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, Sarah A. Tabacco, Kimberly L. Berkowski, Anne M. Rachupka and John J. Dolhun

IAP 2013

5.301: Chemistry Laboratory Techniques

IAP January 2013

Faculty & Staff	Duties
John J. Dolhun, Ph.D.	Instructor for Course 5.301
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Lynn Marie Guthrie	Administrative Assistant
Randall A. Scanga	Technical Associate

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* *

Experiment Techniques Covered:

1. Transfer and Extraction Techniques
2. Purification of Solids by Recrystallization
3. Purification of Liquids by Distillation
4. Protein Assays and Error Analysis
5. Introduction to Original Research

Major Equipment Students will be Trained on:

1. Automated 5890 Series II Gas Chromatograph
2. Automated Perkin Elmer Lambda 35 UV-VIS Spectrometers
3. Varian Saturn 2000 Gas Chromatograph-Mass Spectrometer
4. Perkin Elmer Spectrum 100 Series FT-IR
5. Varian Mercury 300MHz NMR Spectrometer
6. Rotary Evaporators
7. Auto Abbe Automatic Refractometer

5.301 Chemistry Laboratory Techniques

IAP 2013

Instructor: John J. Dolhun

TA's: TBA

January 8, 2013 thru January 28, 2013
Lecture: MTWRF 10-11 AM
Laboratory: MTWRF 12-6:00 PM

Course Highlights

5.301 includes a series of chemistry laboratory instructional videos called the Digital Lab Techniques Manual (DLTM), used as supplementary material for this course as well as other courses offered by the Chemistry department.
This course is offered during MIT's Independent Activities Period (IAP) - a special 4-week term that runs the full month of January.

Course Description

This course is an intensive introduction to the techniques of experimental chemistry and gives first year students an opportunity to learn and master the basic chemistry lab techniques for carrying out experiments. Students who successfully complete the course and obtain a "Competent Chemist" (CC) or "Expert Experimentalist" (EE) rating are guaranteed to secure opportunities for research work in a chemistry lab at MIT.

Acknowledgements

The course was invented by Professor Rick Danheiser (1997) and the laboratory manual and materials for this course were developed under his direction by then graduate students Dr. Katherine J. Franz (Lippard) and Dr. Kevin M. Shea (Danheiser) with the assistance of Professors Rick L. Danheiser and Timothy M. Swager. Materials have been revised by J. Haseltine, Kevin M. Shea, Sarah A. Tabacco, Kimberly L. Berkowski and John J. Dolhun; The idea for the current original research project was synthesized by John J. Dolhun

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7. Introduction to Original Research

8. Technique Guides

9. Instrument Operation Guides

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, which you will try to answer in the lab. The experiments you will run require many of the techniques you will learn during the first two 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

Three texts have been chosen for 5.301: *The Organic Chem Lab Survival Manual: A Student's Guide to Techniques*, Eighth Edition (2012), by James Zubrick (referred to as Zubrick), *Techniques in Organic Chemistry*, Third Edition (2010), by Mohrig, Hammond & Schatz (referred to as Mohrig), 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 all were designed particularly for organic chemistry lab students, the techniques described are equally relevant in inorganic and organometallic research, and include many of the techniques important in biological and physical chemistry labs as well.

The texts by Zubrick & Mohrig are extremely readable and were designed specifically for the introductory organic lab student. They have excellent practical advice, nice illustrations, and Zubrick 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 and Mohrig. This text can supplement Zubrick & Mohrig 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 Tuesday, January 8th, there are several chapters in the texts 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 & Mohrig. These texts were selected because they’re 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. Also, at the

beginning of each lab period, there will be a short overview of that day's topic where Dr. Dolhun and your TA will facilitate a discussion of the assigned reading and the actual lab experiment. Much of this 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 Tuesday January 8th 10 AM and again that same day at NOON for the Safety Lecture then proceed to the lab for an NMR Demonstration and laboratory check in at 1:00 PM.

Zubrick - ***The Organic Chem Lab Survival Manual (Eighth Ed., 2012)***
Chapters: 1 - Safety, 2 - Notebooks, 4 - Jointware, 6 - Interesting Equipment, 9 - Clean and Dry, 10 - Drying Agents, 11 - On Products, 15 - Extraction and Washing, 17 - Heat, 18 - Clamps, 27 – TLC, 28 - Column Chromatography, 30 - Gas Chromatography, 32 - Infrared Spectroscopy, 33 – Nuclear Magnetic Resonance 34 - Distillation,

Mohrig, Hammond, & Schatz – ***Techniques in Organic Chemistry (Third Ed., 2010)***
Part 1: Basic Techniques: Chapters 1 thru 16 pages 1-197 Part 3: Spectroscopic Methods: Chapters 20-24 pages 275-438.

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 so 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 be ready to start a UROP, and will not receive a pass. 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 three+ weeks together, you will encounter five technique modules and one introduction to an original research project. You will work on the technique challenges during the first two weeks, with the final week set aside for the research project. Each 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 CC level experiments, two EE challenges (you are encouraged to complete them all, but required to complete two), and run at least one epoxidation reaction during the original research project.

1.5 - 5.301 CALENDAR
January 2013

Su	Monday	Tuesday	Wednesday	Thursday	Friday	Sa
		1	2	3	4	5
6	7	8 Day #1 10-5 Safety Instruments & Check In	9 Day #2 CC: Transfer & Manipulation NMR, IR	10 Day #3 EE: Acids, Bases & In Between	11 Day #4 CC: Recrystallization Mothball	12
13	14 Day #5 EE: Recrystallization Single Crystal	15 Day #6 CC: Distillation GC and MS	16 Day #7 EE: Distillation RI & IR	17 Day #8 CC/EE: Column Chromatography	18 Day #9 CC: Biochemistry	19
20	21 HOLIDAY	22 Day #10 EE: Biochemistry	23 Day #11 Orig Research: Synthesis Penicillin	24 Day #12 Purify product Column Chromatogrpahy	25 Day #13 Test Penicillin with E. Coli	26
27	28 Day #14 Complete Research Labwork	29 Work on Lab Report	30 Work on Lab Report	31 5.301 Lab Reports Due in Collection Box		

	2 CC	2 EE	3 CC	3 EE	4 CC	4 EE	5 CC	5 EE	6 CC	6 EE
1. Volumetric Techniques										
2. Titration										
3. TLC Basics							X	X		
4. TLC Advanced										
5. Reaction Work-Up I	X	X								
6. Reaction Work-Up II	X	X								
7. Filtration	X	X	X	X						
8. Sublimation										
9. Recrystallization			X	X						
10. Column Chromatography							X	X		
11. Using a Balance	X	X	X							
12. Melting Point Determination	X	X	X							
13. Using an Automatic Pipet									X	X
14. Making Buffers and Using a pH Meter										
15. Distillation I					X					
16. Distillation II						X				
17. Reflux				X						

1.7 - 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, but will instead concentrate on practical aspects of chemistry. To facilitate this practical learning, this manual has been divided into nine sections, which will briefly be explained here.

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 and mass spectroscopy (MS).

Third, section seven discusses the introduction to the original research project that you will encounter in the third 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 comfortable operating the NMR, GC, IR, MS and UV-Vis instruments.

1.8 - INTRODUCTION TO THE LABORATORY

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 Tuesday, January 5. 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. 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 dustpan and brush for broken glass can be checked out of Lab Supplies. 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 checkout of the laboratory (see Item 3 below).

3. CHECK-OUT PROCEDURE AND CHARGES

Checkout will be on **Monday, January 28, 2013 at 4:00PM**. 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. <u>Chemicals and Solvents</u>

Organics and Inorganics

Acids and Bases - under hood

Solvents - on shelves at end of benches

b. <u>Ovens and Refrigerators</u>

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. 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 dustpan 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 Safety Officer, Jim Doughty, 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. Dolhun'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, and 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 bench top areas clean and workable space maximized.

5.301 Lecture Syllabus January IAP 2013

The laboratory lectures are an essential part of 5.301. Attendance at all lectures is expected. Students may be prohibited from doing an experiment if we believe they are unprepared because of abscence from a lecture pertinent to that experiment. Unless otherwise indicated, the lectures will be on M-F at 10:00 AM.

Date	Lecture Schedule Groups A, B, & C
T Jan 8	Introductory Lecture to 5.301 Pre-Lab Reports and Lab Notebooks 10:00 AM (Lecture is followed by lunch at 11:00 room TBA)
T Jan 10	Mandatory Safety Lecture 12:00 NOON Jim Doughty
T Jan 8	Live NMR Demonstrations 1:00-3:00 PM Undergraduate Labs NMR
T Jan 8	Laboaratory Check In 3:30 PM Undergraduate Labs
W Jan 9	NMR Spectroscopy 10:00 AM
TH Jan 10	Extraction Techniques & IR Spectroscopy 10:00 AM
F Jan 11	Recrystallization Techniques 10:00 AM
M Jan 14	Mass Spectroscopy 10:00 AM

T Jan 15	Atmospheric & Vacuum Distillation 10:00 AM
W Jan 16	TLC, Column & Gas Chromatography 10:00 AM
TH Jan 17	Protein Assays 10:00 AM
F Jan 18	Student Presentations 10:00 AM
M Jan 21	HOLIDAY
T Jan 22	Original Research Project Charge Synthesis of Penicillin Derivatives 10:00 AM
W Jan 23	Error Analysis 10:00 AM
TH Jan 24	Students Present Results of Synthesis and Schemes 10:00 AM
F Jan 25	Writing Up The Lab Report 10:00 AM

1.10. The Laboratory Notebook

GENERAL GUIDELINES:

1. Use a ballpoint pen (press hard if duplicate pages).
2. Write on one side only.
3. Do not erase or use whiteout. If you make a mistake, draw a single line through the error and write the correct entry on the top or side of it.
4. Do not remove an original page. If the entire page is incorrect, draw a single diagonal line through the page and state the reason for this line.
5. Record all data and results (with units) directly into your notebook.
    - DO NOT record data on scrap paper, your hand, etc., to be transferred later.
    - If you need to enter remote data (printout from computer, etc.), date, sign, and tape it into your notebook.
6. Start a new page for each new experiment.
7. Write the title of the experiment, date, and your name at the top of each page.
8. Indicate if a page is continued from the previous page.
9. NEVER skip a space for later additions.
10. Be neat and thorough! Someone should be able to pick up your notebook twenty years from now and be able to repeat your experiments.

COMPONENTS:

A. Pre-Lab – a detailed plan of the work that you will be doing

1. Brief statement of purpose.
2. Paragraph discussion of the safety and environmental issues (ex. waste generation).
3. Step-by-step procedure in your own words. Be **concise** and **complete**, but DO NOT copy the lab manual. Use diagrams and sketches when necessary. Reference all sources of information.

NOTE: The lab manual may not be brought into the laboratory or consulted during the laboratory session. However, the appendices are allowed.

B. Factual Record – what to record

1. Keep a running account of all procedures carried out and observations made during experimental work.
2. Record observations such as physical appearance, color, odor, and physical properties.
3. Sketch apparatuses and label parts.
4. Use a table to record all information about reactants (see below).

	Source	Molecular Formula	Molecular Weight	Equiv.	Moles	Amount (Vol/Mass)
Reagent 1						
Reagent 2						
Product						

5. Record all data and results, including the crude yield of products and mixtures. Use tables when possible.
6. All of the reactants must be accounted for in the factual record. For example, if you started with 1.0 mol of Reactant 1, you must account for the fate of all 1.0 moles at the end of the reaction. Simply describing the isolated 0.25 mol of product at the end (for example) will not be acceptable.
7. For calculations, show the formula and a sample calculation. If the calculation is repeated, use a table to report your results.

8. All graphs should be constructed with a graphing program. Label all axes and provide a title for each graph. Reference the data on the graph (XXX-I-005).
9. Attach all spectra to your notebook, label the axes, and reference the spectra in the procedure section.

C. Data Analysis/Conclusions:

1. Examine and discuss the accuracy and precision of your data. Is the precision reasonable? Discuss possible systematic and random errors.
2. Summarize the key results and provide a conclusion. Describe any difficulties that you had. Discuss which results are poor and provide explanations. Provide suggestions for improvement.

Transfer and Extraction Techniques

2.1 - Competent Chemist Rating

"Ethyl Ester's Excellent Adventure"

Techniques Checklist

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

Pre-lab Discussion

- Extraction - Reading: Zubrick ch 15, LLP chapter 10, Mohrig ch 11
- Theory of extraction - Reading: Zubrick chapter 35
- Melting point determination - Reading: Zubrick pages 88-103, Mohrig 176-180
- NMR theory and operation -Reading: Zubrick ch 33, LLP ch 15.2, Mohrig ch 21
- IR theory and operation - Reading: Zubrick ch 32, LLP ch 15.3, Mohrig ch 20

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- Reaction Work-Up I #5 (Extracting, Washing and Drying)
- Reaction Work-Up II #6 (Using the Rotovap)
- Using a balance #11
- Melting Point Determination #12

Equipment

- Separatory funnel - 250 or 500-mL
- Graduated Cylinder – 100-mL
- Erlenmeyer flasks - 2x250-mL, 1x500-mL
- Beaker 150-mL
- Round-bottomed flasks - 50-mL, 100-mL
- NMR tubes
- IR cards

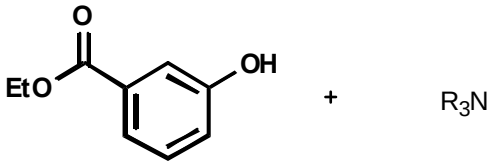
- Funnels - large, with long neck
- Filter Paper
- Rotary evaporator

Goal

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

Experiment Outline

- You will receive a vial containing 100 mg of ethyl 3-hydroxybenzoate (mp 72-74 ^{o}C) contaminated with triethylamine. You will also receive four different ^{1}H NMR spectra of: the mixture in your vial, pure ethyl 3-hydroxybenzoate, pure triethylamine, and diethyl ether.



- Dissolve the sample in 50-75 mL of ether in a separatory funnel.
- Remove the amine by extraction with a 10% HCl solution.
- Continue with the "standard aqueous work-up" (including an ether back- extraction)- see Extraction and Washing Guide.
- Remove the solvent by rotary evaporation and concentration under vacuum to a constant weight and obtain a mass.
- Take an NMR spectrum of the compound and compare to your earlier spectra.
- Recombine the NMR sample with the remainder of the purified sample.
- Obtain an IR spectrum using your IR card - see IR Sample Prep. Guide.
- Remove the solvent for the final time to a constant weight.
- Obtain a mass and a melting point.

Notes

- When removing solvent with the rotary evaporator, keep the receiving flask cold and the water bath warm. Otherwise, your product will never solidify.
- If you have trouble getting your product to solidify, add a few milliliters of methylene chloride to your flask and return 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 IR and ^{1}H NMR analysis (spectra should show only negligible amounts of impurities in the judgment of the professor and TA) and by melting point measurement (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:

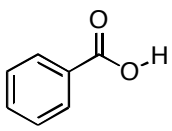
- 5. Reaction Work-Up I: Extracting, Washing & Drying
- 6. Extraction Work-Up II: Using the Rotavap
- 11. Using a Balance
- 12. Melting Point Determination

Equipment:

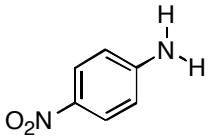
- Graduated Cylinder (100-mL)
- Separatory funnel (125-mL)
- Erlenmeyer flasks (4x250-mL)
- Beaker (150-mL)
- Round-bottomed flask (100-mL)
- pH paper
- NMR tube
- Funnel
- Filter paper
- Rotary evaporator

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

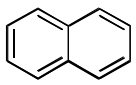
Experiment Outline: You will receive a vial containing 100 mg each of benzoic acid, 4-nitroaniline, and naphthalene. Using the *pKa*, values of these molecules, carefully devise an extraction and washing sequence that will selectively separate the three components.[1]



Benzoic Acid
MP 122–123 °C
pKa 4.2



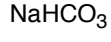
4-Nitroaniline
MP 149–151 °C
pKa (RNH_3^+) 1.0



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:
  - 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 addition, the isolated compounds should melt over no more than three degrees, with the range beginning no lower than two degrees below the melting point values given above.

---

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

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

- Theory of recrystallization Reading: Zubrick ch 13; LLP ch 11.2, Mohrig ch 15

Equipment

- Test tubes - five 13x100 mm
- 2x50-mL 1x 125-mL Erlenmeyer flasks
- Small magnetic stir bars
- Stemless funnel and fluted filter paper
- Büchner funnel and filter paper
- Magnetic stirring/hot plate
- 250-mL Filter flask and aspirator stopper
- Rubber filter adapters
- Large vial with white cap
- Solid #7 rubber stopper
- 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]

Experiment Outline

Part 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 Zubrick or Mohrig.

Part 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 crystals (no traces of yellow) weighing at least 1.30 g (well dried!) and melting 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.

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

Purification of Solids by Recrystallization

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

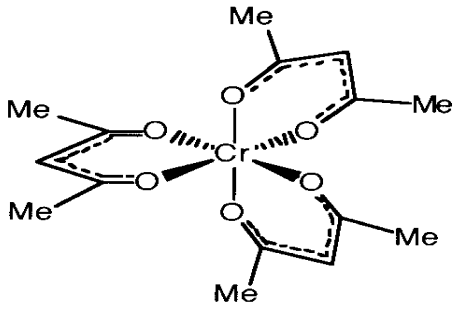
- Use of different recrystallization techniques: vapor diffusion, solvent layering, temperature variation

Digital Lab Techniques Manual

- Reflux #17

Equipment

- Magnetic stirring hot plate
- 50-mL Round-bottomed flask
- Condenser
- Stir bar
- Vials (3 large, 4 small) + 2 Glass jars
- Glass frit (D)
- Side-arm 250 mL Erlenmeyer flask / rubber filter adaptor
- 2-mL Glass syringe
- Variac and heating mantle



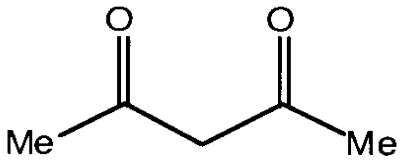
Chromium Acetylacetonate
$Cr(acac)_3$

Goal

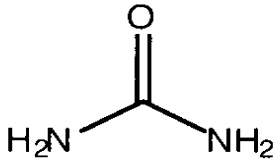
Synthesize $Cr(acac)_3$,[2] then perform several slow recrystallizations to obtain a single crystal of satisfactory quality.

Experiment Outline

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



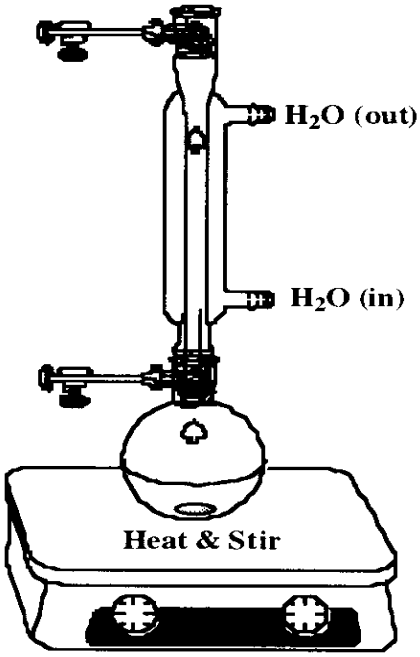
2,4-Pentanedione



Urea

reagent	source	Form. Wt.	density	mass/vol.	mmoles	equiv
$CrCl_3 \cdot 6H_2O$					1.00 mmol	1
Urea						17
2,4-pentanedione						8
$Cr(acac)_3$	Product					

[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.



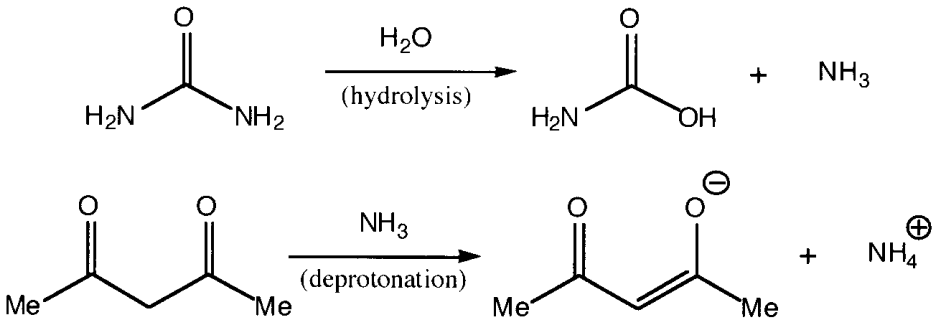
Reaction Setup

Experimental

- 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 with cold water.
- Dry the product in vacuo (using the high-vac. desiccator provided by the TAs), and obtain a yield or dry the product in your desiccator overnight, and obtain a yield and melting point of the product.
- Set up multiple crystallizations to grow single crystals - see Growing a Single Crystal Guide.

Note

- Urea slowly hydrolyzes in the acidic solution used for this reaction, 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 acac (acetylacetonate, also known as 2,4-pentanedione); it is the acac anion which then 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, filter the solution through a plug of glass wool in a pipette before setting up the crystallization.

Results

- To obtain your "EE Rating," you must obtain ≥ 45% yield of $Cr(acac)_3$ and you must produce at least one single crystal that is suitable for X-ray analysis.

Purification of Liquids by Distillation

4.1 - Competent Chemist Rating

"How Did the Peach Get Inside the Banana?"

Techniques Checklist

- Setting up distillation glassware correctly
- Performing atmospheric pressure distillations
- Using Gas Chromatography and Mass Spectrometry GC-MS to analyze samples

Pre-lab Discussion

- Theory of distillation - Reading: Zubrick ch 34, LLP ch 11.3, Mohrig ch 13
- Distillation glassware and how to set it up - Reading: Zubrick chapter 19
- Use of the GC - Reading: Zubrick chapter 30, GC-MS- Mohrig ch 19

Digital Lab Techniques Manual:

- Using a balance #11
- Distillation I #15

Equipment

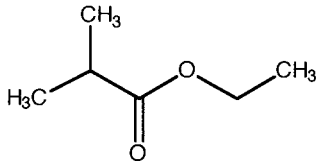
- 25-mL & 50-mL round-bottomed flask with stir bar
- 3 Scintillation Vials or flasks that fit short path
- Distillation Kits (distillation head)
- Ground glass Thermometer and adapter
- Keck clips
- Glass wool and aluminum foil (optional)
- Heating mantle w/sand
- Variac

Goal

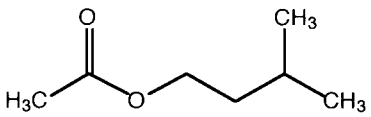
- To purify a mixture of two liquids using distillation.

Experiment Outline

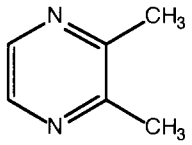
- You will receive a vial containing 11.20 g of a mixture of two compounds whose boiling points differ by about 40^0C (See possible compounds below).
- Analyze the mixture using the GC - *see GC Sample Preparation and GC Operation Guides.*
- Perform atmospheric pressure distillation - *see Distillation Guide*.
- Prepare a GC sample of your purified, low-boiling fraction.
- Obtain a mass spectrum and a gas chromatogram of your purified low-boiling compound.



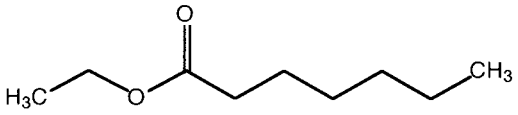
Ethyl Isobutyrate (Peach)
(BP 112–113 °C)



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



2,3-Dimethylpyrazine
(BP 156 °C)



Ethyl Heptanoate
(BP 188–189 °C)

Helpful Hints:

- Make sure all your joints are lightly greased and sealed well. Otherwise, you will lose your product into the atmosphere.
- Do not heat your mixture too fast, or your entire sample may end up in your collection flask.
- Insulate your distillation head with cotton and foil to increase the rate of distillation.
- 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.

Purification of Liquids by Distillation

4.2 - Expert Experimentalist Rating

"What's With Those High-Altitude Recipes Anyway?"

Techniques Checklist

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

Pre-lab Discussion

- Differences between atmospheric pressure and reduced pressure distillation

Digital Lab Techniques Manual:

- Distillation II #16

Equipment

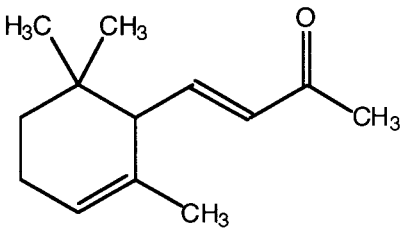
- 25-mL Round-bottomed flask
- 3x10-mL Pear-shaped flasks
- Vigreux column-Vacuum Distillation Kits
- Short path distillation head
- Ground glass Thermometer and cow adapter
- Keck clips
- Glass wool and aluminum foil
- Heating Mantle (w/sand) and Variac

Goal

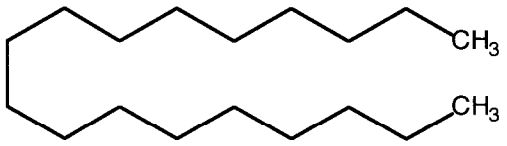
- To purify a mixture of two liquids by reduced pressure distillation.

Experiment Outline

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



a-Ionone
(BP ~ 265 °C)

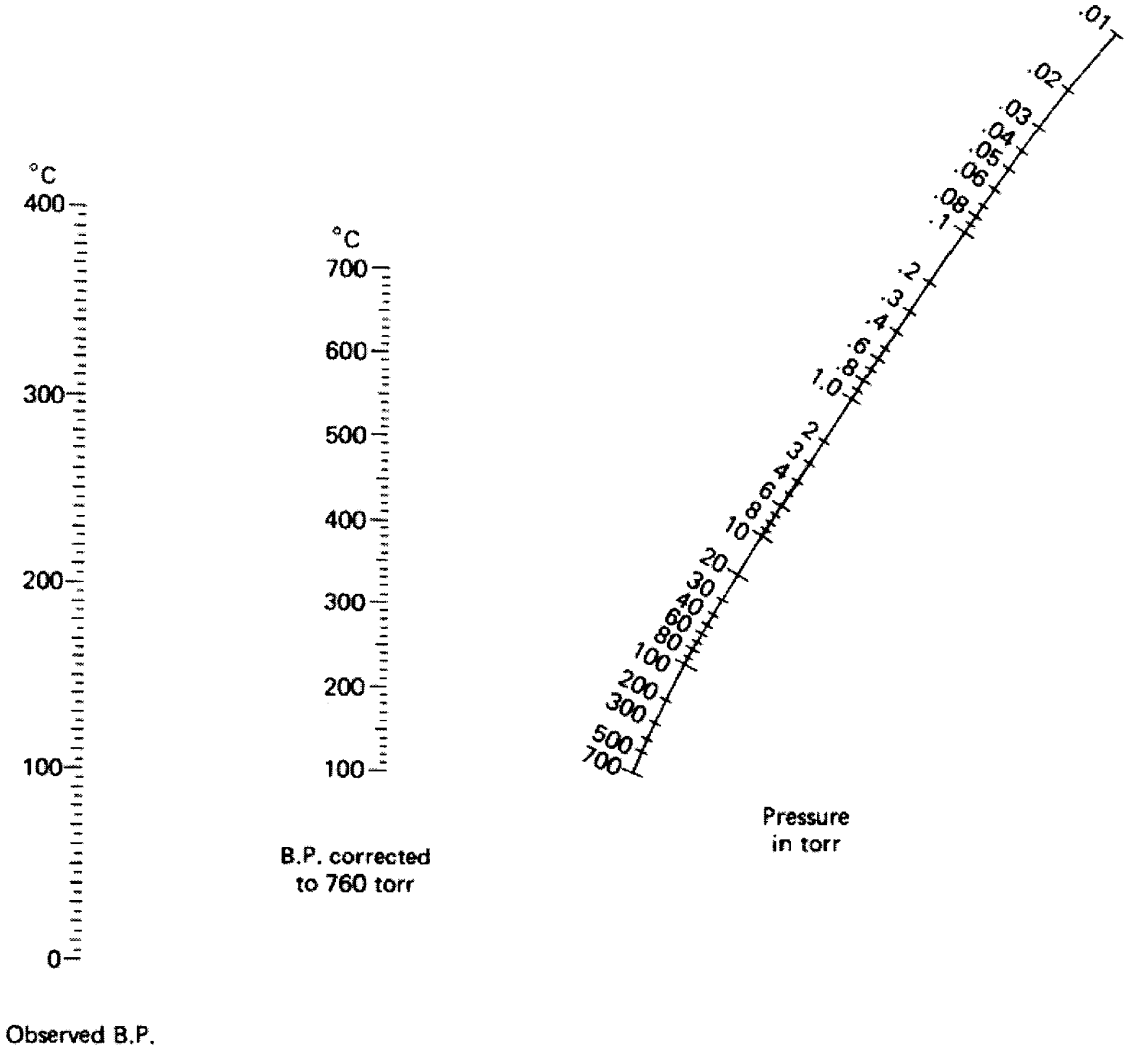


Octadecane
(BP 317 °C)

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 alpha-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.



Purification by Flash Column Chromatography

5.1 - Competent Chemist Rating

"Looks Are Sometimes 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

- Theory of column chromatography - Reading Zubrick ch 28, Mohrig ch 18
- TLC - polarity/solvent sys - Reading Zubrick ch 27, LLP ch 9.3.1, Mohrig ch 17
- Setting up silica gel column Reading Zubrick ch 31, LLP ch 11.6, Mohrig ch 18
- Applying crude mixtures to the column
- Running a flash column

Digital Lab Techniques Manual

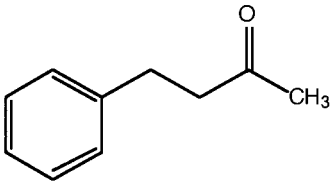
- TLC: The Basics #3
- Column Chromatography #10

Equipment

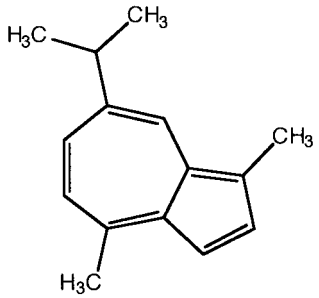
- Flash Chromatography Column
- Air Flow apparatus (Stopper, T-valve, Screw clamp, tubing)
- Round-bottomed flasks - 1x100-mL, 2x500-mL
- Test tubes - 18x150 mm
- Test tube racks
- TLC plates - cut silica/glass plates and UV lamp
- Large plastic funnels

Goal

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



Benzylacetone



Guaiazulene

Experiment Outline

- You will be given 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 - *see TLC Guide*, using 10% ethyl acetate/hexanes as the solvent system.
- Record the Rf 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 10mL 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, monitoring the fractions by TLCð *See Flash Chromatography Guide and TLC Guide*
- Concentrate the set of fractions containing pure benzylacetone.
- Weigh the purified compound and prepare a GC and GC-MS sample.
- Check product with TLC and obtain a GC and GC-MS spectrum.

Results

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

Purification by Flash Column Chromatography

5.2 - Expert Experimentalist

Techniques Checklist

- Picking the correct eluent then 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

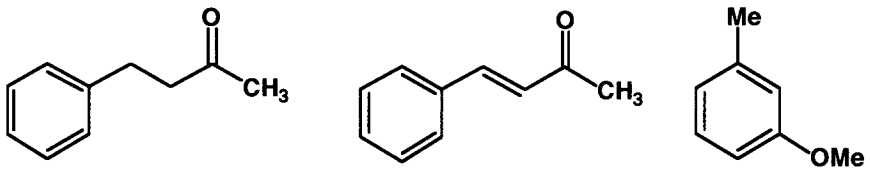
- Identical to CC Level

Goal

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

Chemical Data

- Benzylacetone- FW 148.21, bp 235 0C, d 0.989
- Benzylideneacetone- FW 146.19, mp 39-41 0C
- 3-Methylanisole – FW 122.17, bp 175-176 0C, d 0.969



Experiment Outline

- You will be given 1.00 g of a mixture containing 0.60 g of the major ketone, 0.20g of the minor ketone, and 0.20g of methylanisole in 20 ml of an ether/hexane solution.

- Analyze this mixture by TLC using various solvent systems - *see TLC Guide for hints.*
- Pick an eluent.
- Decide on the silica gel to compound ratio.
- Prepare the column.
- Deposit the mixture on silica gel, dry completely, then apply to the column.
- Run the column.
- Concentrate pure fractions.
- Weigh the purified compounds.
- Analyze the pure ketones by NMR and TLC.

Note

- The ketones are somewhat volatile, and 3-methylanisole, with its low molecular weight, is much more so. Therefore, do not concentrate it (or a mixture containing it) using the vacuum pump.

Results

- To obtain your "EE Rating" in Purification by Flash Column Chromatography, you should get at least 0.45 g of the major ketone and 0.13 g of the minor ketone, and 0.13 g of methylanisole. These samples must be at least 95% pure as demonstrated by NMR spectroscopy. Your samples must also be submitted to the TA for possible weight and purity verification.

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

- Pipettmen: 100 P, 1000 P
- Pipette tips - large and small, 8 Test tubes
- Eppendorf tubes and holders
- Disposable UV-Vis cuvettes - 5 mL

Goal

- You will be given a sample solution of bovine heart cytochrome c. You will use the Coomassie® Plus Protein Assay from Pierce to determine the protein concentration of 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 and then empty it into a tared container. You can then obtain a weight and, 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 the 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

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**

- Pipette 0.05 mL of each standard or unknown sample into appropriately labeled Eppendorf tubes. Prepare 3 unknown samples.
- Use 0.05 mL of the diluent (25 mM MOPS buffer, pH 7. Provided by TA) to prepare one blank tube.
- Add 1.5 mL of the Coomassie® Plus reagent to each tube including the blank, mix well. Allow 10 minutes at RT for color to develop.
- Transfer standards, unknowns and blank to 1.5 mL UV cuvettes. Measure the absorbance at 595 nm of each tube *vs.* blank.
- The computer will subtract the average 595 nm reading for the blank from the 595 nm reading for each standard or unknown sample. Follow the guidelines for opening the program and operating the UV (see 9.4 UV Operation Guide)
- 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 re-do 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.

Protein Assays and Error Analysis

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)
- Pipettmen: 20 P, 100 P, 1000 P
- Pipette tips
- Eppendorf tubes (safe-lock)
- Centrifuge
- Boiling plate or rack to hold Eppendorf tubes
- Large crystallizing dish

Goals

- From the CC-level experiment, you should 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

-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 3 tubes with 300 μL of your unknown protein sample.

μL of Fe AA standard (100 μg/mL)	μL of Buffer to add
0	300
3	297
6	294
12	288
18	282
24	276
30	270

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

• Place the closed Eppendorf tubes in a rack, and boil them for 15 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 three samples against a milli-q water blank.
• 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.

7.1 - Introduction to Original Research[1]

Introduction:

Your advisor has carefully monitored your progress in 5.301. 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 lab mates will learn to work as a research group in order to reach a definitive goal in a short period of time.

Overview:

Our research group has a longstanding interest in the synthesis of derivatives of penicillin, which is classified into the β-lactam class of antibiotics having a common structural backbone as illustrated in Figure 1.

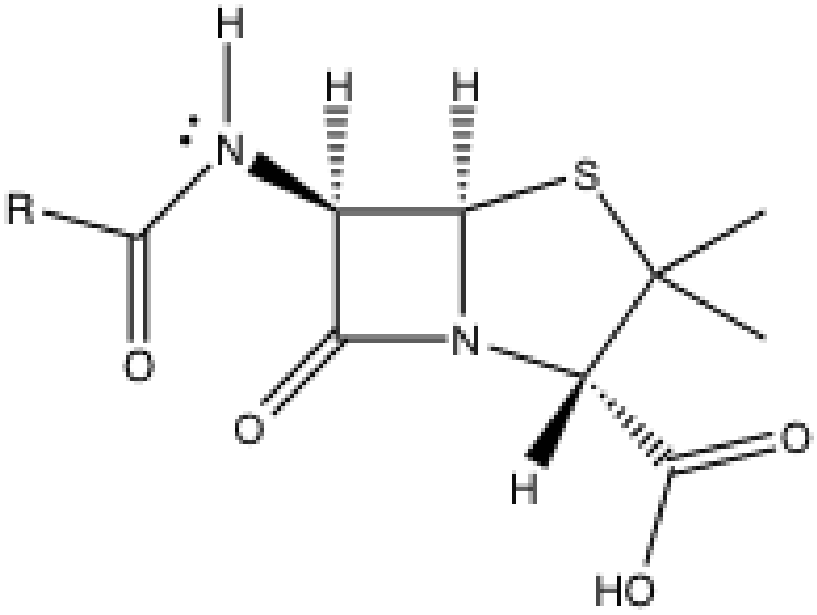
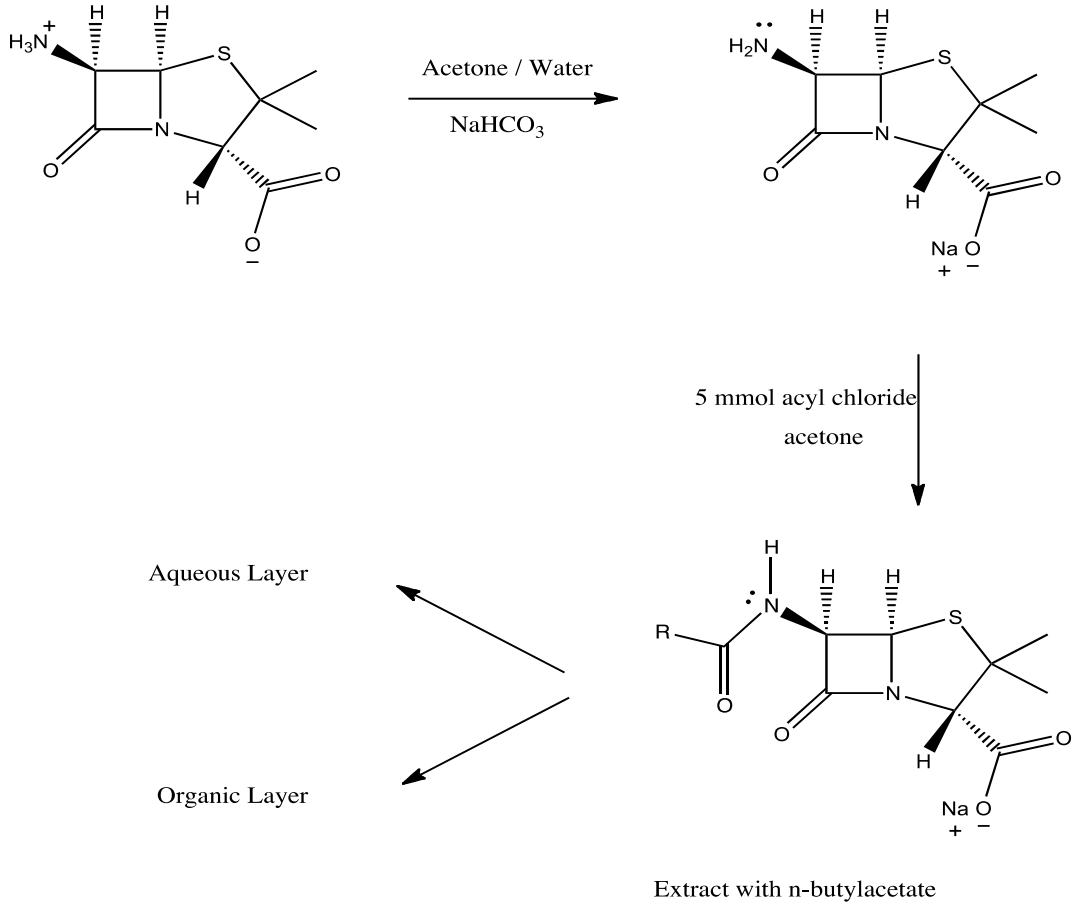


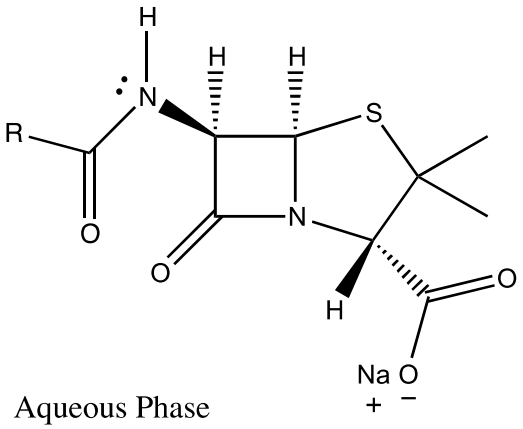
Figure 1: β-lactam Shell of a Penicillin molecule which has been acylated at the 6- amino position resulting in a variable R-group

[1] Adapted from: Whitaker, D. R.; Truhlar M. L.; Yuksel D.; Walt, R. D. *Journal of Chemical Education*, **2010,** 87: 634-636.

Penicillin is used to treat many types of bacterial infections mostly Gram + and sometimes Gram - organisms. Since the discovery of penicillin there has been an ever-increasing resistance by the bacteria to many of the penicillin molecules presently on the market. In this research project, you will be synthesizing a new group of penicillin derivatives in order to overcome the antibiotic resistance to various strains of bacteria. Our research group will begin a comprehensive study, which will afford each student an opportunity to synthesize a fresh penicillin derivative. Go to the library and look through the literature, talk to the department graduate students on this project about the reactions that you will be running, and be sure to organize your efforts with your fellow lab mates.

In this research project you will each synthesize a penicillin derivative through acylation of 6-aminopenicillanic acid (Scheme 1):





Aqueous Phase

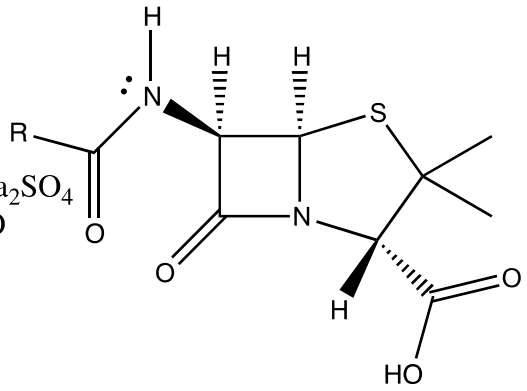
1. cold n-butylacetate

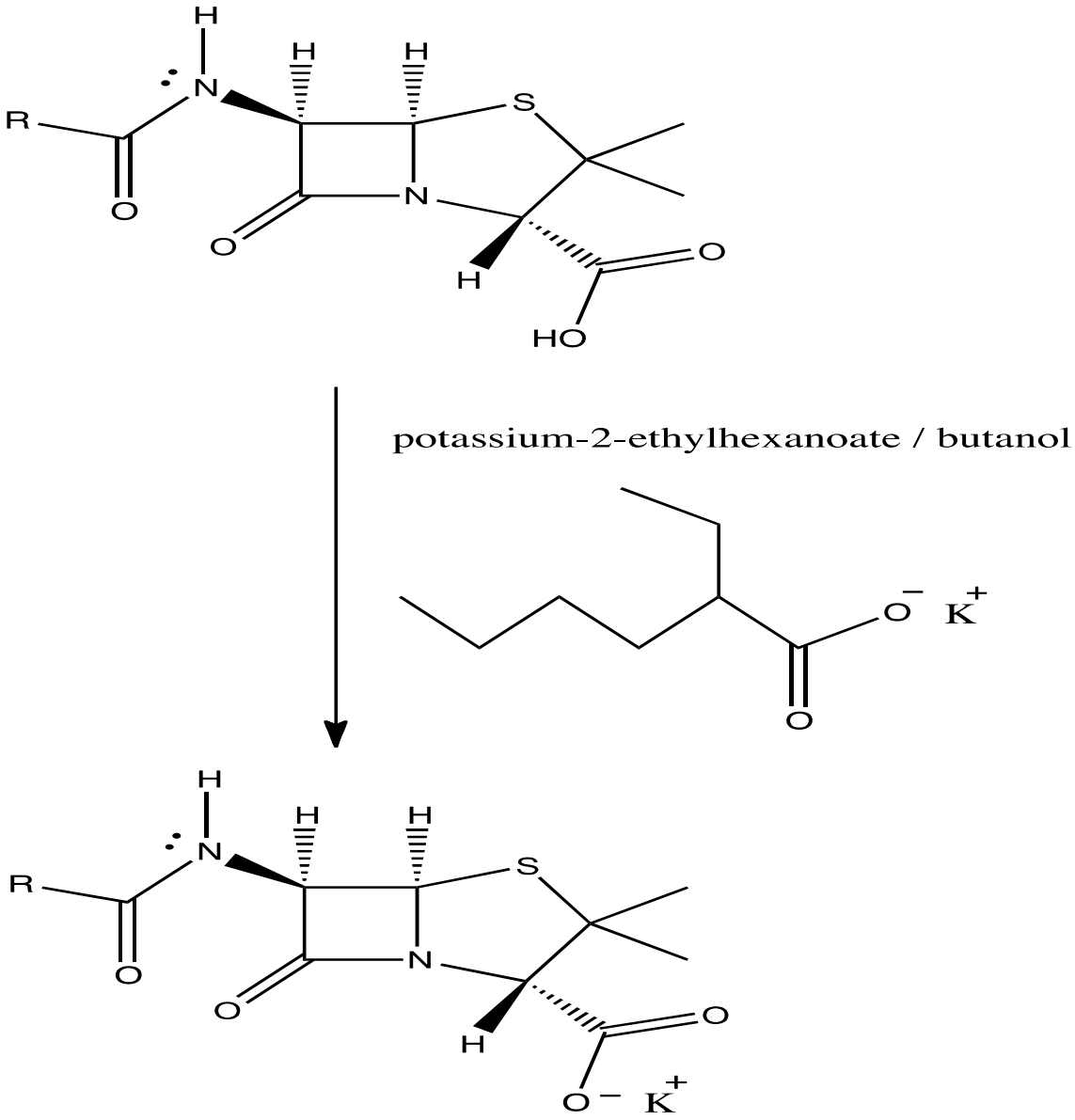
2. 5M H_2SO_4 to pH 2.0

Aqueous Phase

Organic Phase

1. wash with water
2. pour over anhydrous Na_2SO_4
3. filter off Na_2SO_4 x H_2O





A wide selection of acyl chlorides will be made available to choose from. Progress of the reaction will be followed using TLC plates with additional characterization by NMR and MASS SPECTROSCOPY. The penicillin derivatives once synthesized will be purified by column chromatography and tested in a bioassay to determine and quantify their ability to kill bacteria. A broth dilution method will be developed to determine the concentration of penicillin that inhibits the growth of 50% of bacteria in vitro. Each of the penicillins will be tested using broth diluted with E. coli a Gram - bacteria. After

incubation of the diluted samples optical density (OD) measurements will be made measuring their absorption at 600 nm on a Perkin-Elmer UV-VIS spectrometer. The results of the entire class will be made available to all participants so that the students in their final reports can develop structural and functional relationships among the various penicillin derivatives and discuss trends used in ranking the various derivatives based on their effectiveness at inhibiting bacterial growth. We will be synthesizing penicillins that may not yet be commercially available. The goal is to create and identify new antibiotics to stave off biological resistance by the bacteria.

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 CH 35 and Mohrig CH 21 are relatively useful, but you should follow the specifics in this handout. Also, keep in mind, 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_3COCD_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 TA will prepare the bottle of $CDCl_3$ that you will use for the course. 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 Ångstrom 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 for making a standard: 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 (or other holder).
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 the sample. Capillary action will draw approximately 10 mg into the pipet.
5. Place this on top of the pipet filter and rinse it into the NMR tube with your 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. (The colored caps are 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. Do steps 1+3 above.
2. Place approximately 10 mg of your sample into a vial.
3. Dissolve your compound in about 1 mL of your NMR solvent.
4. Using a pipet, transfer the liquid through the pipet filter into the NMR tube.
5. See 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 Ch 32 and Mohrig Ch 19

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 - pentane, dichloromethane, ether, etc.
3. Insert the tip of a pipet into this liquid.
4. Rinse this 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. See 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), and a mobile phase, some 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 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, while non-polar substances will diffuse into the solvent and travel large distances on the plate. The measure of the distance a compound travels is called R_f. This number, between zero and one, is determined by measuring the distance the compound moved from the baseline (where it was originally spotted) divided by the distance the solvent moved from the baseline.

Reference:

For a thorough discussion see LLP pages 145-152, Mohrig Ch 17, Zubrick Ch 28

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 the 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's a list of some standard solvents and their 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/Pentane - 5-30% useful for very polar compounds

Dichloromethane:Hexane/Pentane - 5-30% sometimes useful

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, (your plate should be dry) pick up the 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 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.
10. **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.
11. **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 Ch 15, Mohrig Ch 11.

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** - usually 125 or 250-mL, large scale reactions (1-10 g) 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. **Washing 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. Also, 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. **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 a significant

loss to the aqueous phase. TLC can be used to determine when all of your compound has been removed from the water, (see TLC Guide for details).

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 to 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 116-119 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 rotovap, do not fill the flask more than half full.
10. **Concentrate the solution on the rotovap 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 rotovap 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 rotovap, not the vacuum pump.
13. **Obtain a constant weight.** Weigh the flask after leaving it on the vacuum pump (or rotovap), then return to the pump (or rotovap) 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 your solvent and cap with a rubber septum. Secure the septum with copper wire.
3. Purge the solution by injecting a needle through the septum and placing it directly in the solvent. Vent the flask with another vent 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 (over 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 the 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 filtrations 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 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 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, re-apply a positive N_2 flow 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 105-106, Mohrig 183-197

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 this case) and heat to boiling on a stir/hot

plate. The excess solvent is used to keep your compound from precipitating during the filtration.

3. Filter off the insoluble contaminant through fluted filter paper in a pre-warmed stemless funnel (pre-warm by adding some hot solvent just before you filter your 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 your solution to about 15 mL by boiling off the excess solvent.
6. Cool to room temperature. At this point, you probably do not have 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 the 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 in solution: "super-saturated". If this happens, add a drop of your first 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, then place in an ice bath.
9. Chill a mixture of your solvent system (in about the same ratio you used to obtain a saturated solution), which will be used to wash your crystals.
10. Collect your 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. It also 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.

Tips - 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 your material dissolves, then allowing it to cool down.

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 your saturated 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 your saturated solution in a small vial that sits inside another larger vial. Add the second solvent to this outer vial and cap. The second solvent should slowly diffuse into the saturated solution, and your 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

water/methanol

THF/ether or pentane

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, receiver 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 ÁC.*
3. **Record weight of labeled receiving flasks.**
4. **Put compound to be distilled in a round bottom 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 using 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 system 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 your 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 oil bath 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. T**ry to keep the apparatus at a constant temperature**—at least within 5 degrees of the apparatus 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, lower 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 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 45-47 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 oil bath 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 can be used on a preparative scale. We are running flash columns since we will use compressed air to push the solvent through the column. This not only helps provide better separation, but also cuts down 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 (see TLC Handout). You should aim for Rf values between 0.2 and 0.3. If your mixture is complicated then this may not be possible. In complex cases, you will probably have to resort to gradient

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 your solvent system, 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, you can tap the sides of the column gently with a rubber stopper on 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 your 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 your eluent. At first, add the solvent via 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 your 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! So, keep the pressure on and change the test tubes once they become filled. Remember to replenish the solvent in the column frequently.

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 your 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 your column is perfectly vertical - crooked columns make separation more difficult.
9. Close the stopcock and add a few inches of your solvent (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 which will help prevent the silica from ending up in your 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 we 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 your 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 your solvent system, 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, you can tap the sides of the column gently with a rubber stopper on 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.*
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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 your initial solvent system until the higher Rf compounds have come off the column. Once they are safely in your collection flasks, you can begin to 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 your 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 attach your 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 bottom flasks and concentrate them on the rotovap. 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

Robert Kennedy Ph.D. and Anne Gorham M.S. **20101117**

Objective: Obtain a well shimmed NMR spectra to determine: chemical shifts, integration(1H only), splitting patterns, coupling constants.

Contents:

This document is intended as an introductory guide to the Varian VNMR NMR acquisition and processing software catering to the needs of the MIT Undergraduate Lab. For those interested, a large amount of additional NMR related information can be found on the DCIF website at http://web.mit.edu/speclab/www/ulab.html.

Conventions used in this guide are as follows:

- **Boldface** type indicates commands that are typed into the VNMR input window or in a terminal window
- Italic ***[Boldface]*** type with square brackets indicates a button in the VNMR menu that is to be pushed.
- **<Boldface>** type surrounded by a bracket indicate keyboard strokes.
- **LMB** indicates the Left Mouse Button
- **MMB** indicates the Middle Mouse Button
- **RMB** indicates the Right Mouse Button
- Unless otherwise noted, all commands typed into the input window are be followed by an <↵ Enter> keystroke.

Magnet Safety:

- CAUTION: NMR Superconducting Magnets are ALWAYS ON!
- People with pacemakers, cochlear and other medical implants should not enter the NMR room without a doctor's permission.
- Keep all metal objects, electronic and magnetic devices, at least 5 feet from the magnet. This includes cell phones, iPods, jump drives, chairs, wallets, keys, hair clips and pins.
- If a tube breaks inside the magnet, contact the Undergraduate Lab Staff immediately.

Figure 1

NMR Equipment: (A) Computer Workstation, (B) NMR Console, generates and receives RF, (C) NMR Magnet.

Sample Preparation:

- Use a clean and dry 5 mm NMR tube that is free of cracks and chips.
- Choose an NMR solvent by testing the solubility of the compound in the non-deuterated solvent.
- Dissolve 20-100 mg of the compound in 0.7 mL of the chosen deuterated[4] solvent.
- If the solution is clear, add the solution to the NMR tube.
- If the solution is cloudy, filter using a small piece if a Kim Wipe inserted into a glass Pasteur pipette.
- Cap and label the NMR tube.

[4] Deuterated solvent is used for two purposes: 1) Serve as internal reference (lock) for the spectrometer to correct for magnetic field drift. 2) Prevent large solvent 1H signals.

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 your initial solvent system until the higher Rf compounds have come off the column. Once they are safely in your collection flasks, you can begin to 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 your 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 attach your 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 bottom flasks and concentrate them on the rotovap. 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.

VNMR Software Overview

Before we get started, we must first introduce some of the general features of the VNMR software interface. Upon initially opening the program (done by **single** clicking the VNMR application icon in the Toolbar shown at the right), the VNMR software display screen is divided into four distinct windows. These windows are called:

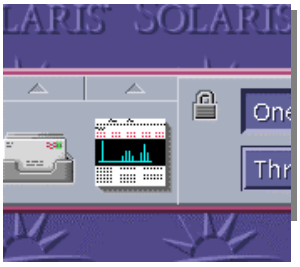


Figure 4: Small section of the toolbar showing the VNMR

1. The Input Window
2. The Graphics Window
3. The Text Window
4. The Acquisition Status Window.

The significance of each of these windows will be discussed.

1. **The Input Window:**

   The Input Window is typically located at the top of the display and is sub-divided into three subsections. A graphics representation can be seen in Figure 5 below. VNMR system messages will be displayed in the Status Window (topmost portion), commands and parameters are typically input into the Input Window (middlemost portion), and menu selections can be made through the Menu Button Window (bottommost portion of the window).

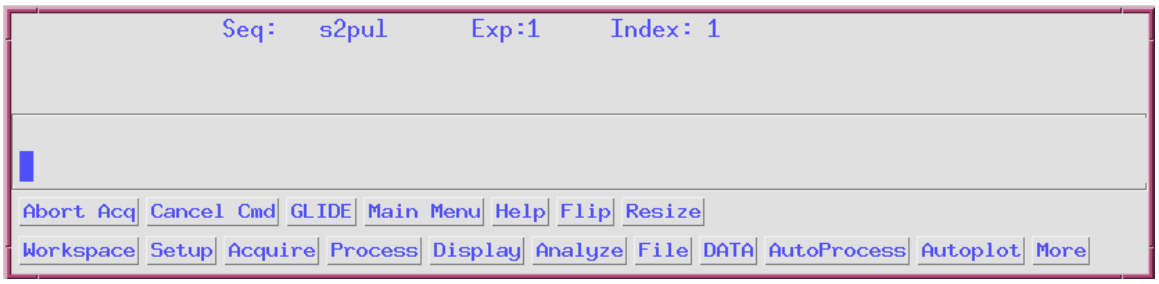


Figure 5: The VNMR Input Window. This is divided into the Status Window (Top), the Input Window (Middle), and the Menu Button Window (Bottom).

Most of the user input in this tutorial will be made through the Input Window and the Menu Button Window.

2. **The Graphics Window:**

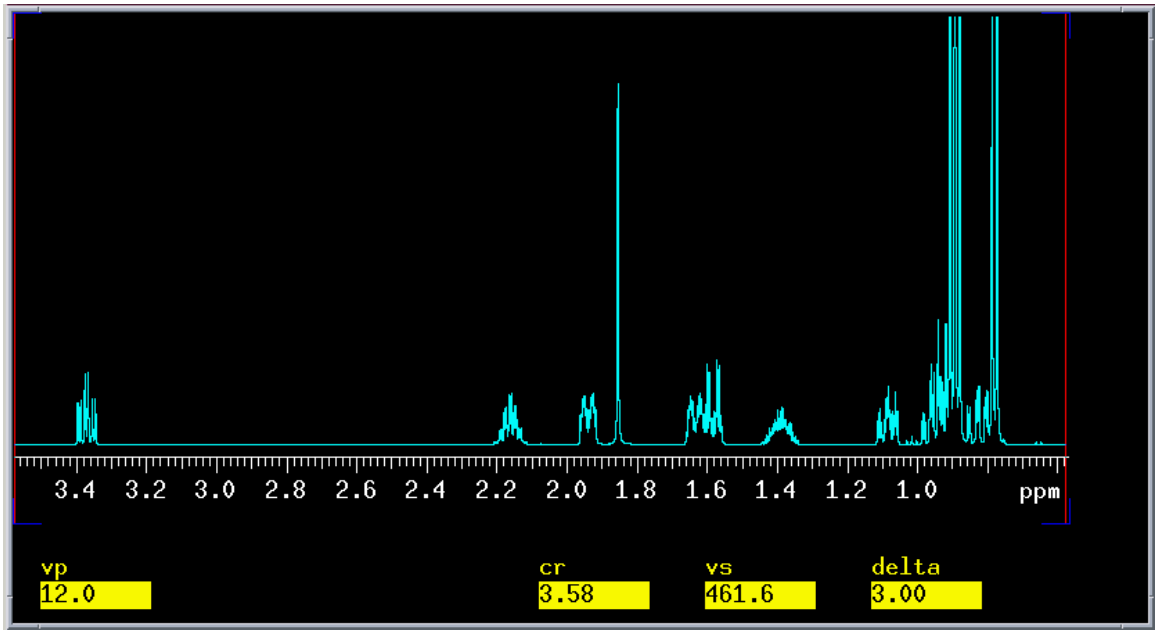


Figure 6: The VNMR Graphics Window

Here the user can graphically process visualize the newly acquired data. As an example we have shown here a small portion of a simple 1D ^{1}H spectrum of menthol ($C_{10}H_{20}O$) in deuterated chloroform ($CDCl_3$). Included in the window is the reference scale, vertical position (vp in mm) with respect to the bottom of the display, cursor position (cr in ppm), vertical scale (vs arbitrary units), and distance between the cursors (delta in ppm). This window can be thought of as a WYSIWYG (what you see is what you get) type of interface. Under normal circumstances most plots are created just as they are seen in the Graphics Window. Here the user can process, zoom, expand, and compare the data they've acquired.

3. **The Text Window:** The Text Window can have many different faces. Here text messages, manual pages, and certain analysis output can be displayed.

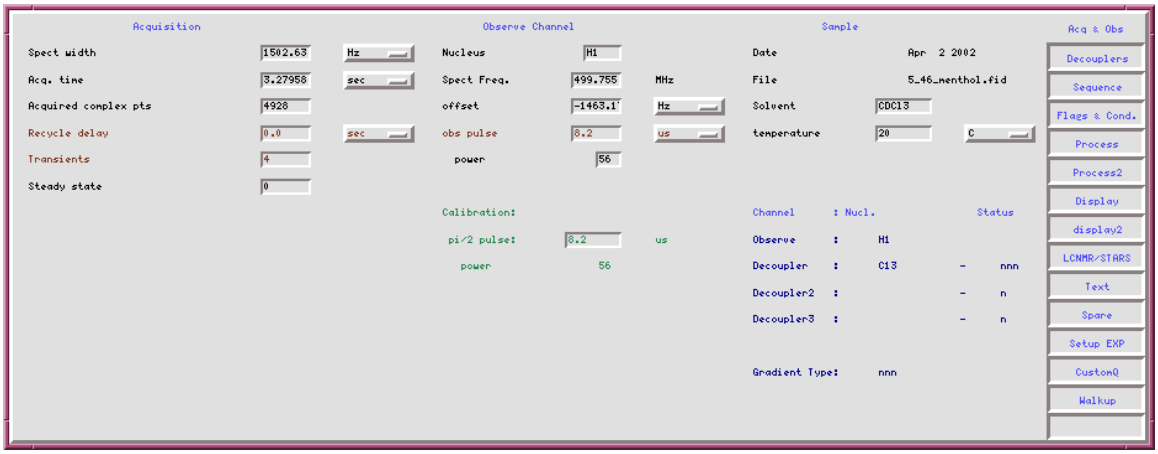


Figure 7: The Text Window. Note the buttons on the right-hand side. These allow for the easy visualization and changing of various parameters.

The Acquisition Status Window:

The VNMR software also has a small window called the Acquisition Status window, which is only available, when the workstation is connected to a spectrometer. The Acquisition Status window is shown in Figure 8 and displays information about the experiment being acquired.

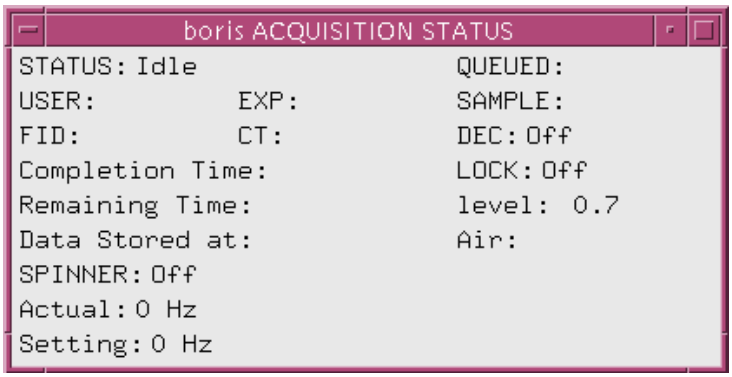
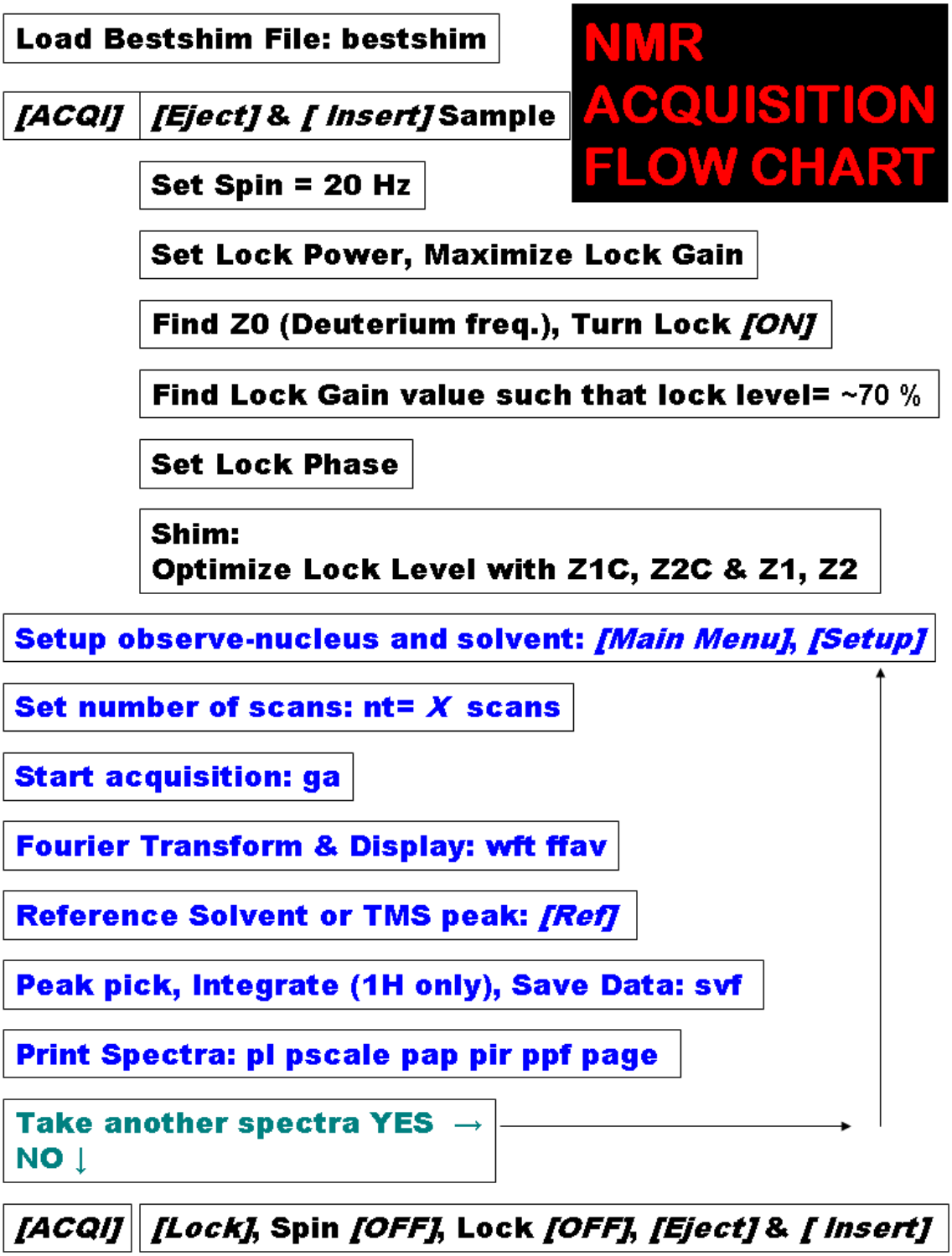


Figure 8: The Acquisition Status window. Note that the status here is 'idle'.

Acquisition Flow Chart

I. Instrument Setup
II. Experiment Setup & Execution (repeat II if desired)
III. Sample removal

NMR ACQUISITION FLOW CHART



I. Instrument Setup: Bestshim, Insert, Spin, Lock & Shim

Log onto the spectrometer workstation

Open the VNMR program by either clicking on the VNMR icon in the CDE toolbar.

1. Load Bestshim File

These Staff assigned shim values are only intended as a reasonable starting place to lock and shim.

Type **bestshim** in the input window. The **bestshim** command is a combination of two commands, **rts('best')** and **su**. You can also load other shim files using **rts('*file name*')**. Your TA may suggest other shim files to use. Remember to type **su** after you have loaded a shim file.

2. Set Sample Depth in Spinner (as directed above)and Insert Sample

Click ***[acqi]*** in the Menu Button window to connect to the acquisition interface. The Interactive Acquisition window will open, Figure 9.

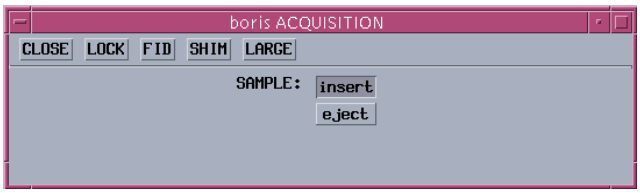


Figure 9: The Interactive Acquisition Window.

Click ***[lock]*** and make sure that the spin is set to zero or turned ***[off]*** and lock is turned ***[off]***. A recessed button indicates the command or parameter is selected. A graphic representation is shown in Figure 10 below with spin set to 20 but turned off and the lock turned off.

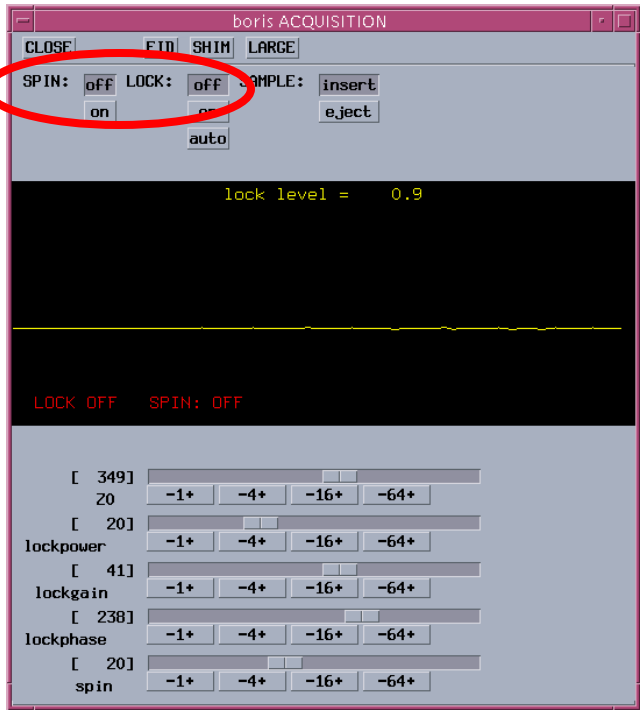


Figure 10: The 'Lock' window within the acquisition interface. The adjustable parameters here are Z0 (magnetic field offset), lock power, lock gain, lock phase, and spin. Each of these may be adjusted by using either the slider bar (very coarse) of stepwise in increments of *[±1]*, *[±4]*, *[±16]*, or *[±64]*. (LMB: moves −, RMB: moves +)

3. Set Spin

Spinning the sample improves the effective magnetic field homogeneity for the bulk of the spins in the sample. Narrower line widths result. If the sample spin is not stable or does not spin at all contact a staff member for assistance.

Use the slider bar to set the spin rate to 20 Hz and Click spin ***[on]***.

4. Set Lock Power

The magnetic field will drift and shift during data collection. A good experiment must compensate for these instabilities and this is why we lock. The “lock” may be considered a separate referencing experiment (in this case, deuterium). This deuterium experiment serves as a feedback loop to repetitively compensate for the magnetic field drift to keep the field constant (and keep good line resolution).

Be sure the lock is clicked ***[off].***
Use the slider bar to set the lock power to the solvent dependent value listed below

These values have been determined sufficient for the Mercury UL1 and UL2.[6]

- $CDCl_3$ (chloroform) use a lock power of 27
- D_2O (water) use a lock power of 18
- CD_3OD (methanol) use a lock power of 12
- C_2D_6O (acetone) use a lock power of 12

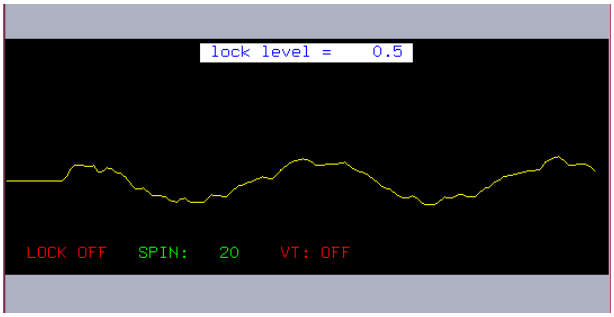
For solvents not listed, ask a Staff member for assistance.

5. Find On-Resonance Z0, the Deuterium Resonance Frequency

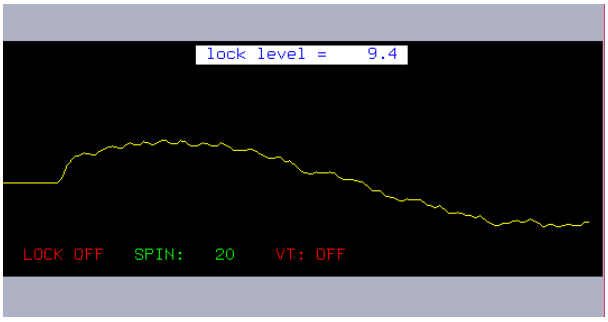
Use the slider bar to set the lock gain to maximum value.

Use the slider bar to set Z0 to the solvent dependent[7] approximate value listed on the chart on the spectrometer.[6]

Increase (**RMB)** or decrease (**LMB)** the ***[-4+]*** button until the frequency of the sine wave decreases and then becomes zero (looks like a step function or a square wave) as shown in Figure 11. Use the ***[-1+]*** button to optimize Z0 until the maximum lock level (square wave) is obtained. See Figure 11



Z0 off resonance



Z0 approaching resonance

[6] The following variables are instrument dependent: Z0, lock power, lock gain, lock phase. Z0 and lock phase also vary over time. The spectrometer chart will be updated periodically by the Staff.

[7] Z0 is directly related to the chemical shift of protonated solvent.

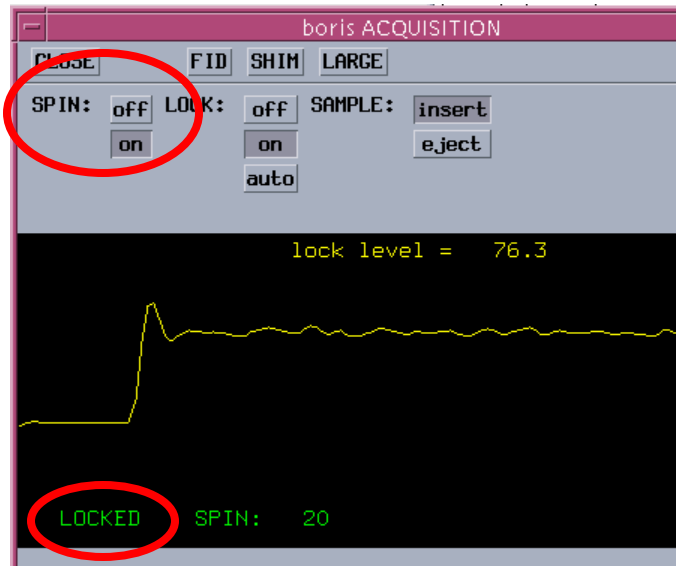


Figure 11: Sample pictures of the lock signal as Z0 approaches the on-resonance step-function.

After Z0 is optimized (see a step signal with the maximum lock level), turn the lock: ***[on]***.

6. Find a Lock Gain

Adjust the lock gain using ***[-1+]*** until the lock level is ~70 %.

7. Set Lock Phase Value

Use the ***[-16+]*** to adjust the lock phase to produce the maximum lock level.[6]

Click ***[Close]*** to exit the acquisition interface window.

8. Shim Sample:

Each sample perturbs the magnetic field differently. Shim adjustments are required to change the currents in 'corrective' coils surrounding the sample. These coils then perturb the magnetic field in such a way to (hopefully) make the field more homogeneous, producing better spectra. These coils are wrapped in such a way that they each affect the magnetic field *reasonably* independently, Unfortunately, physical constraints introduce "impurities" where the shim wires are physically positioned close together and consequently are influenced by the currents flowing through neighboring coils. Recall the importance of positioning the sample in the spinner. Correct and consistent volumes in the NMR tubes will be easier to shim.

Shimming can be performed by observing the lock level (higher ≡ more homogeneous) or shimming on the FID or spectrum. For now we will concentrate on shimming to increase the lock level. The lock level can be thought of as the *height* of the deuterium peak on which you are locked. The more homogeneous the magnetic field, the narrower the peak becomes. Since the

area under the peak is constant, the peak height and lock level increase, as the field becomes more homogeneous.

The UL Mercury NMRs have 13 shims that adjust the magnetic field within the X, Y, Z, vector space. Many shims that have already been optimized for you when you type **bestshim** (assuming your sample volume (0.7 mL), tube quality, etc. are correct). Most of the shims should not be adjusted for typical samples. If you are spinning, the shims with x- and y-axis components must not be touched; only the z-axis shims should be adjusted. How many of the z-axis shims you should adjust depend on the sample's specific needs.

Everyone should probably touch up the Z1C (course Z1) and Z2C (course Z2) shims. For some synthetic chemists with non-crowded spectra, this may be enough to see what is needed. If the spectrum is crowded or if the line shape is important, Z1 (Z1 fine) and Z2 (Z2 fine) should be adjusted very carefully. Then if the spectrum indicates that more shimming is needed, the higher-order Z-axis shims may need to be carefully adjusted in a systematic fashion. Ask for Staff assistance if you need to shim to shim Z3-Z5. [8]

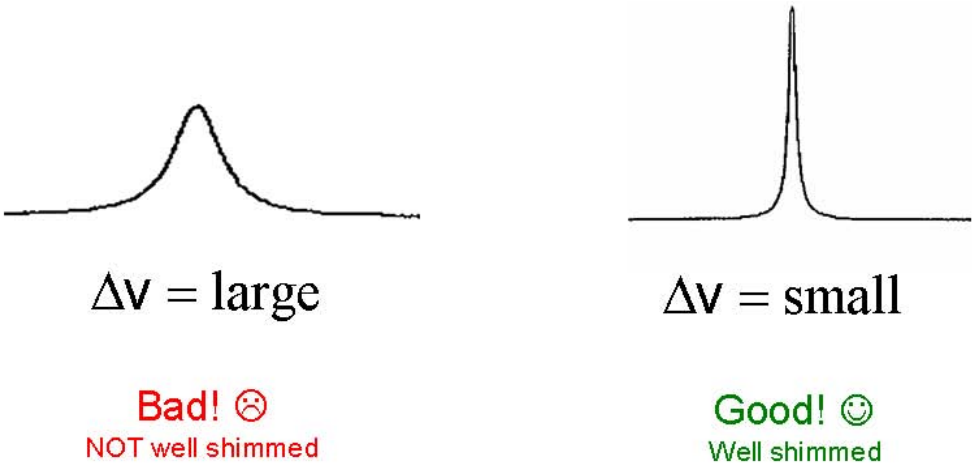


Figure 12 When am I finished shimming? Well optimized shims will produce sharp, symmetrical peaks.[9]

Directions for shimming:

From the Interactive Acquisition window press ***[shim]***

[8] If you would simply like more information about NMR spectroscopy and shimming check out the Acorn NMR website at http://www.acornnmr.com.

[9] Notice that a well shimmed peak is more intense, illustrating why lock level intensity can be used to measure magnetic field homogeneity.

The Interactive Acquisition window will now be displayed in 'shim mode'. See Figure 13. The window displays two bar graphs labeled 'coarse' and 'fine'.

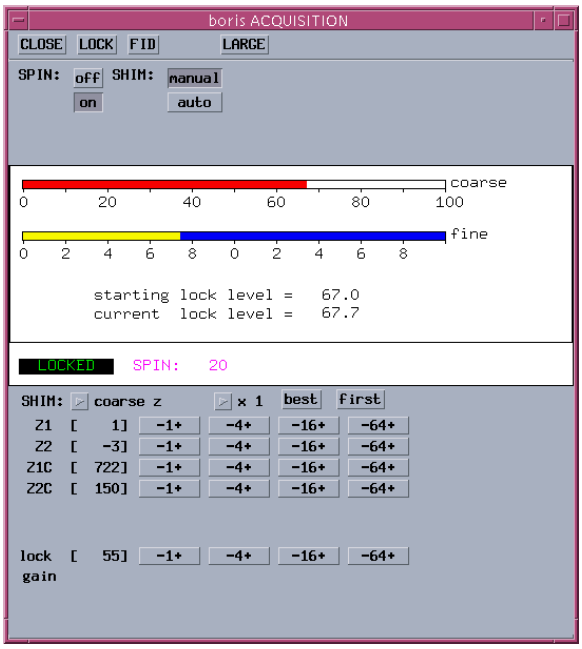


Figure 13: The 'shim' window of the Interactive Acquisition window. Close inspection shows that the 'fine' scale is nothing more than an expansion of the twenty-unit range where the current lock level resides. The name of the game now is to adjust the shims in order to maximize the lock level.

Start by clicking the ***[-1+]*** button of Z1C until the lock level is maximized.

The lock gain may need to be decreased (any time the lock level approaches 100, decrease the lock gain until the lock level is ~70 using the ***[-1+]*** button).

Maximize the lock level with the ***[-1+]*** button of Z2C and repeat Z1C.

Next, use the ***[-64+]*** button of Z1 until the lock level is maximized. Do the same with the ***[-64+]*** button of Z2. As these two shims are coupled, iterate back and forth between Z1 and Z2 using the ***[-16+]*** button, until a global maximum is achieved.

After shimming, adjust the lock gain until the lock level is ~70.

Click ***[close]*** in the Interactive Acquisition window.

NOTE: If the NMR spectrum does not have good line shape, start over: type **bestshim** in the input window and re-optimize the Z1C, Z2C, Z1, and Z2 shims as directed.

II. Proton Experiment Setup, Execution and Printing

1. Setup observe nucleus (pulse program) and solvent

Click ***[main menu]***, click ***[setup]*** and then ***[Nucleus, Solvent]***

Click ***[1H]***

Click a ***[solvent] button***

2. Set the Number of Scans

Type **nt=8** for 1H.

Type **time** in the input window to calculate the acquisition time required.

Type **su** to initialize the parameters with the spectrometer.

3. Start Acquisition, Stop

Type **ga** to start the acquisition

At any time, you can stop the acquisition by typing **sa** (**s**top **a**cquisition after next transient is collected) or **aa** (**a**bort **a**cquisition, now!).

4. Fourier Transform and Display

Type **wft ffav** in the Input window

Type **vp=12** in the Input window

If the baseline is not flat on both sides of each peak, seek help from your TA.

To Zoom-In:
Select a region in the Graphics Window by defining the region with the **LMB** and then the **RMB** (two red cursors will define the region), then click ***[expand]***. The vertical scale can be adjusted, by dragging the **MMB** on top of a peak. Type **ffav** to return to the full view.

5. Reference Solvent Peak

To reference the spectrum, zoom-in on the solvent peak.

Click the spectrum with the **LMB** near the solvent peak.

Type **nl** (**n**earest **l**ine), then click **[Ref]** and enter the reference value from the CIL NMR Solvent data chart

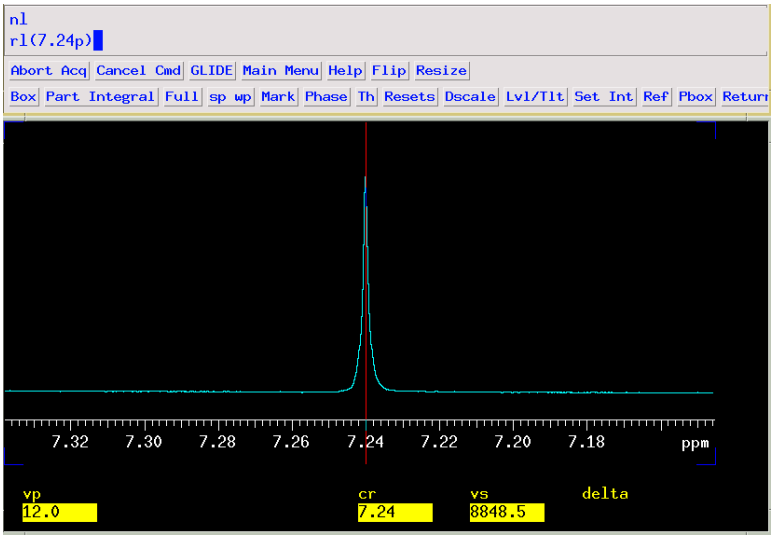


Figure 14: Referencing residual $CHCl_3$

6. Peak Pick

Zoom in on each peak

Click the threshold button ***[Th]*** and use the **MMB** to set the line at the lowest point for peak picking.

Print the peak expansions with chemical shift values in ppm.

Type: **pl pscale ppf page**

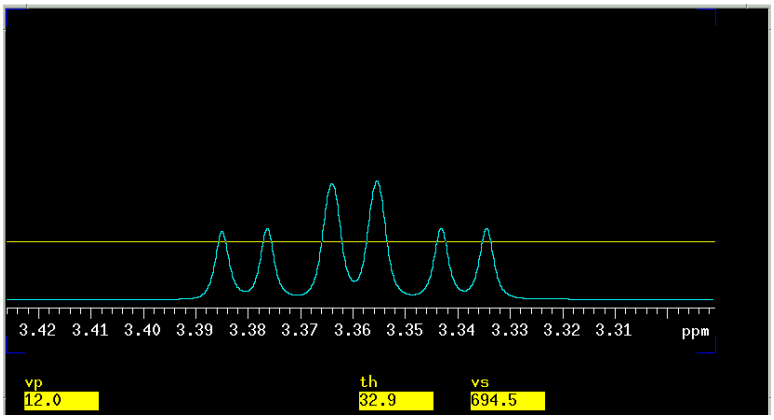


Figure 15: Setting threshold for peak picking

Print the peak expansions with chemical shift values in Hz

Type: **pl pscale ppf page**

Use these values to calculate the coupling constants.

Optional: To view the peak picking on the monitor use the command **dpf** or **dpfhz**. Then type **ds** to return to the spectrum.

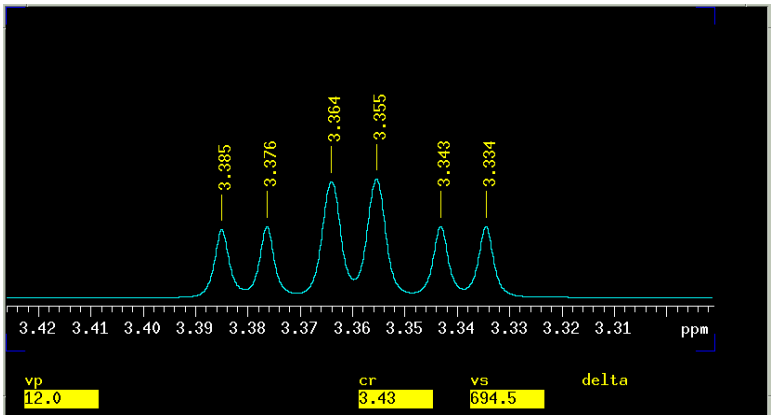


Figure 16: Peaking picking with dpf

7. Integrate (Proton Data Only)

Type **intmod='partial'** to display the green integral line.

If the integral is not flat where the baseline is flat, use the ***[Lvl/Tlt]*** button and the **LMB** and **RMB** to adjust the integration.

Type **cz** (clear zeros from memory), then click ***[resets]***. Use the **LMB** to define those regions you wish to integrate by clicking on both sides a peak. The **RMB** can be used to undo mistakes. Use the **MMB** to adjust the **is** (integral scale).

To assign an integral value to a particular region, type **ds** to regain cursor control then use the **LMB** to place the cursor over the desired region and click the ***[Set int]*** button. You will now be queried for an integral value. Type **ds dpir** (display peak integral regions) to display the integral values under the spectrum.

Print your spectrum with integration values:
pl pscale pir page (Zoom in and repeat printing if necessary)

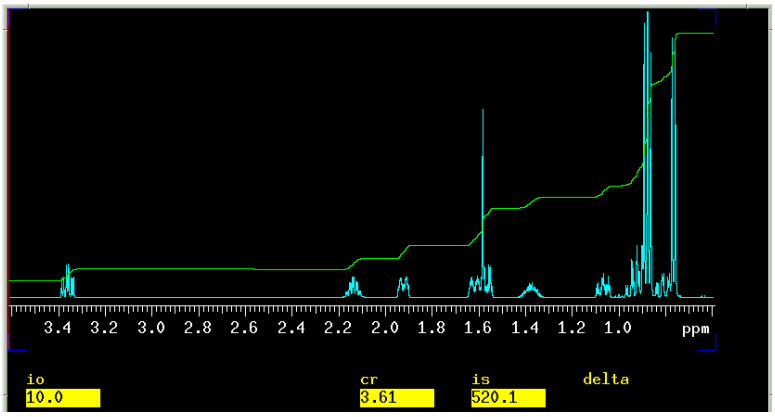


Figure 17: 1H spectra of menthol with intmod='partial' and using the *[Lvl/Tlt]* button to level the integration.

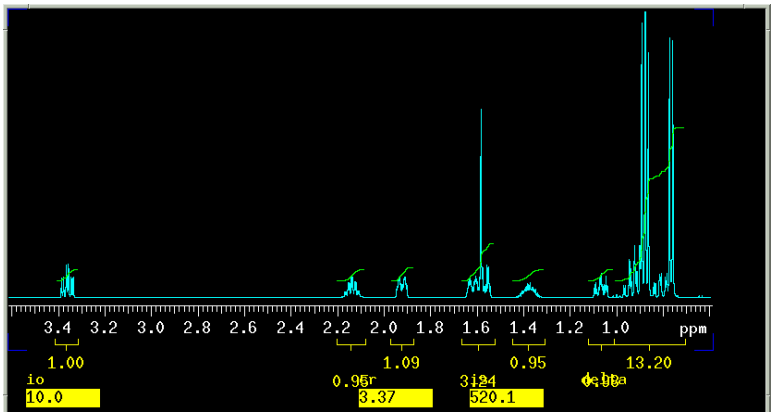


Figure 18: 1H spectra of menthol after setting the *[resets]* and calibrating with *[Set int]* the peak at 3.36 ppm as equivalent to 1 proton. The command dpir was used to display the integral values below the x-axis.

8. Save Data

Type **svf < enter> ,** then type a ***filename*** at the prompt to save the fid to the disk. Do not use spaces or punctuation in the file name.

9. Review of Print Commands

To print your results, create the desired view of the spectrum within the Graphics Window. Then select which print commands you desire.

Type **pl pscale page**
This basic print command will print the spectrum (**pl**), scale (**pscale**) and **page** sends the plot to the printer.

Type **pl pscale pir ppfhz pap page**
Will print the spectrum (**pl**), the scale (**pscale**), the integral resets (**pir**), the peak picking in Hz (**ppfhz**) and the acquisition parameters (**pap**); **page** sends the plot to the printer.

Omit any command that is not desired. Add additional commands (ie **ppf** to print peak frequencies in ppm) before the **page** command.

III. Sample Removal

1. Stop acquiring data **sa**
2. Open ***[ACQI]*** choose the ***[Lock]*** Menu
3. Turn Spin ***[Off]***
4. Turn Lock ***[Off]***
5. ***[Eject]*** to turn on air and remove sample
6. ***[Insert]*** to shut off air
7. Click ***[Close]*** to exit the acquisition interface window.
8. Type **exit** in the command window to exit VNMR
9. Log off the computer **RMB**, Select logout
10. Leave at login screen for next user. DO NOT POWER OFF the workstation.

Appendix 1

Quick guide to performing a 1D 13C spectrum and a DEPT
(Distortionless Enhancement by Polarization Transfer) experiment.

Experiment Setup and Acquisition

The basis for the DEPT experiment is simple. The transfer of polarization from protons to carbon enhances the carbon signal strength. By carefully calibrating the decoupler power, it is possible

to distinguish CH_3 (methyl), CH_2 (methylene), and CH (methine) groups using three pulse widths of 45, 90, and 135 degrees. Since quaternary carbons have no attached protons, they are not detected.

- Use all of your of material (100-200 mg) in 0.7mL of deuterated solvent. If you have a low amount (less then 75mg), you may want to increase **nt** to 64 or 128 for the DEPT experiment. *Remember that you will need to recover your material from solution AFTER you have finished your DEPT.*
- Follow the NMR Instrument Setup guidelines to insert, lock and shim the sample.
- Turn spin off.

- Acquire a 13C spectrum, reference the solvent, peak pick and print the spectrum.

  1. *Click **[main menu]***, then ***[setup],** then **[C13,CDCl3]***
  2. Type: **nt= 64** (nt = 64 should take about 3 minutes)
  3. Type: **su**
  4. Type: **ga**
  5. Type: **ffav**
  6. Reference the spectrum, zoom-in on the solvent peak. Click the spectrum with the **LMB** near the solvent peak. Type **nl** (**n**earest **l**ine), then click **[Ref]** and enter the reference value from the CIL NMR Solvent data chart
  7. Type **ffav**
  8. Click the threshold button ***[Th]*** and use the **MMB** to set the line at the lowest point for peak picking.
  9. Plot your carbon spectra **pl pscale ppf page**

- Acquire a DEPT experiment

  1. Type: **dept su** which will load the 'dept' parameters.
  2. Set **nt=32** to this value (or a multiple of 8). (nt=32 should take 10 minutes, nt=64 about 18 minutes, and nt=128 about 34 minutes).
  3. Type **time**, if you wish to see how long this experiment will take.
  4. Type **au** (submit experiment to acquisition and process data). Once completed, the processed data will automatically be sent to the printer.

- If the automatic processing fails, type **ds(1)** to display the first spectrum. Rephase and expand region of interest. Place a threshold line just below the lowest peak.
- Type **adept dssa** to analyze the data
- Type **pldept** to plot the DEPT spectra

9.2 Perkin Elmer FT-IR Operation Guide[10]

1. Turn on Computer, Screen and Printer. IR stays on all the time do not turn IR off.
2. Click FTIR
3. On right side double click spectrum express
4. Put in User name and Password students/students
5. In left margin click on sample view
6. Place a background sample in machine IR card if you are using one
7. Click background after it runs prepare your sample on an IR card using a glass pipette add one tiny drop of sample gently spreading it on the IR card and let it dry briefly then pull up on the instrument door and insert the IR card into the card holder pushing it down gently all the way then shutting the instrument door (cover). *Check with the TA for workup and preparation of a solid sample.*
8. Click scan at top right
9. Click on text to add a name to the spectrum
10. Click on label to mark peaks with wave numbers
11. Use left mouse to move labels around on the spectrum
12. Right click on spectrum then appearance to change color for printing
13. Click on file then print
14. Delete all your sample files
15. Remove your samples from IR
16. Clean area around IR and take all your samples with you
17. X out of the menu
18. Choose turn off computer from start menu
19. Shut of printer and screen
20. IR REMAINS ON AT ALL TIMES

9.3 Auto Abbe Refractometer Operation Guide[11]

1. Turn on unit from switch on back of instrument.
2. Turn on water pump next to instrument
3. Let instrument warm up for at least 10 minutes
4. Select Mode “nD” or nD-TC (refractive index temperature compensated
5. Calibrate place water on prism using plastic pipette and close cover wait for 2 minutes select then press Calibrate

[10] Written by John J. Dolhun

[11] Written by John J. Dolhun

6. Press read switch should give you an nD value for water
7. Machine is now ready for routine operation
8. Place sample in the sample well use plastic pipette, press read switch to get a reading
9. Immediately clean off your sample wipe off gently with dry lens tissue. Then use methanol. Finally do a water wash and dry off with lens tissue.
10. To check automatic temperature compensation
11. Again place water to sample well
12. Close cover and wait 2 minutes
13. Put instrument in nD mode
14. Press read switch and record value
15. Press temperature switch and record value
16. Compare water refractive index at that temperature see manual by machine
17. Turning off machine please turn off the water pump next to the machine first, then, the refractometer.
18. Any other questions read manual by instrument.

9.4 UV-Vis Operation Guide[12]

Guidelines for measuring a UV-Vis spectrum using the Perkin Elmer Lambda 35 UV/VIS Spectrometer for the Protein Assay Experiments with Automatic Cell Feeder

1. Turn on computer
2. Insert your personal jump drive into the top USB port on the front of computer
3. On the main screen double click on the Perkin Elmer UV WinLab icon top right corner of the screen
4. At Perkin Elmer login message User name must say student or students click OK and you will see the Base Methods appear in the left hand part of the screen
5. Left click on Protein Assay Method Module you are running. Sample info window then loads. Samples box at top of the screen should read 0. Do not change the cell or carousel numbers; Insert a blank UV cuvette into the cell changer position 1. When ready press Start button at top of page. This is your air blank, which is running.
6. Message appears to remove sample. Open door remove the empty cuvette and place your distilled water/buffer blank in position 1 (extreme lower left port on cell changer) this will be scanned as a blank to produce a spectral background. Never touch the clear sides of the cuvette since it will always be in the path of the light beam. Keep the exterior of the cuvette clean and dry before inserting it. Close the door and say OK to the message.
7. After about a minute another message appears asking you to insert your samples into cells 2 thru 8 respectively depending you can run up to seven standards/samples simultaneously. Open the door insert the samples in correct order. Close the door and say OK. Machine begins to scan samples and overlay the graphs one at a time. Once the standards/samples have been run it will ask you to load the final samples follow the instructions then say OK.
8. Message then appears that all samples in the table have been run say OK. Calibration message appears answer it OK.

[12] Written by John J. Dolhun

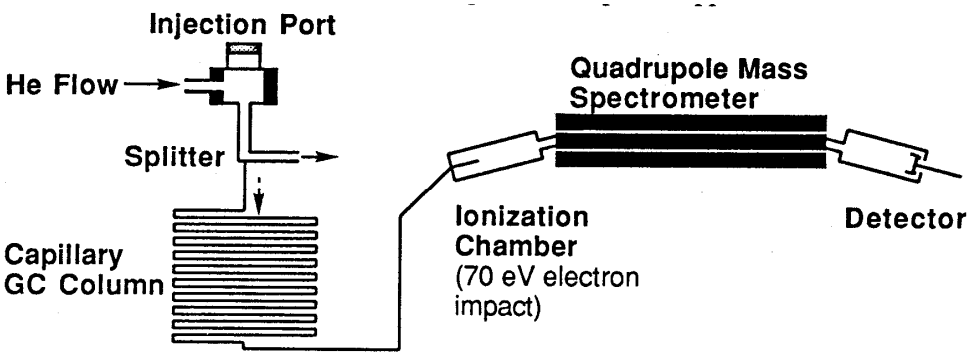
9. Now on the left side of screen click on the word Output then go up to the top and click on file then select report. Say OK to Print and it will print your graph and or your list of absorbances v. concentration values. We are using a quadratic graph for the method.
10. Now Save Data to your jump drive which you inserted previously, go to File then select Save Results then choose As a New Task you will then name your file and save task message. The computer creates a new folder by that name on your removable jump drive
11. To safely remove your hardware device from the computer look for the green icon on the lower bottom right side called Safely Remove Hardware Icon click it and a Safely remove hardware message opens select the task and click Stop a hardware device window opens select your device from the menu and say OK. Message appears at bottom of screen that it is safe to remove Hardware. You can now pull out your USB device.
12. TAs only clear out the TASK folder on a weekly basis deleting the files.

9.5 Hewlett Packard GC/Mass Spectrometer and Varian Saturn 2200 GC/MS/MS Operational Guide and Technique Manual

The Use of Mass Spectrometry as a Laboratory Tool for Confirming the Molecular Mass and Structure of a Reaction Product

The GC-Mass Spectrometer has become on of the most sensitive and powerful instruments for the identification of organic compounds. Analysis of trace quantities of organic compounds has advanced to a stage greater than 1 part in 10^{15}. Many impurities and contaminants found in food and water can now be identified unambiguously in trace amounts. To make the process easier many thousands of spectra including insecticides and industrial pollutants have been compiled into databases and are now readily available as packages that can be purchased and downloaded to computers hooked directly to GC/MS systems.

The liquid organic products from our distillations will be injected neat into the heated injector port of the GC/MS using a special custom-designed 0.01 microliter syringe. The volatilized compound is carried in the helium flow to a splitter with a split out ration set at 1:300 whereby only 0.33% of the sample is diverted to the GC column (the rest is vaporized and exits the system). The GC column used in this instrument is a capillary column, which is 10-30 meters long and only 0.4 mm in diameter. The conditions above insure that no more than 10^{-9} gram of the sample is applied to this column.



Schematic Diagram of HP GC/MS Quadrupole Mass Spectrometer

In the Hewlett Packard GC/MS as the organic compounds elute from the GC, they are introduced immediately into a Quadrupole Mass Spectrometer and a series of mass spectra are repeatedly recorded for each two-second interval. In the ionization chamber, electrons traveling across a 70 eV potential bombard the molecules. When a molecule is struck by one of these high-energy electrons, it loses one of its own electrons and becomes a radical cation $M^{\cdot +}$. Many of the molecular ions will be formed with enough excess energy to undergo subsequent reactions in the ionization chamber. From the ionization chamber, the positively charged ions are attracted through the magnetic field of a quadrupole ion chamber maintained under high vacuum. For a given strength of the magnetic field, only those ions having the correct charge/mass ratio (m/e) will be deflected through the magnetic field and reach the detector. The instrument varies the magnetic field continuously to produce a spectrum of m/e peaks.

Photo of Varian Saturn 2000 GC/MS

The Varian Saturn 2000 GC/MS analyzes the gas-phase ions formed from a sample in terms of their mass-to-charge (m/z) ratios and their relative abundances in the resulting spectra. The Saturn GC/MS creates the mass spectrum with an ion-trap analyzer. This type of device differs from the beam transport analyzer in the HP Quadropole GC/MS where the ions to be separated pass through a series of fixed electromagnetic fields. Instead, the ion trap confines the ions within a single region where

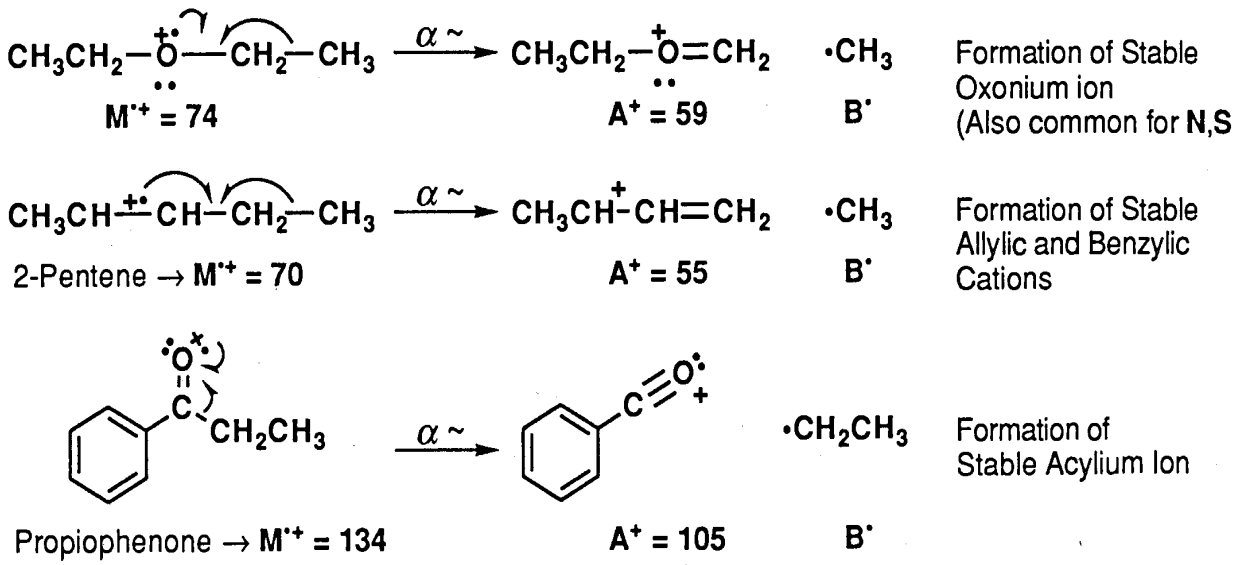
they experience time-dependent electromagnetic fields. The sample is first introduced into the ion trap analyzer through the directly coupled capillary column. The sample is ionized in the mass spectrometer by causing an electron to be removed from somewhere in the molecule. Depending on the compounds original structure and the excess energy present internally in the structure from the destabilizing process, the molecular ion or ionized compound undergoes further fragmentation and breaks apart. The process forms fragment ions and neutral fragments. The molecular and fragment ions, which are formed, are stored inside the ion-trap cavity, where they travel around in defined orbits. Helium gas is also present inside the ion trap. It acts as a buffer and helps to focus the ions into more compact orbits, which then produce sharper mass peaks as they are scanned out. Helium is used because it does not ionize as readily as the analyte molecules. Helium ions end up being the most dominant ions inside the trap, but are immediately pumped away as soon as they are formed. Ion analysis takes place by applying a radio frequency (rf) (1.1 MHz) voltage to the ring electrode that encircles the trap cavity. As the voltage increases on the ring electrode, ions are sequentially sucked out and ejected from the trap according to their mass-to-charge ratio.

In the next section are detailed mechanisms for common fragmentation and ionization reactions. It is much more difficult to solve the structure of an organic compound from the mass spectrum alone than it is to match the spectrum with that of the known compound. With the help of NMR and IR spectroscopy, mass spectrometry can play an important role in structure determination.

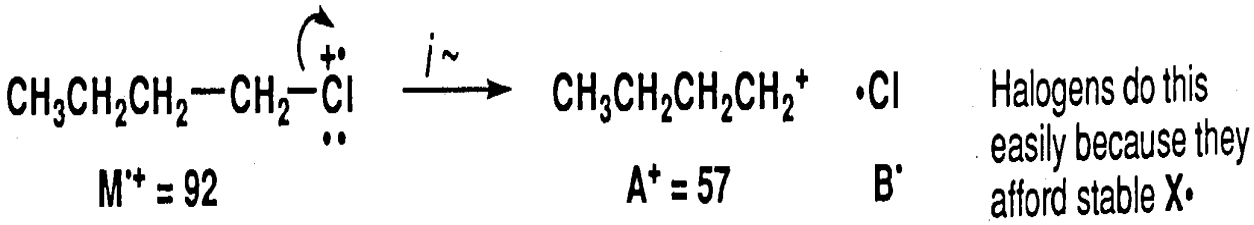
Reactions Observed in Mass Spectroscopy

A Fragmentation Reaction involves the cleavage of one bond of the radical cation $\mathbf{M}^{\cdot+}$ to form a cation $\mathbf{A}^{+}$ and a radical $\mathbf{B}^{\cdot}$. The reaction is especially facilitated by substituents, which stabilize the cation $\mathbf{A}^{+}$.

α-Cleavage Reaction. The α-cleavage reaction is driven by the formation of a stable carbocation as shown in the examples listed below. In the first case, ethers readily undergo the α-cleavage reaction forming a stable oxonium ion (also common for similar N,S compounds). In the second example, alkenes undergo α-cleavage reaction to form a stable allylic cation (also common for aryl compounds and alkynes). In the third example, ketones readily undergo α-cleavage to produce stable acylium ions.



Inductive Cleavage Reaction. Alkyl halides often undergo the inductive cleavage reaction in which the halogen atom (e.g. **Cl**·) simply breaks off the initial radical cation. This is most prevalent for bromides and iodides and for compounds, which can produce a stable carbocation.

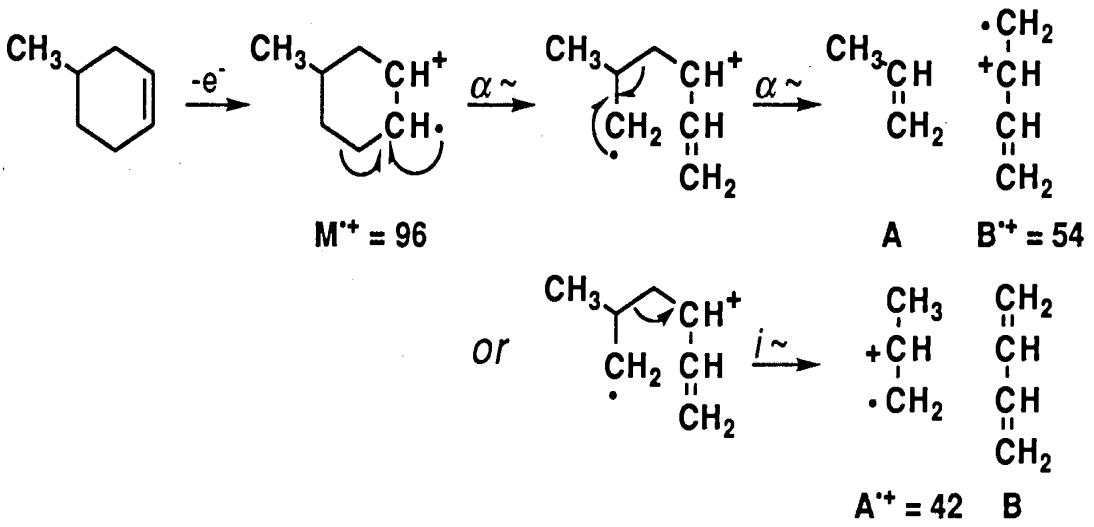


In each case, fragmentation reactions above produce both a radical and a cation from the initial radical cation $\mathbf{M}^{\cdot+}$. Also, as a consequence of the nitrogen rule: For molecules that do not contain nitrogen, fragmentation reactions of the even-massed radical cations will produce odd-massed $\mathbf{A}^{+}$ ions. This can be a useful method for finding which ions are produced by one of the above reactions.

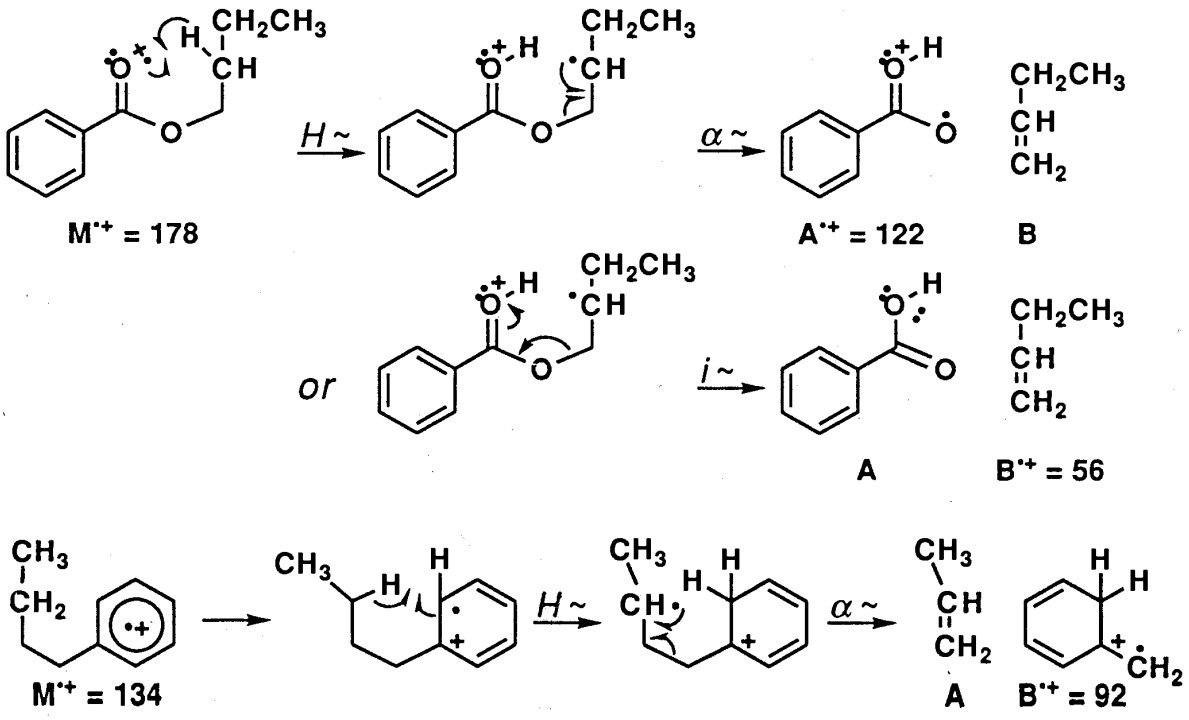
A Rearrangement Reaction involves the cleavage of more that one bond of the radical cation $\mathbf{M}^{\cdot+}$ to ultimately form a new radical cation $\mathbf{A}^{\cdot+}$
and a neutral compound **B**. Often, rearrangement reactions may proceed by complementary pathways so that the mass numbers for both parts of the molecule can be found in the mass spectrum.

$$M^{\cdot+} \rightarrow A^{+} + B \text{ or } A + B^{\cdot+}$$

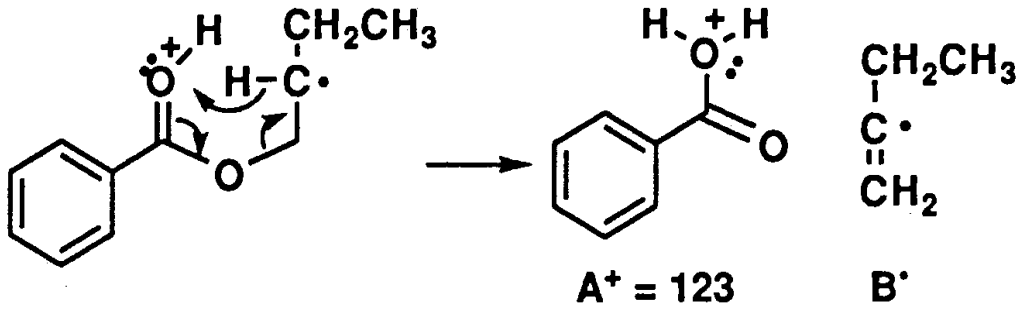
Rearrangement of Cyclic Structures. When a cyclohexene derivative is ionized in the mass spectrum, a particularly facile cleavage of the ring is reminiscent of a retro-Diels-Alder reaction.



Radical Site Rearrangements (McLafferty Rearrangement). Carbonyl derivatives readily undergo a rearrangement reaction in which the $\mathbf{M}^{+\cdot}$ undergoes intramolecular H atom transfer to the carbonyl oxygen atom. The best geometry involves a six-membered transition state and subsequent α-cleavage or inductive cleavage leads to the fragment ions of each piece.



For each example of rearrangement reactions, the observed ions are radical cations. For a molecule which does not contain nitrogen, the McLafferty rearrangement of the even-massed $\mathbf{M}^{+\cdot}$ ion produces an even mass $\mathbf{A}^{+\cdot}$ ion and so are easily distinguished from the odd-massed ions from a simple fragmentation reaction. Also, many molecules display a peak in the mass spectrum arising from a McLafferty +1 rearrangement. For the ester above, the mechanism involves a second H atom transfer.


H
CH2CH3
H–C•
O
H
+
H
O
O
CH2CH3
C•
CH2
A+ = 123
B•

APPENDIX I[1]

Procedure for Operating the Hewlett Packard GC-MS

1. Turn on the computer (Pump and GC should already be on) if its already on go to step 6 skip steps 1-5.
2. Strike F1 key when prompted on computer screen
3. When program manager appears left mousclick on chemstation then restore or maximize
4. Left click to highlight MS Top then go to file and select open
5. Top Default screen should now be open
6. Go to methods and Load and Run Methods Select Chem532m and OK
7. Fill in your last name followed by (.D) for the file name, operator, sample unknown #, and any notes, then click on Run Method
8. Now take a 0.01 micro liter syringe and fill with your sample (neat) with one swift movement inject the sample into the GC and quickly press start on the GC until the solid green light appears on GC console. The spectrum appears on the computer and will be blank for the first 2.00 minutes as the solvent delay keeps the MS from turning on when the bulk of the sample hits the source. After 2.00 minutes you should see the tail end of your sample appear on the GC trace.
9. A message appears Override Solvent Delay 2.00 minutes Select "NO" and enter to get rid of the message
10. Once spectrum finishes running it disappears into the data file and you are returned to TOP Chem 532.m
11. Click on the link data analysis and select main panel from drop down menu
12. From file choose load data file click on your file name and open
13. Move you cursor to a point under your peak (2.20 minutes) and right click twice and your mass spectrum appears under your GC trace, you can move it to another location on the GC trace and right double click until you get the cleanest looking mass spectrum that you want to print from
14. Go to file and select Print Trace + Spectrum click only once and wait after a minute you will see message in lower left of computer Printout

[1] Written by John J. Dolhun

sent to background spooler be patient it takes a few minutes and then prints

15. Go to the link Spectra then select tabulate spectra then Print and then click Done
16. Go to Integrate select Generate % report respond generate report then Done this may have nothing on it depending on your spectrum
17. You should leave the machine with the MS Top screen open for the next student

APPENDIX II[2]

Procedure for Operating the Saturn 2000 GC/MS

1. Open computer and click upper left icon which reads System Control/Automation
2. Allow box to load and you should see System Control-Varian Saturn 2200-Not Ready
3. In lower left corner of computer open the box 2000.40 by clicking in the white box to expand page. This is your system control panel.
4. Click on the folder to the right- Activate a Method (this opens activate a system control method file). Find folder SaturnWS then Service then CHEM532_CI&EI then click on EI_JDquick this loads the method you will use to run your GC/MS
5. Click on the Acquisition tab upper right corner and wait until both green lights appear Ready and No Faults. The "Red" Not Ready light will continue to flash at the top of the screen.
6. When both green lights appear click on the word ENDTIME on left side of screen and set the minutes to 10.00 minutes then click OK.
7. Prepare your sample for injection. Make sure the syringe has been cleaned and baked out in the syringe heating block allow time for it to cool completely before drawing your sample. Draw up 0.01 microliters into your syringe. Note the three-pronged injector on the top of the instrument. Insert the syringe and with one quick sequence inject the sample and simultaneously push down on the three-pronged injector switch. The "Red" Not Ready light changes to "Running" and the GC Green run light comes on and the chromatogram begins to record.
8. Once the Chromatogram completes after 10.00 minutes click on the last colored icon box in the middle of the screen above the spectrum, which opens up the GC spectrum in a window called MultiChro1. Click anywhere in the spectrum

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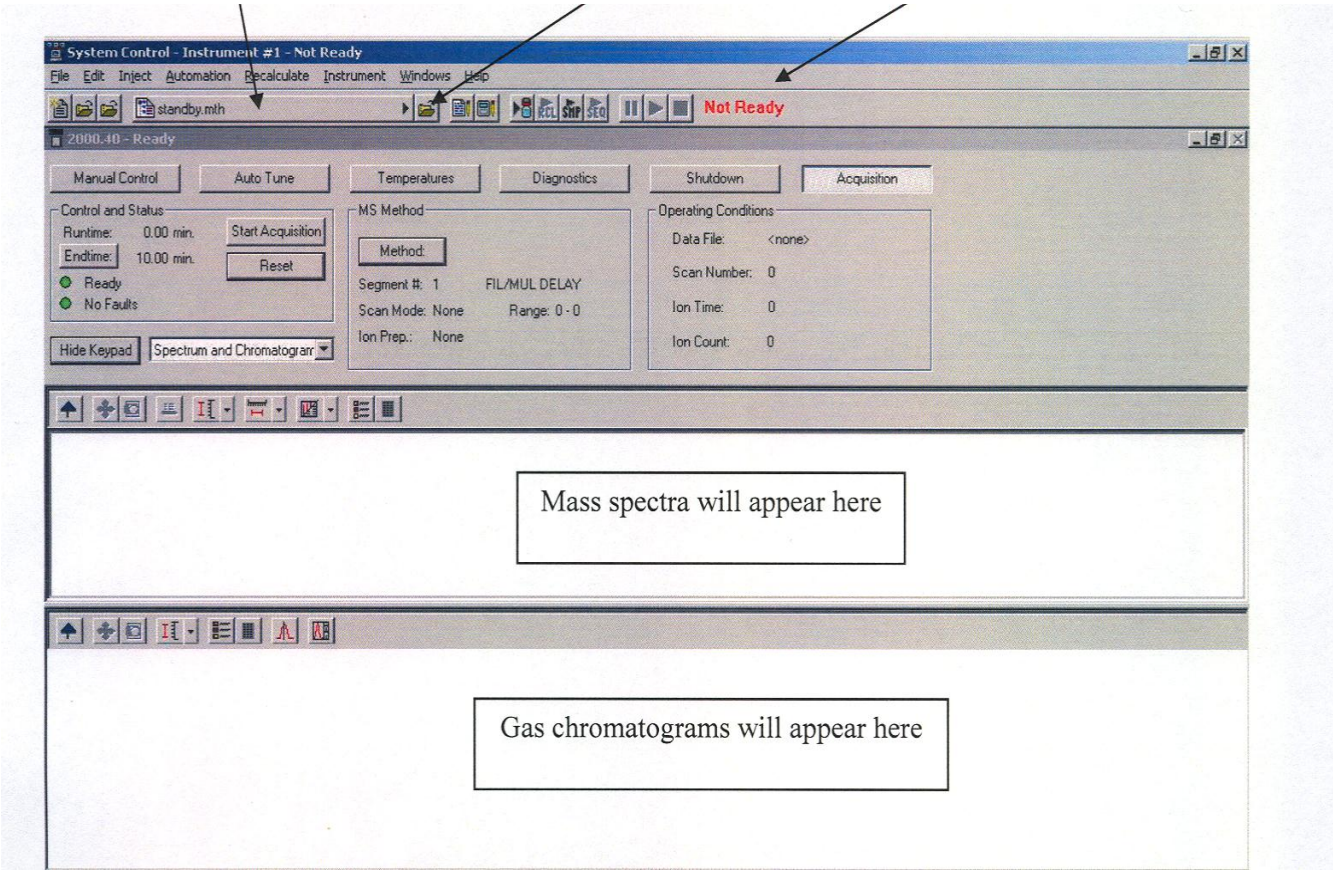
and the screen divides in half GC on the left and MS on the right.

9. Click on the GC Spectrum at a point where you want to see a corresponding mass spectrum.
10. Go to file and select print chromatogram and you will get a printout of the Mass Spectrum above and the gas chromatogram below.
11. Select Search from the menu bar and click on Library Search then at bottom left click Saturn Search. Type in parameters molecular weight range and Target ion range then hit search and print out sheet of your possible hits. A list of probable compounds prints.
12. Click on Spectra and Edit Spectrum to get a list of Mass Ions and their intensities. Click on Print button lower left on screen. You should be able to import data to a computer diskette.

System Control Varian Saturn 2200

Method: EI_JDquick — File Folder: Allows selection of Method — Injection Status: Changes to Running after Injection



9.6 - 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.

GC Operation Instructions [15]

1. Open valves for hydrogen, air and helium
2. Turn on computer and printer
3. Open hydrogen, air and helium on GC left side front
4. Turn on GC from lower right side
5. Set up solvent wash bottles near needle
6. Allow machine time to warm up
7. Open top and blow out hydrogen block with air
8. Press hydrogen ignition button
9. Set up all vials to be run with bar codes
10. Select Method Experiment
11. Select Sequence from computer
12. Edit sequence table
13. Type in From 1 to # of samples you have loaded
14. Say OK
15. Select Run Control
16. Select run sequence
17. Message will appear answer OK
18. Once first run has ran use cursor to pull down screen
19. Continue this process until all samples have ran
20. Click upper left corner dash to shut down computer
21. Turn off GC
22. Turn off printer and computer
23. Shut off air, hydrogen and helium gas valves
24. Shut off air, hydrogen, and helium knobs on left side front top corner of GC.
25. Clean area and dump all sample tubes into white Bucket below GC.

[15] Written by John J. Dolhun

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