EXPERIMENT 3: Practical NMR and IR Spectroscopy and Mass Spectroscopy

Relevant parts of the text:

Chapter 12: Infrared Spectroscopy and Mass Spectrometry
Chapter 13: NMR, ^{13}C and ^{1}H

General Concepts

Nuclear Magnetic Resonance (NMR) Spectroscopy is the most widely used and powerful analytical tool for structural elucidation of molecules. It is used extensively by chemists to follow the course of reactions and to properly identify the structure of molecules. It is also commonly used in natural product chemistry to solve the structure of unknown molecules obtained by isolation from natural sources. In addition to its use to elucidate structure, the technique is also utilized to study dynamic processes such as kinetics and chemical equilibrium processes. Outside the realm of chemistry, biochemists make use of NMR spectroscopy to solve the 3-D structure of proteins, peptides, DNA and polysaccharides. The principles of NMR are identical to those of Magnetic Resonance Imaging (MRI), which is a well-known technique used in medicine.

The purpose of this experiment is to learn and apply the principles of NMR spectroscopy. You will be given an unknown organic molecule for which you will obtain the ^{13}C, ^{1}H NMR and IR spectra and be provided with its molecular formula mass spectrum and then you will analyze the data to solve the structure.

As part of the experiment, you will prepare the NMR samples and the spectra will be collected at the NMR facility. You will also have the opportunity to tour of the department's multimillion dollar NMR and MS facilities, information on the facility can be found at:

http://publish.uwo.ca/~chemnmr/

http://www.uwo.ca/chem/resources/MassSpectrometry.htm

Experimental Procedure

Obtain an unknown compound from your TA and record the unknown number. You will be given the mass spectrum. Your compound contains only C, H and O. You will obtain Infrared Spectra and NMR Spectra (^{13}C, ^{1}H) of your unknown. For this experiment, all samples are liquids but the details for both solids and liquids are provided for future reference.

Part A: Proton and Carbon NMR Spectroscopy

(i) Preparing the NMR Sample

- Obtain an NMR tube. These tubes are precision pieces of glassware and cannot be chipped or broken.
- To run a ^{1}H NMR spectrum typically about 10-15 mg of a solid (for liquids 1-2 drops) are dissolved in an NMR solvent so there is 50 mm (in terms of the height) of solvent (approximately 0.6 mL, or 5 cm of liquid) in the NMR tube.
- Check to make sure that your compound is soluble in the NMR solvent before you prepare your tube. Because in this laboratory you want to measure both a ^{1}H and ^{13}C NMR spectra, you will need about 5 times as much material (5-10 drops) with the same amount of solvent (^{13}C NMR spectra are less sensitive and take more sample to measure in a reasonable amount of time – why?). There should not be any floating particles in the NMR sample solution!
- ***In this experiment you are going to run both a ^{1}H and ^{13}C NMR spectrum on the same sample. This will take ca. 5 drops of your unknown. Then fill your NMR tube up to 5 cm using $CDCl_3$.***

There are many NMR solvents to choose from, but the key considerations are solubility (your compound must be soluble) and then cost of the solvent. The NMR solvent must be perdeuterated, so that the protons of the solvent do not overwhelm the spectrum. Deuterated solvents are expensive. A typical NMR solvent that has good solubility properties and has a relatively low cost is $CDCl_3$ –deuterated chloroform. This solvent is still expensive so do not waste it. Also, it is important not to contaminate the solvent bottle. Any impurities will appear in the NMR spectra and complicate your analysis.

- The NMR tube must be clearly labeled on the side of the tube using **only** the labels provided and a pen. The label should include your initials, class number, and unknown number. For example Superb O. Chemist is in Chemistry 2273 and is running her unknown # 12 so she would label her tube as: *SOC227312*. This is important so that you can identify and process the data from your unknown.

(ii) Running NMR experiments:

Routine proton and carbon spectra samples are run by an automated sample changer attached to the Varian Mercury 400 MHz spectrometer. The robot places all of the samples into the magnet and the spectra are run automatically. We have already toured the NMR facility, so you should be familiar with the instrument configuration.

You will process your own NMR data using the instructions below.

(iii) Retrieving NMR data:

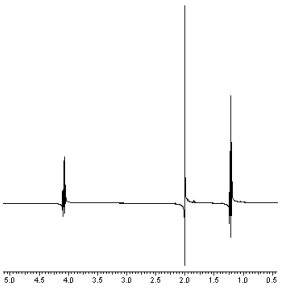
The NMR data will be transferred from the Mercury 400 computer to a PC in the lab using an FTP program. Please do not change directories - the software is setup for a specific location on the disk so that bookkeeping is manageable.

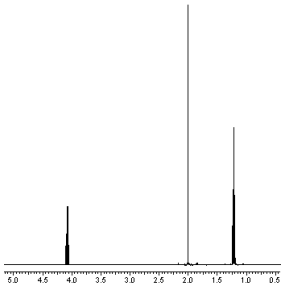
A) Click on the **Start** button.
B) Click **NMR FTP** (upper left of start panel) to load the software
C) Press **OK**. This connects your PC to the NMR computer.
D) Find your file name in the list in the right window and click on it.
E) Press the ←button to transfer the data to the PC.
F) Press **Yes** to transfer the folder and its contents.
G) After you have all of the files you need, press the **Exit** button to quit FTP.

(iv) Processing NMR data:

The data transferred from the spectrometer computer to the PC are in a raw unprocessed form (fid). In the end, you will want to have a spectrum with chemical shifts, integrals (if ^{1}H), peak lists, and possibly expansions of regions. This can be accomplished using the instructions below. You will want to print off expansions of key parts of your spectra to make your spectra easier to see and solve.

A) Click on the **Start** button.
B) Click **NMR Processor** (upper left of start panel) to load the software.
C) Click File → Import (not open, open is for already processed NMR data) → From a 1D NMR directory.
D) Find your file name and double click on it. In this directory you will find a directory called **PROTON.fid** (and/or **CARBON.fid** if a carbon was run). Double click on this folder. Double click on **fid** in the left window to load the raw data.
E) **FOUREIR TRANSFORM:**
   - Press the **Fourier Tr.** button to Fourier transform the data from the time domain to the frequency domain.
F) **PHASE CORRECTION:**
   a. If phasing is good, move to part G. If phasing is bad, continue to next point.
   b. Press the **Phase Corr.** (correction) button to phase the spectrum. Press the **Auto Simple** button. This should fix the phase distortions in 99% of your spectra. Once you have finished phasing the spectrum press the green checkmark on the far left of the toolbar to save the changes to the phase constants.


BAD PHASING

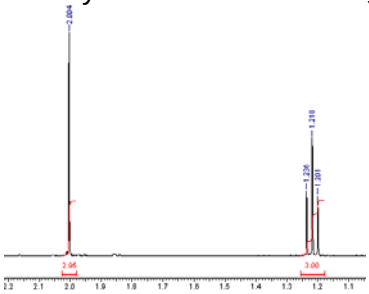

GOOD PHASING

G) **BASELINE CORRECTION:**
   - Press the **BLine Corr.** (baseline correction) button to improve the baseline of the spectrum. Press the **Auto** button followed by the **Result** button. Once you have finished the baseline correction, press the green checkmark to save.
H) **REFERENCE:**
   - Press the **Reference** button. Use the cursor to select the signal closest to 0 ppm. This is TMS (tetramethylsilane), which is a reference compound included in the chloroform-d. Select TMS from the list of solvents that appears and click OK. This should put the signal at exactly 0.00 ppm. You should also have a singlet at 7.25 ppm (^{1}H NMR Spectrum) or a triplet at 77.00 (^{13}C NMR Spectrum), which is your chloroform-d signal.
I) **PEAK PICKING:**

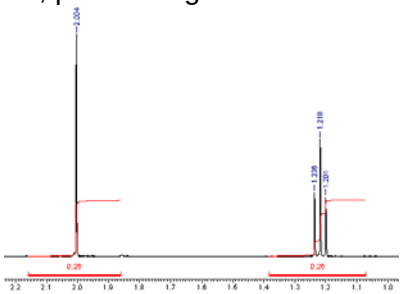
- To perform peak picking (to get chemical shifts and J-couplings) press the **Peak Picking** button. Press the **Peak Level** button. Now place your cursor on the spectrum to the height of the smallest peak you want to select (pick) and press the mouse button. All peaks of this height and higher will be picked. If you pick too high, just move the cursor down a bit and press the mouse again. If you have picked too low and have selected too many peaks, press the **Clear All** button and start over. Once you have finished peak picking, press the green checkmark to save.

J) **INTEGRATION (^{1}H NMR SPECTRA ONLY):**

- Integration is done to determine the relative area of each signal. Integration is best done as follows:
- First **zoom in** on the peak you wish to integrate. To do this, move the cursor to the left hand side of the peak (or peaks) you wish to zoom in on. Right click the mouse – a magnifying glass will replace your cursor. Then left click, hold, and drag to the right hand side of the peak(s) and release the button. A magnified view of that peak(s) will now appear on the screen.
- Press the **Integration** button. Press the **Manual** button. Move the cursor to the left hand side of the peak you want to integrate. Click and hold the mouse button. Move the cursor to the other side (right) of the peak and release the button. This will integrate over the area that you held the mouse.
- To **zoom out** back to the full spectrum (in order to integrate other signals), click on the picture of a magnifying glass with a minus sign inside it (toolbar menu). Zoom in and integrate all the remaining signals.
- Integration values should be expressed as whole numbers, your TA will show you how to do this.
- Once you have finished integration, press the green checkmark to save.



GOOD INTEGRATION



BAD INTEGRATION

K) **PRINT (SAVE AS .PDF):**

- To print (save as .pdf and print at home) the spectrum, press the icon that looks like a printer. There are a number of options that you can change depending on what you want for output (including peak lists, integration tables, titles, etc.). Under the “TEXT” tab, input your name in the “USER INFORMATION” box. Press the **OK** button. This will call up the “SAVE AS” window, save your spectrum to your USB key.
- In addition to printing your whole spectrum, you should also print expansions. Zoom in on the desired areas (as described above) and repeat the printing/saving procedure.

L) When you have finished processing and saving your data, double click on the **X** in the upper right hand side of the software to close the program and press **NO** to saving the data.

(v) Solving the Structure:

Once you have obtained your spectra you can begin to solve the structure of your unknown. Use the molecular formula to determine the sites of unsaturation of your molecule. This may aid in finalizing a structure.

When writing up your experiment you will need to present your data in such a way as to convince the reader how you have solved the structure from the spectra. Assign the IR spectrum, being sure to indicate the key absorptions for functional group analysis.
For the NMR spectra you will need to report *Chemical Shifts* and the structural information obtained from it. For the proton spectra, in addition to reporting chemical shifts, you need to report and discuss *integration* and *splitting (coupling).* This might be best accomplished by setting up a chart/table. No formal introduction, experimental is required. Your report should be a clear and concise analysis of your spectra and a proposed structure of your unknown.

The chemical shifts of solvent signals observed for ^{1}H NMR and ^{13}C NMR spectra are listed in the following table. The multiplicity is shown in parentheses as 1 for singlet, 2 for doublet, 3 for triplet, etc.

Table 1: NMR Solvent Signals

Solvent	**^{1}H NMR Chemical Shift**	**^{13}C NMR Chemical Shift**
Acetic Acid	11.65 (1) , 2.04 (5)	179.0 (1) , 20.0 (7)
Acetone	2.05 (5)	206.7 (13) , 29.9 (7)
Acetonitrile	1.94 (5)	118.7 (1) , 1.39 (7)
Benzene	7.16 (1)	128.4 (3)
Chloroform	**7.26 (1)**	**77.2 (3)**
Dimethyl Sulfoxide	2.50 (5)	39.5 (7)
Methanol	4.87 (1) , 3.31 (5)	49.1 (7)
Methylene Chloride	5.32 (3)	54.00 (5)
Pyridine	8.74 (1) , 7.58 (1) , 7.22 (1)	150.3 (1) , 135.9 (3) , 123.9 (5)
Water (D_2O)	4.8	

Listed below are the chemical shift positions of the water signal in several common solvents. Note that water is seen in aprotic solvents, while HOD is seen in protic solvents due to exchange with the solvent deuteriums.

Table 2: NMR Water Signals

Solvent	**Chemical Shift of H_2O (or HOD)**
Acetone	2.8
Acetonitrile	2.1
Benzene	0.4
Chloroform	**1.6**
Dimethyl Sulfoxide	3.3

Part B: Infrared Spectra

During the course of the experiment you need to obtain an IR spectrum of your unknown. Since all unknowns in this experiment are liquids you will obtain a spectrum of a thin film. Your TA will remind you how to properly obtain an IR spectrum.

(i) Sample Preparation for Infrared Spectroscopy

- **Liquids:** Place a drop (or less) of the neat liquid directly onto an IR salt plate to form a thin continuous film. Measure the spectrum, ensuring that you wipe off any excess compound (with a Kimwipe) if the absorptions are too intense.
- **Solids:** Solid samples are often difficult to analyze, particularly if they cannot be reduced to a fine powder or are not soluble in common infrared solvents. The solid sample (powder form) must be distributed evenly in the spectrometer in order to minimize light scattering effects and to eliminate distorted band shapes.
- **Cast Films:** A cast film is used if a solid material cannot be easily reduced to a fine powder but can be dissolved in a volatile solvent, such as acetone or chloroform. The sample is dissolved in a minimum amount of solvent and the solution is spread over a window where the solvent is evaporated, leaving only the solid sample.

- **Mulls:** An oil mull is prepared by grinding the solid to a very fine particle size and suspended in Nujol (spectra between 1330 cm^{-1} to lower frequencies) or Halocarbon oil (4000 cm^{-1} to about 1330 cm^{-1}). The solution is spread between two infrared windows, forming a thin continuous film that produces a spectrum virtually free of interfering bands of the mulling agents.

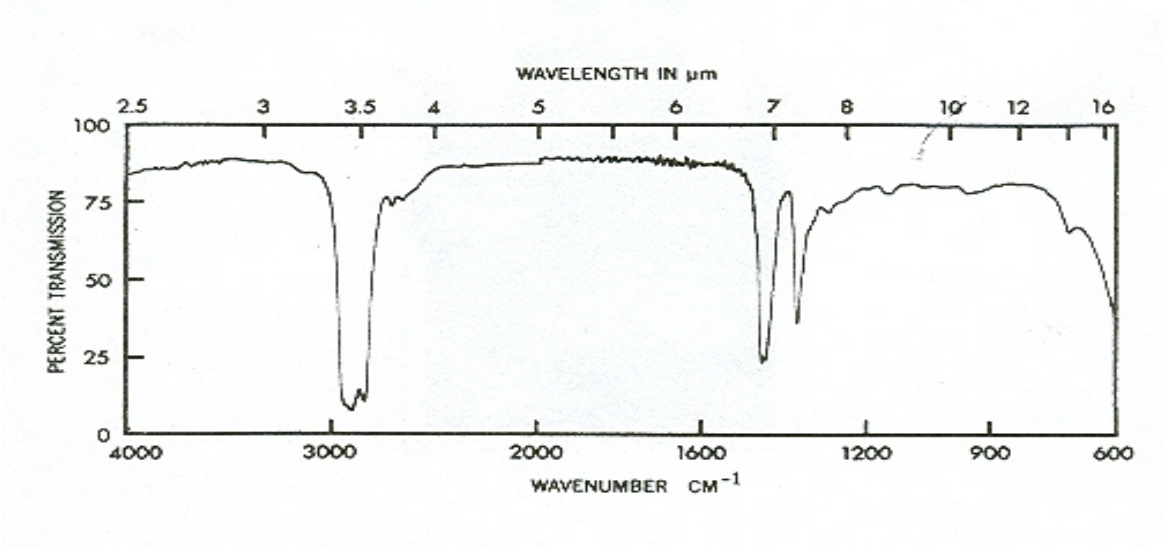


Figure 1: Spectrum of Nujol

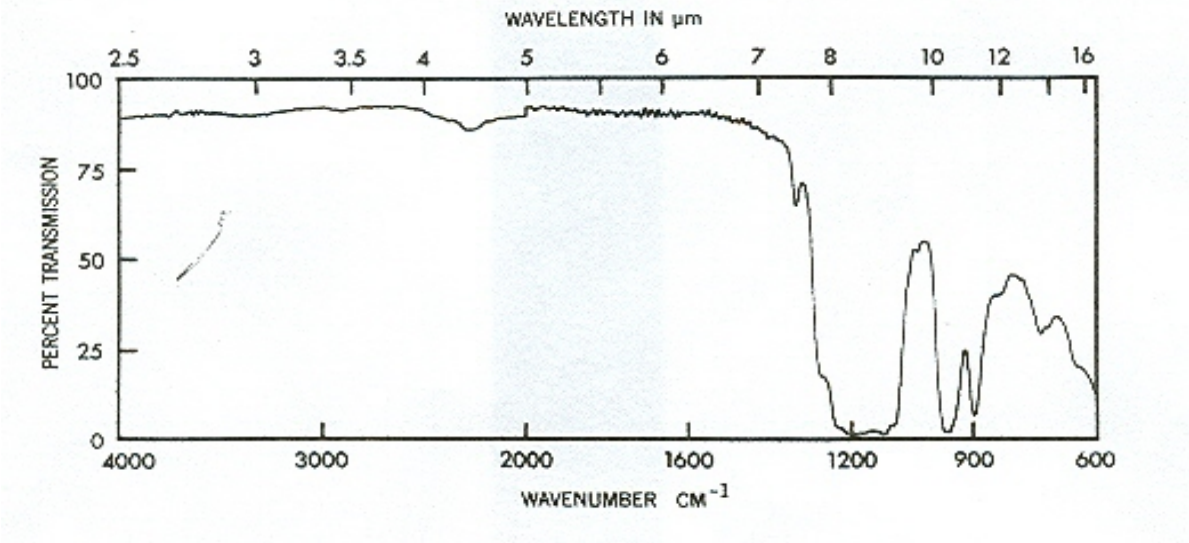


Figure 2: Spectrum of Halocarbon

(ii) Operating Instruction for FTIR Spectrometers

- If the software is not already loaded click on the ‘Start” key and select the appropriate icon to activate the spectrometer’s software.
- Click on [Edit] in the top toolbar and [Clear] to remove any existing spectra.
- It is always good practice to measure a background spectrum prior to running the spectrum of your sample.
- To measure a spectrum, place your sample into the instrument by sliding the top door back and click on the [Col Smp] button on the toolbar.

***Note*:** Wait several minutes before recording your spectrum to allow the atmosphere in the spectrometer to equilibrate.

- Enter your sample name and click [OK]. Although a spectrum will appear immediately on the screen, data collection continues for about 30 seconds. Before processing your spectrum ensure the instrument has stopped collecting. This is indicated at the bottom of the screen, by following the progress of data acquisition.
- If the instrument asks for a background, remove your sample, close the door and wait several minutes for the sample compartment to equilibrate and then click [OK] twice. Reinsert your sample and click [OK] to record your spectrum.

The absorptions of a good quality spectrum fills the spectral window and uses the full scale of % transmittance. If the signal is too weak or strong, simply add or remove sample and re-measure by following the directions above.

- When data collection is complete, click [YES] to place the spectrum in a window for data manipulation and printing.
- Peaks may be labeled individually by using the [T] annotation button in the lower left toolbar or you can use the [Find Peaks] icon on the main toolbar and use the cursor to set a peak threshold below which all peaks will be labeled. *After setting a peak threshold <u>be sure</u> to click on the [Replace] button just above the spectral window.*

(iii) Printing

- Use the [Print] icon on the main toolbar to get a paper copy of your spectra.

Note: ***Attendance at Week 2 of the laboratory is REQUIRED, even if you have obtained all your spectra. The time will be used to start to solve your spectra. The TAs will be available to help you work through the data. They will not know the identity of your unknown.***

Part C:

Make a photocopy (or print out a duplicate) of all your spectra before writing on them. If you have an even unknown number, swap your copy with a copy with someone who has an odd unknown number and vice versa.

NOTE – ONLY SWAP THE SPECTRA (NOT THE SOLUTION), YOU ARE RESPONSIBLE FOR SOLVING THE STRUCTURE FOR TWO COMPOUNDS

YOU WILL NEED TO HAND IN THE SOLUTION TO BOTH UNKNOWNS.