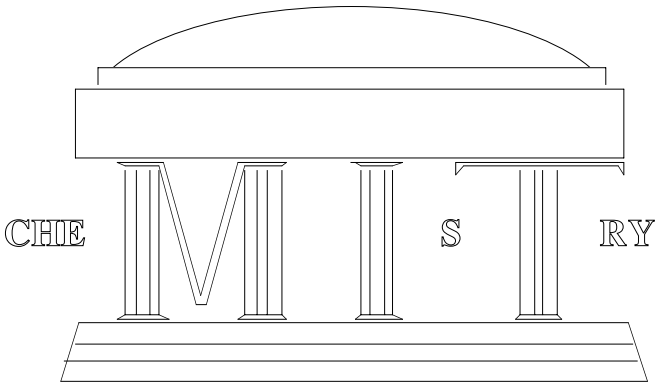
Massachusetts Institute of Technology

Department of Chemistry



Laboratory Manual

5.301
Chemistry Laboratory Techniques

Prepared by Katherine J. Franz and Kevin M. Shea
with the assistance of Professors Rick L. Danheiser and Timothy M. Swager

Revised by J. Haseltine, Kevin M. Shea, and Sarah A. Tabacco

IAP 2004

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1.1. Overview

Welcome to 5.301! This course has been specially designed as an intensive introduction to the techniques of experimental chemistry. Our goals in this class are twofold. First, since freshmen cannot enroll in any of the regular chemistry lab courses, 5.301 has been created to give interested first-year students an opportunity to get "hands-on experience" with chemistry. A second aim of 5.301 is to prepare freshmen for UROP in the Chemistry Department. Freshmen often have a difficult time finding a UROP position in our department because they don't yet have the experimental skills and experience developed in our regular chemistry lab course sequence. During the next month, you will mix, stir, and measure until you reach a "professional level" of skill in various techniques fundamental to chemical research. Unlike other laboratory classes, the goal is not just to successfully perform an experiment and write a report; instead, the focus will be on mastering the techniques and skills necessary to carry out experiments.

The techniques we will study in 5.301 are divided into five different modules, each consisting of two sections: “Competent Chemist” and “Expert Experimentalist.” To obtain your "Competent Chemist Rating" for each technique, you will be required to develop a certain level of proficiency with that skill as demonstrated by obtaining a minimum amount and minimum purity of a specific product. After you have attained your "CC Rating" for a particular section, you may then move to the more challenging "Expert Experimentalist" level technique where you will need to demonstrate an even higher level of skill to obtain your "EE Rating." To help gauge your personal progress, after completing each module you should review the "Techniques Checklist" at the beginning of the section and ask yourself whether you now feel comfortable performing that set of laboratory operations. Remember that you can be comfortable with a technique while not being a true expert. It is too ambitious for us to imagine that after 5.301 you will be able to independently solve any problem that comes your way in the research lab. This will come after much more experience and practice. Our goal is for you to reach a professional level of comfort and understanding so that you can seek the proper advice when confronted with unfamiliar problems or techniques.

In the final week of the course, you will be introduced to original research. Your "advisor" will pose a problem that you will try to answer in the lab. The experiments you will run require many of the techniques you will learn during the first three weeks of the class. If these skills are applied correctly, you will be able to provide your "advisor" with an experimentally determined answer.

When you have completed 5.301, you will have acquired many of the fundamentals of laboratory practice, and you will be ready to attack more challenging problems. Good luck!

1.2. The Texts

Two texts have been chosen for 5.301: *The Organic Chem Lab Survival Manual: A Student's Guide to Techniques*, Sixth Edition, by James Zubrick (referred to as Zubrick) and *Advanced Practical Organic Chemistry*, Second Edition, by J. Leonard, B. Lygo, and G. Procter (referred to as LLP). These texts complement each other nicely. Although both texts were designed particularly for organic chemistry lab students, the techniques described are equally relevant in inorganic and organometallic research. Many of the included techniques are important in biological and physical chemistry labs as well.

The text by Zubrick is extremely readable and was designed specifically for the introductory organic lab student. It has excellent practical advice, nice illustrations, and is actually quite funny. This is a good place to start when learning about unfamiliar techniques. One note of caution, however, is that some of Zubrick's discussions are either dated or a bit below the level of 5.301. This is where LLP comes in.

The text by Leonard, Lygo, and Procter, while still readable, was (as the title indicates) designed for a more advanced audience than Zubrick. This text can supplement Zubrick by explaining subjects in more detail and describing the true research lab, as opposed to the undergraduate teaching lab. LLP can aid you in your goal of becoming comfortable not only in the teaching lab, but also in the research environment.

1.3. Introductory Reading

Before we get started in the lab on January 5th, there are several chapters in the text that you must read first. Our time in the lab will be intense, but of limited duration, so it is important that you complete the readings on time. Not only is the reading essential to your success in 5.301, but it also will help in your development as an experimental chemist.

So, before you begin your experiments, take some time to read over the following chapters in Zubrick. This text was selected because it's easy to read and very practical. For more in-depth reading on these and related topics, we recommend the listed selections in the text by Leonard, Lygo, and Procter. A copy of this text will be available in the Reserve Book Room of the Science library. It is often difficult to fully grasp a laboratory concept by simply reading about it, but using the strategy of introductory reading, practicing in the lab, and post-lab review reading you will retain most of what we will cover in 5.301.

At the beginning of each lab period, there will be a short overview of that day's topic where Dr. Tabacco and your TAs will facilitate a discussion of the assigned reading and the actual lab experiment. You will also view relevant portions of *The Digital Lab Techniques Manual* to give you a visual representation of the important techniques. Time will be set aside to answer questions that you have from the readings.

The following list is the bulk of the reading for the course. There will also be additional reading during IAP, but this introductory reading is meant to familiarize you with the typical chemistry laboratory.

We'll see you on January 5th at noon!

Zubrick - ***The Organic Chem Lab Survival Manual (Sixth Ed.)***

Chapters: **1**- Safety, **2**- Notebooks, **4**- Jointware, **6**- Interesting Equipment, **9**- Clean and Dry, **10**- Drying Agents, **11**- On Products, **15**- Extraction and Washing, **18**- Heat, **19**- Clamps, **31**- Instrumentation in the Lab

Leonard, Lygo, and Procter - ***Advanced Practical Organic Chemistry (Second Ed.)***

Chapters: **1**- Introduction, **2**- Safety, **3**- Keeping Records, **4**- Equipping the Lab, **8**- Vacuum Pumps

1.4. Grading

Overview:

This class will be graded strictly on a pass/no record basis. It has been constructed such that, if you complete a predetermined number of experiments, you will pass the class. In 5.301, a pass means that you are qualified to begin UROP in a chemistry research laboratory. If you do not complete the required experiments, then you will not receive a pass and will not be ready to start a UROP. However, this class has been designed so that talented, dedicated, and enthusiastic students should not find it difficult to successfully complete the requirements.

During our four weeks together, you will encounter five technique modules and an introduction to an original research project. You will work on the technique challenges during the first three weeks, with the final week set aside for the research project. Each technique module has two exercises rated at different levels of technical difficulty. Successfully completing the first level will earn you a "competent chemist" rating, denoting that you have achieved a sufficient level of expertise in this technique area to allow you to carry out research requiring this experimental technique. Successfully completing the second exercise in each technique area will gain you the coveted "expert experimentalist" rating, identifying you as having an advanced level of skill in that technique.

Requirements:

All technique modules, CC and EE, come complete with standards that you must meet to earn your rating in that experiment. If, on the first try, you do not meet these standards, then you should repeat the experiment until you obtain the desired result. Keep in mind that experimental chemistry is both a craft as well as a science, and in some cases considerable practice is necessary before chemists can reach a certain level of expertise.

To successfully complete 5.301, you must pass all five CC level experiments and two EE challenges. You are encouraged to complete them all, but required to complete only two. You must also run at least one epoxidation reaction during the original research project .

1.5. 5.301 CALENDAR
January 2004

Sun	Monday	Tuesday	Wednesday	Thursday	Friday	Sat
				1	**2**	**3**
4	**5 Day #1** Lab Safety; Overview; Instruments	**6 Day #2** CC: Transfer & Manipulation	**7 Day #3** CC: Recrystallization	**8 Day #4** EE: T&M or Recrystallization	**9** Optional: Re-do CC or Finish EE	**10**
11	**12 Day #5** CC: Distillation	**13 Day #6** EE: Distillation	**14 Day #7** CC: Column Chromatography	**15 Day #8** EE: Distillation or Column (day 1)	**16** Optional: Re-do CC or Column (day 2)	**17**
18	**19** Holiday	**20 Day #9** CC: Biochemistry	**21 Day #10** EE: Biochemistry	**22 Day #11** Original Research: Make salen ligand	**23** Original Research: Make Mn Catalyst	**24**
25	**26 Day #12** Original Research: Run Epoxidation	**27 Day #13** Original Research: Purify & Analyze	**28 Day #14** Original Research: Compile Data	**29** Finish Up & Check Out	**30** Original Research Report Due	**31**

1.6. How to Use This Manual

Like the entire class, this manual has been designed to introduce you to the chemistry research environment. We will spend very little time discussing theory and concepts, and will instead concentrate on practical aspects of chemistry. To facilitate practical learning, this manual has been divided into nine sections that will be explained here in brief.

First, the section that you are currently in—The Introduction—will get you acquainted with the goals and philosophy of 5.301.

Second, sections 2–6 cover technique modules that make up the bulk of the class. The five topics included here are "Transfer and Extraction," "Purification by Crystallization," "Purification by Distillation," "Purification by Flash Column Chromatography," and "Protein Assays and Error Analysis." It is important to note that the manual does not contain all of the information that you will need to complete these experiments. Some important information will be found in your pre-lab reading, while the rest will be covered during the pre-lab discussion. This three-pronged approach (the texts, the manual, and the discussions) will prepare you to tackle the experiments outlined in the technique modules.

An important part of sections 2-6 is the techniques checklist. Each section begins with a list of techniques that you will encounter during the experiment. When you have completed a technique module, you should return to the techniques checklist and check off all of the techniques that you have mastered. If you are still uncomfortable with a specific skill then you should practice it until you feel confident that you could apply it in a different experiment. In addition to various purification and manipulation techniques, this section will also introduce you to spectroscopic techniques like nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy, gas chromatography (GC), and ultraviolet-visible (UV-Vis) spectroscopy.

Third, section seven discusses the original research project that you will encounter in the fourth week of IAP. This brief set of experiments will introduce you to some of the realities and excitement of performing original research.

Fourth, section eight, entitled "Technique Guides," will provide you with step-by-step instructions for some of the more common techniques encountered in a chemistry laboratory. These guides will prove useful not only in 5.301, but also in your research, where many of these techniques will be encountered again.

Finally, section nine will instruct you on the operation of the instruments we will use on a regular basis in 5.301. These detailed instructions will help you become familiar with the operation of the NMR, GC, IR, and UV-Vis instruments.

1.7. INTRODUCTION TO THE LABORATORY

Prepared with the help of Dr. Mircea Gheorghiu and Professor Scott Virgil

1. SAFETY

Be sure that you are familiar with the locations and use of the following safety equipment:

1. Fire extinguishers, mounted in various locations in the lab.
2. Showers, one in each of the labs near the corridors.
3. Eye wash stations/face sprays, one at each sink in the center aisle.
4. Fire blankets, at each end of the lab near the corridors and near the power control panel.
5. Telephone - to be used for emergency calls only - **DIAL 100**.

Only CO_2 and dry-chemical fire extinguishers should be used on chemical or electrical fires. Water faucets at sinks may be used to wash skin exposed to corrosive chemicals. You should note the location of this safety equipment in your working areas and be sure (even rehearse) what you would do in the case of a fire or other accident. However, in the event of fire or other accident, do not take any action that would risk the safety of yourself or others. Most importantly, make any emergency known as soon as possible to a TA or staff member.

You must wear **safety goggles** in the laboratory at all times. *This is a Massachusetts state law, not just a lab regulation*. Although radios and musical instruments are not technically considered safety hazards, they will not be allowed in the laboratory.

Learning about the hazards of materials, equipment, and procedures used in chemical laboratories is a part of the educational objective of this subject. We will discuss matters of safety pertaining specifically to this course during our first meeting on Monday, January 6. The discussion will prepare you for hazards encountered in an undergraduate lab. At this time, you will also receive a copy of the MIT Chemistry Department's *Chemical Hygiene Plan and Safety Manual*, which will serve as your safety reference throughout your MIT career.

Disposal of solvents, chemicals and other materials:

Never pour solvents or reactive chemicals down a drain. Such careless handling of flammable or toxic liquids presents a serious hazard in the laboratory. Also, never keep an open beaker of such solvents outside a hood. Chlorinated solvents are poured into solvent waste containers kept inside the hoods in 4-454 and 4-460. When in doubt about how to dispose of something, ask a TA. If drain disposal is necessary and acceptable, always flush the drain before, during, and afterwards with a lot of water, always using the drains in the hoods. **All glass must be discarded in the specially designed containers.** A dust pan and brush for broken glass can be checked out of Lab Supplies (4-450). Spilled mercury is a special safety hazard and should be reported to your TA for cleanup.

2. CHECK-IN PROCEDURE

After a brief tour of the undergraduate labs (including instrumentation and safety equipment), the Lab Check-In Procedure will begin. You will be assigned a lab bench and should obtain the following items:

1. A sheet of safety regulations - you must read, sign, and turn in this sheet.
2. Desk assignment and key, a list of desk equipment and Check-In Sheet
3. Safety goggles, lab coat, and a lab notebook (required for 5.301).
4. A list of 5.301 specific equipment.

Check the equipment in your cabinet against the list given to you by the TA. Report any discrepancies to the TA, who will either give you the missing item or instruct you to obtain it at the Laboratory Supplies Stockroom. Once you have signed the Check-In Sheet, you are responsible for the items in your desk. At the termination of the course, even if the course is dropped the following day, it is your responsibility to check-out of the laboratory (see Item 3 below).

3. CHECK-OUT PROCEDURE AND CHARGES

Check-out will be on **Thursday, January 30, 2002**. Students who do not check out as scheduled will be checked-out by the Office of Laboratory Supplies. For this service, the student's personal account will be charged $35.00.

4. LOCATIONS OF LABORATORY EQUIPMENT

a. Chemicals and Solvents

Organics and Inorganics - 4-457

Acids and Bases - under hood in 4-457

Solvents - 4-454 and 4-460, on shelves at end of benches

b. Ovens and Refrigerators

Ovens are located in 4-454 and 4-460. Each oven is designated for a specific purpose. Do not place any plastic items in the ovens. All samples must be clearly labeled with the identity of compound, your name and date. Ovens will be cleared weekly and improperly labeled samples will be removed. Refrigerators - 4-454. Samples must be clearly labeled.

c. Balances. Abuse of balances and littering of the area will not be tolerated.

d. Common Laboratory Items

The following items are available from LS (LS = Lab Supplies):

vials and labels for submitting samples

filter paper, 17 mm, 5 1/2 cm, 11 cm

rubber stoppers, rubber septa and rubber bands

pliers, needle-nosed, file, glass tubing and other hardware

sponge, spill pillow absorbent dust pan and brush

5. SAFETY IN THE M.I.T. UNDERGRADUATE CHEMISTRY LABORATORIES

Protection of the health and safety of individuals in the laboratory and respect for preservation of the environment are regarded by the Chemistry Department as moral imperatives. A good safety program requires everyone to share the responsibility - faculty, staff, and students. The safety program in these laboratories is headed by the Undergraduate Laboratory Director, Dr. Mircea Gheorghiu, and includes an Undergraduate Laboratory Safety Committee composed of faculty, teaching assistants and students.

Safety information will be provided in a number of ways. Each laboratory subject begins with a mandatory safety lecture to provide general information and advice. In addition, the instructions for each experiment and the accompanying TA presentations will contain safety information specific to each experiment. Reference works with various sorts of data on chemicals used in the laboratory will be on file and available in the reference room outside Dr. Gheorghiu's office. One of these, *Prudent Practices in the Laboratory*, is especially recommended as a readable comprehensive document on the subject.

The laboratory policy regarding toxic substances is to design experiments and procedures that keep levels of exposure below the threshold limit values (TLV's) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). This is a conservative policy, since these TLV's are regarded as safe for indefinite periods of exposure for 40 hours a week in the work place. Copies of the ACGIH-recommended TLV's are available for reference.

Notwithstanding the department's unswerving commitment to safe undergraduate laboratories, it is important to bear in mind that an absolutely *risk-free* teaching environment is neither possible nor desirable. Hazards abound in daily life. Gasoline, for example, is both explosive and toxic, yet most car-driving citizens are confident that they know how to handle it safely. Anyone considering a career in the experimental sciences or in medicine needs to learn how to handle a great variety of potentially dangerous substances with informed caution and competence. One of the objectives of the undergraduate laboratory subjects is to provide that kind of education for safe behavior and practices in the laboratory and in the outside world.

A list of basic rules for safety in the laboratory, which you should be familiar with, is appended. It is also imperative that you become familiar with your copy of *The Chemical Hygiene Plan and Safety Manual*. Strict adherence to the guidelines outlined in both of these references will promote a safe and successful lab experience.

6. GENERAL SAFETY RULES FOR THE UNDERGRADUATE LABS

1. The safe way is the right way to do your job. Plan your work. Follow instructions. If you do not know how to do the experiment safely, ask your teaching assistant.

2. Be able to use all safety devices and protective equipment provided for your use and *know their location* (eyewash fountain, shower, fire blanket, fire extinguisher).

3. Safety goggles must be worn at all times.

4. *Do not* eat or drink in the laboratory (and do not store food in the refrigerators). Smoking in the laboratory is absolutely forbidden.

5. Personal effects: wear proper clothing (including protective clothing when handling corrosive, toxic, or flammable materials). Avoid loose sleeves, loose cuffs, bracelets. Be careful with long hair. Proper shoes are required (no sandals).

6. Horseplay in any form is dangerous and prohibited. Do not run in laboratory areas.

7. If you see a colleague doing something dangerous, point it out to him or her and to the TA.

8. Report to your TA all unsafe conditions, unsafe acts, and "near misses" that might cause future accidents. Report any accident or fire, no matter how trivial, to the TA.

9. Hazardous Chemicals:
   a. Be especially mindful of fire hazards when you *or your lab neighbors* are working with flammable liquids.
   b. Hazardous Substances: Know common explosive, toxic, and carcinogenic materials and use them only with adequate safeguards.

10. Never leave a reaction or experiment running unattended, unless you have told your lab partners enough about it to deal with potential hazards while you are away.

11. Keep hood and benchtop areas clean and workable space maximized.

2. Transfer and Extraction Techniques

2.1. Competent Chemist Rating: *"Ethyl Ester's Excellent Adventure"*

Techniques Checklist:

- Extraction and Washing ❑
- Careful transfer of solutions without loss of material ❑
- Solvent drying and concentration ❑
- Melting point determination ❑
- Nuclear Magnetic Resonance (NMR) spectrometer operation ❑

Pre-lab Discussion and Required Reading:

- Extraction : Zubrick Ch. 15, LLP Ch. 10
- Theory of extraction: Zubrick Ch. 37
- Melting point determination : Zubrick pp. 87-92
- NMR theory and operation : Zubrick Ch. 35, LLP Ch. 15.2

Digital Lab Techniques Manual:

- 5. Reaction Work-Up I: Extracting, Washing & Drying
- 6. Extraction Work-Up II: Using the Rotavap

Equipment:

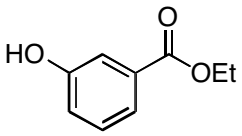
- Graduated Cylinder (100-mL)
- Separatory funnel (125-mL)
- Erlenmeyer flasks (2x250-mL)
- Beaker (150-mL)
- Round-bottomed flask (100-mL)
- NMR tube
- Funnel
- Filter Paper
- Rotary evaporator

Goal:

To manipulate and purify a known amount of a contaminated sample and to record its ^{1}H NMR spectrum, all with minimal loss of material.

Experimental Outline:

• You will receive a vial containing 100 mg of ethyl 3-hydroxybenzoate contaminated with triethylamine. You will also receive four different ^{1}H NMR spectra: one of the mixture in your vial, and one each of pure ethyl 3-hydroxybenzoate, triethylamine, and diethyl ether.



Ethyl 3-Hydroxybenzoate
MP 72–74 °C

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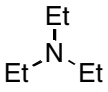


Triethylamine

• Dissolve your sample in 50–75 mL of ether in a separatory funnel.
• Remove the amine by extraction with a 10% HCl solution.
• Continue with a standard aqueous work-up, including an ether back-extraction - *see Extraction and Washing Guide*.
• Remove the solvent by rotary evaporation to a constant weight, and obtain a mass.
• Take a ^{1}H NMR spectrum of the compound and compare to the other spectra.
• Recombine the NMR sample with the remainder of the purified sample.
• Remove the solvent for the final time to a constant weight.
• Obtain a mass and a melting point.

Helpful Hints:

• When removing solvent with the rotary evaporator, make sure the receiving flask is cold and the water bath is warm. Otherwise, your product will never solidify.
• If you have trouble getting your product to solidify, try adding a few milliliters of methylene chloride to your flask and returning it to the rotary evaporator.

Results:

• To obtain your "CC Rating" in Transfer and Extraction Techniques, you must end with at least 90 mg of ethyl 3-hydroxybenzoate. Additionally, this material must be of adequate purity as determined by ^{1}H NMR analysis. This means that the spectra should show only negligible amounts of impurities as judged by the professor and TA. In addition, the purified material should melt over no more than three degrees, with the lower range beginning no lower than 69 °C and the upper range ending no higher than 73 °C. This material must also be submitted to the TA for possible weight and melting point confirmation measurements.

2.2. Expert Experimentalist Rating: *"Acid, Base, and in Between"*

Techniques Checklist:

- Separation of multi-component mixture using pK_a ❑
- Planning an extraction and washing sequence ❑
- Careful transfer of solutions without loss of material ❑
- Solvent drying and concentration ❑
- Melting point determination ❑

Pre-Lab Discussion and Required Reading:

- Same as CC

Digital Lab Techniques Manual:

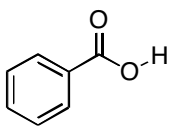
- Same as CC

Equipment:

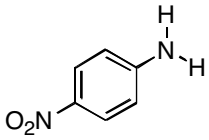
- Graduated Cylinder (100-mL)
- Separatory funnel (125-mL)
- Erlenmeyer flasks (4x250-mL)
- Beaker (150-mL)
- Round-bottomed flask (100-mL)
- pH paper
- NMR tube
- Funnel
- Filter Paper
- Rotary evaporator

Goal: To separate a three-component mixture, using differences in *pKa*, with minimal loss of material.

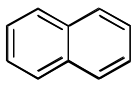
Experiment Outline: You will receive a vial containing 100 mg each of benzoic acid, 4-nitroaniline, and naphthalene. Using the pK_a values of these molecules, carefully devise an extraction and washing sequence that will selectively separate the three components.[1]



Benzoic Acid
MP 122–123 °C
pKa 4.2



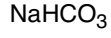
4-Nitroaniline
MP 149–151 °C
pKa (RNH_3^+) 1.0



Naphthalene
MP 80–82 °C
(no acidic protons)

Water
pK_a 15.7

Hydrochloric Acid
pK_a –7



Sodium Bicarbonate
pK_a (H_2CO_3) 6.4

- Before beginning your extraction sequence, check with your TA or professor to make sure that it will work.
- You are free to use any or all of the following solvents and solutions:
  - Diethyl ether
  - Methanol
  - Water
  - Saturated Sodium Bicarbonate Solution (Aqueous)
  - 6 M HCl
  - 1 M NaOH
- Carry out your extraction and washing sequence, isolating each of the three components.
- For each compound, remove the solvent by rotary evaporation to a constant weight, and obtain a mass.
- Obtain a melting point for each compound.

Results: To obtain your "EE Rating" in Transfer and Extraction Techniques, you must isolate at least 90 mg of two of the three compounds in the mixture. In addition, the isolated compounds should melt over no more than three degrees, with the range beginning no lower than two degrees below the literature melting point values given above.

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[1] Adapted from Gilbert, J. C; Martin, S. F. *Experimental Organic Chemistry: A Miniscale & Microscale Approach*; 3rd ed.; Brooks/Cole: Pacific Grove, CA 93950; p.141.

3. Purification of Solids by Recrystallization

3.1. Competent Chemist Rating: *"How do you recrystallize a Mothball?"*

Techniques Checklist:

- Solubility tests ❑
- Choosing a good solvent system ❑
- Decolorization ❑
- Inducing crystallization ❑
- Filtration ❑

Pre-Lab Discussion and Required Reading:

- Theory of recrystallization : Zubrick Ch. 13, LLP Ch. 11.2

Digital Lab Techniques Manual:

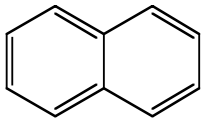
- 9. Recrystallization

Equipment:

- Test tubes (five 13x100 mm)
- Erlenmeyer flasks (2x50-mL, 1x125-mL)
- Small magnetic stir bar
- Funnel
- Filter paper
- Büchner funnel and filter paper
- Magnetic stirring/hot plate
- Filter flask (250-mL) and aspirator stopper
- Rubber filter adapter
- Large vial with white cap
- Test tube rack
- Large crystallizing dish
- Desiccator

Goal:

You will be given 2.00 g of impure naphthalene (mothballs!). Your job is to purify the naphthalene by recrystallization without losing a significant amount of your sample![1]



Naphthalene

Experiment Outline:

I. Solubility Tests

• Determine an appropriate solvent system for the recrystallization of naphthalene. For your tests try: **water, methanol, acetone, hexane, and toluene.** To understand how to find the appropriate solvent or solvent mixture for recrystallization, see pages 90 and 99-102 in Zubrick.

II. Recrystallization of Naphthalene

• Transfer the material to a 50-mL Erlenmeyer flask equipped with a stir bar. Add about 20 mL of the solvent (determined in Part I) and heat to boiling on a stir/hot plate.

• Remove any insoluble impurities by filtration, and recrystallize your product - *see Two-Solvent Recrystallization Guide*.

• Collect your crystals on a small Büchner funnel by vacuum filtration, and rinse with the cold solvent mixture.

• Your crystals should be colorless. If some orange or yellow color persists, wash your material with cold hexane. (*Be careful: What is the solubility of naphthalene in hexane?*).

• Dry your compound well - *see Two-Solvent Recrystallization Guide for tips*.

• Determine the yield, and obtain a melting point.

Results:

• To obtain your "CC Rating" in Purification of Solids by Crystallization, you must obtain colorless, well dried crystals weighing at least 1.30 g (no traces of yellow!). The purified material must melt over no more than three degrees with the lower range beginning no lower than 77 °C and the upper range ending no higher than 83 °C. This material must also be submitted to the TA for possible weight and melting point verification.

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[1]Adapted from Fieser, L. F.; Williamson, K. L. *Organic Experiments;* 7th ed.; D. C. Heath and Company: Lexington, MA, 1992; p. 40.

3.2. Expert Experimentalist Rating: *"The Single-Crystal Shakedown"*

Overview:

X-Ray diffraction is an important and powerful tool for determining the solid state structure of compounds. Modern advances have made data collection and structure solution almost routine for many small molecules. To use this technique, however, good quality single crystals are still needed. In this exercise, you will experiment with the art of growing single crystals.

Techniques Checklist:

- Manipulation of milligram quantities of material ❑
- Syringe use ❑
- Crystallization techniques for growing good quality single crystals ❑

Pre-Lab Discussion:

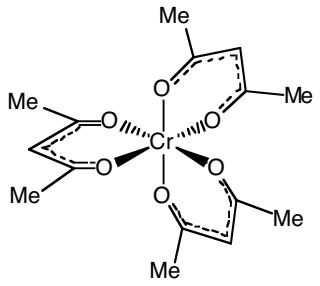
- Use of different recrystallization techniques: vapor diffusion, solvent layering, temperature variation

Goal:

Synthesize $Cr(acac)_3$,[2] then perform several slow recrystallizations to obtain a single crystal of satisfactory quality.

Equipment:

- Magnetic stir plate
- Heating mantle and Variac
- 50-mL Round-bottomed flask
- 2-mL Glass syringe
- Condenser
- Stir bar
- Glass frit (D)
- 250-mL Filter flask and rubber filter adaptor
- Vials (3 large, 4 small)
- 2 Glass jars

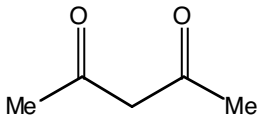


Chromium Acetylacetonate
$Cr(acac)_3$

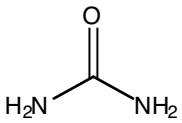
[2]Adapted from Szafran, Z.; Pike, R. M.; Sing, M. M. *Microscale Inorganic Chemistry: A Comprehensive Laboratory Experience*; Wiley: New York, 1991; "Synthesis of Metal Acetylacetonates" p. 224-229.

Experiment Outline:

• ***Before coming to the lab***, perform the necessary calculations to fill in the following table.



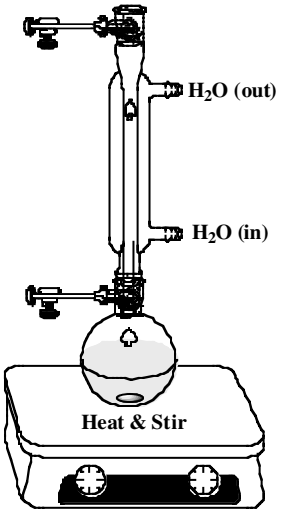
2,4-Pentanedione



Urea

Reagent	Source	F.W.	Density	Mass or Volume	mmols	Equiv
$CrCl_3 \cdot 6H_2O$					1.00 mmol	1
Urea						17
2,4-Pentanedione						8
$Cr(acac)_3$	Product					

• Dissolve $CrCl_3 \cdot 6H_2O$ in 2 mL of distilled water in a 50-mL round-bottomed flask, equipped with a stir bar.
• Add the urea in one portion to the flask, and stir until completely dissolved.
• Add the 2,4-pentanedione dropwise via syringe.
• Attach the condenser to the flask, and heat the mixture to vigorous reflux (*this is important!*), with stirring, for about 1 hour.
• Cool the reaction flask to room temperature, and collect the product by vacuum filtration on a size D glass frit funnel, washing the product with cold water.
• Dry the product in your desiccator overnight, and obtain a yield.
• Set up multiple recrystallizations to grow X-ray qualitysingle crystals - *see Growing a Single Crystal Guide*.

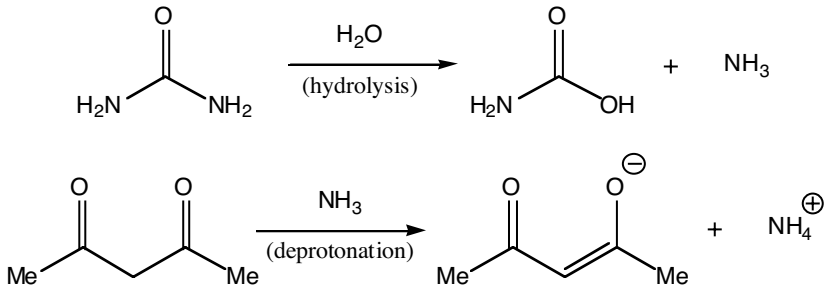


Reaction Setup

Note:

• Urea slowly hydrolyzes under the reaction conditions, liberating ammonia (NH_3), which controls the pH of the reaction. As more NH_3 is generated, the solution becomes more basic, making it easier to remove the proton from the acetylacetonate (acac), also known as 2,4-pentanedione. It is the anion of the acetylacetonate that coordinates to the

metal to form the desired $Cr(acac)_3$ complex. What is the limiting reagent? Calculate your percent yield.



Helpful Hints:

• When using a saturated solution to grow crystals, it is important that you filter the solution through a plug of glass wool in a pipet before setting up the crystallization.

Results:

• To obtain your "EE Rating," you must obtain ≥ 50% yield of $Cr(acac)_3$, and you must produce at least one single crystal that is suitable for X-ray analysis.

4. Purification of Liquids by Distillation

4.1. Competent Chemist Rating: *"How Did the Peach Get in the Banana?"*

Techniques Checklist:

- Setting up distillation glassware correctly ❑
- Performing atmospheric pressure distillations ❑
- Using Gas Chromatography (GC) to analyze samples ❑

Pre-lab Discussion and Required Reading:

- Theory of distillation: Zubrick Ch. 36, LLP Ch. 11.3
- Distillation glassware and how to set it up: Zubrick Ch. 20
- Use of the GC: Zubrick Ch. 32

Equipment:

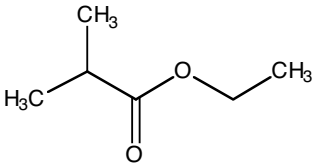
- Round-bottomed flasks (1x25-mL, 1x50-mL)
- Magnetic stir bar
- Distillation kit (distillation head)
- Ground glass thermometer
- Keck clips
- Glass wool and aluminum foil (optional)
- Heating mantle (w/ sand) and variac

Goal:

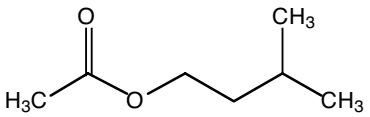
- To purify a mixture of two liquids using distillation.

Experiment Outline:

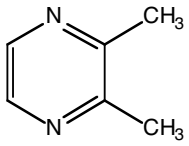
- You will receive a vial containing 11.20 g of a mixture of 2 compounds whose boiling points differ by about 40 °C (See possible compounds below.).
- Analyze the mixture using the GC - *see GC Sample Preparation and GC Operation Guides*.
- Perform atmospheric pressure distillation - *see Distillation Guide*.
- Prepare a GC sample of your purified low-boiling product.
- Obtain a mass and a gas chromatogram of your purified low-boiling compound.



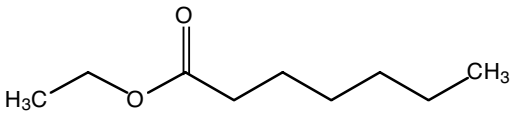
Ethyl Isobutyrate (Peach)
(BP 112–113 °C)



Isoamyl Acetate (Banana)
(BP 142–143 °C)



2,3-Dimethylpyrazine
(BP 156 °C)



Ethyl Heptanoate
(BP 188–189 °C)

Helpful Hints:

• Make sure all of your joints are sealed well. Otherwise, you will lose your product into the atmosphere.

• Do not heat your mixture too fast, or your entire sample will end up in your collection flask.

• Be aware that the temperature reading on the thermometer may not correlate accurately with the boiling point of the distilling liquid.

Results:

• To obtain your "CC Rating" in Purification of Liquids by Distillation, you must obtain at least 7.00 g of the low-boiling material that is 92% pure or better as determined using GC analysis. You must also correctly identify the two components of your mixture. Think boiling points and smell!

4.2. Expert Experimentalist Rating: *"What's With Those High-Altitude Recipes?"*

Techniques Checklist:

- Glassware setup for reduced pressure distillation ❑
- Running reduced pressure distillation ❑

Pre-lab Discussion:

- Differences between atmospheric pressure and reduced pressure distillation

Equipment:

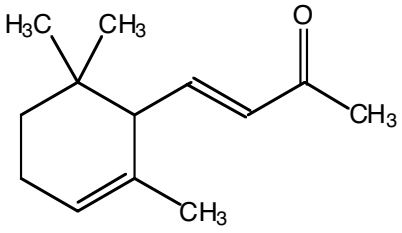
- 25-mL Round-bottomed flask
- Vacuum distillation kit (distillation head, cow adaptor)
- Collection flasks
- Ground glass thermometer
- Keck clips
- Glass wool and aluminum foil
- Heating mantle (w/ sand) and Variac

Goal:

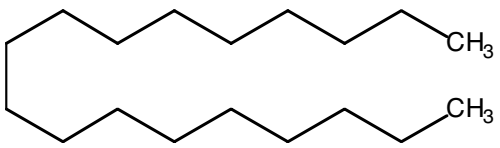
- To purify a mixture of two liquids by reduced pressure distillation.

Experiment Outline:

- You will receive a vial containing 7.50 g of a mixture of α-ionone and octadecane.
- Repeat procedure for CC level distillation using a Vigreux column and the high vacuum line - *see Distillation Guide*.



a-Ionone
(BP ~ 265 °C)

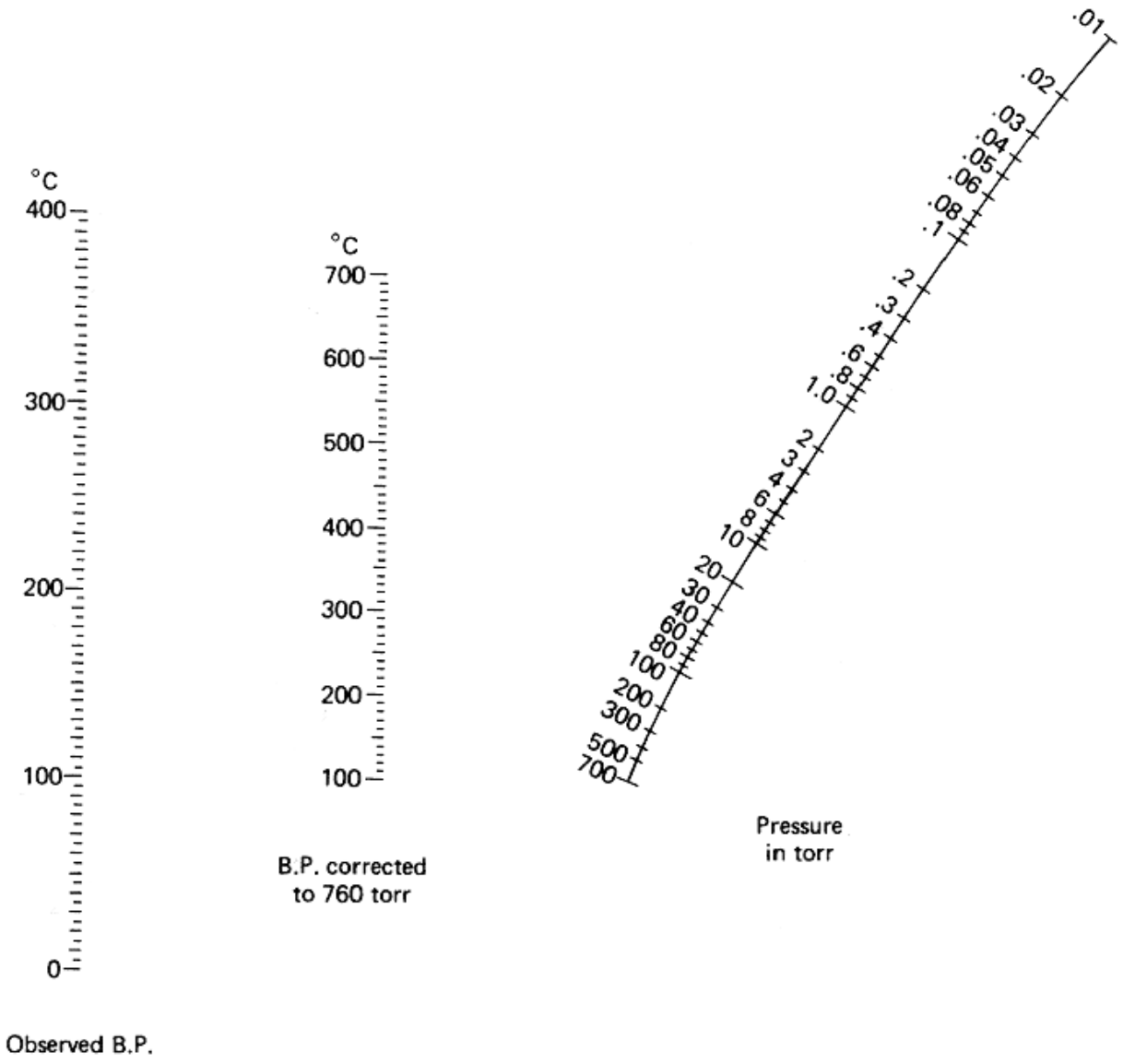


Octadecane
(BP 317 °C)

Results:

• To obtain your "EE Rating" in Purification of Liquids by Distillation you must predict the boiling points of the compounds in your mixture at 0.5 torr. You must also obtain at least 4.00 g of α-ionone that is 93% pure or better as determined using GC analysis.

The picture below is a **nomograph**. Using it and a ruler, you can determine at what temperature a liquid will boil under vacuum.



5. Purification by Flash Column Chromatography

5.1. Competent Chemist Rating: *"Looks Can Be Deceiving"*

Techniques Checklist:

- Analyzing mixtures by TLC ❑
- Assembling a silica gel column ❑
- Applying crude mixtures to a silica gel column ❑
- Separating simple mixtures with a silica gel column ❑

Pre-lab Discussion and Required Reading:

- Theory of column chromatography : Zubrick Ch. 27
- TLC - polarity/solvent systems: Zubrick Ch. 28, LLP Ch. 9.3.1
- Setting up a silica gel column: Zubrick Ch. 29, LLP Ch. 11.6
- Applying crude mixtures to the column
- Running a flash column

Equipment:

- Flash Chromatography Column
- Air flow apparatus (stopper, T-valve, screw clamp, tubing)
- 100-mL Round-bottomed flask
- Test tubes - 18x150 mm
- Test tube rack
- TLC plates and spotters
- UV lamp
- Large plastic funnels

Digital Lab Techniques Manual:

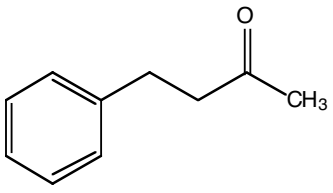
- 3. TLC: The Basics
- 10. Column Chromatography

Goal:

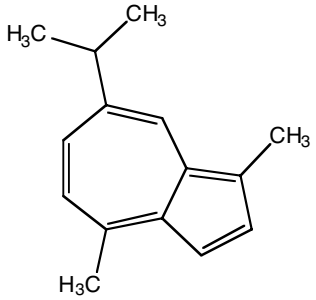
- Purify a contaminated compound using silica gel flash column chromatography.

Experiment Outline:

- You will receive 2 mL of an ether/pentane solution containing 1.00 g of benzylacetone contaminated with a small amount of guaiazulene.
- Analyze this mixture by TLC, using 10% ethyl acetate/hexanes as the solvent system - *see TLC Guide*.
- Record the R_f values.
- Prepare the column in the hood, using 10% ether/pentane and 50 g (about 5'') of silica gel - *see Flash Column Chromatography Guide*.
- Elute the column with 10 mL of pentane.
- Apply your sample to the column, being careful not to disturb the top layer of sand. Rinse the sample flask three times with 1 mL pentane each, and use the rinses to wash the sides of the column.
- Run the column - *See Flash Column Chromatography Guide*.
- Monitor the fractions by TLC - *See TLC Guide*.
- Concentrate the set of fractions containing pure benzylacetone.
- Weigh the purified compound and prepare a GC sample.
- Obtain a TLC and a gas chromatogram of the purified compound.



Benzylacetone



Guaiazulene

Results:

- To obtain your "CC Rating" in Purification by Flash Column Chromatography, you must collect at least 0.95 g of benzylacetone. This sample must be at least 95% pure as demonstrated using ^{1}H NMR spectroscopy. Your sample must also be submitted to the TA for possible weight and purity verification.

5.2. Expert Experimentalist: *"Setting the Pace"*

***** This is a two-day experiment. Do not start this experiment unless you have two free lab days to complete it.** ***

Techniques Checklist:

- Picking the correct eluent ❑
- Adsorbtion of a crude mixture onto silica gel ❑
- Separating complex mixtures using gradient elution ❑

Pre-lab Discussion:

- Suggest limited list of eluent solvent systems
- Discuss sample adsorption and gradient elution strategies

Equipment:

- Identical to CC Level

Goal:

- Separate a complex mixture of three compounds using gradient elution flash column chromatography.

Experiment Outline:

- You will receive 20 mL of an ether/pentane solution containing 0.20 g of guaiazulene, 0.20 g of 9-fluorenone, and 0.20 g of 3-methylanisole.
- Analyze the mixture by TLC using the solvent systems discussed in the pre-lab lecture - *see TLC Guide*.
- Decide on an appropriate starting eluent.
- Decide on the silica gel to compound ratio.
- Prepare the column - *See Flash Column Chromatography Guide*.
- Adsorb the mixture onto a small amount of silica gel, according to the instructions provided in the pre-lab lecture and the DLTM.
- Apply the mixture to the column, being sure to rinse the sides and apply an extra layer of sand to the top of the column.
- Run the column.

• TLC all of the fractions from your column, have your TLC plates checked by an instructor, and reproduce them in your lab notebook.

• Calculate R_f values for the three compounds in the chosen TLC solvent mixture.

Results:

• To obtain your "EE Rating" in Purification by Flash Column Chromatography, you must successfully separate all three components of the mixture by TLC and correctly calculate the corresponding R_f values.

6. Protein Assays and Error Analysis

6.1. Competent Chemist Rating: *"What's in a Cow's Heart Anyway?"*

Techniques Checklist:

- Pipetting with pipetman ❑
- Calibrating pipetman ❑
- Preparation of a standard curve ❑
- Serial dilution ❑
- UV-Vis Spectroscopy ❑

Pre-Lab:

- Discussion of Protein Assays

Equipment:

- Pipetman: 100 P, 1000 P
- Pipet tips - large and small
- 8 Test tubes
- Eppendorf tubes and holder
- Disposable UV-Vis cuvettes - 5 mL

Goal:

- You will be given a sample of a solution of bovine heart cytochrome c. You will use the Coomassie® Plus Protein Assay from Pierce to determine the concentration of protein in the sample.

Note:

- You will receive a tray of Eppendorf tubes: one containing stock solution, three containing 50 μL each of bovine heart cytochrome c, and several empty tubes for mixing solutions. You will also be provided with a bottle of 25mM MOPS buffer, pH 7.

Experiment Outline:

Pipetman Calibration

Prior to beginning any experiment with a pipetman, it is necessary to first calibrate it. This procedure will determine exactly how much liquid is delivered when a certain amount is "dialed-in" to the instrument. To calibrate your pipetman, simply draw up a certain amount of water, empty it into a tared container, and obtain a weight. Knowing that water has a density of 1.00 g/mL, you can perform a calculation to tell you the accuracy of your pipetman. Most instruments will need no correction, and ones that are incorrect will usually be off by no more than 1 μL.

The Coomassie®-Protein Reaction Scheme

This protein assay works by forming a complex between the protein and the Coomassie® dye. When bound to the protein, the absorbance of the dye shifts from 465 nm to 595 nm (A_{595}). You will first generate a standard curve using the protein Bovine Serum Albumin (BSA) by measuring the absorbance at 595 nm of a series of standards of known concentration. Next, you will measure the A_{595} of your sample and determine its concentration by comparison to the standard curve.

Protein + Coomassie®G-250 in acidic medium---> Protein-Dye complex (blue; measured at 595 nm)

1. Preparation of diluted BSA standards

• Prepare a fresh set of protein standards by diluting the 2.0 mg/mL BSA stock standard (Stock) as illustrated below. There will be sufficient volume for three replications of each diluted BSA standard, if necessary.

Vol of the BSA to Add	Vol of Diluent (buffer) to Add	Final BSA Conc.
300 µL of Stock	0 µL	**Stock** - 2000 µg/mL
375 µL of Stock	125 µL	**A** - 1500 µg/mL
325 µL of Stock	325 µL	**B** - 1000 µg/mL
175 µL of A	175 µL	**C** - 750 µg/mL
325 µL of B	325 µL	**D** - 500 µg/mL
325 µL of D	325 µL	**E** - 250 µg/mL
325 µL of E	325 µL	**F** - 125 µg/mL
100 µL of F	400 µL	**G** - 25 µg/mL

2. Mixing of the Coomassie® Plus Protein Assay Reagent:

• Allow the Coomassie® Plus reagent to come to room temperature. Mix the Coomassie® Plus reagent solution just prior to use by gently inverting the bottle several times. Do not shake.

3. The Standard Protocol

• Pipet 0.05 mL of each standard solution into appropriately labeled Eppendorf tubes. Prepare at least three samples of your unknown solution. Use 0.05 mL of the diluent (25 mM MOPS buffer, pH 7, provided by TA) to prepare two blank tubes.

• Add 1.5 mL of the Coomassie® Plus reagent to each tube, mix well.

• Measure the absorbance at 595 nm of each tube *versus* a water reference.

• Subtract the average 595 nm reading for the blanks from the 595 nm reading for each standard or unknown sample.

• Prepare a standard curve by plotting the average blank corrected 595 nm reading for each BSA standard *versus* its concentration in μg/mL. Using the standard curve, determine the protein concentration for each unknown sample.

Helpful Hints:

• Keep all of your solutions until after you have plotted and analyzed your data. You may need to redo some of your UV absorptions.

Results:

• To obtain your "CC Rating" in Protein Assays and Error Analysis, the line fit for your standard curve must have a 0.930 correlation coefficient (R value) or higher. Additionally, the results from your absorbance values of the unknown should have a standard deviation of less than 0.048. Finally, you must determine the concentration of your unknown protein.

6.2. Expert Experimentalist Rating: *"A Heart as Strong as Iron"*

Techniques Checklist:

- Use of a centrifuge ❑

Equipment:

- Disposable UV-Vis cuvettes (1-mL capacity)
- Pipetmen: 20 P, 100 P, 1000 P
- Pipet tips
- Eppendorf tubes (safe-lock)
- Hot plate
- Centrifuge
- Boiling plate or rack to hold Eppendorf tubes
- Large crystallizing dish

Goals:

• From the CC-level experiment, you know the concentration of protein in your sample. Now you will determine the concentration of iron in bovine heart cytochrome c.

Experiment Outline:

The Ferrozine Assay

Ferrozine is an iron-chelating agent. When it forms a complex with ferrous iron (Fe^{II}), it shows a characteristic UV-Vis absorption at 562 nm. By comparing the A_{562} of your sample to a calibration curve of iron standards, you will determine the concentration of iron in your protein sample.

Solutions provided by your TA:

-Fe AA standard (AA = atomic absorption)

-Buffer - 25 mM MOPS, pH 7

-Ultrapure HNO_3 (5 M)

-75 mM Ascorbic acid solution

-10 mM Ferrozine solution

-Saturated ammonium acetate solution

1. Preparation of Standards:

• Prepare a fresh set of iron standards in 2 mL Eppendorf tubes, as illustrated below. Carefully label each tube. Also fill 2 tubes with 300 μL of your protein sample.

μL of Fe AA standard (99 μg/mL)	μL of Buffer to add
0	300
6	294
12	288
18	282
24	276

• Add 30 μL of ultrapure HNO_3 (5 M) to each standard and sample tube.

• Place the closed Eppendorf tubes in a rack, and boil them for 30 minutes in a hot water bath (a large Pyrex dish over a heating plate).

• Centrifuge for 1-2 minutes, making sure the centrifuge is properly balanced.

• Remove 300 μL of the supernatant liquid from each tube, and transfer to fresh tubes (labeled!).

• Add 1020 μL of distilled water.

• Add 60 μL of 75 mM ascorbic acid.

• Add 60 μL of 10 mM ferrozine.

• Add 60 μL of saturated ammonium acetate.

• Shake each tube and wait 10-15 minutes, (the solutions should become purplish in color).

• Transfer to a 1.5 mL cuvette, and determine the A_{562} for each standard and your two samples.

• Generate a calibration curve of A_{562} *vs.* [Fe] from your standards.

• Determine the [Fe] in your unknown.

Results

• To obtain your "EE Rating" in Protein Assays and Error Analysis, the line fit for your standard curve must have a 0.995 correlation coefficient or higher. Additionally, the absorbance values for your unknown samples must have a standard deviation of 0.035 or less. Finally, you must determine the number of molecules of iron per molecule of protein.

7. Introduction to Original Research

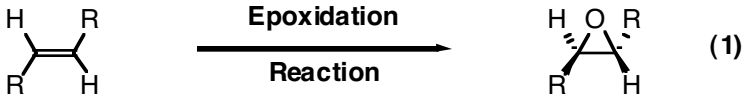
7.1. Olefin Epoxidation with Mn(salen) Complexes

Introduction:

Your "advisor" has carefully monitored your progress in 5.301 and believes that you are ready to move from the technique modules to an actual project. This project will require you to use many of the skills that you have learned over the past three weeks to address a specific question. In addition, you and your labmates will learn to work as a research group in order to reach an ambitious goal in a short period of time.

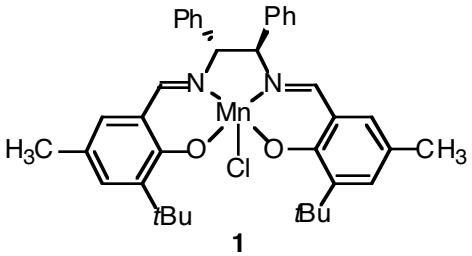
Overview: (written by your advisor)

Our group has had a longstanding interest in the epoxidation of olefins (eq 1), and would like to take another look at an important report from a decade ago. Eric Jacobsen (former MIT Post-doc and current Harvard Professor) showed that Mn(salen) complexes such as **1** are effective epoxidation catalysts (eq 1). Because Mn(salen) complexes are easy to make, I am interested in probing the relative reactivity of a series of different Mn(salen) derivatives. A graduate student in the lab has investigated several Mn(salen) complexes, but I want you and your labmates to complete a more comprehensive study. So, look over the attached Jacobsen paper, talk to the graduate student on the project about the reactions you will be running, and be sure to organize your efforts with your fellow labmates. Let me know what trends you discover.

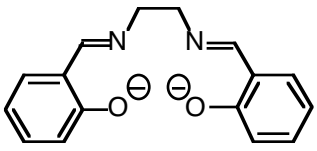


(1)

Olefin Epoxide



1
(one of Jacobsen's Mn(salen) catalysts)



salen =
bis(salicylaldehyde)ethylenediamine

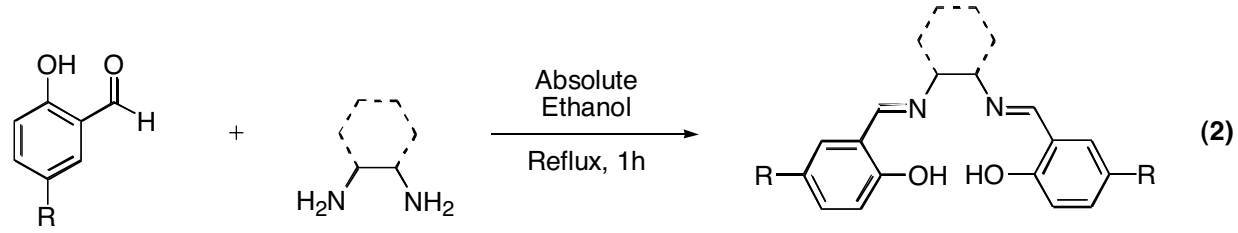
Goals:

- Synthesize a new salen complex (eq 2), and obtain a ^{1}H NMR spectra.
- Synthesize the corresponding Mn(salen) complex (eq 3).
- Compare isolated yields of the catalyst with your labmates.
- Epoxidize stilbene using the published Jacobsen procedure and your catalyst (eq 4).
- Determine the conversion of your reaction using ^{1}H NMR analysis.
- Purify your crude reaction mixture by flash column chromatography, and obtain a yield.
- Obtain a ^{1}H NMR spectrum of the pure epoxidation product.
- Compare conversions and isolated yields with your labmates.
- Use your results to make predictions about the correlation between catalyst structure and catalyst efficiency. This will help us to plan better catalysts in the future.

Hints from Your Graduate Student Mentor:

1. Making the Salen Ligand (eq 2)

- Reactions that you shouldn't try: (I had problems making the Mn(salen) complex with these ligands.)
    1) ethylenediamine + salicylaldehyde
    2) *trans*-diaminocyclohexane + 3,5-di-*tert*-butyl, 2-hydroxybenzaldehyde
    3) ethylenediamine + nitrosalicylaldehyde
- You should aim to make 1.00 g of the Mn(salen) complex. Do your calculations based on the two reactions proceeding in 70% overall yield.
- There will be a large amount of solid produced, so use a large stir bar and fast stirring rate.
- Don't forget that after the reaction finishes you'll need to add water to precipitate the product, so don't start with too small a reaction flask.
- When you first isolate the ligands (and later the metal complexes), they will be very wet, so dry them under a good vacuum before you determine their weight.
- Note the difference between ethanol and *absolute* ethanol.

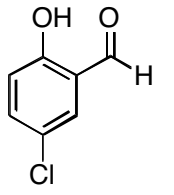


(2)

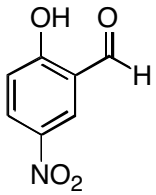
R = H, OMe, NO_2, or Cl

Starting Materials:

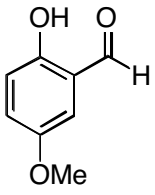
Salicylaldehyde Derivatives:



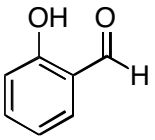
FW = 156.57



FW = 167.12

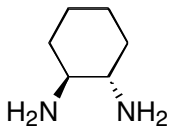


FW = 152.15
d = 1.219

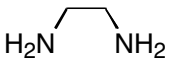


FW = 122.12
d = 1.146

Diamines:



FW = 114.19
d = 0.951

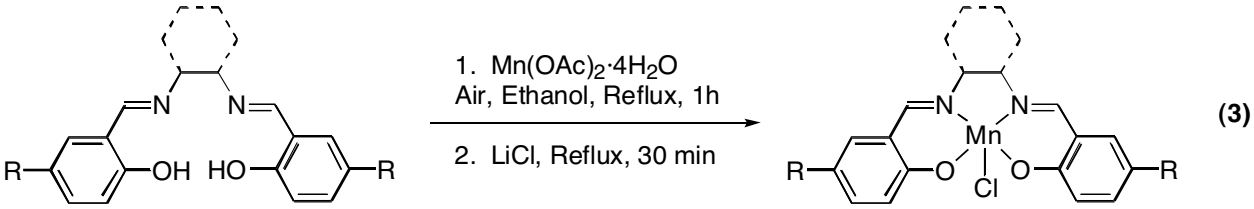


FW = 60.10
d = 0.899

2. Making the Mn(salen) Complex (eq 3)

• Some of our salen ligands behave a little differently than the ones Jacobsen describes. For some cases toluene needs to be added to the hot ethanol solution to dissolve the ligand.

• After the reaction is complete, it is crucial to cool the solution down to promote the precipitation of the Mn(salen) complex. It's easiest to put the flask in an ice-bath for about an hour, then filter the mixture. If no solids form after the ice-bath cooling, it may be necessary to leave the solution in the fridge overnight.



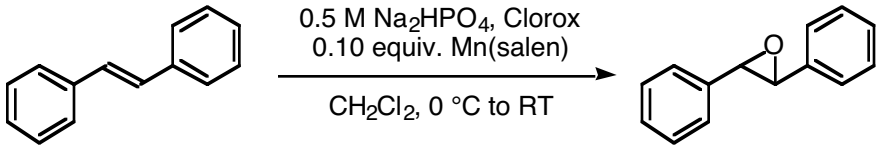
(3)

Zhang, Wei, and Eric N. Jacobsen. “Asymmetric Olefin Epoxidation with Sodium Hypochlorite Catalyzed by Easily Prepared Chiral Manganese(III) Salen Complexes.” *J. Org. Chem*. 56, 7 (1991): 2296-2298.

3. Epoxidizing Stilbene (eq 4)

• The best way to get the proper pH for this reaction is to use a pH meter. When it comes time to do this step, the TAs can show you how to use our meter.

• Monitoring this reaction by TLC can be tricky since the organic layer is on the bottom. The best way to do it is to dip a pipet as deep as possible into the mixture. The liquid drawn into the pipet by capillary action can then be rinsed into a vial using pentane. The less dense organic phase will now be present on top of the aqueous phase and will be easy to obtain using your TLC spotter. Note: we are only using this extravagant dilution procedure because this is a biphasic solution in dichloromethane. Most homogeneous reaction solutions (or biphasic mixtures with less dense organic solvents) can be directly spotted onto TLC plates for analysis.



(4)

8.1. FT-NMR Sample Preparation Guide

Overview:

A good ^1H NMR sample contains about 10 mg of compound. The solution should contain no solids or paramagnetic impurities. Your deuterated NMR solvent should be free of water, and your NMR spectrum should contain no solvent peaks.

Reference:

Zubrick page 295 is relatively useful, but you should follow the specifics in this handout. Also, keep in mind that we won't be running continuous wave spectrometers, so you should disregard any discussion of them.

NMR Solvents:

Typical deuterated solvents include chloroform ($CDCl_3$), water (D_2O), benzene (C_6D_6), acetone ($CD_3C(O)CD_3$), acetonitrile (CD_3CN), and tetrahydrofuran (C_4D_8O). Chloroform is by far the most popular and will be used exclusively in 5.301. The bottle of CDCl3 that you will use for the course will be prepared by the TA. In the future, when you purchase bottles of $CDCl_3$, you will have to prepare them for use. There are typically three things that must be done before your deuterated chloroform is ready for the NMR. First, a few drops of a standard (tetramethylsilane (TMS)) are usually added. Second, any residual water in the solvent is removed by the addition of activated 4 Å molecular sieves. Third, the acidic nature of the $CDCl_3$ (and the molecular sieves) is sometimes neutralized by the addition of anhydrous, granular K_2CO_3 (a weak base). The chloroform that we will use in 5.301 has been treated with molecular sieves and TMS has been added, but, since we won't use any acid sensitive compounds, K_2CO_3 has not been added. (Note: Remember that you do not want water getting into your chloroform, so keep the bottle open to the atmosphere as little as possible. As long as it's open, water from the air will dissolve in your NMR solvent.)

Before Preparing the Sample:

1) Determine the minimum height of a sample by checking the depth gauge in the NMR room.

2) Make a measuring standard to ensure that your samples will always have enough solvent. (Hint: Use a 10-mL graduated cylinder to hold your NMR tube when filling it. Mark the outside of the graduated cylinder with a Sharpie at the minimum height level.)

Preparing NMR Samples of Liquids:

1) Dry and remove all solvent from your compound.

2) Take a clean, dry NMR tube and place it in a 10-mL graduated cylinder.

3) Place a Kimwipe pipet filter on top of the NMR tube. This is constructed by taking a small piece of a Kimwipe and stuffing it into a small Pasteur pipet. It can be tamped into place using the tip of a large Pasteur pipet. (This filter will remove any insoluble impurities.)

4) Dip the tip of a different pipet into your sample. Capillary action will draw approximately 10 mg of the compound into the pipet.

5) Insert this pipet into the pipet filter and rinse the sample into the NMR tube with deuterated solvent.

6) Check to see that you have enough solvent.

7) Cap your NMR tube and record the sample number if running more than one spectrum. (Writing on the colored cap is the easiest way to do this.)

8) After running the NMR, rinse the sample back into the flask containing your compound and concentrate it to remove the solvent.

Preparing NMR Samples of Solids:

1) Perform steps 1–3 above.

2) Place approximately 10 mg of your sample into a vial.

3) Dissolve your compound in about 1 mL of deuterated solvent.

4) Using a pipet, transfer the liquid through the pipet filter into the NMR tube.

5) Perform steps 6–8 above.

Cleaning NMR Tubes:

1) Rinse the tube thoroughly with acetone.

2) Place the tube in a drying oven for about one hour.

3) Store the tube in a desiccator at room temperature.

8.2. GC Sample Preparation Guide

Overview:

This handout describes how to prepare a standard gas chromatograph sample. It involves preparing a dilute solution of a somewhat volatile compound and using the GC to assess its purity.

Reference:

Zubrick page 252

Liquid Sample Preparation:

1) Insert the tip of a Pasteur pipet into the liquid. Capillary action will draw approximately 10 mg of the liquid into the pipet.

2) Rinse this into a vial using 1 mL of a volatile solvent – ether, ethyl acetate, pentane, etc.

3) Insert the tip of a pipet into this liquid.

4) Rinse this through a pipet filter into another vial using 1 mL of the same solvent.

5) Your sample is ready to be injected!

Solid Sample Preparation:

1) Dissolve approximately 10 mg of the compound in 1 mL of one of the volatile solvents listed above.

2) Perform steps 3–5 above

8.3. Thin layer Chromatography (TLC) Guide

Overview:

Thin Layer Chromatography (TLC) is an extremely useful technique for monitoring reactions. It is also used to determine the proper solvent system for performing separations using column chromatography. TLC uses a stationary phase, usually alumina or silica, that is highly polar (standard) or non-polar (reverse phase). The mobile phase is a solvent whose polarity you will choose. In 5.301, and in most lab applications, you will use standard phase silica plates. You will apply your reaction mixture in solution to the plate and then "run" the plate by allowing a solvent (or combination of solvents) to move up the plate by capillary action. Depending on the polarity of the components of the mixture, different compounds will travel different distances up the plate. More polar compounds will "stick" to the polar silica gel and travel short distances on the plate. Non-polar substances will spend more time in the mobile solvent phase and travel larger distances on the plate. The measure of the distance a compound travels is called the R_f value. This number, between zero and one, is defined as the distance the compound moved from the baseline (where it was originally spotted) divided by the distance the solvent front moved from the baseline.

Reference:

For a thorough discussion see LLP pages 145-152.

Steps for TLC:

1) Cut TLC plates. Usually silica plates are bought as square glass pieces that must be cut using a diamond tipped glass cutter and following a template. Before scoring the glass, use a ruler and a pencil to lightly mark baselines on the silica side of the plate (be careful not to remove any silica from the plate). Using a sharp glass cutter and a ruler as a guide, you should have no problem scoring the glass. Once the entire plate is scored, you can then break the glass into individual pieces. (In the beginning this may be frustrating, but after some practice, you should become comfortable with this technique.)

2) Determine an appropriate solvent system. Your compounds will travel different distances up the plate depending on the solvent you choose. In non-polar solvents like pentane and hexane, most polar compounds will not move, while non-polar compounds will travel some distance up the plate. In contrast, polar solvents will usually move non-polar compounds to the solvent front and push the polar compounds off of the baseline. A good solvent system is one that moves all components of your mixture off the baseline, but does not put anything on the solvent front - R_f values between 0.15 and 0.85. This is not always

possible, but should be your goal when running a TLC. (For column chromatography the correct solvent system should give an R_f between 0.2 and 0.3.) Now, which solvents to pick? Here is a list of some standard solvents and their relative polarity (from LLP):

Very polar additives:
Methanol > Ethanol > Isopropanol
Moderately polar additives:
Acetonitrile > Ethyl Acetate > Chloroform > Dichloromethane > Diethyl Ether > Toluene
Non-polar additives:
Cyclohexane, Petroleum Ether, Hexane, Pentane
Common solvent combinations:
Ethyl Acetate/Hexane : 0–30% most popular combination, sometimes tough to remove solvents completely on rotary evaporator
Ether/Pentane: 0–40% very popular, easy to remove on the rotary evaporator
Ethanol/Hexane or Pentane: 5–30% useful for very polar compounds
Dichloromethane/Hexane or Pentane : 5–30% sometimes useful when other mixtures fail

3) Fill TLC chamber with 1–2 mL of the desired solvent system. Place a large piece of cut filter paper in the chamber as well.

4) Spot the compound on the baseline of the TLC plate. We will use commercial spotters, but spotters can be pulled from hot Pasteur pipets (you may see this in your UROP). If you are monitoring a reaction, make sure to spot the starting material, the reaction mixture, and a co-spot of both.

5) Run the TLC. Let the solvent go about 90% of the way up the plate.

6) Remove the plate from the chamber and mark the solvent front immediately with a pencil. You will use this to calculate the R_f.

7) Let the solvent dry off of the plate.

8) Visualize the TLC using non-destructive technique(s). The best non-destructive method is the UV lamp. Place your plate under the UV lamp and circle any UV active spots with your pencil. Although we won't do this in 5.301, another popular non-destructive method is staining with iodine. (You might see this in your UROP.)

9) Visualize the TLC using a destructive method. This will be critical for compounds that are not UV-active. There are several varieties of stains that are very useful and will be available to you in 5.301. To use the stain, pick up the dried TLC plate with a pair of tweezers and dip it into the stain, making sure to cover the area from the baseline to the solvent front. Completely dry the back of the plate with a paper towel. Place on a hot plate and watch the development of the spots. Remove the TLC plate from the heat once the spots are visible and before the background color obscures the spots.

9) Revise your choice of solvent system based on the results of your initial TLC. Make the solvent system more polar if you want a larger R_f or make it less polar if you want to decrease the R_f. Also, if there is "streaking" of your compound on the plate - basically you see large streaks instead of sharp circles - your sample is probably too concentrated. Try diluting your sample and running the TLC again. If this doesn't work, you will have to move to a different solvent system.

10) Label your TLC, calculate the R_f for each spot and draw a picture of it in your notebook.

8.4. Extraction and Washing Guide

Overview:

This handout describes standard extraction and washing protocols that can be applied to virtually any crude reaction mixture. Aqueous washings are done to remove water soluble impurities from organic products since normally the compound that you desire will be dissolved in the organic layer.

Reference:

For an excellent discussion, read Zubrick pages 127–138.

Standard Aqueous Workup Protocol:

1) Pick an organic solvent. Ether is the most popular because it can be removed easily on the rotary evaporator, ethyl acetate also works well but is harder to remove, dichloromethane is a poor choice and should be avoided, if possible, since it often forms nasty emulsions and complicates matters because it is heavier than water.

2) Pick the size of your separatory (sep.) funnel. You will usually use 125 or 250-mL, large scale reactions (1–10 g) can require 500-mL or 1-L sizes. Remember that your sep. funnel will contain the solvent and wash liquid which must be thoroughly mixed.

3) Dilute the crude reaction mixture with your solvent of choice and transfer to your chosen sep. funnel. Large amounts of material require large amounts of solvent. Normal reactions (50–500 mg of product) can be diluted with between 25–100 mL of solvent.

4) Wash the organic layer to remove impurities. The volume of a wash phase is typically one tenth to one half the volume of the organic phase. It is sometimes best to repeat a wash two or three times. An acid wash (usually 10% HCl) is used to remove amines, while a basic wash (usually sat. $NaHCO_3$ or 10% NaOH) is used to remove unwanted acids. In most cases, when neither acidic nor basic impurities are an issue, the solution is washed with distilled water to remove any non-organic compounds. *(Note: When shaking mixtures in a sep. funnel be sure to vent it regularly by holding it upside-*

down, pointing it up and to the back of your hood, then opening the stopcock. This will release any pressure that has built up during mixing. Additionally, when draining liquids out of the sep. funnel, be sure to first remove the stopper.)

5) Back-extract to recover lost product. If your compound is somewhat water soluble (has several polar functional groups), you may need to back-extract the water layers with ether or ethyl acetate to avoid significant loss of compound in the aqueous phase. TLC can be used to determine when all of your compound has been removed from the water.

6) Finish with a brine (saturated NaCl solution) wash. This helps disrupt any emulsions and will "dry" the organic layer by extracting water that may have dissolved in the organic phase.

7) Dry the organic layer. After removing your solution from the aqueous phase, a drying agent is added to remove all traces of water. This is usually $MgSO_4$, more effective and faster, but slightly acidic; or Na_2SO_4, less effective and slower, but neutral. These compounds bind to any water remaining in the organic solution, forming clumps when they react. A decent amount of drying agent should be added, but as long as some solid is not clumped, no more needs to be added. (This will make sense once you've done this a couple of times.)

8) While the compound is drying, it is time to flute the filter paper. Refer to Zubrick page 136-138 for directions. Some chemists prefer to use a Büchner funnel and unfluted filter paper (or a fritted funnel) under mild vacuum as their standard filtration method. Their motive is a slightly higher yield of product.

9) Filter the solution into a large round bottom flask using your expertly fluted filter paper and a large funnel (or the Büchner method). To guard against bumping on the rotavap, do not fill the flask more than half full.

10) Concentrate the solution on the rotavap, then dissolve the compound in a small amount of solvent and transfer to a small pre-weighed (tared) flask.

11) Concentrate the solution on the rotavap again. Higher boiling solvents are more effectively removed by concentrating, adding dichloromethane then repeating once more.

12) Use the vacuum pump to remove residual solvent. For non-volatile compounds, residual solvent is most effectively removed by using the vacuum pump. One useful trick to speed up this process is the following: evacuate the flask and vent to N_2, repeat this again, then pump on the flask for 30 minutes. If your compound is volatile—low molecular weight and/or low boiling point—obtain a constant weight using the rotavap, not the vacuum pump.

13) Obtain a constant weight. Weigh the flask after leaving it on the vacuum pump (or rotavap), then return to the pump (or rotavap) for 15 to 30 minutes and weigh again. Once two weights in a row are the same, you're ready to take an NMR.

8.5. No-Air Techniques Guide

Working on a vacuum manifold:

See LLP chapter 9.2 for an excellent discussion on working with air-sensitive reagents, including how to use a two-way manifold.

De-gassing solvents:

The best way to remove water and oxygen from a solvent is to distill it over an appropriate drying agent (such as sodium). This can sometimes be a lengthy (and dangerous) task. Since we will only need a small amount of oxygen-free solvent in 5.301, it is more efficient to de-gas the solvent by purging with an inert gas, in this case N_2.

1) Place some activated molecular sieves in a hot round-bottomed flask, and purge with nitrogen until cooled to room temperature. Glass readily adsorbs moisture from the air, so it is important to thoroughly oven (or flame) dry all glassware. The sieves will act as a sponge to pick up water dissolved in the solvent.

2) When the flask has cooled, add the solvent and cap with a rubber septum. Secure the septum with copper wire.

3) Purge the solution by injecting a clean needle through the septum and placing it directly in the solvent. Vent the flask with another needle. You should see bubbles.

4) 15–20 minutes should be sufficient.

Another method of degassing a solution is "freeze-pump-thaw." The solution is frozen (using liquid nitrogen, for example), then vacuum is applied for several minutes. The vacuum line is closed, and the solvent is allowed to slowly warm to room temperature. Repeat this procedure at least two more times. *Caution: Some polar solvents like water, methanol, and acetonitrile expand with freezing and can break your glassware*. We won't be using this method in 5.301, but you may encounter it in the future.

Cannula Transfer:

See LLP chapter 6.4

Filtering a Solution:

Your filtration apparatus will consist of three parts: the Schlenk flask containing the sample, the Schlenk frit, and the receiving Schlenk flask.

1) While the glass is still hot, grease both ends of the frit. Attach one end to the receiving flask (don't forget the stir bar!), and cap the other end with another small flask that has a 14/20 opening. Secure with Keck clips. Evacuate the set-up. Refill with nitrogen (be careful not to suck oil from the bubbler into your manifold!). Repeat the vac-fill cycle at least three times.

2) When you are ready to filter, place both the sample flask and the receiving flask under a positive nitrogen flow. Quickly remove the small capping flask from the frit and the septum from the sample flask. Connect the two components, and invert.

3) Close off the original sample flask from N_2, and pull a slight vacuum on the receiving flask (make sure you have a cold trap set up on your vacuum line, to prevent any solvent vapors from destroying the expensive pump!). You can then close off the vacuum, since filtration should occur under static vacuum.

4) When the solution has been transferred, apply positive N_2 flow to the receiving flask and replace the frit with a rubber septum.

8.6. Two-Solvent Recrystallization Guide

Overview:

For a two-solvent recrystallization, you should have one solvent (solvent #1) in which your desired compound is soluble at the boiling point. The second solvent (solvent #2) should induce crystallization when added to a saturated solution of your compound in the primary solvent.

Reference:

See Zubrick pages 114–117.

Recrystallization Steps:

1) The first step is to remove insoluble material from your compound by filtration.

2) Transfer the material to a 50-mL Erlenmeyer flask, equipped with a stir bar. Add an excess amount of solvent #1 (about 20 mL in experiment 3.1) and heat to boiling on a stir/hot plate. The excess solvent is used to keep the compound from precipitating during the filtration.

3) Filter off any insoluble contaminants through fluted filter paper in a pre-warmed stemless funnel (pre-warm by adding some hot solvent just before you filter the solution, thus preventing loss of material on the filter paper.)

4) Wash the flask and filter paper with about 2 mL of hot solvent.

5) Reduce the volume of the solution (to about 15 mL) by boiling off the excess solvent.

6) Cool to room temperature. At this point, it is probably not a saturated solution, so crystallization will not occur.

7) Add solvent #2 dropwise until the solution just becomes cloudy. Again heat the solution to the boiling point (with stirring!) and continue addition of solvent #2. After each drop, you will notice a cloudiness that dissolves away. Continue dropwise addition of solvent #2 until the solution is saturated (i.e. if you were to add one more drop, the cloudiness would

persist, and the solution would be super-saturated.) If this happens, add a drop of solvent #1 to return to a clear solution.

8) Remove the flask from heat, fish out the stir bar with a magnet, allow to cool undisturbed to room temperature before placing in an ice bath.

9) Chill a mixture of the solvent system (in about the same ratio you used to obtain a saturated solution). This will be used to wash your crystals.

10) Collect the crystals on a small Büchner funnel by vacuum filtration, and rinse with the cold solvent mixture.

11) Pull air through the filter cake, then dry thoroughly *in vacuo* before obtaining a yield. One option to dry your product is to place it in a pre-weighed vial, and place the vial in a vacuum desiccator. You can cover the vial by fastening a Kimwipe on top with a rubber band.

8.7. Guide to Growing a Single Crystal

Overview:

As you may discover, growing single crystals takes patience as well as an artful hand. Crystallizations can be very sensitive to temperature and minor disturbances. Therefore, you will be encouraged to try several different temperatures, using otherwise identical conditions, and to always find a quiet undisturbed location to promote crystal growth. Here are some tried and true tips to get you started.

Option #1

• Sometimes nice crystals will grow simply by cooling your solution. You can also try supersaturating a solution by heating it until all of the material dissolves, then allowing it to cool down slowly.

Option #2

1) Find a solvent that your compound is soluble in, and make a saturated solution.

2) If necessary, perform a filtration to remove insoluble impurities. For such small scales, a good filter can be made by plugging a disposable Pasteur pipet with glass wool (or even a bit of Kimwipe), then filling (about an inch) with a filtering aid such as Celite. Moisten the Celite with fresh solvent, then filter your solution by forcing it through the pipet with a pipet bulb.

3) Find another solvent, in which your compound is NOT soluble (or only slightly soluble), and which is miscible and less dense than the first solvent.

4) Carefully layer the second solvent onto the staurated solution in a small vial. You may see some turbidity at the interface. Your crystals should grow along this interface.

Option #3

• Another option is to place the saturated solution in a small vial that sits inside another larger vial. Add the second solvent to the outer vial and cap. The second solvent should slowly diffuse into the saturated solution, and crystals should appear! To slow the process even further, place the diffusion set-up in the fridge.

Solvent systems to try:

CH_2Cl_2/ether or pentane
toluene/ether or pentane
$CHCl_3$/*n*-heptane
THF/ether or pentane
water/methanol

8.8. Distillation Guide

Overview:

Distillation is an extremely useful technique that is used to purify reagents and separate crude product mixtures. There are two varieties of distillation - atmospheric pressure and reduced pressure. The former is easier, while the latter involves some more complicated techniques. Both types will be explored in 5.301.

Glassware:

Distillations require special glassware that is unique to this technique. There are several types of set-ups, but we will use only two. In both cases we will use a short path distillation head, varying only in the use of a Vigreux column. Even though we won't use other set-ups in 5.301, you should become familiar with them through your reading.

Steps for Running an Atmospheric Pressure Distillation:

1) Collect the necessary glassware: short path distillation head, thermometer and adapter, receiving flasks (at least two), Vigreux column (optional - consult LLP page 196).

2) Preheat oil bath or heating mantle. If the boiling point is unknown, this step should be omitted. Keep in mind that for most distillations the heating apparatus must be 20–30 °C higher than the boiling point of the distillate. *Note: Due to thermal breakdown and possible ignition, oil baths are only useful for temperatures below 200 °C.*

3) Record weight of labeled receiving flasks.

4) Put compound to be distilled in a round-bottomed flask with stir bar. (The stir bar will prevent bumping.) The size of the round bottom flask is very important. It should be roughly half to two-thirds full; any higher and it may boil over prematurely, any less and it may take too long to distill.

5) Assemble glassware, making sure all joints are snug. A large assembly of glassware should involve at least two clamps - when in doubt use more clamps! Also, no joint grease is necessary for atmospheric pressure distillations. (Note: for air or water sensitive compounds the apparatus should be flame dried and distilled under N_2 or Ar. We won't do this in 5.301, but you may encounter this in your UROP.)

6) Insulate the column. When using a Vigreux column, it should be wrapped with glass wool and aluminum foil. Without insulation, these set-ups tend to take a very long time.

7) Connect the condenser to the water lines, turn on the water, and check for leaks.

8) Raise the stir plate and heating apparatus to the flask and begin heating. Note: Variac gauges do not correspond to temperature. Setting the dial at 70 will not heat your oil bath to 70 °C - it will actually go much higher. Also, different oil baths and heating mantles will heat to different temperatures at the same Variac settings.

9) Lower the hood sash. This is always a good practice in case of accident, but it also keeps the distillation apparatus away from the air conditioning of the lab. This will cool your set-up and make your distillation take longer.

10) DO NOT HEAT TOO QUICKLY!!! Patience is the key to distillation.

11) Slowly increase the temperature of the heating apparatus until the solution is refluxing.

12) Wait to see the distillation thermometer respond. If nothing happens after about 10 minutes then raise the temperature *slightly*.

13) Repeat step #12 until you see the distillation thermometer respond. Once this happens, prepare to collect.

14) Try to keep the apparatus at a constant temperature - at least within 5 degrees of the temperature when the distillation thermometer registered.

15) Collect until a dramatic change in temperature occurs. Usually the temperature of the distillation thermometer will drop when one fraction is done distilling. At this point you should change receiver flasks or stop the distillation entirely.

16) When you have collected everything you want, drop the heating apparatus and let the entire apparatus cool.

17) Weigh the collection flask(s) and obtain the weight of your product(s).

Steps for a Reduced Pressure Distillation:

1) Collect the glassware - the same as above except this time make sure to include a pig (3-neck) or cow (4-neck) adapter. Another useful piece of glassware that we won't use in 5.301 is a Perkin Triangle. This is described in your texts and may be useful later in your chemistry career.

2) Perform steps 2–4 above.

3) Assemble the glassware, making sure to grease all of the joints. Be sparing with the vacuum grease - it's expensive and you don't want it getting into your compound. See Zubrick pages 53-55 for a discussion of joint greasing.

4) Perform steps 6–7 above.

5) DO NOT START HEATING!!!

6) SLOWLY open the distillation apparatus to vacuum. You should see the liquid begin to bubble. Don't worry, this is normal. Excess solvent or low boiling impurities will often boil away under vacuum at room temperature. (This is a good example of why you need to keep your trap full of liquid nitrogen, otherwise these compounds will go directly into your pump oil!)

7) Once the bubbling subsides, or slows almost to a stop, then you can start heating the flask.

8) Perform steps 9–15 above.

9) Release the vacuum. When you are done collecting, it is not quite time to cool the apparatus. First, you must release the vacuum. Before you do this, however, make sure that all of your collection flasks are secured to the apparatus by clamps, joint clips, your hand, etc. You do not want to release the vacuum then see your product flask shatter on the

bottom of the hood! Once everything is secure, vent the apparatus to nitrogen and then remove the heating apparatus and let the set-up cool to room temperature

10) Once everything has cooled, record the weight of your tared collection flask(s) and calculate the weight of your product(s).

8.9. Flash Column Chromatography Guide

Overview:

Flash column chromatography is a quick and (usually) easy way to separate complex mixtures of compounds. We will be performing relatively large scale separations in 5.301, around 1.0 g of compound. Columns are often smaller in scale than this and some of you will experience these once you move into the research lab. Column chromatography uses the same principles discussed in the TLC Handout, but it can be used on a preparative scale. We will be running flash columns since we will use compressed air to push the solvent through the column. This not only helps provide better separation, but it also cuts down on the amount of time required to run a column.

Reading:

For an excellent description, see LLP pages 205–214.

Preparing and Running a Flash Column:

1) Determine the dry, solvent-free weight of the mixture that you want to separate.

2) Determine the solvent system for the column by using TLC. You should aim for R_f values between 0.2 and 0.3. If your mixture is complicated, this may not be possible. In complex cases, you will probably have to resort to gradient elution. This simply means that you increase the polarity of the solvent running through the column (eluent) throughout the course of the purification. This technique will be described in more detail later in the handout, but for the TLC analysis you should determine which solvent systems put each of the different spots in the 0.2 to 0.3 R_f range.

3) Determine the method of application to the column. You have three choices: neat, in solution, or deposited on silica.

Neat: If your compound is a non-viscous oil, it is sometimes easiest to apply it neat. You can use a long Pasteur pipet to apply the liquid to the column and then rinse any traces of it into the column using the predetermined solvent system.

In Solution: Neat application can sometimes lead to column cracking, so a more common method for liquids, as well as solids, is to dissolve the sample in a solvent and apply it to the column in solution. In the best case, all of the components of your mixture should have an R_f of zero in this solvent - usually pentane or hexane. In many situations

this is not possible, so a solvent that moves only one compound in the mixture can be used, or you can simply use the chosen eluent. Keep in mind that these last two options are risky for difficult purifications.

Adsorption onto Silica: The final technique that is useful for some liquids and all solids is to deposit (adsorb) the compound on silica. *Caution: silica gel is acidic, and this procedure can destroy acid-sensitive compounds that normally survive on silica gel columns*. First, using a round-bottomed flask, dissolve the mixture in dichloromethane and add silica gel (double the weight of your compound to determine the weight of silica). Concentrate the solution on the rotary evaporator. *Caution: Silica gel is a very fine powder and can easily get sucked into the rotavap*. Plug the opening of the adapter or bump guard with glass wool to prevent "bumping" of the solid. Fast rotation also helps prevent this problem. Once the solid is mostly dry (you will know it's dry when most of the solid has fallen off the sides of the flask), remove the flask from the rotavap and finish removing the solvent using the vacuum pump - assuming nothing in your mixture is volatile. *Caution: Use a glass wool plug in the vacuum adapter, or you will find silica gel (and your compound) throughout the vacuum tubing and manifold*. Once it is totally dry (no more bubbling from the solid), remove the flask from the vacuum line and scrape the sides clean with a spatula. The solid can now be added to the top of the column by simply using a powder funnel followed by a few 1.5-mL rinses with the eluent.

4) Determine the appropriate silica gel to compound ratio. Easy separations require ratios between 30-50:1 (by weight), while harder separations call for ratios of up to 120:1. The reading in LLP and discussions with more experienced colleagues can help you make this tough decision.

5) Pick the appropriate column. The amount of silica gel you are going to use determines the size of your column. There is an ongoing debate about whether to use silica columns that are short and wide or ones that are tall and skinny. In 5.301 we will argue that the short, wide columns provide better separation, but this statement may be challenged by some of your future co-workers. When you are first starting, the best way to select the correct column for a given amount of silica gel is to ask other members of your lab which column they would use and record this in your notebook. (This is much easier than measuring column diameters.) In 5.301 we will only have one size to choose from, so the choice will be fairly straightforward!

6) Pick appropriate collection test tubes. This is also a good time to consult your more experienced colleagues, but a simple guideline is to divide the volume of silica that you used by four and pick test tubes that can accommodate this volume. (200 mL of silica = 50 mL fractions)

7) Once you have selected a column, you need to plug the stopcock end so that the silica will not drain out. This is normally done with a small piece of cotton or glass wool and a long stick or glass rod.

8) Mount the column in the hood - due to the large volumes of volatile solvents used and the health risks associated with dry silica gel, you should never run a column outside of the hood. Check to see that the column is perfectly vertical - crooked columns make separation more difficult.

9) Close the stopcock and add a few inches of eluent.

10) Add sand (dried and washed) to the column using a funnel. Your goal is to produce a thin layer of sand (no more than 1 cm) above the plug that will help prevent the silica from ending up in the collection flasks.

11) Measure out the correct volume of silica. The safest way to do this is by volume in the hood. Silica gel has a density equal to 0.5 g/mL so you can use an Erlenmeyer flask to measure it out (100 g = 200 mL). Don't fill the Erlenmeyer more than one third full of silica since you will be adding solvent to the flask as well.

12) Make a slurry of the silica by adding at least 1.5 times the volume of solvent as silica you just measured out. Mix it thoroughly by swirling or stirring vigorously to remove all the air from the silica. (Air bubbles will compromise the effectiveness of the column.)

13) Using a powder funnel, carefully and slowly pour the slurry into the column making sure not to disturb the layer of sand. Stop pouring frequently to swirl the slurry so that the silica is evenly mixed. Once you've finished pouring, rinse the Erlenmeyer several times with the eluent and add the remaining solvent/silica mixture to the column.

14) Using a pipet and eluent, rinse any silica stuck to the sides of the top of the column into the solvent layer.

15) Once all of the silica is off the sides of the column, open the stopcock and use the compressed air to pack the column. The silica level in the column will shrink to about half of its original height. Check to make sure that the top of the column is flat. If not, it must be stirred up and allowed to settle undisturbed. As the excess solvent elutes under the applied pressure, tap the sides of the column *gently* with a rubber stopper or the end of a pencil. This will improve the packing of the silica particles. Collect all the solvent that elutes from the column and recycle it for use after your compound has been added. *Caution: Never let the solvent level drop below the top of the column.*

16) Once the column is packed, add a protective layer of sand to the top of the silica. This should be relatively level and about 2 cm thick. This will protect the column when you are adding solvent - if you add solvent too fast, it can destroy a flat column (thus hurting separation) unless it is protected by sand.

17) Using the compressed air, lower the solvent level until it is even with the height of the sand.

18) Close the stopcock and put the first test tube under the column outlet.

19) Carefully add your compound to the column - when adding liquids be sure to drip them down the sides of the glass, not directly onto the top of the column. When rinsing the flasks that contained the mixture, carefully add a one pipet-full of the rinse solution to the column at a time. Then open the stopcock and drain the liquid level down to the top of the column and close the stopcock. Rinse the flask three times using this procedure. For mixtures that were deposited on silica gel, an additional 2 cm layer of sand is now added.

20) Carefully fill the column with eluent. At first, add the solvent by Pasteur pipet. Once 1 cm of solvent has been added, the stopcock can be opened for good. Keep adding the solvent by pipet until a few centimeters of solvent are above the column. Now add the solvent from an Erlenmeyer through a powder funnel—*slowly*—letting it first run down the side of the column. Be patient, you do not want to disturb the top of the column.

21) Once you have filled the column with eluent, you are ready to "run" the column. Remember that a quick flow rate helps to give good separation. Adjust the air pressure to

give a swift flow rate—no fire-hoses, though! Keep the pressure on and change the test tubes once they become filled. *Remember to replenish the solvent in the column frequently*.

22) Monitor the column's progress by TLC - this can get a little hectic, trying to run TLCs and collect your fractions, so in the beginning you might want to decrease the air pressure (or remove it entirely) when you are checking the progress of the column.

23) When running a gradient elution column, use the initial solvent system until the higher R_f compounds have come off the column. Once they are safely in collection flasks, increase the polarity of the eluent. *Caution: Increase the polarity gradually*. Drastic polarity changes can "crack" the silica gel - sending fissures through the column like in a bad earthquake movie. This cannot help your separation! Instead, increase the polarity by about 5% for every 100 mL (or more) until you reach the desired solvent system. Then, stay with this eluent until your desired compound has eluted. At this point, you can either change eluents again or proceed to the next step.

24) Once you have determined that all of the compounds you are interested in have eluted from the column, you are ready to wrap everything up. First, put a large Erlenmeyer flask underneath the column, and use a green Keck clip to attached the compressed air source to the column. Allow the air to push all of the remaining solvent out of the column and then to dry the silica gel. (It's difficult to remove the silica from the column until it is completely dry.) This will take at least one hour for large columns.

25) While the column is drying, start to combine fractions. Using TLC, determine which test tubes contain your pure compound(s). Combine fractions of similar purity in large round-bottomed flasks and concentrate them on the rotavap. For longer duration columns, combine fractions while the column is still going to expedite the process.

26) Once the solvent is completely removed, analyze the compounds by NMR.

9.1. NMR Operation Guide

Operation of the Varian Mercury 300 Plus FT NMR Spectrometer

(a complete set of instructions is provided near the instrument)

The NMR spectrometer is the most complex (and expensive) piece of equipment that you will use in 5.301. Therefore, it is essential that you take time to familiarize yourself with its correct operation. This section contains an outline of the steps required to obtain a simple ^{1}H NMR spectrum; however, you will notice that instructions corresponding to steps 2–7 have been left out. On Tuesday, January 7, you will be required to obtain your first ^{1}H NMR, with the help of your TA. At that time, your TA will demonstrate correct usage of the spectrometer, giving you a chance to take detailed notes and ask questions about what you are doing or why. By the end of IAP, you should be relatively proficient at taking ^{1}H NMR spectra. With adequate practice, you will eventually be able to acquire a ^{1}H NMR spectrum in less than ten minutes by performing these seven steps:

1) Loading the Sample
2) Obtaining Deuterium Lock
3) Shimming
4) Acquiring the FID of the Spectrum
5) Processing the Data
6) Integrating
7) Plotting and Finishing Up

1) Loading the Sample:

- Using a Kimwipe, clean the outside of the capped NMR tube that contains your sample.
- Insert the tube into the spinner turbine using the depth gauge provided.
- Clean the tube again.
- Click **[eject]**. **You must hear the ejection air before proceeding.** If there's no sound, check the valve on the N_2 tank.
- Place the tube and spinner in the bore tube at the top of the magnet, and click **[insert]** to lower your sample into the magnet.
- Wait until you hear a click, then click spin **[on]**.

• Notes:

2) Obtaining Deuterium Lock:

The Purpose of Locking Your Sample: The frequencies of protons in ^{1}H NMR spectra vary only by *parts per million* (ppm) or less, so the individual FIDs which are added together must coincide exactly. Small deviations in the strength of the magnetic field would make this a big problem, so the spectrometer must maintain a constant frequency reference with which the individual FIDs are modified so that they add correctly. This is the role of the deuterium lock. The deuterium atoms contained in the solvent molecules resonate at a completely different frequency from the protons of the sample, and they give only one signal. The spectrometer follows the deuterium signal and makes the appropriate changes to the ^{1}H NMR signals so that any drift in magnetic field is compensated.

• Instructions:

3) Shimming:

The purpose of shimming. The word "shim" was used to describe a bell tuner who would remove shims of metal from different places on the bell so that the bell produced a single sharp tone. Our goal is to remove (or add) shims of magnetic field to the main magnetic field so that everywhere in the sample the magnetic field is exactly the same strength. Otherwise broad peaks would be produced because identical protons of the molecule would resonate at different frequencies depending on which part of the NMR tube they occupied. Spinning the NMR tube at 20 rev/s is one way to simulate *homogeneity* in the XY plane.

We will be working on the Z shims, which shim the magnetic field in the vertical direction. We are always trying to establish a maximum lock signal. Other than the solvent, the major factor in determining how easily your sample will be shimmed is the **sample height**. If too little solvent is used (<4 cm), you will have a very difficult time shimming because the air/solvent interface is close to the coils which are acquiring the NMR signals. In order to make shimming easier, good shims for your solvent have been recently given to the computer, and you recalled them above.

- Instructions:

4) Acquiring the FID (Free Induction Decay) of the Spectrum:

- Instructions:

5) Processing the Data:

- Instructions:

6) Integrating:

You can integrate the peaks in your NMR spectrum accurately to establish the relative numbers of each kind of proton.

- Instructions:

7) Plotting and Finishing Up:

- Instructions:

9.2. IR Operation Guide

These instructions are posted on the wall next to the FT-IR we will be using in 5.301.

Background Scans:

First increase the screen illumination using the knob at the back of the instrument. Then place the background card in its slot and enter the following commands

- Background
- Scan
- Number of scans - usually four

When scanning is complete, the spectrum will appear. You can now exchange the background card for your sample.

Scanning Sample:

(Scan will be stored in buffer X, Y, or Z.)
Enter the following commands

- X, Y, or Z (Pick the buffer to store your spectrum, will overwrite whatever else is in that buffer)
- Scan
- Number of scans - usually four

Preparing the Plotter:

- Turn the plotter on using the toggle switch on the back left-hand side
- Put both pens into the plotter
- Position paper properly - flush against the left side and touching the white guide above
- Lock the paper using the small black handle on the left side
- Press *Plot* on the IR console

Wait for the plotter to finish

- Remove and cap pens
- Turn plotter off

9.3. GC Operation Guide

Overview:

In 5.301 we will use a state-of-the-art HP 5890 Series II Gas Chromatograph equipped with an HP 7673 Automatic Sampler and an HP 18576 Bar Code Reader, all controlled by an HP 3365 Chemstation. This simply means that you are using great equipment that is easy to operate.

Preparing a Sample for the Autosampler:

Unlike most research labs, in 5.301 we will not manually inject our samples onto the GC column. Fortunately, you can let the autosampler do all of the necessary syringe work. So, all you have to do to run the GC is to transfer your sample into a special autosampler vial.

1) Using the special dispenser, affix a bar code label to an autosampler vial. (Record this number in your notebook so that you don't later become confused about which number corresponds to which sample.) Check the diagram on the dispenser to make sure the vial is oriented correctly. Check to ensure the entire label is sticking to the vial.

2) Pipet about 1 mL of your GC sample (prepared using the GC Sample Prep. Guide) into the vial. It is properly filled if the solvent level is just below the bar code - avoid over or under filling the vial.

3) Using the crimper, affix a crimp cap to the vial. Do not over-crimp - moderate pressure should be adequate. Check to make sure that the crimp cap cannot be turned by hand, that there are no metal folds around the neck of the vial, and that the septum is flat and *centered*. If there are any defects, do not use this vial - the syringe could easily become damaged. Instead, remove the defective cap with wire cutters and replace it with an acceptable cap.

4) When all of your vials are ready and acceptable, get the TA who will instruct you on the operation of the instrument.

9.4. UV-Vis Operation Guide

Guidelines for measuring a UV-Vis spectrum using the HP8542 diode-array spectrophotometer.

For the Protein Assay Experiments:

1) At the main menu of the HP89532A software, select **Kinetics** from the program selector.

2) Several of the default parameters must be changed. Select **Time/Cell**, then set the cycle time to 2 seconds and the runtime to 6 seconds. Next, select **Functions**, then choose single wavelength. Enter 596 or 562, depending on which experiment you are doing.

3) Fill a cuvette with deionized water. This will be scanned as a blank to provide a spectral background. *Note: Never touch the clear sides of the cuvette, since this will disrupt the path of the light beam.* Keep the exterior clean and dry using care and Kimwipes. Place the cuvette filled with water in the holder, and push the black lever down to lock it in place. Select **Scan Screen**, **Pre-Run**, and **Meas. Blank.** After about five seconds, the blank scan will appear as a very noisy line. Don't worry, this is normal and results from the computer's autoscaling feature.

4) Unlock the cell holder and remove the cuvette used for the blank. Insert a plastic cuvette filled with your first sample to be measured. Select **Start Run**, then **Begin**, and the instrument will measure the absorbance value four separate times.

5) To obtain a hard copy of the absorbance values, the following sequence must be entered. Select **Return**, **Tabulate**, and **Time Traces.** This will print out all of the absorbance values. It is often advisable to measure the absorbance value of each sample more than once to check the precision of the instrument. You can simply remove the cuvette from the holder, wipe it quickly with a Kimwipe, lock it back in place, and run another scan. If the values are much different from the first numbers, run more scans until at least three different rounds of numbers are relatively close together.

6) Once your data have printed, to run another scan, hit *Escape* and then select **Scan Screen.**

7) When you are satisfied with the data from one sample, repeat steps 4–6 with the remainder of your samples.