Contemporary Topics in Undergraduate Experimental Organic Chemistry

A supplementary OER booklet for Undergraduate Organic Chemistry Laboratory Courses offered by the Dept. of Chemistry, Colorado State University.

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Safety in an Organic Chemistry Laboratory

1. Introduction

The organic chemistry laboratory courses are designed to offer students the true joy of chemistry, that is, the practical/experimental applications. In the organic chemistry lecture, you learn the theories that allow you to understand and predict the results of chemical reactions, but it is the laboratory where you learn how to carry out reactions in practice, which is perceived as the most fun part of chemistry by many! Some of the important questions that are addressed in an organic chemistry lab include,

- How are the reagents combined? Can two compounds simply be mixed together or is a solvent necessary? Can the reaction be done at room temperature or is it necessary to heat or cool the reaction?

- What side reactions are possible, and can they be minimized?
- How is the product isolated and purified from the solvent and side products?
- How can the product be analyzed to determine its purity and confirm its identity?

Generally, the first few experiments of an introductory/intermediate-level organic chemistry laboratory course are dedicated to illustrating common lab techniques such as recrystallization, distillation, extraction, and chromatography. These techniques are useful when the focus shifts to organic syntheses, along with spectroscopic methods of analyzing organic compounds (e.g. IR and NMR spectroscopy), as the course progresses. Advanced organic laboratory courses dive into organic syntheses and unique, complex lab techniques right from the beginning, since the foundation for them is already provided by the introductory or intermediate-level courses. The organic syntheses and reaction chemistry covered in laboratory courses provide valuable opportunities to reinforce the theories that you learn in the lecture courses.

Safety:

There can be many hazards associated with the practice of organic chemistry in the laboratory. Therefore, it is extremely important to follow the safety precautions and guidelines. Let's take a look at some of the basic lab safety requirements in the next few subtopics; other specific safety details are discussed in lab modules and pre-lab lectures.

In our organic chemistry lab manuals, you can often notice instructions marked with this attention symbol:

Any time you see this symbol, pay special attention to the information next to it, since they convey a safety warning. Warnings range from process hazards (i.e. *be sure to follow this instruction, or your lab won't work*) to safety hazards (i.e. *be sure to follow this instruction, or you or your neighbors could get hurt*).

Many lab courses offer points for adhering to the safety rules and requirements since any and all efforts toward working safely and efficiently in a lab deserve commendation. Well, what that means is if you do not pay attention to those rules, you are going to lose points, and worst yet, you could be putting yourself and everybody else in the lab in harm's way. So, always keep in mind that safety is a must, not an option.

2. Organic Chemistry Laboratory Safety Practices

Let's take a look at common safety practices followed in the lab.

Laboratory Attire:

The Irish proverb, better safe than sorry, is our guiding principle here. When in the lab, all students must wear appropriate clothing and protective eyewear at all times.
This includes:

- Safety goggles (also known as splash goggles)
- Closed-toed shoes that cover the foot entirely.
- Long sleeve shirts or lab coats (no holes in clothing)
- Long pants or ankle-length skirts
- Gloves (provided in lab)

The primary goal of the above clothing requirements is to minimize the exposure of skin and vital sensory organs (such as eyes) to the surrounding where hazardous chemicals and supplies are in use and stored. Hence, ripped clothing (ripped jeans, distressed t-shirts, etc.) and shorts, sleeveless or short-sleeve shirts, sandals, etc. are strictly *NOT* allowed in an organic chemistry laboratory setting. Also, given the various dangers that they can pose (catch on glassware/ equipment, drag through spills, fire hazard, etc.), loose and/or dangling clothes are not allowed in the lab as well. Students are required to tie their hair back and restrain or remove jewelry before starting lab work, for the same reasons.

Eye Safety:

Eyes unarguably are one of the vital sensory organs we have, and among the most vulnerable organs to injuries in the lab. Therefore, eye protection plays a key role in lab safety.

Safety goggles must be worn in the laboratory at all times. Prescription glasses are not sufficient for eye protection in the laboratory. Contact lenses may pose hazards in the laboratory due to the ability of certain organic solvents used in the lab to dissolve lens material. Additionally, they reduce the effectiveness of the eyewash, should chemicals come in contact with the eye and may be difficult to remove due to involuntary eye spasms. Therefore, students must take extreme care when wearing contact lenses in the lab, and should always wear safety goggles as well.

Chemical Fume Hoods:

The most noticeable difference between an organic chemistry laboratory and any general chemistry laboratory setting probably is the use of chemical fume hoods. You are always required to conduct experiments in a chemical fume hood, instead of on a benchtop, in the lab, especially given the types of volatile chemicals handled in organic chemistry lab experiments. Chemical fume hoods draw air and solvent vapors away from the space in the hood, so that you and your fellow students do not end up inhaling them.

So, it is important to know how to use a fume hood properly. Here's what you need to pay attention to, in that regard:

1. Maintain the sash height at the position designated by arrows on your hood. The hood does not function properly if the sash is raised above the position marked by the arrows.
2. Perform all procedures at least 6 inches behind (inside) the plane of the sash.
3. Keep your head outside of the fume hood.
4. Keep the inside of the hood clean and uncluttered.
5. Do not rely on the fume hood to protect you from splashes or flying glass: wear safety goggles.

Supervision:

You sign up for laboratory classes to learn and gain hands-on lab experience. Hence, all laboratory courses are supervised and students are not allowed to enter the laboratory without the presence of a Teaching Assistant (TA**).** This is a step towards ensuring a safe learning environment for everybody in the lab, so, always make sure that a teaching assistant (or a person of similar or higher responsibility) is present in the lab from start to finish of your lab session.

Food and drink:

Food and drink are *NOT* permitted in the laboratory due to the high risk of contamination by chemicals. This applies to any and all food and beverage items, including chewing gum and tobacco. Depending on the layout of the laboratory, if sealed in an air-tight container, you may keep your food and beverages in your backpack in the cubbyholes/shelves reserved for student belongings. However, you must *NEVER* open those containers inside the lab! If you are desperate, seek permission from your TA to walk outside of the lab to consume your food/beverage.

Keep in mind, leaving your experiment unattended, even for a very short period of time, could pose a safety hazard. So, if you must walk outside to get a sip of water or a snack, make sure that you notify your TA and get their permission first.

Additionally, always store your backpacks and belongings in the designated cubbyholes/shelves for them; leaving them on the floor or a chemical countertop leads to the risk of chemical contamination and tripping hazards.

Safety Equipment:

One of the most important things you must do during the safety orientation (usually the first day of labs) is learning the location of common lab safety equipment such as the fire extinguisher, shower, and eyewash. Do not hesitate to seek help from your TA to locate them.

Eyewash:

- If a particulate matter, droplet, liquid, etc. gets in your eye while working in the lab, immediately wash it out using the eyewash, holding your eyelids open. Then, seek medical attention depending on the incident/injury.

Fire Extinguisher:

- A small, localized fire may be extinguished by inverting a large watch glass or beaker over the fire and quickly closing the hood sash. If that is not practical, immediately seek help from your TA. If that does not work either, notify the lab preparation room staff, and depending on the severity of the fire, evacuate the building.

  If the fire spreads to your clothing, call for help while you *stop-drop-and-roll*.

Shower:

- In some instances of fire or large chemical spills, the safety shower may be advised, but if you are not sure and you (and others around you) are not in immediate danger, seek quick help from your TA. In the case of a chemical spill, all contaminated clothing should be removed as quickly as possible, followed by the use of the shower. The lab preparation room usually is able to provide temporary clothing in situations of this sort.

Fire Safety:

It is important to turn off all electrical equipment (transformers/Variacs, heating mantles, hot plates, stir plates, centrifuges, Mel-temps, roto-vaps) before unplugging them, especially since a spark from the outlet to the plug may ignite flammable vapors, starting a fire. You should also allow those equipment to cool down before wrapping the cords and returning them to the storage shelves, to prevent the insulation on the cord from melting. Electrical equipment should be distanced from water sources as much as possible to minimize the chances of electrical shocks.

Thermal burns can be avoided by exercising caution with hot equipment (handling hot glassware with tongs or towels, cooling electrical equipment, not boiling solutions to dryness, etc.). In case of a thermal burn, wash the area with cold running water for 10-15 minutes and seek medical help as needed.

Chemical Safety:

Attending each lab well prepared is the key to preventing lab accidents: *know what you are doing, know what you are working with, know what to expect, and be calm and alert while you are in the lab.* As we discussed in the previous chapter, hazards, handling, and other information about the chemicals you use in the lab can be found in safety data sheets (SDS).

Exposure to chemical vapors may be hazardous, and you can easily and effectively reduce this hazard by working in the hood with the sash lowered down appropriately. When in doubt, all reagents and solvents should be treated as if they are toxic, especially since some hazards may be unknown.

If you spill a chemical on your skin, flush the area with cold, running water for at least 10 minutes (and longer if needed). If you spill a caustic chemical, such as concentrated acid, on your clothing and it penetrates or soaks through to your skin, remove or cut off the affected article of clothing and flush the area with cold running water for at least 10 minutes. Ask your TA immediately for help at the same time!

To minimize the risk of contamination on your hands, use nitrile gloves (usually provided in the lab), which are slightly more protective than latex gloves, and have minimal risk of an allergic reaction. Correct usage of gloves means replacing them periodically as needed (they will eventually be permeated by chemicals). When they are contaminated, remove the glove by turning it inside out.

Do not wear gloves when you leave the lab! Remove your gloves and wash your hands before touching the door knobs and stepping outside the lab for any reason. You can easily reduce the risk of most contaminations by maintaining a neat workspace.

Also, clean any spills in your workstation/lab as soon as possible. If you are unsure of how to proceed with the cleaning, do not hesitate to seek guidance from your TA. An acid spill can be neutralized easily with sodium bicarbonate (baking soda), and a base spill can be neutralized with a mild acid such as citric acid. All laboratory spaces are supplied with spill kits, labeled broken glass containers, waste containers, brushes, and dustpans.

Non-Obvious Safety Practices:

- Concentrated acids often give off acidic fumes, which can cause pain when inhaled. These reagents should only be handled in fume hoods.
- Reagents must be added slowly to keep the reaction under control, otherwise, a violent reaction may result. When mixing two reagents, the stronger reagent should be added to the weaker (i.e. acid to water) so that any violent reaction will involve a mixture containing mostly the weaker reagent.
- Never heat a closed system! heat causes vapor and air to expand and will eventually vent itself by breaking a seal or glass. Avoid this by ensuring that the system is open to outside air before applying heat.
- The instrument room houses a Nuclear Magnetic Resonance (NMR) instrument, which has a relatively large magnetic field. Students with *pacemakers* or *metal prostheses* should consult their TA and the course instructor, and should not enter the instrument room without further counsel. To avoid potential injuries due to ferromagnetic objects, do not bring them inside the restricted area in the room, usually marked as such.
- Walking or turning around abruptly, especially in a crowded teaching laboratory, could lead to bumping into another person, and cause accidents. Use extreme caution.
- Any accident resulting in an injury, in which you might go to the health center or a doctor, requires the completion of an accident report by the TA or the course instructor, with the help of the injured person (when possible) and the best witness to the accident.
- You can only stay in the lab during its scheduled period. So, you must begin cleaning up 10-15 minutes before the end of the lab, especially since poor time management and rushing at the end is a recipe for disaster 😊. When you finish, the lab needs to be ready for the next student/class. So, remember to clean any spills, stains, etc. in your workstation and remove any and all glassware that you have clamped to the metal frame *(known as monkey bars)*. Additionally, with guidance from your TA, be sure to empty the solvent traps in the vacuum filtration system in your workstation.

3. Hazardous Waste Disposal

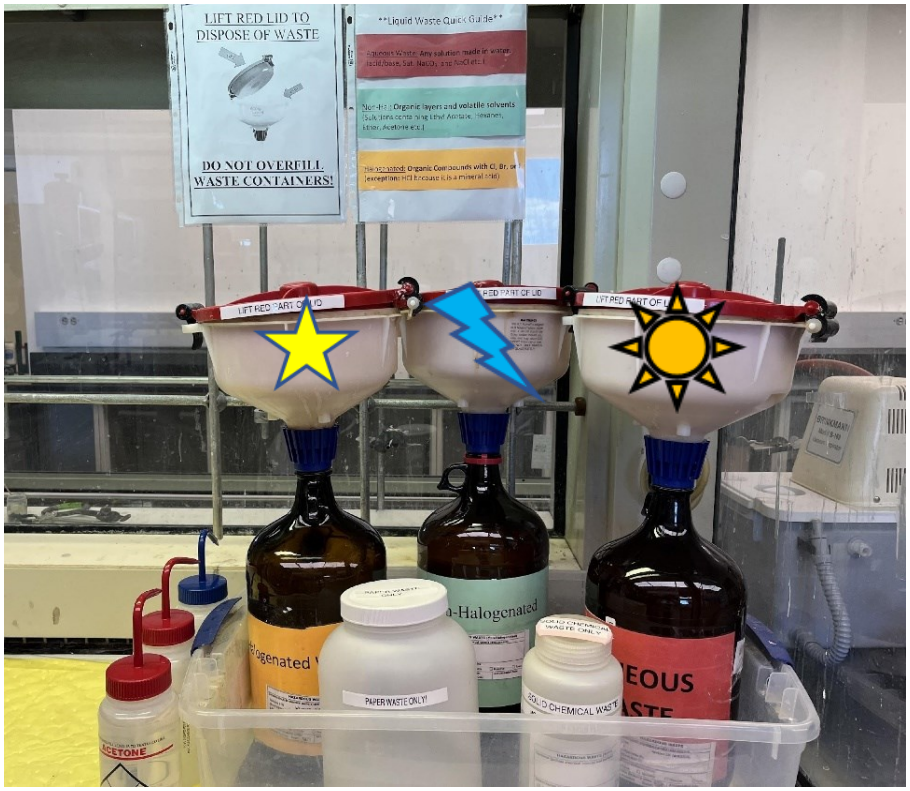
Proper disposal of waste generated in the lab is as important as the actual lab experiment itself! It is a responsibility that we cannot take lightly, especially since the chemicals and supplies we use in the lab have a high impact on our environment. So, we all must do our part to protect our environment while making the best use of learning opportunities presented in the lab, by adhering to proper waste disposal rules and guidelines.

In our lab manuals, instructions on waste disposal for each experiment are indicated by the following symbol:

Hazardous waste disposal rules are generally mandated by federal law, and violations can result in heavy fines. You must be aware of the rules and follow them each and every time you dispose of a chemical. So, always pay attention to the information in the waste disposal section of each lab manual, and carefully follow the directions.

General Instructions for Waste Disposal:

A typical waste station in an organic chemistry lab looks like this:



Liquid Waste

There are several labeled bottles placed in the reagent/waste hood for liquid waste disposal.

(i) **NON-HALOGENATED WASTE**: Only organic liquid compounds that do not contain a halogen atom (fluorine, chlorine, bromine, or iodine), and liquid compounds that were not in contact with halogenated compounds should be disposed into this bottle. Be careful *NOT* to discard water in this waste container.

(ii) **HALOGENATED WASTE**: Organic liquid compounds that contain halogen atom(s) and compounds that were in contact with halogenated compounds should be disposed into this bottle. Some examples include bromoheptane, t-butyl chloride, bromobenzene, etc.

NOTE: HCl (hydrochloric acid) is not a halogenated organic compound! it is a mineral acid

(iii) **AQUEOUS WASTE:** Water that may have some organic solid or liquid dissolved in it.

Solid Waste

Solid chemical waste should be disposed of in the *Solid Chemical Waste* container in the hood. Filter papers, when contaminated with solid chemicals, should be discarded in the *Paper Waste* container.

Paper towels should be discarded in the regular trash, and *NOT* in the solid waste or paper waste; paper products in solid waste interfere with the disposal of solid chemicals, and could lead to fire hazards!

If intended to reuse, syringes and needles should be stored carefully in your drawer. Otherwise, there is a separate Sharps Container in the lab for the safe disposal of syringe needles.

Broken Glassware

All glass waste products that include broken glassware, capillary tubes, Pasteur pipettes, disposable test tubes, and glass-backed TLC plates should *only* be disposed of in the container labeled *"GLASS WASTE"*. As much as possible, try to rinse off any chemicals in them, before discarding them in the glass waste bin/box.

Image Credits

Safety First Sign: https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcSZoFgA6qr-EGpGsH7pkYx-x2qWoRAHaVuMeQ&usqp=CAU

Safety goggle photos: https://tar97125.wordpress.com/2012/12/20/all-you-need-to-know-about-lab-safety/#jp-carousel-47

Fume Hood: Stock photos from the undergraduate organic chemistry lab program at Colorado State University

Food and Drink Sign: https://www.mydoorsign.com/img/lg/L/no-food-or-drink-glass-door-decal-lb-2898.png

Tripping Hazard Sign: https://www.emedco.com/iso-warning-symbol-labels-tripping-hazard-sym43.html

Fire: http://dnrc.mt.gov/divisions/water/operations/images/floodplain/Fire_Icon.png

Flask: https://cdn3.vectorstock.com/i/1000x1000/43/47/chemistry-flask-school-on-white-background-vector-26164347.jpg

Question Mark: http://www.clker.com/cliparts/D/A/w/N/h/p/question-mark-red-hi.png

Acing the Skill of Scientific Report Writing

1. Introduction

The lab report is a precious opportunity for you to properly document what you did in the lab, explain what you did clearly and concisely so that *anyone* can follow it, and show, with evidence, that you understood what you did. Generally, there are two types of lab experiments in organic chemistry laboratory courses – technique experiments and preparatory experiments.

Technique Experiments:

These experiments involve making observations and learning techniques that are common to organic chemistry laboratories. Generally, these experiments do not involve major transformations of compounds such as synthesizing an alkene from an alcohol, etc. Examples of technique experiments include recrystallization, distillation, liquid extraction, and chromatography.

Preparatory Experiments (Organic Syntheses):

These experiments involve converting one compound into another as you would see in a synthesis reaction.

Lab report is a comprehensive document of your work in the lab, so, understandably, the format of it will differ slightly depending on the experiment. Let's go over the key sections that you need to include in a lab report, and how they differ between technique and preparatory lab reports.

2. Key Sections of a Lab Report

It is not uncommon at all for laboratory courses to ask students to maintain a physical lab notebook. When that's the case, depending on the requirements of the lab course, you can submit the lab notebook pages with complete information as the lab report, or use the information/notes/data written down by you in the lab notebook to prepare a well-formatted and word-processed lab report. Let's talk about the former, since it is easy to apply it to the latter.

1. **Heading:**
   Use a new page of the notebook to start a new experiment. Include your name, the date, the title of the experiment, and any references as applicable.

2. **Introduction:**
   This is where you give a brief description of the experiment, but do not try to write a long paragraph for this section. Instead, clearly state the purpose(s) of the experiment and how you are going to achieve them, in a couple of sentences.

3. **Pre-Lab Write-up**:
   The pre-lab write-up is what you do *before* showing up for the lab. It generally includes the following sub-topics, and helps you immensely to get a grasp of what you are going to do in the lab. It also prepares you well to answer the pre-lab quiz questions.

- **Main Reactions:** When applicable, write the balanced equation for the conversion of starting material to product. If it is a preparatory experiment, be sure to include a detailed, arrow-pushing mechanism. You will be amazed to realize how helpful drawing a mechanism is to actually understand the purpose of each reagent in that particular experiment and why they are used in a certain order, etc.

- **Table of physical constants:** This is where you summarize the information about each chemical/reagent that you are above to use in the lab. This table should contain the chemical name, molecular weight, and pertinent physical constants such as melting/boiling point(s), density, specific rotation, etc.) of all reagents that you use in the lab, that includes the reactants, catalysts, and products. This information is important for some of the calculations you do and also to interpret some of the observations you make in the lab. Chemical vendors such as Fisher Scientific, Sigma-Aldrich, and scientific websites such as https://pubchem.ncbi.nlm.nih.gov/ are great sources to find the physical constants of a given chemical. When you use those sources, be sure to cite them in your lab report (see page 6 for more details).

- **Chemical Hazards/Safety Information:** A wise person once said *better safe than sorry*, which is very much applicable to science. But how do we work safely in the lab? The simplest answer is by knowing what hazards are associated with the chemicals we use and the processes we follow, and taking appropriate safety measures accordingly. Therefore, the *Chemical Hazards* section is an extremely important part of your pre-lab notes. It is the one place where you list the safety information of *all* chemicals that you use in a given experiment. In other words, this is where you should include information regarding toxicity (e.g. carcinogenic, teratogenic, irritant, etc.), flammability, and corrosivity of each chemical for easy reference. Information of this type can easily be found in open-access safety data sheets (SDS) available online through chemical vendors such as www.sigmaaldrich.com. When you use those sources, be sure to cite them in your lab report.

- **Experimental Procedure:** Write the procedure in point form with enough information and clarity so that you are able to follow it easily. This doesn't mean that you should copy and paste the steps exactly as they are written in the lab manual! You can take your liberties and write the procedure in your own words while preserving accuracy and comprehensiveness. If provided, check the sample lab reports/notebook pages in the Canvas module to get a rough idea of how to organize your notes with adequate space to note down measurements, observations, data, and any changes to particular steps. If you are required to write a separate lab report at the end, this organization of notes, observations, data, etc. in your lab notebook will make that task much easier.

  Additionally, this section is a great place to talk about proper waste disposal. Lab manuals provide general instructions for it, but you can easily figure out which chemicals/reagents are going to constitute the aqueous waste and which ones constitute the halogenated or non-halogenated organic waste, just by looking at the lab procedure. We take a bit more detailed look at different types of waste in the next chapter. However, when in doubt, always seek guidance from the TA to make sure that you accurately identify the type of waste.

4. **Observations:** Observations play a vital role in learning how a particular experiment proceeded, and arriving at conclusions. More importantly, whenever a reaction doesn't work as expected, observations provide useful evidence for troubleshooting. Therefore, one must make it a habit to note down *any and all* observations, in real-time, while performing the experiment. So, record everything you do (mass and volume measurements, temperature readings, physical appearance of the final product, color changes, pH readings, deviations from procedure, etc.) *directly* in your notebook.

   Do not use scrap paper to note down measurements and observations with the hope of transferring them to your notebook later; it is poor practice and a guaranteed way to lose information.

5. **Results, Calculations, and Analysis:** This is the section where you actually analyze the observations, measurements, and data gathered during the experiment. Hence, this usually happens after the lab. Percent yield/recovery calculations, TLC R_f calculations, relative abundance calculations based on chromatographic data, and NMR and IR spectral data interpretation are among the common analysis done in this section. Whenever applicable, it is extremely important that you show the steps of calculations, draw/attach properly and accurately labeled diagrams, chromatograms, spectra in a sequential manner, and use complete sentences for reasoning. It will earn you partial credit even when the final answers are inaccurate.

6. **Discussion and Conclusions:** This section is your opportunity to convince the reader that you understood the experiment. So, you must discuss the observations and data analysis from the section above. Keep in mind that you should draw conclusions *only* from the observations you gathered and the data analysis you did. In other words, you must use the observations and data analysis to explain what actually happened, as opposed to what you thought should have happened. If the experiment involved identification of an unknown, this is the section where you explain how your data supports the identifications you made (well, don't forget to state your identifications first). If you conducted instrumental analysis such as polarimetry, GC, IR, and NMR, this section is the place to explain what conclusions you can arrive at from those results, and how those results support your conclusions. If the experiment is designed to carry out an investigation and answer a specific question, make sure you answer that question here, based on your results.

7. **Post-lab questions:** Post-lab questions are generally designed to test your knowledge and see how well you can apply what you learned in the lab to solve a problem. When you answer them, provide logical and concise explanations; do not hesitate to use chemical reaction equations and mechanisms, where applicable, to support your answers.

8. **References:** Generally, you state your references for information such as physical constants of chemicals, when you prepare your pre-lab notes. However, if you use scientific papers and other resources to compare and contrast your results and data analysis, or simply just to help understand what your data analysis means, it is extremely important that you give credit to those resources by properly citing them. You can do that in this section, and it is always a great practice to adhere to a consistent format for those citations.

   See some examples below:

   - **Journal article:**

   Hu, X.; Zhang, G.; Bu, F.; Lei, A. *ACS Catal.* **2017**, *7*, 1432.

   Note that the minimum required information for a journal is author, abbreviated journal title, year, publication, volume number, and initial page# of the cited article. The journal abbreviation and volume are italicized. The Year of publication is bolded.

- **Books and book chapters:**

Anastas, P. T.; Warner, J. C. Green Chemistry: Theory and Practice; Oxford University Press: Oxford, 1998.

Note that the minimum required information for a book is author or editor, book title, publisher, city of publication, and year of publication.

- **Online scientific resource:**

Freudenrich, C. How Lead Works. http://science.howstuffworks.com/lead.htm (accessed May 29, 2014).

Note that the minimum required information for a website is the site title, URL, and date accessed. Include the author's name if one is listed.

- **Lab manual:**

Piyaratne. P.; CHEM 344 Modern Organic Chemistry Laboratory Manual. **2021**, *3*, 1-8

Note that the author, title of the lab manual, year, experiment number, and pages of manual are included in the citation.

Keep these guidelines in mind when you construct your notes and prepare your lab report next time. Remember, writing a proper lab report/scientific communication is a skill that takes time to master; the training you receive now in these lab courses will definitely help you ace that skill.

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Report Graphic: http://clipart-library.com/weekly-reports-cliparts.html

SDS Graphic: https://www.kha.com/wp-content/uploads/2019/11/sds-254x318.png

Laboratory Practices in NMR and IR Spectroscopy

Spectroscopic analysis of samples has become an integral part of many organic chemistry laboratory courses. In the lecture and recitation, you usually only see perfect spectra where there are no interferences from starting materials, by-products, etc. However, when you actually conduct an experiment/synthesis in the lab, there is always a chance of residual reactants, by-products, and other impurities showing up in your spectra. So, let's revisit the basics of NMR and IR spectroscopy, and take a look at deciphering *real-life* spectral information.

^{1}H NMR Spectroscopy

As new organic compounds are synthesized in pharmaceutical and chemical research, or even in teaching labs, it is essential to be able to identify/verify their chemical structure. Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful tools employed in labs to determine the identity of organic molecules. Particularly, ^{1}H NMR gives you information about the structure of the compound by indicating the number of different hydrogen atoms and their surroundings in the molecule.

This is what a typical ^{1}H NMR spectrum looks like:

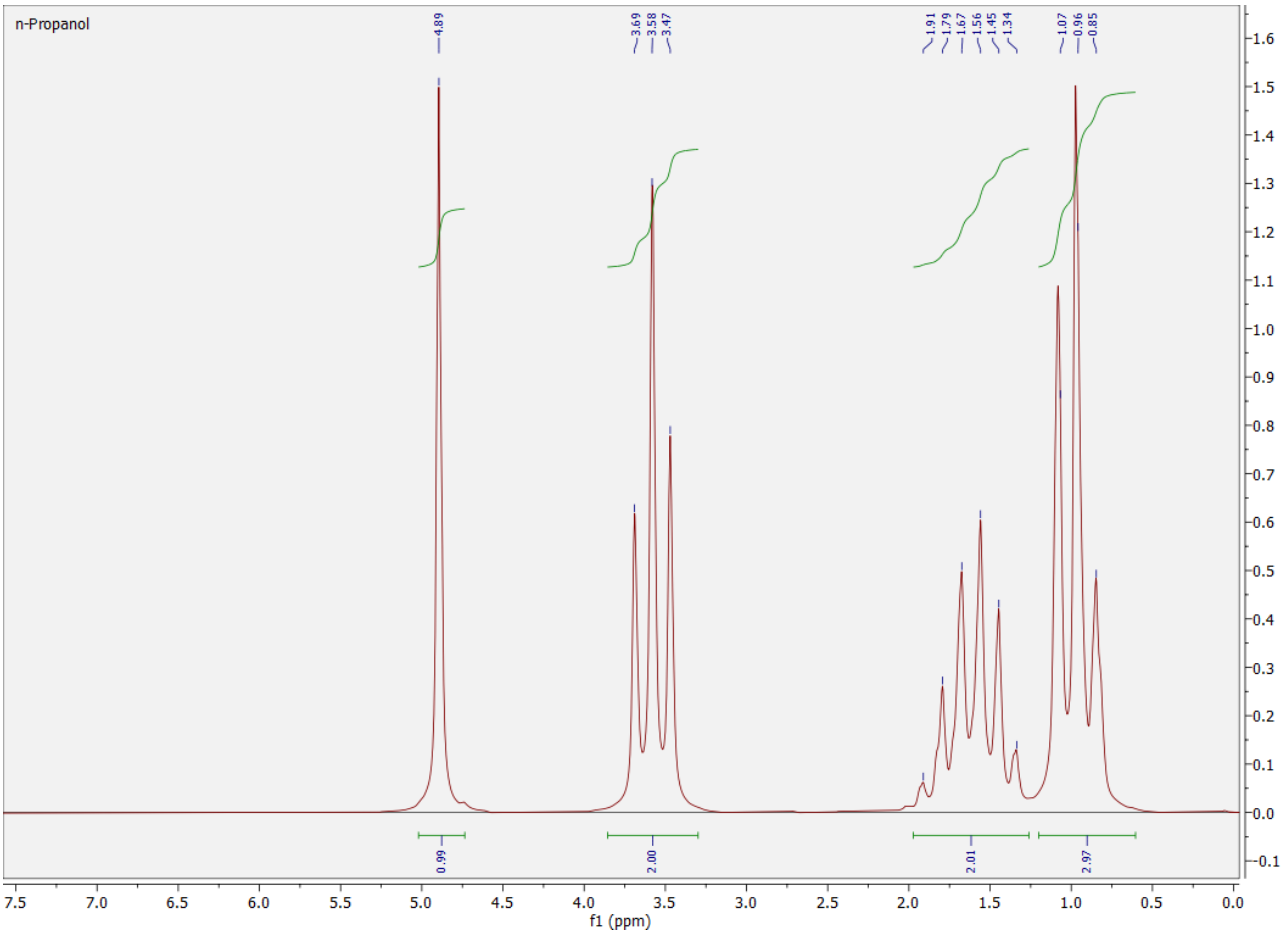


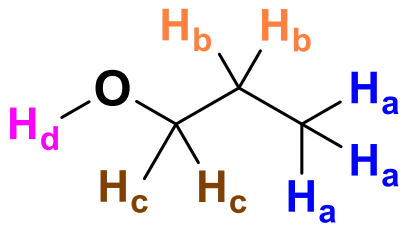
Figure 1: ^{1}H NMR of n-propanol

Now, to read and understand the above spectrum, you will probably have to review the concepts of proton NMR from your organic chemistry textbook. Here is a summary of information that can be obtained from a ^{1}H NMR spectrum:

- **How many types of H?** Indicated by how many groups of signals there are in the spectra.
- **What types of H?** Indicated by the chemical shift of each group. Also termed shielded or deshielded.
- **How many H of each type are there?** Indicated by the integration (relative area under the peak) of the signal for each group.
- **What is the connectivity?** Look at the splitting pattern of each signal. This is a result of coupling, and it tells you what is next to each group.

The above spectrum of n-propanol was obtained using a Nanalysis 60MHz benchtop NMR and processed using MestreNova software. We can use a spectral data analysis table, as shown below, to summarize and present the information in it.

Table-1: Spectral data analysis table for n-propanol

Labeled structure of n-propanol: (*^{1}H-NMR is given on page 1*)				
Chemical Shift (ppm)	**Integration**	**Splitting *(n+1 rule)***	**Coupling Constant (J-value)**	**Structural Assignment (label of the corresponding proton)**
0.96 ppm	2.97	3 (*Triplet*)	6.6	H_a
1.56 ppm	2.01	6 (*Sextet*, but more precisely, "*triplet of quartets*")	7.2	H_b
3.58 ppm	2.00	3 (*Triplet*)	6.6	H_c
4.89 ppm	0.99	1 (*Singlet*)	n/a, since no splitting	H_d

Now let's see whether the information in this table is in agreement with what we know from the theory.

Chemical Shifts

Looking at the chemical structure of n-propanol, the first thing one would realize is that the H_c and H_d protons are the most deshielded, given their proximity to the electronegative oxygen atom. Following that logic, H_as are the least deshielded (or most shielded), and H_bs are the next most shielded.

The NMR chemical shift chart (provided on Canvas) predicts the *ppm* ranges where these different protons show up in the spectrum, and you will notice that the alcohol -O**H** proton can show up anywhere between ~1 ppm and ~5.5 ppm, which does not seem very helpful. So, the take-home message here is that chemical shift alone is not adequate information when you are tasked with deciphering a proton NMR spectrum; you must consider other factors too, such as the integration, splitting patterns, and also the J-values, and evaluate all of them collectively to arrive at an accurate interpretation.

Integration

The integration simply gives you the ratio of protons in different chemical environments. For n-propanol, the NMR above shows you four sets of peaks for the four different chemical environments. Notice that the ratio between the integrations in the spectrum is *2.97* to *2.01* to *2.00* to *0.99*, which translates to the whole number ratio of 3 to 2 to 2 to 1. When you are tasked with assigning peaks to a known chemical structure, this simplest whole-number ratio gives you half of the answer already! The peak that integrates to 2.97 represents the methyl group (H_a), and the two peaks that integrate to 2.00 and 2.01 represent the two -CH_2 groups (H_b and H_c, and the chemical shift values help you figure out which one is which), and the peak integrates to 0.99 corresponds to H_d.

Don't forget, it may look like the integration values give you the exact number of protons in each environment, since our example here (n-propanol) is a very simple molecule. However, in reality, the integrations truly only give you the ratio of protons in different chemical environments.

Splitting

Peak splitting tells you the number of protons in neighboring groups. For example, the methyl group (-CH_3, protons denoted by H_a), is coupled with the protons in the adjacent -CH_2 group (protons denoted by H_b), which splits the peak corresponding to the methyl group (at 0.96 ppm) into a triplet, adhering to the $n+1$ rule.

When the -CH_2 group in the middle (protons denoted by H_b) is considered, the splitting gets a bit complicated. That -CH_2 group can couple with the methyl group (protons denoted by H_a) as well as the other -CH2 group (protons denoted by H_c). But those two groups are in slightly different chemical environments, so the magnitude of coupling is not the same. Ideally, the peak corresponding to the middle -CH_2 group (protons denoted by H_b), will be split by the methyl

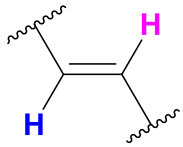
group into a quartet (*n+1* rule), which will then be split into a triplet by the adjacent -CH_2 group. So, the overall splitting can be identified as a *triplet of quartets*. However, the 60MHz NMR that we use does not offer enough resolution to see those fine differences between coupling, so you only see a sextet for that peak at 1.56 ppm.

The -CH_2 group next to the O atom (protons denoted by H_c) couples with the adjacent -CH_2 group, which splits its peak into a triplet. NMR instruments with higher resolution might allow you to observe long-range coupling coming from the -OH proton (H_d), which clearly is not the case with the 60MHz NMR we used. The -OH proton appears as a singlet for the same reason.

While we are on this topic, it is definitely worth mentioning that -OH protons do not always show up as sharp peaks. They can exchange the proton with deuterium in the solvent (if a deuterated solvent is used to prepare the NMR sample), and depending on the extent of this exchange, the -OH proton may show up as a small bump as opposed to a sharp peak, bury in the area of another prominent peak (which can usually be detected by looking at the peak integration), or not show up at all. This is why I personally think that NMR data interpretation has many similarities to detective work 😊

Coupling Constants *(J-values)*

In the previous section, we talked a little bit about how coupling makes the peaks split, and how coupling from protons in slightly different chemical environments can lead to complex splitting patterns such as *triplet of quartets,* etc. When you know the chemical structure of the compound, it is much easier to interpret the NMR data of it, but imagine a situation where you are not sure what exactly is the chemical structure of the compound you synthesized in the lab. When that is the case, knowing the magnitude of coupling by the neighboring protons in the NMR spectrum offers valuable information! You can compare the coupling constants (also known as J-values) that you observe in the NMR spectrum to known J-value ranges in the literature to get a rough idea of the neighboring protons. For example, a trans-alkene bond usually has the two protons coupling with each other with a J value that falls in the range of 11-18 Hz.

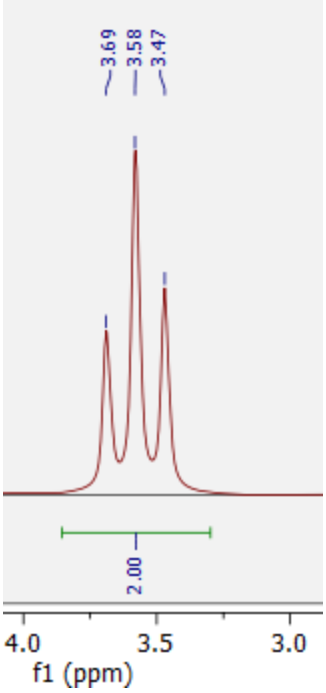


J-value range: 11 - 18 Hz

So, knowing how to calculate the J-value rewards you with fine details of the compound you are analyzing. All you need to do is taking the *immediate* difference of the chemical shift values of the split peak, and multiplying it by the frequency of the NMR.

For example, H_a protons in the methyl group in 1-propanol are coupled with H_b protons, so the methyl group in the NMR spectrum shows up as a triplet. The individual chemical shifts of that triplet are listed in the spectrum as 1.07 ppm, 0.96 ppm, and 0.85 ppm. So, the difference between the two adjacent chemical shift values is 1.07 – 0.96 = 0.11 ppm. To calculate the J value, you simply have to multiply that difference by 60 MHz, which is the frequency of the

NMR we used. What it tells you is that the H_a and H_b protons couple with each other with a magnitude of, 0.11 ppm x 60 MHz = 6.6 Hz. This is generally denoted as $J_{Ha\text{-}Hb}$ = 6.6 Hz. Similarly, the coupling constant between **H_c** and **H_b** in 1-propanol can be calculated as follows:

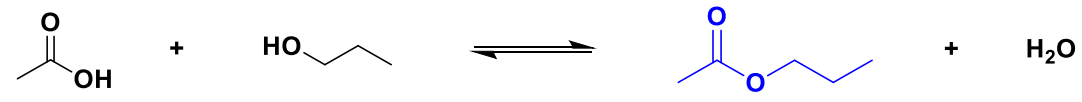


$$J_{Hc\text{-}Hb} = (3.69 - 3.58) \text{ ppm} \times 60 \text{ MHz}$$
$$= 6.6 \text{ Hz}$$

Interpretation of NMR spectra - *Clean vs. Contaminated*

We talked quite a bit about NMR data interpretation in the previous section, and now it is time to take a look at a *real-life* ^{1}H NMR where the unreacted materials are present along with the intended product, and compare it to a ^{1}H NMR of the fine product. The goal of this exercise is to learn how to extract the relevant data accurately when the data set (spectrum, in this case) is complicated.

Let's consider the following simple esterification reaction between n-propanol and acetic acid.



Acetic Acid n-Propanol n-Propyl acetate

We already know how the ^{1}H NMR of n-propanol look, but if you ever wondered how that of acetic acid would look, it is shown in Figure 2.

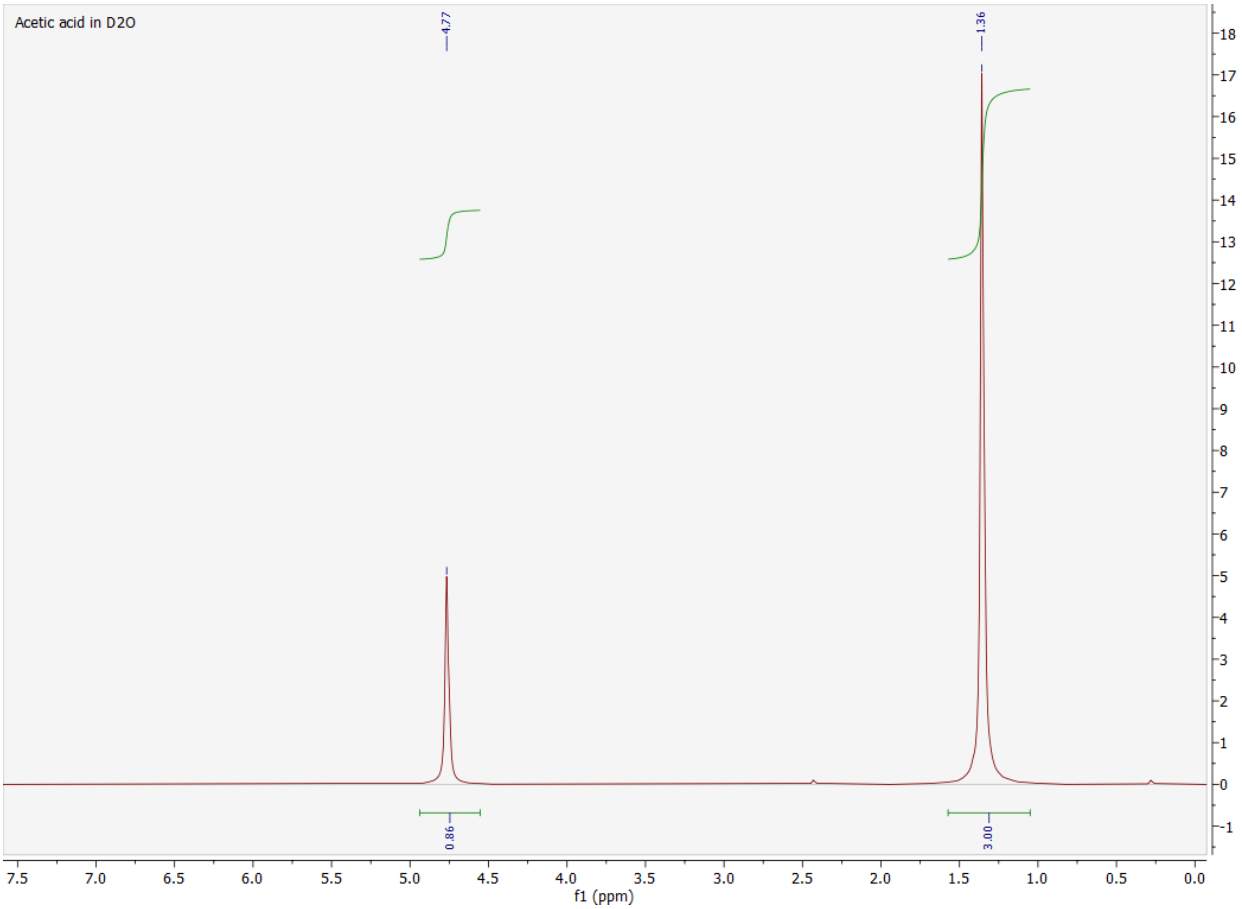
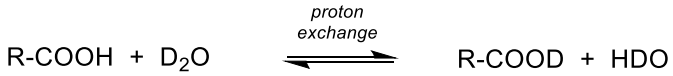


Figure 2: ^{1}H NMR of acetic acid in D_2O

The singlet at around 1.3 ppm with an integration value of 3.00 is obviously the -CH_3 group of acetic acid. But what is the peak at around 4.75 ppm with integration of 0.86? It is <u>not</u> the -COOH proton; it actually is D_2O, the solvent used for preparing this NMR sample. As you know, deuterium in D_2O cannot show up in ^{1}H NMR, and that is the reason why it is used as the solvent for preparing this sample. However, D_2O shows up in this spectrum due to a very unique reason, and that is the ability of the -COOH group in acetic acid to exchange its proton with D_2O to form a very small amount of HDO, and -COOD.



That is also the reason why there is no peak in this NMR spectrum for the -COOH proton of acetic acid. This proton exchange between NMR solvents and *acidic* protons of compounds is a well-known phenomenon, which is sometimes advantageous since you can use the resulting solvent peak as your reference peak.

Now let's look at the ^{1}H- NMR of the product, n-propyl acetate (Figure 3). When the n-propyl acetate sample was prepared for obtaining the NMR, we added a drop of reagent-grade acetone

so that we can use the acetone peak (a singlet that appears usually at around 2.1 ppm) as our reference. In other words, we can set the x-axis value of acetone peak to 2.1 ppm, which moves the rest of the peaks of the spectrum to their accurate x-axis (ppm) positions (values).

Although we used acetone as the reference peak for convenience, that's not the only option; you can use the solvent peak for referencing, if you use a deuterated solvent for the sample preparation and enough proton exchange happens between the deuterated solvent and acidic protons of the sample *(well, for esters, proton exchange with the solvent is not very obvious)*. The other most common option is the use of an extremely small amount of tetramethylsilane (TMS) in the sample, and referencing that peak to 0.0 ppm.

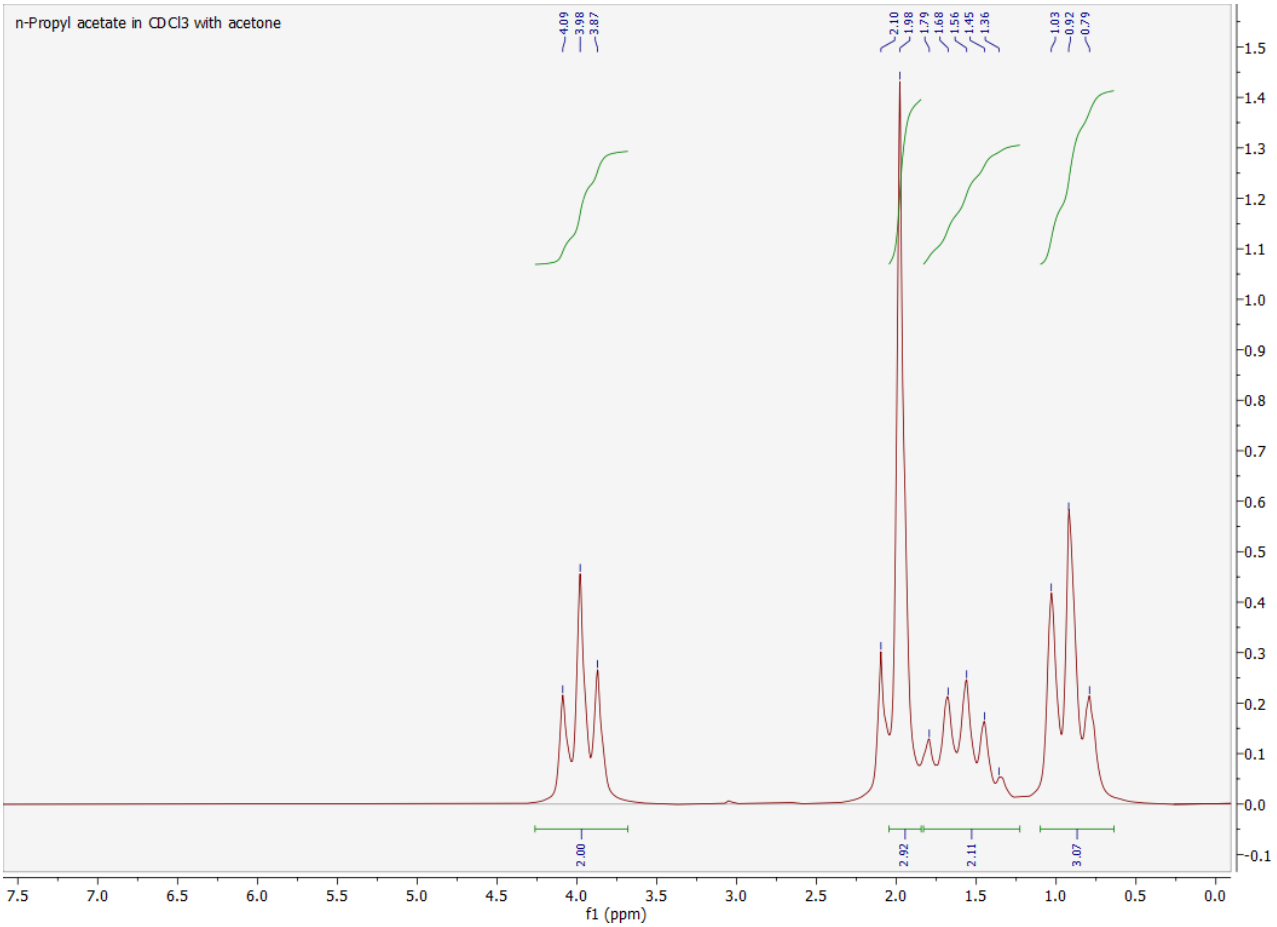
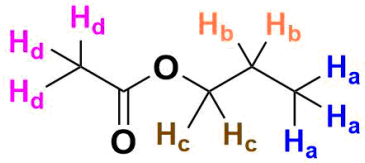


Figure 3: ^{1}H NMR of n-propyl acetate in $CDCl_3$

Now let's use a spectral data analysis table to list the information from the above spectrum and interpret the data. As you can see in Table 2, the chemical shift values, integration ratio, and splitting collectively are in agreement with the chemical structure of n-propyl acetate.

Table-2: Spectral data analysis table for n-propyl acetate

Labeled chemical structure: 

Chemical Shift (ppm)	Integration	Splitting *(n+1 rule)*	Coupling Constant (J-value)	Structural Assignment
0.92 ppm	3.07	3 (Triplet)	6.6	H_a
1.56 ppm	2.11	6 (Sextet) *(partially overlaps with the base of the singlet next to it)*	7.2	H_b
1.98 ppm	2.92	1 (Singlet)	n/a, since no splitting	H_d
3.98 ppm	2.00	3 (Triplet)	6.6	H_c

There is one peak however, a small singlet, right next to the singlet corresponding to H_d, with no integration value. This *mysterious* peak is coming from the drop of acetone that we added to the sample. As expected, it is a singlet, but it may or may not integrate into 3, given the very small proportion of it in the sample, compared to n-propyl acetate. However, it is used as the reference peak in this spectrum, and its ppm value is set to 2.1 ppm.

Well, everything looks nice and pretty when your NMR sample is clean (i.e. only the intended compound is present in very high abundance). What if your NMR sample has unreacted material? Now that is more of a real-life situation we have to deal with! Examine the ^{1}H-NMR of n-propyl acetate below (Figure 4). When there is contamination from unreacted material (or pretty much anything) the spectrum could get really complicated really fast! With careful comparison of *what should be in your spectrum* (since you already know the structure of the compound), and *what is actually in the spectrum*, you will be able to notice the following information that suggests the presence of unreacted n-propanol in the sample.

- The triplet at around 4 ppm, which usually corresponds to the deshielded -CH_2 group, now integrates *almost* to three protons (integration value: 2.62). This is due to the overlap of the singlet coming from the -OH in n-propanol.
- A second triplet at around 3.58 ppm that integrates to 2 protons (integration value: 2.00). This is coming from the deshielded -CH_2 group of n-propanol.
- The sextet at around 1.5 ppm, which usually corresponds to the shielded -CH_2 group, now integrates *close* to four protons (integration value: 3.78). This is due to the overlap of the sextet coming from the shielded -CH_2 group of n-propanol.
- The triplet at around 0.9 ppm, which usually corresponds to the shielded -CH_3 group, now integrates *almost* to six protons (integration value: 5.54) and has split tips. This is due to the overlap of the triplet coming from the shielded -CH_3 group of n-propanol.

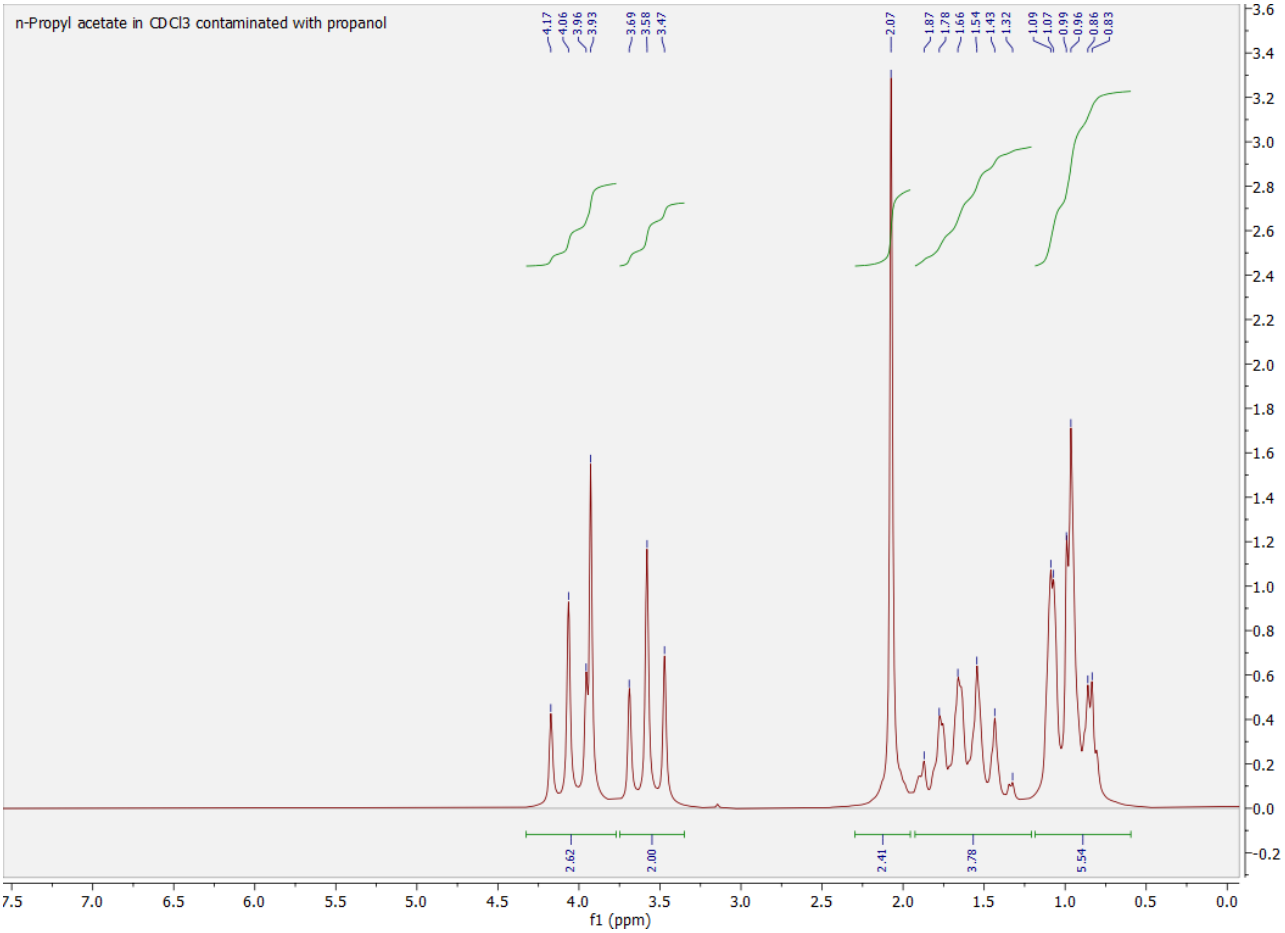


Figure 4: ^{1}H NMR of n-propyl acetate in $CDCl_3$, with unreacted starting materials

The above example walked you through a very common situation any student can run into in the lab, that is, the presence of unreacted materials and/or contaminants in their final product. While the goal of a lab course is to give you adequate opportunities to learn and master the lab techniques and skills, it is equally important to be able to critically analyze the data and figure out what happened when your experiment and spectral data do not turn out the way you expect them to be. Remember, mistakes can happen, but we must learn from them so that we can do a better job next time.

Preparing an NMR sample - *A few things to think about*

We discussed quite a bit about NMR data interpretation in the previous section. Now let's take a brief look at NMR sample preparation.

(A) Use the Correct Quantity of Materials.

In general, for ^{1}H spectra of organic compounds the quantity of material required is only just a few milligrams. Unless you are going to do quantitative calculations using the NMR spectrum

(very unlikely in our lab experiments/applications), there is absolutely no need to weigh the sample; just eyeball it. You definitely can obtain spectra from even an extremely small amount of sample, however, you must take precautions to minimize any contaminations, since the peaks from those contaminants can very easily dominate the spectrum and truncate the peaks of the actual compound of interest to you.

(B) Remove Any and All Solid Particles.

Particulates suspended in the NMR sample warp the magnetic field homogeneity because the magnetic susceptibility of a particle is different from that of the sample solution. This causes broad lines and indistinct spectra that cannot be corrected and makes analysis more difficult, if not impossible *(you can read more about this from https://nmr.chem.umn.edu/samprep.html, https://www.cif.iastate.edu/nmr/nmr-tutorials/sample-preparation).*

So, when feasible, it is a good practice to filter samples into the NMR tube. You can filter your samples through a small plug of glass wool tightly packed into a Pasteur pipette. If the plug is not tight enough, the solid particles can pass through and the filtration will fail; if the plug is too big, some of your sample will remain trapped in it. This is where the lab skills and practice help you out 😊 The goal is to have your sample as clear as possible (i.e. no cloudiness/turbidity).

(C) Prepare Samples to the Desired Volume.

For the NMR that we use in the lab, the optimal range of sample volume is 0.5-0.7mL, which translates to a minimum height of 4 cm and a maximum height of 5 cm. After filtration, prepare your samples so that they physically fall within this range. Samples that are below 4 cm are very difficult to shim and cause considerable delay in recording the spectrum. Samples that are above 5 cm can also be difficult to shim. So, always check your sample depth using a ruler and stay at ~ 4 cm range. When dealing with a liquid sample, if you do not have enough sample to bring the volume to ~ 4 cm height range in the tube, consult your TA; they will help you overcome that challenge (see next section for a brief explanation).

After preparation, make sure that the cap is tightly applied on the tube, to minimize solvent loss through evaporation.

(D) Use Deuterated Solvents.

In general, samples are prepared using solvents that contain deuterium in place of hydrogen. The NMR signal from the deuterium nuclei is called the NMR lock and is used by the spectrometer for stabilization *(you can read more about this from https://nmr.chem.umn.edu/samprep.html, https://www.cif.iastate.edu/nmr/nmr-tutorials/sample-preparation).*

However, a deuterated solvent is not strictly required by the benchtop NMR we use in our lab. So, if your final purified product is a liquid, we can most probably analyze it as is, with maybe a drop of acetone to serve as the reference peak (be careful not to add more than a drop of acetone; the acetone peak will truncate everything else in your spectrum otherwise). If you have a solid final product, or not enough volume in your liquid sample to prepare the NMR sample, we can use a deuterated solvent. If this is the case, please consult your TA for instructions on how to use a deuterated solvent.

(E) Use Clean Tubes and Caps.

Use clean NMR tubes and caps. Prior to preparing your sample, always inspect your NMR tube for visible chips, cracks, or broken tops. If your NMR tube is damaged or dirty, please consult your TA. Tubes must be capped, and caps should be treated the same way as tubes, to prevent/minimize any contaminations.

(F) Label Your Samples.

This is best done with a permanent marker directly on the cap or on the side of the tube. Remember, the label should be readable, and serve as the identifier of the sample you prepared.

FT-IR (Fourier Transform Infrared) Spectroscopy

The atoms of molecules are in constant motion relative to one another – covalent bonds are constantly stretching and bending. Each unique bond stretches and bends with a particular frequency. If the molecule is irradiated with light from the infrared region of the electromagnetic spectrum, the molecule absorbs light at specific frequencies that correspond to the energy required for its bonds to bend and stretch. Different types of atoms, and bonds with different bond orders, bend and stretch at different frequencies.

What does this tell you about the structure of an organic compound? It tells you which ***functional groups*** are present in your compound. For example, because oxygen-carbon single bonds can stretch more easily than oxygen-carbon double bonds, C-O single bonds absorb light at a different frequency than C=O double bonds, and you can use that to figure out whether your compound has a carbonyl functional group or an alcohol functional group, or both. That type of information is very helpful when faced with the task of figuring out a molecular structure.

Since we have been talking about the reaction of n-propanol and acetic acid to form n-propyl acetate, let's take a look at the FT-IR spectrum of n-propyl acetate, first to familiarize ourselves with it, and then learn how to interpret it.

Figure 5 shows what a typical FT-IR spectrum looks like; unlike the NMR, IR spectra do not need extensive processing of the raw data file – you can pretty much directly read the output from the instrument.

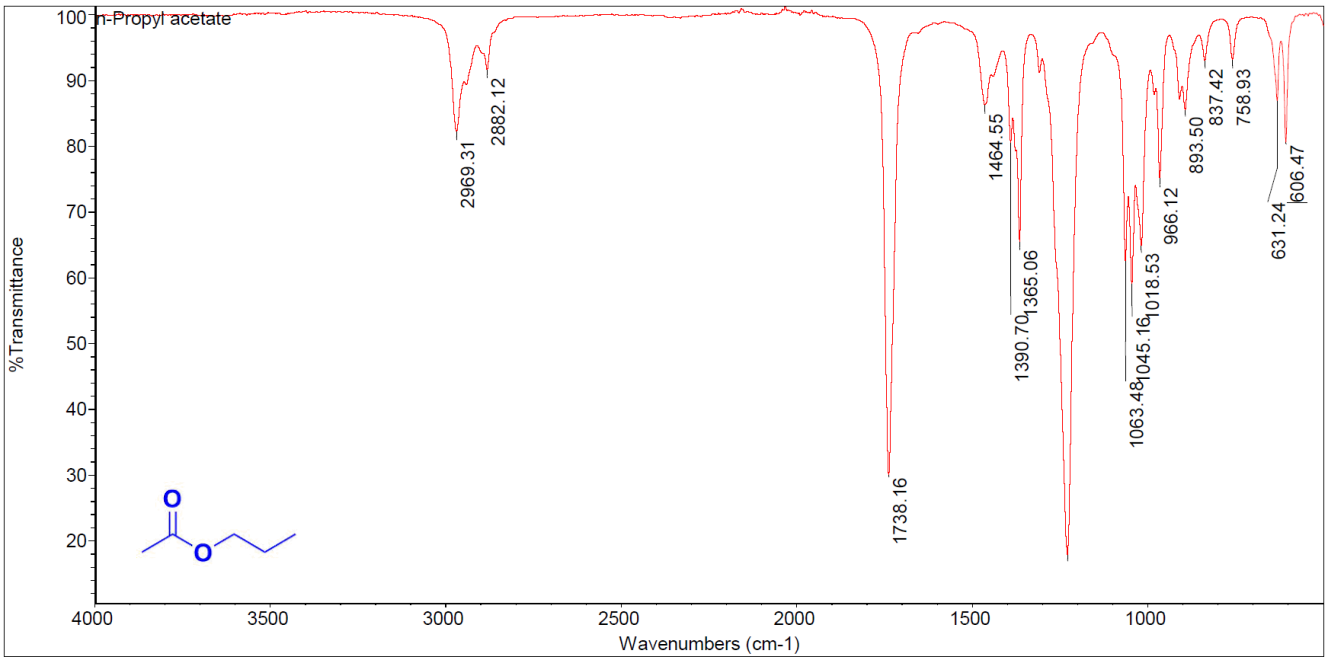


Figure 5: FT-IR spectrum of n-propyl acetate

As shown in Figure 5, the IR spectra we use for our data interpretation purposes have inverted peaks, and % transmittance as the y-axis. The x-axis is defined in cm^{-1} units for wavenumber. Just like the NMR chemical shift chart, the IR absorption chart (provided on Canvas) tells you how the wavenumber of each peak can be related to the chemical structure of the compound.

For example,

- The prominent peak at 1738.16 cm^{-1} can be attributed to the C=O bond of n-propyl acetate, since it falls within the wavenumber range of -C=O stretch of esters in the IR absorption chart (1750-1735 cm^{-1}).
- The other most prominent peak at around 1250 cm^{-1} can be attributed to the C-O single bond of n-propyl acetate, since it falls within the wavenumber range of -C-O stretch of esters in the IR absorption chart (1300-1100 cm^{-1}).
- The slightly broadened/overlapped less-prominent peaks at 2969.31 cm^{-1} can be attributed to the -C-H stretch of *sp3* hybridized carbon-hydrogen bonds in the compound, since it falls within the wavenumber range of *sp3* -C-H stretch in the IR absorption chart (2980-2850 cm^{-1}).

What about the other peaks? Well, this is where the FT-IR data interpretation significantly differs from NMR data interpretation; we do *not* try to assign each and every peak in the IR spectrum to the chemical structure of the compound. Although you can assign each and every peak, what makes more sense is looking for the most prominent and key peaks in the spectrum (like the ones in the bullet points above) to find out whether your sample has the compound that you suspect, which in this case is n-propyl acetate.

To elaborate this approach a little further, you can confirm that the sample you analyzed in FT-IR has the intended product (n-propyl acetate) in high purity if,

- ✓ The carboxylic acid -O-H stretch (broad peak, 3200-2800 cm^{-1}) that belongs to the reactant, acetic acid, is absent in the IR spectrum.
- ✓ The alcohol -O-H stretch (moderately broad peak, 3550-3300 cm^{-1}) that belongs to the reactant, n-propanol, is absent in the IR spectrum.
- ✓ Two prominent peaks, at around 1740 cm^{-1} and 1250 cm^{-1}, which corresponds to ester C=O stretch and -C-O stretch respectively, are present in the IR spectrum.

With that said, remember, carboxylic acids too have one or more -C=O bonds and -C-O bonds that show up in 1740 cm^{-1} and 1250 cm^{-1} ranges, but if the carboxylic acid is present in the sample, the spectrum should also show the characteristic broad peak of the -CO**O-H** stretch in the 3200-2800 cm^{-1} wavelength range. So, whenever applicable, look for all relevant evidence before making a determination.

Assume your final sample has unreacted n-propanol left in it. If that's the case, your IR spectrum will have a broad peak corresponding to the alcohol -O-H stretch, somewhere in the 3550-3300 cm^{-1} range, as shown in Figure 6 below.

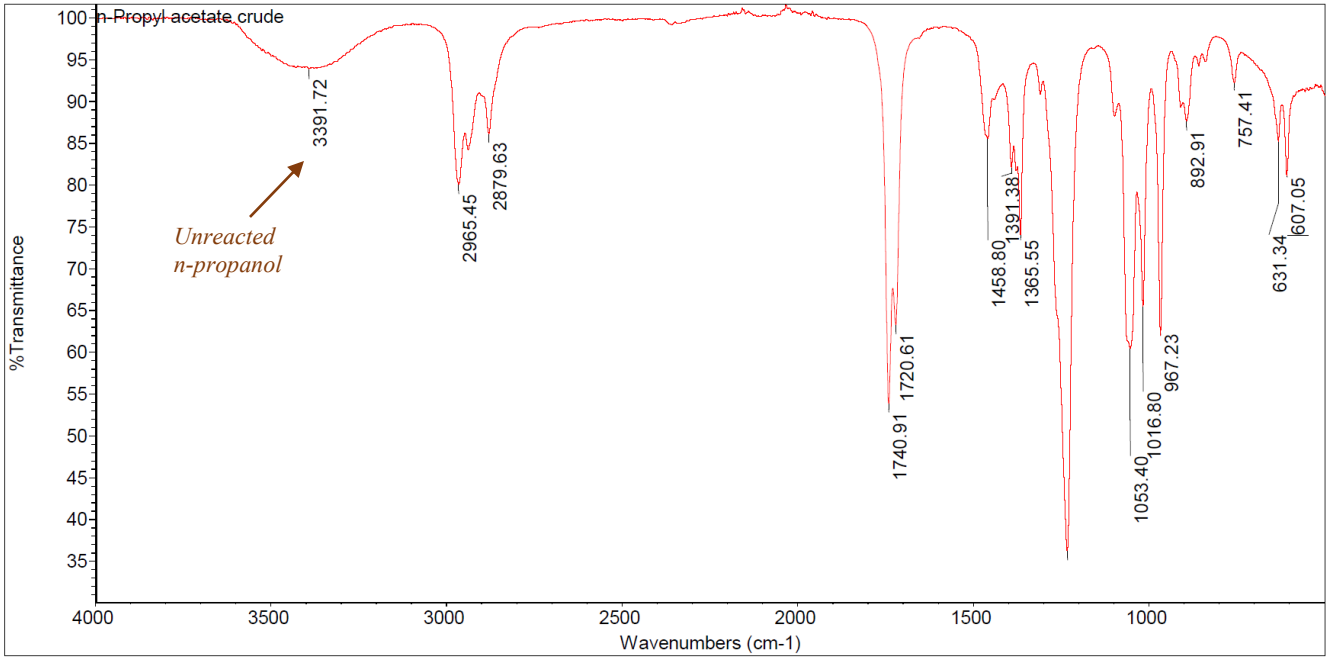


Figure 6: FT-IR spectrum of crude n-propyl acetate

So, in a nutshell, you go about utilizing IR spectral data to determine whether you have the intended final product in high purity, by looking for the presence of the peaks that correspond to the product you expect to have in your sample, and the absence of the peaks that correspond to the starting materials (or other possible contaminants). The FT-IR offers a quick and reliable method to get a *first look* at your reaction success, while more conclusive data can be obtained by NMR.