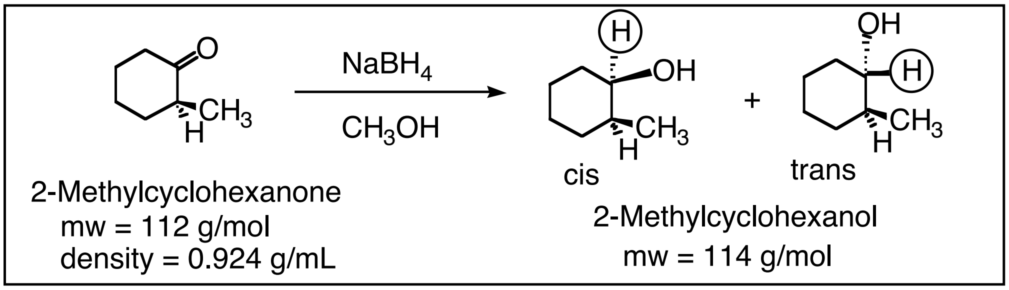
5. Your yield was considerably less than 100%. Discuss where you think things might have come up short. You may wish to differentiate reaction things (reasons or evidence that you didn't have complete chemical conversion) versus isolation things (reasons or evidence that you didn't isolate all of the product that was actually made chemically). (It's possible that your TLC may support or disprove some possible explanations.)

6. Given the quantities of chemicals used in this recipe, one could conceivably have gotten a 100% chemical yield without having completely reacted all of the magnesium, or without having completely reacted all of the bromobenzene. But it would not have been possible to get 100% chemical yield if the methyl benzoate didn't react completely. Explain.

$NaBH_4$ Reduction of 2-Methylcyclohexanone. H-NMR for Analysis of Isomeric Product Ratios

BACKGROUND Hydrogen-NMR is useful for analyzing a pure sample, and one of the pieces of information is the **integration** of hydrogen signal sets. Integration of hydrogen signal sets measures the area, and this area is proportional to the number of hydrogens causing the particular signal. Thus in a pure compound, a CH_3 group would give an integral 1.5 times as large as a CH_2 group.

In today's experiment, we will apply integration in a related but different way: to measure the ratio of two different products formed in a single reaction mixture. The chemical experiment will be a standard $NaBH_4$ reduction of a ketone to produce alcohol. Due to the chirality of the starting ketone, two diastereotopic cis/trans alcohols are produced. Attack of the hydride from the back face, trans to the methyl group, produces the cis product alcohol. Attack of the hydride from the front face, cist to the methyl group, produces the trans alcohol. The labeled hydrogens on the oxygen-bearing carbons of the alcohol products give NMR signals with different chemical shifts. By integrating the sizes of their signals, we will be able to determine a product ratio.



Chair Conformations and NMR Interpretation Summary: How do we know which product is cis and which is trans?

We know that a cyclohexane ring has two chair conformations of unequal energy.

- You will want to draw both chairs for the cis isomer, and identify which is the more stable.
- You will then want to draw both chairs for the trans, and identify which of those is more stable.
- **By comparing the best cis chair with the best trans chair, you should be able to recognize which of the two products is more stable overall, cis or trans**.
- **By looking at your models/drawings, you should also be able to recognize whether the best cis chair has an axial or equatorial "feature H" (the hydrogen attached to the oxygen bearing carbon, which will give a signal in the 3's.)**
- **Likewise you can determine whether the trans isomer should have its "feature H" equatorial or axial. (It will be axial in one of the isomers and equatorial in the other.)**

Fact: **An axial hydrogen has a chemical shift further to the right ("upfield", lower number) relative to otherwise analogous equatorial hydrogens** in an H-NMR spectrum. (The reason for this is that an axial hydrogen is more crowded, and closer to electron clouds around other atoms. The greater crowding/proximity to electron clouds causes the upfield shift.)

Application: Your drawing/model-building should tell you whether the axial "feature H" correlates to the cis or trans product.

- By integrating the axial (upfield) to equatorial (downfield) signals, you will thus be measuring the ratio of the two isomers.

"Thermodynamic Product-Stablity Control" versus "Kinetic Control"

When the same starting material can give two different products, we say that the reaction is either under "product stability" control or under "kinetic control". "Product stability" control usually applies, because factors that stabilize the product often stabilize the transition state as well. But this is not always true: sometimes steric crowding can destabilize a transition state without destabilizing a product. If a reaction does not preferentially produce the most stable product, the reaction is said to be under "kinetic control" rather than product stability control. In today's experiment, might the methyl group obstruct the front face and thus destabilize the transition state leading to the trans product?

Experimental Procedure

1. To a large test tube, add 4 mL (or two full pipet squirts) of methanol
2. Add 0.9 mL of 2-methylcyclohexanone.
   - Use density and molecular weight information to calculate how many moles are involved
   - density = 0.924 g/mL
   - mw = 112 g/mol)
3. Prepare an ice-water bath in your 150-mL beaker
4. Place the test tube into the ice-water bath
5. Weigh out 0.15 g of $NaBH_4$ (mw = 38 g/mol)
6. Carefully add the $NaBH_4$ to the test-tube. (The $NaBH_4$ is in excess, so if some sticks on the walls of the tube, it isn't a problem).
7. After the vigorous bubbling subsides, remove the test tube from the icewater and let it stand at room temperature for 20 minutes.
8. Clamp your smallest iron ring to a vertical rod, and insert your separatory funnel
9. Pour your test tube solution into the separatory funnel
10. Rinse test tube with an additional two pipets of dichloromethane and add this to the separatory funnel
11. Add two pipets of tap water, and then two full pipets of 3 M sodium hydroxide solution (purpose: to decompose the borate salts and move them into the aqueous phase)
12. Shake the mixture, then let it settle
    - Question: which layer is organic and which is aqueous? If in doubt, add some addition water and watch to see which layer it falls into, and which layer grows!
13. Drain the dichloromethane layer into a 50-mL Erlenmeyer flask
14. Wash the aqueous layer in the sep funnel with an additional pipet of dichloromethane, let settle, and drain the organic layer into the Erlenmeyer flask.
15. Repeat the last step again.
16. Add a large scoop of anhydrous sodium sulfate to the Erlenmeyer flask to "dry" your organic solvent. If the sodium sulfate all clumps, add more until at least some does not clump up.
17. Pre-weigh a 50-mL round-bottomed flask, and then clamp it onto a vertical rod.
18. Take your long stem funnel and push a little glass wool into the neck.
19. Pour the organic solution from the Erlenmeyer through the funnel into the round-bottomed flask. The wool should be sufficient to filter off the solid sodium sulfate, and only allow the solution to get into the flask.
20. Rinse the Erlenmeyer with additional dichloromethane, and pour the rinse through the funnel into the round-bottomed flask.
21. At this point, there should be only dichloromethane and alcohol products in your flask.
22. Concentrate the organic solution by rotary evaporation. Be sure the aspirator power is on; that the top air valve is closed; and that you have an adapter for a good glass seal. Make sure that the spinner is also turned on.
23. Once the sample has concentrated to a residual oil, weight the flask and calculate your mass yield.
24. Prepare and run an NMR.

Model Building (Can share this groups of students.)

1. Build a model of both cis and trans 2-methylcyclohexanol.
2. Chair-flip both
3. For the cis isomer, which chair if more stable?
4. In the more stable cis chair, is the "feature hydrogen" axial or equatorial?
5. For the trans isomer, which chair is more stable?
6. In the more stable trans chair, is the "feature hydrogen" axial or equatorial?
7. Which is more stable, the best cis chair or the best trans chair?
8. Draw your best cis and best trans chairs.

Name:

Sodium Borohydride Lab Report

1. Use Standard Synthesis Format:
    a. Illustrate the Chemical Reaction
    b. Summarize the Chemicals Used
    - Include mole Calculation for 2-methylcyclohexanone
    c. Calculate the theoretical yield
    d. Write up the procedure, **including observations**
    e. Analysis:
    - Include actual yield, and percent yield

2. Take H-NMR
    - Print full spectrum
    - Print horizontal expansion from about 4.0-2.8
    - Notes: CH_2Cl_2 solvent, if not evaporated completely, will give a singlet at 5.3; and methanol, if not completely extracted/evaporated will give a singlet around 3.5.

3. Discussion/interpretation
    - Draw both cis chairs
      -Identify the better of the two
      –is the "feature" H axial or equatorial?

    - Draw both trans chairs
      -Identify the better of the two
      –is the "feature" H axial or equatorial?

    - Would the cis chair or the trans chair be most stable overall?

    - From the NMR integration and chemical shifts, determine the trans/cis ratio.

    - Was the major product formed via "product-stability control" (the most stable product is formed preferentially) or "kinetic control" (for some steric reason, the fastest reaction/lowest transition state did not lead to the most stable product)?

Summary of 1H-NMR Interpretation

I. Number of Signal Sets

II. "Chemical Shifts" of the Signal Sets

9's (9.0-10.0)	**Aldehyde** sp^2 hybridized C-H's
7's (6.5-8.4)	**Aromatic** sp^2 hybridized C-H's
5's (4.8-6.8)	**Alkene** sp^2 hybridized C-H's
3's (2.8-4.5)	**Oxygenated** or **Halogenated** sp^3 hybridized C-H's (halogenated and nitrogenated alkyl C-H's will also come in this window, although no candidates for today's lab). Oxygenated sp^3–carbons are routinely present for the following functional groups that contain oxygen single bonds: • **alcohols**, • **ethers**, or • **esters**
2's (1.8-2.8)	**Allylic** sp^3 hybridized C-H's (sp^3 hybridized C-H's that has a double bond attached to the sp^3 hybridized C). Allylic signals routinely appear when one of the following double-bonded functional groups is present: • **carbonyls**, (ketones, esters, aldehydes, acids, amides) • **alkenes**, or • **aromatics**
1's (0.7-2.0)	sp^3 hybridized C-H's, with **no attached Functional Groups** • **Note:** Many molecules with non-functional alkyl portions will give a lot of signal in this area.
0-12 (anywhere!)	**Alcohol/Acid** O-H hydrogens (N-H hydrogens likewise) • **alcohols**, • **carboxylic acids**

8. Recognize OH's..

Check each of the zones. Each one gives you a yes or no answer about the presence of absence of the featured group.

9. End-Check: Check that the functional groups indicated by your chemical shift information match with the structure you believe you actually have! If not, structure needs correction!
10. The regions are somewhat approximate, and have some spillover.
11. For multi-functional complex molecules, there are more complex ways for a C-H to come in some of the above window. For example, an sp^3-hybridized C-H with two attached oxygens can come in the 5's, or an sp^3-hybridized C-H that is doubly allylic can come in the 3's. In other words, the impact of functional groups is roughly additive.

III. Integration These **must be simple whole-number ratios** (2:1, 3:1, 3:2, etc..)

IV. Splitting

- **N-1 Rule: N lines ⇒ N-1 neighbor H's** (H's directly attached to carbons attached to the C-H group causing the signal)
  - The N-1 Rule is useful when working from spectrum to actual structure
- **N+1 Rule: N neighbor H's ⇒ N+1 lines**
  - The N+1 Rule is useful when working from structure to actual spectrum

Note: OH hydrogens don't participate in splitting (normally)

Summary of C13-NMR Interpretation

6. **Count how many lines** you have. **This will tell you how many types of carbons** you have. (Symmetry equivalent carbons can at times cause the number of lines to be less than the number of carbons in your structure.)
   a. Each "unique"carbon gives a separate line.
   b. Symmetry duplicates give the same line.
   c. If there are more carbons in your formula than there are lines in your spectrum, it means you have symmetry.

7. **Check diagnostic frequency windows** ("chemical shift windows") of the lines **to provide yes-or-no answers regarding the presence or absence of key functional groups** in your molecule.

220-160	C=O carbonyl carbons, sp^2 hybridized
160-100	C alkene or aromatic carbons, sp^2 hybridized
100-50	C-O oxygen-bearing carbons, single bonds only, sp^3 hybridized
50-0	C alkyl carbons, no oxygens attached, sp^3 hybridized

8. **Check Splitting**. C13 NMR's are often acquired as "decoupled" spectra, in which each carbon signal appears as a singlet. This is the way our laboratory C13 NMR's come out. However, at the cost of extra time it is also possible to get "coupled" C13 NMR's with splitting. These splitting values are very useful, and follow the N+1/N-1 rules (the number of lines is one greater than the number of attached H's).

Quartert (q)	CH_3
Triplet (t)	CH_2
Doublet (d)	CH
Singlet (s)	C (no attached hydrogens).

9. **Signal Height/Size**
   a. Carbons without any attached H's are short. This is common for carbonyls (aldehydes are the only carbonyl carbons that have hydrogens attached) and for substituted carbons in a benzene ring.
   b. Symmetry duplication multiplies signal height (if you have two copies of a carbon, the line will probably be taller than normal!)

10. **Aromatics, Symmetry, and C-13 Signals**. Most aromatics have symmetry, and both the number of aromatic lines and the splitting of the aromatic lines can be indicative of the substitution pattern on a benzene. Mono- and para-disubstituted benzenes have symmetry.

4 lines	s, d, d, d	Monosubstituted benzene. (Has symmetry).
4 lines	s, s, d, d	Para-disubstituted benzene. (Has symmetry).
6 lines	s, s, d, d, d, d	Ortho- or meta-disubstituted benzene. (Has no symmetry).

Summary of IR (Infrared) Interpretation

1. **Check for Diagnostic Signals**

3500-3200	OH or NH
1800-1640	C=O
3500-2500 + 1800-1640	CO_2H

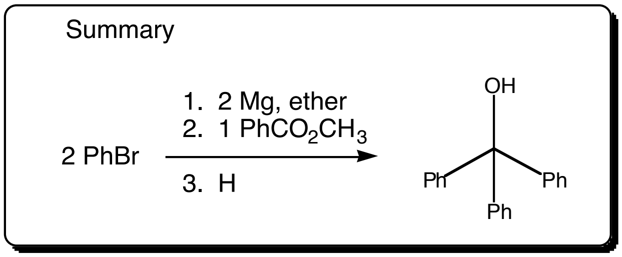
2. **Further Information in the "Carbonyl Zone"**

<1700	Unsaturated C=O
>1700	Saturated C=O
1720-1700	Saturated ketones, aldehydes, acids
1750-1735	Saturated ester

Standard Synthesis Laboratory Report Format: The following layout is standard for a "synthesis reaction" report. Provide the parts and information in the sequence specified.

1. Title = Reaction Summary

For an organic reaction, there is no point in having a Worded Title: The chemical reaction is the best title summary of what you did!



2. Listing of all Chemicals Used

- This should include all chemicals used, including solvents.
- For each chemical, you should include the actual quantity used and measured. For example, with the methyl benzoate you measured a volume by syringe, rather than by weighing on a balance. So you should list the volume you actually used rather than just the weight.
- For reactants that might possibly be limiting reactants and might possibly factor into calculation of the theoretical yield, you must include more than just the quantity of chemical used. You should also include a conversion from what you measured into the number of moles used.
- In some cases, there may be considerable roundoff (you needn't keep precise record of the quantity of solvent that was used, for example, or of sodium sulfate drying agent…)
- If a person was later to repeat your experiment, they should be able to look at this list and know all the chemicals they'd need to have on hand and in what quantities, in order to complete the experiment.

3. Calculation of Theoretical Yield

- Specify which chemical is the limiting reactant
- Given moles of limiting reactant, calculate theoretical moles of product
- Given moles of product, calculate theoretical grams of product.
- Note: Why do this so early in report?
    - First, because it fits in near your mole calculations above.
    - Second, if calculated in advance. as with most research, you know which chemical is limiting and thus must be measured most carefully, but you also know which are in excess and thus need not be measured with equal precision.
    - Third, it's nice to know approximately how much material is expected, so you can recognize whether your actual results are reasonable or problematic.

4. Writeup of Actual Procedure.

- For this particular experiment, the "procedure" section will be by far the biggest portion of your report.
- This should be a concise but detailed description of things, including:
    - What you actually did (even if not recommended or not from recipe)
    - All observations should be included. These include all observed changes, such as:
        - Changes in **color**
        - Changes in **solubility** (formation of precipitate or cloudiness…)
        - Changes in **temperature** (like, reaction became hot…)
        - Formation of **bubbles**
    - Time and temperature details:
        - Whenever you heat something or cool something, the procedure should specify
        - Specify times. Whether you boiled for 5 minutes or 5 hours matters!
- Writing details: As a record of what actually happened, the report must be written in **past tense**, not **command tense**. (Rather than "Add this", should read "I added this", or "I dropped that…")
    - Use of personal pronouns is accepted in this class. You may use "I" or "we" to simplify writing.

5. Product Analysis

- Any NMR, mp, bp, TLC information. For this report, mp and TLC information must be included.
- Final yield and percent yield information.

6. Discussion/Summary. Need not be long, but any conclusions or excuses would go here…

7. Answers to any assigned Questions

User's Guide to ^{1}H, ^{13}C NMR

- Note: the default mouse button is the left button. Always use the left one unless told otherwise.
- For help, see Dr. Jasperse, Hagen 411-I.

1. Login
    a. double click on **jasperse** icon (or type in "jasperse")
    (Research Users: You should use your boss's login.)
    b. Password "**chem355**" (need underline in between)
    c. double click on "**xwinnmr**"

2. Sample Insertion/Lock/Tune
    a. remove cap from spectrometer if needed, and then click **LIFT ON/OFF** key on upper lefthand corner of SCM keyboard (to right of computer) to lift lock sample
    b. place your sample in sample holder, adjust position using depth gage, and place in spectrometer [DO NOT PUT SAMPLE IN WITHOUT THE SAMPLE HOLDER! YOU WILL BREAK YOUR SAMPLE AND WRECK THE INSTRUMENT!]
    c. click **LIFT ON/OFF** key on SCM keyboard to lower sample
3. Acquiring the spectrum
    a. type "**xmac**"
        - a listing of suggestions will come up
        - at present, all of these assume $CDCl_3$ as solvent
        - instructors/researchers, these can be easily customized for you needs. See Jasperse
    b. select the experiment of interest, normally **ah1-tune** or **c13-tune** or **c13-notune**
        - Note: if you are going to run both ^{1}H and ^{13}C on the same sample, you don't need to tune twice. Run "ah1-tune" first for hydrogen, then "c13-notune"
    c. when asked for file name information, type your name into the name box and
    d. type "**chem355**" into the "user" box.
    e. click **SAVE**
    f. click **COPY ALL** when the box comes up
        - The computer will now do everything for you: read in the correct parameters, lock, tune if specified, adjust the receiver gain, acquire the spectrum, phase the spectrum, and store the phasing information for automatic integration.
    g. When "ns = 8 (or 128)" box pops up, hit return to accept default, or else enter something different for the number of scans
    h. wait patiently until either an "**xmac:finished**" or "**abs finished**" message appears
        - Hopefully this whole process will take less than 5 minutes for proton or less than 8 minutes for a carbon spectrum. If 8 minutes have passed and still incomplete, see Jasperse...
    i. click on the ⊼ icon (upper right corner of the icon group) to adjust the vertical scale of the viewed spectrum. (For example, if your baseline looks flat, this will fix it!)

4. Plot
    a. click **PLOT** icon
    b. hit return in response to any boxes that appear
    c. To do **horizontal expansions**, **manual integrations** or **vertical expansions**, see instructions on page 2.

5. Exiting:
    a. Replace your sample with the default sample, as described in part 3.
    b. Type "lock cdcl3"
    c. type "exit"
    d. Say OK if it asks you anything about closing things
    e. put cursor outside of any boxes into the blue area, then press the *right* mouse button, click **Logout** and click **Yes**.

1. Plotting **Horizontal Expansions**
   a. Make sure that the cursor is somewhere on the spectrum.
   b. Click the **left mouse button**. You will now get a doubled arrow.
   c. Move the doubled arrow to the left end of the area you want to expand and click the **center mouse button** do define the **left boundary**.
   d. Move the doubled arrow to the right end of the area you want to expand and again click the **center mouse button** to define the **right boundary**.
   e. click **PLOT** icon
   f. To get back to the full expansion, click the |◁▷| icon
   g. To get out of the "doubled arrow" mode, click the **left mouse** button

2. **Manual Integration**
   a. click **INTEGRATE**
   b. define the regions of interest (see horizontal expansion instructions above)
   c. click **RETURN** and save your integral regions
      - Sometimes you may wish to improve the "flatness" of the integral, or you may wish to assign calibration values of your own choosing. Do the following:
   d. put the arrow within the region of your integral, and click the **<u>left</u> mouse button**. The integral under consideration will then get a star by it.
   e. click **CALIBRATE** and respond accordingly
   f. adjust the **BIAS** in order to get the left side of the integral level
   g. then adjust the **SLOPE** to get the right side of the integral level

3. Reducing the noise in noisy, dilute ^{13}C spectra. "**Power Spectrum**" .
   [**<u>Do not use for 1H spectra!</u>]**
   a. After getting the normal spectrum, type "**ps**"
   b. click on the ⏶ icon (upper right corner of the icon group) to adjust the vertical scale of the viewed spectrum.
   c. click **PLOT** icon

   - Note: The "ps" command can make plots look prettier, by de-emphasizing noise. It does so by squaring all signals, however, so it will also de-emphasize small peaks that are real. In addition, by changing the relative sizes of peaks, it is incompatible with integration.

4. **Vertical Expansions**
   a. Type "**cy**" and increase or decrease the default value as you see fit. Doubling will double the printed heights, tripling will triple the printed heights, etc.
   - At default, cy=14, and is set so that the tallest peak in the spectrum will be 14cm tall. Thus, if you are wanting to expand a peak that is too tall, you need to multiply the cy as needed.

5. Manual Phasing
   a. click **PHASE**
   b. Click **BIGGEST**
   c. Click **PHO**, and keep finger held down
   d. -drag, to adjust phase of biggest, marked peak
   e. Click **PH1**, and keep finger held down
   f. -drag, to adjust phase of peaks distant from biggest
   g. click **RETURN**
   h. type "<u>abs</u>" if you want integrations to be automatically printed as a result

6. Printing Titles
   a. type "**setti**" (for "set title") b. delete existing title and type in new one
   c. click **<u>save</u>** d. click **<u>quit</u>** e. type "**title**" f. choose **<u>yes</u>**