NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

INTRO: UNDERSTANDING THE THEORY

NOTE: Turn on the PicoSpin 45 immediately and leave it on for the duration of this experiment.

GOALS

This lab is often seen as a continuation of the pulse nuclear magnetic resonance lab. As before, this lab will look primarily at liquid phase proton nuclear magnetic resonance of various molecules. This time around, we will be dealing primarily with organic molecules. While the PNMR allows the user to study magnetic resonance of independent nuclear spins, the NMR Spectroscopy lab uses an apparatus that examines interacting nuclear spins, which we can only observe with a piece of equipment that has a much greater resolution. That is, the PNMR allows us to observe resonance over a range of thousands of hertz, high hundreds if the experiment is done with extreme care and precision. This experiment has a resolution of several hertz, allowing the observer to see differences within a molecule. The three primary objectives for this lab are as follows:

- Proficiency with the equipment
    - PicoSpin 45
    - MNOVA program
- New skills to apply from Lab
    - Understanding the theory and concept of scanning tunneling microscopes.
    - Performing scans at different magnifications.
    - Identifying underlying atomic structure.
- Experimental design
    - Understand how the equipment and theory work.
    - Design your own experiment!

BACKGROUND

In 1946, the phenomenon of nuclear magnetic resonance in matter was observed by two different, independent groups: Felix Bloch of Stanford and Edward Purcell of Harvard. While both groups were working with different equipment and employing different techniques, the effect they observed was identical. When magnetic nuclei in matter were subjected to a magnetic field, the nuclei responded to a continuous radio frequency magnetic field as the field was tuned through resonance.

When placed in an external magnetic field, the protons in a liquid phase sample will align with the direction of magnetization. By applying a radio frequency, the frequency is dependent on the magnitude of the magnetic field, the protons will resonate. Using a high resolution spectrometer such as the PicoSpin 45, the intramolecular magnetic field associated with the molecule will shift the resonance frequency. This shift is often unique to the

type of molecule, and therefore can be used to identify the type of molecule, as well as glean details about the molecular structure, such as j-coupled groups for example.

THEORY (DENSITY OPERATOR)

To start this lab, consider a sample. The spin states of the system will start is some equilibrium state. (It may be helpful to review the Theory Section of the Pulse Nuclear Magnetic Resonance lab, specifically the section on magnetization). Since there is a superposition of multiple states, we can construct a density operator and consider our system in terms of quantum mechanics.

Start by considering a pure state denoted by the ket $|\psi\rangle$. Thinking back to quantum mechanics, the expectation value of some Hermitian operator $\boldsymbol{Q}$ is define as $\langle \boldsymbol{Q} \rangle = \langle \psi | \boldsymbol{Q} | \psi \rangle$. We can also represent this state as a projection operator $\boldsymbol{P} = |\psi\rangle\langle\psi|$. Using this, we can rewrite our expectation value as

$$\langle \boldsymbol{Q} \rangle = \mathrm{tr}(\boldsymbol{QP}) \qquad \textbf{(1)}$$

In which tr means trace. This allows us to construct our $\boldsymbol{QP}$ operator as a matrix that contains an orthonormal basis $|i\rangle$ and then sum the diagonal entries. This is equivalent to computing the expectation value:

$$\langle \boldsymbol{O} \rangle = tr(\boldsymbol{OP}) = \sum_i \langle i|\boldsymbol{O}|\psi\rangle\langle\psi|i\rangle = \sum_i \langle\psi|i\rangle\langle i|\boldsymbol{O}|\psi\rangle = \langle\psi|\boldsymbol{O}|\psi\rangle \qquad \textbf{(2)}$$

So. We have a hypothetical result with a superposition of many different states $|\psi_i\rangle$ and each state appears with some probability p_j. We define a density operator as the average projection operator:

$$\boldsymbol{\rho} \equiv \sum_j p_j |\psi_j\rangle\langle\psi_j| \qquad \textbf{(3)}$$

Now, if we compute the trace of the operator product $\boldsymbol{O\rho}$, we get the average expectation values from the entire superposition which is

$$\langle \boldsymbol{Q} \rangle = \mathrm{tr}(\boldsymbol{\rho P}) = \sum_j p_j \langle \psi_j | \boldsymbol{O} | \psi_j \rangle \qquad \textbf{(4)}$$

So the density operator is a way to express the quantum aspects of an expectation value that includes coherence and interference effects, as well as the averaging associated with any statistical superposition.

For an NMR, we next need to know how this density operator evolves with time. For a time-independent Hamiltonian, the associated Schrodinger equation is

$$|\psi(t)\rangle = e^{-iHt/\hbar}|\psi(0)\rangle \qquad \textbf{(5)}$$

Now, substituting **(5)** in **(3)** gives us the result

$$\boldsymbol{\rho}(t) = e^{-iHt/\hbar}\boldsymbol{\rho}(0)e^{iHt/\hbar} \qquad \textbf{(6)}$$

This result is similar to the motion of an operator in Heisenberg representation, though it isn't. This is till the Schrodinger representation in which states evolve in time and operators are time independent, other than the density operator

THEORY (EQUILIBRIUM STATE)

When considering an NMR experiment, the spin system will start in a thermal equilibrium state. Suppose the system Hamiltonian is $\boldsymbol{H}$ and has exact energies and eigenstates E_j and $|j\rangle$ respectively. The Boltzmann probability p_j to find the system in these states in thermal equilibrium will be proportional to

$$\rho_j \propto e^{-E_j/kT} \qquad \textbf{(7)}$$

using **(3)**, the equilibrium density operator is

$$\rho_{eq} \propto \sum_j e^{-E_j/kT} |j\rangle\langle j| = \propto \sum_j e^{-\boldsymbol{H}/kT} |j\rangle\langle j| = e^{-\boldsymbol{H}/kT} \qquad \textbf{(8)}$$

THEORY (SPIN HAMILTONIAN)

Placing a sample in the PicoSpin 45, there is a strong, very uniform magnetic field, B_o, which, by convention, is in the +z-direction. Consider only NMR of spin ½ protons, the nucleus of the hydrogen atom. The Hamiltonian is a sum of two parts: One describing the Zeeman interaction of the spins with the applied field, and the other describing interactions between the spins themselves. The Zeeman term for N spins can be written as

$$\boldsymbol{H_Z} = \sum_i^N (-\omega_0 + \omega_i) \boldsymbol{S}_i^Z \qquad \textbf{(9)}$$

This is just the energy of N spin ½ magnetic moments in an applied magnetic field. The subscript i on the spin operators $\boldsymbol{S}_i^Z$ describe the spin in which they act upon. It is important to note that i starts with 1 and not 0 for this equation. The spin Larmor precession frequency ω_0 is

$$\omega_0 = \gamma B_o \qquad \textbf{(10)}$$

you should remember this equation from the pulsed nuclear magnetic resonance lab (and from your quantum mechanics class) in which γ is the Gyromagnetic ratio for protons, in this case $\gamma = 2\pi \bullet 42.48\text{MHz/T}$. For this experiment, the magnetic field will be close to 1.06 T, which gives a Larmor frequency of $\omega_0/2\pi = 45\text{MHz}$. The Hamiltonian is expressed in angular frequency units. To convert to Joules, multiply it by ħ. In order to convert to Hertz, simply divide by 2π.

We include in the Zeeman Hamiltonian a small frequency offset ω_i which can be different for each spin. This is called the “chemical shift”. And it is due to diamagnetic screening of the applied field by the electrons surrounding the nucleus. Its value is usually expressed in parts-per-million (ppm) of the Larmor frequency. For protons in non-magnetic organic molecules, chemical shifts are usually in the range of 0-12 ppm. The resolution of the PicoSpin spectrometer is on the order of 50 parts-per-billion, or around 1 Hz, so this effect can easily be seen.

The equilibrium state can be determined to sufficient accuracy by considering only the largest term in the Hamiltonian, which is the first term in **(9)**. At room temperature, we have $\hbar\omega_0/kT \approx 10^{-5}$ so we can approximate the exponential in the equilibrium density matrix from **(8)** by the first two terms of its Taylor series.

$$\rho_{eq} \propto e^{-\boldsymbol{H}/kT} \approx \| - (\mathbf{H}/kT) \approx \| + \frac{\hbar\omega 0}{kT} \sum_i^N \boldsymbol{S}_i^Z \qquad \textbf{(11)}$$

in this case, the ‖ is the unit operator. The unit operator will not contribute to the expectation value for the observables, we are interested in, and we do not care about overall normalization, so for our purposes, we can reduce **(11)** to simply be

$$\boldsymbol{\rho}_{eq}=\sum_i^N \boldsymbol{S}_i^Z=\mathbf{M}^{\mathbf{Z}} \qquad \textbf{(12)}$$

in which, as you should recall from PNMR, $\mathbf{M}^{\mathbf{Z}}$ is the z-component of the magnetization operator, which is the net sum of the single-spin operators $\boldsymbol{S}_i^Z$.

The full Hamiltonian for liquid-phase NMR is best written in a frame of reference that rotates around the z-axis at the Larmor frequency ω_0. The complete spin Hamiltonian is

$$\boldsymbol{H}=\sum_i^N \omega_i \boldsymbol{S}_i^Z+\sum_{i>k}\boldsymbol{J}ik\mathbf{S_i}\bullet\mathbf{S_k} \qquad \textbf{(13)}$$

In the rotating frame, the term proportional to the Larmor frequency ω_0 does not appear. This is due to the frame itself already is rotating at the Larmor frequency. The chemical shifts ω_i, however, do appear and they cause slow precession of spins at frequencies up to a few hundred Hertz relative to the rotating frame. The interaction term is a double sum over all spins of the dot product of the spin operators for each spin pair. It is restricted to i>k so that the pairs are not double counted. This is called the scalar interaction, J-coupling, or indirect hyperfine interaction, and is due to slight polarization of the electrons by one spin, which then creates a small magnetic field at another nearby spin. The coupling constants $\boldsymbol{J}_{ik}$ can have either sign and their magnitude is usually in the range of $\boldsymbol{J}_{ik}/2\pi\approx$0-20 Hz. The $\boldsymbol{J}_{ik}$ terms do not depend on the strength of the applied field, while the frequencies ω_i are proportional to the field strength. Chemical shifts are expressed in ppm so their values will not depend on the field strength. Rather, they are represented as a proportion of the Larmor frequency.

The two terms in the spin Hamiltonian represent powerful analytical information about the spin system. Briefly, the chemical shift allows one to identify the chemical groups present in the molecule, while the J-coupling reveals the connectivity of the molecule. Together, these two effects make liquid-phase NMR the most important analytical tool in organic chemistry.

THEORY (SPIN SIMULATIONS)

At the beginning of a pulsed NMR experiment, we disturb the equilibrium state $\boldsymbol{M}^Z$ by applying a π/2 radio frequency magnetic field pulse. If the pulse duration and frequency are correct, the magnetization will tip into the xy-plane, and we can suppose that it is initially equal to $\boldsymbol{M}^X$ in the rotating frame. The density matrix will then evolve according to

$$\boldsymbol{\rho}(t)=e^{-i\boldsymbol{H}t}\boldsymbol{M}^x e^{i\boldsymbol{H}t} \qquad \textbf{(14)}$$

in which $\boldsymbol{H}$ is given by **(13)**. A coil with its axis in the xy-plane detects the precessing transverse magnetization by Faraday induction. The signal is amplified, digitized, and then frequency-translated by mixers and low-pass filters. The net result is that the instrument records the free-induction-decay (FID), a complex time-series who's real and imaginary parts are proportional to the x- and y-components of the magnetization in the rotating frame. Our observable is thus $\boldsymbol{M}^x+i\boldsymbol{M}^y$, and the FID $F(t)$ is computed from the density matrix using

$$F(t) = tr((\boldsymbol{M}^x + i\boldsymbol{M}^y)\boldsymbol{\rho}(t)) \qquad \textbf{(15)}$$

The FID can be examined directly, but it is more informative to look at the NMR spectrum, which is the Fourier-transform of the FID.

Equations **(13), (14),** and **(15)** express the theory for liquid-phase NMR spectroscopy of the ½ nuclei. However, these only have included unitary evolution **(14)**, so the oscillations in the FID will not decay away over time as they will in experiments. This can be corrected by including and averaging over "environmental" degrees-of-freedom, such as molecular motions and other spins. Instead of doing this, we can simulate the effects of relaxation simply by multiplying the predicted FID by a decaying exponential function. With this adjustment, our theory gives a very good account of most proton NMR spectra.

Many features of NMR spectra can be understood through analytical calculations, but because this is a laboratory course and an emphasis is placed upon experiments (hence the name) we will rely on a numerical analysis of equations **(13), (14),** and **(15)**. The course provides the MATLAB program liquid_NMR.m, a simple NMR spectrum simulator. The code is brief and heavily commented. This is found on the course website in the NMR Spectroscopy page. You should be able to understand how this works. If you are more familiar with Mathematica, a Mathematica version is also available, though MATLAB is a better choice for problems like this that do not involve symbolic manipulation.

DEFINITIONS

d

Figure 8

WEEK 1: THEORY EXERCISE AND SETTING UP THE NMR SPECTROMETER

First, some theory exercises on your part is essential to quality understanding of NMR and exactly what is happening. It is strongly recommended that you begin this section as part of the prelab, as we will not be turning on the NMR Spectrometer until a later section.

Question 1	Derive equation **(4)** from **(3)**
Question 2	Justify every step needed to get from the left side of equation **(8)** to the right side
Question 3	To construct matrix representation of spin operators such as $\boldsymbol{S}_i^x$ for a multiple spin system, we use matrix direct products, also known as Kronecker products). This is best explained by example Suppose we have a two-spin system and we want to represent $\boldsymbol{S}_2^x$, which acts on the second spin. This is given by the matrix direct product $\mathrm{II} \otimes \boldsymbol{S}^x$ In which II is the 2x2unit operator and the spin matrices are

$S^z = 1/2\begin{pmatrix}1 & 0\\ 0 & -1\end{pmatrix}$, $S^x = 1/2\begin{pmatrix}0 & 1\\ 1 & 0\end{pmatrix}$, $S^y = 1/2\begin{pmatrix}0 & -i\\ i & 0\end{pmatrix}$

To form the direct product, write the first matrix and then place the second matrix at each element of the first matrix, multiplied by the first matrix element:

$$\text{II}\otimes S^x = \begin{pmatrix}1 & 0\\ 0 & 1\end{pmatrix}\otimes\begin{pmatrix}0 & 1/2\\ 1/2 & 0\end{pmatrix} = \begin{pmatrix}1\begin{pmatrix}0 & 1/2\\ 1/2 & 0\end{pmatrix} & 0\begin{pmatrix}0 & 1/2\\ 1/2 & 0\end{pmatrix}\\ 0\begin{pmatrix}0 & 1/2\\ 1/2 & 0\end{pmatrix} & 1\begin{pmatrix}0 & 1/2\\ 1/2 & 0\end{pmatrix}\end{pmatrix} =$$

$$\begin{pmatrix}0 & 1/2 & 0 & 0\\ 1/2 & 0 & 0 & 0\\ 0 & 0 & 0 & 1/2\\ 0 & 0 & 1/2 & 0\end{pmatrix}$$

1. Write the matrix $S^y\otimes$II. To check your result, read the comments in liquid_NMR.m and set it up for a two-spin system (with any values of the chemical shifts and J-coupling). Then run it. (Set your current directory to the folder containing the liquid_NMR.m file and type 'liquid_NMR' at the command prompt.) Now use the MATLAB workspace browser to examine Sy{1}, which is the program's notation for the matrix $S^y\otimes$II that represents the operator S_1^y.(The same quantity in the Mathematica version is Sy[[1]].)
2. Write the matrix II$\otimes S^x\otimes$II. Start at the left and first form the 4x4 direct product II$\otimes S^y$, then form the direct product of the result with II to get an 8x8 matrix. Set liquid_NMR.m up for a three-spin system, run it, and check your result. (type 'clear all' in the command prompt to clear the workspace)
3. This has only dealt with matrices that operate on one spin at a time. Construct the 4x4 matrix $S^x\otimes S^x$ for a two-spin system. To do it the way the program does it, construct the matrices $S^x\otimes$II and II$\otimes S^x$, and then matrix-multiply them together. To check your result, set liquid_NMR.m up for a two-spin system, run it, and then type 'test=Sx{1}*Sx{2}' at the command prompt and examine the variable 'test' in the workspace browser. You can get the same result by forming the matrix direct product of S^x with S^x. Type 'test=kroon(sigma_x,sigma_x)' to do it this way using MATLAB's Kronecker product function.

   Look closely at the lines in liquid_NMR.m where the spin matrices are built, and see if you can understand what is happening. Look at the lines where the Hamiltonian is built to see how the interaction operator is formed for each pair of spins.

4. Two-spin spectrum: Set liquid_NMR.m up for a two-spin system. Set the chemical shift of the first spin to 1.0 ppm and the second spin to 5.0 ppm. (this corresponds to 45 Hz and 225 Hz at a 45 MHz Larmor frequency). Set the J-coupling to zero and leave all other parameters at their default values. Run the program and examine the FID and the spectrum. You should see two single line ("singlets), one at 1.0 ppm and one at 5.0 ppm. This is the spectrum of two independent spins precessing at slightly different frequencies. Now set the J-coupling to 10 Hz and run the program. You should see two doublets. How is the doublet splitting in Hz related to the J-coupling? Set the J-coupling to 300 Hz and run again. Things are more complicated now-the two middle lines are close together and much stronger than the two outer lines. It is a general rule in NMR that simple, well-separated multiplets appear when the J-coupling is much smaller than the chemical shift difference, but not otherwise.

SETTING UP THE NMR SPECTROMETER

NOTE: First of all, this experiment relies on a stable, constant heat within the device in order to keep the magnet stable and provide enough precision to yield usable data. Turn the PicoSpin 45 on immediately and leave it on for the duration of the experiment.

First, this piece of equipment can yield much greater resolution than the TeachSpin PM-1051 Magnet due to the constant magnetic field. This uniform field allows us to see resonance produced by not only the nuclei, but the chemical shifts, J-coupling, and other effects that you may explore. To get your first image, follow the instructions below.

Larmor Frequency	• Begin by turning on the workstation at the experiment. Make sure the Ethernet cable is connected from the PicoSpin45 to the Ethernet port of the computer. • Open a web browser (This should work in either firefox or chrome) and type in the static IP address displayed by the PicoSpin 45 into the address bar of the browser. This model should display 192.168.42.31 • Once the browser redirects to the PicoSpin page, you may really begin! First, click on Temperature in the upper-right corner, and make sure the device's internal temperature is stable at 42^{o}. If you have turned the machine on when you were assigned the lab, it should be fine. • In order to create as uniform magnetic field as possible, you must "shim" the coils that produce your B-field. To begin this, use one of the syringes and fill it with distilled water. Remove the plastic stoppers from "in" and "out" ports on the picoSpin. (a gentle motion is preferred, and forcing water into the syringe or cartridge can fill either with air bubbles or damage the equipment) • Place the tip of the syringe in the "in" port and gently push water into the cartridge. The cartridge is a quartz container that is in the middle of the external magnetic field, and the equipment will detect any resonance from this container. The volume of the cartridge is only 30µL, so not much water is required, however, make sure no air bubbles make it in, or they will interfere with your results. • Keep applying water until water droplets begin to seep out of the "out" port. Once this happens, replace the plastic stoppers on both ports. • Now, the next step is to find the Larmor Frequency. • Click on the "scripts button near the top. The settings on the left side of the screen are typically fine to begin. Make sure test run is checked and click "Start Run" • On the lower graph, you should see a significant amount of noise with a single, large spike. If the spike isn't there, the Larmor Frequency must be changed. The first setting, "tx frequency" is the transmitter frequency. You want this frequency to be within about 500 Hz of the Larmor frequency. The device scans around this frequency over a preset interval, the "bandwidth". If the bandwidth is set at 4kHz, the device will scan over a range of tx frequency±2000Hz. • If a spike is not seen, increase the bandwidth, and increase the "min freq. to plot" and "max freq. to plot" settings near the bottom. This will allow you to see over a larger range. • If you want a better resolution, you can increase "acq. points" setting. This will use a larger number of points, but the "max time to plot" must be adjusted. If you want 1000 points over a bandwidth of 4kHz, you want $\frac{\#\ of\ points}{Bandwidth}$. For our settings of 1000 points with a bandwidth of 4kHz, we need 250 ms. • The lower graph shows the correction from your zero-point, or Larmor frequency. • Change your "tx frequency" so the spike is about 500 Hz away from your zero-point. It is important that you don't set it on the zero-point, as the software runs into issues resolving this. • Figure 1 below is a typical result from an unshimmed magnetic gradient

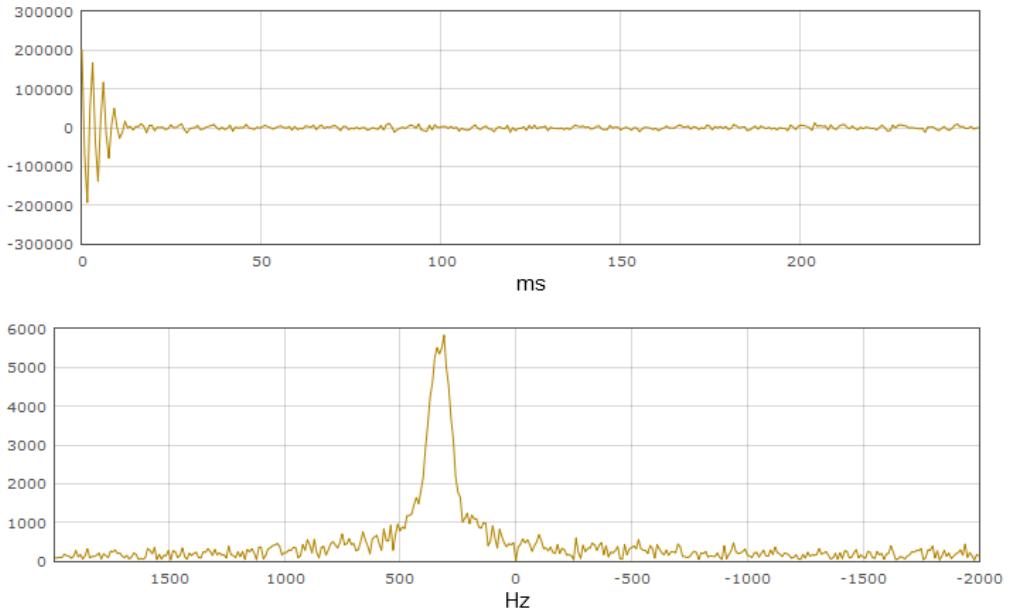

Figure 1

Shimming the Magnetic Gradient	• The value you adjusted your tx frequency is one you should remember. While you are getting a result, the magnetic field gradient is probably quite far off. In order to increase your resolution, uncheck the "test run" box and increase "max iterations" to 50. • This increases the uniformity of the magnetic field by shimming the coils generating the field, and will increase overall resolution. • In order for this lab to be successful, you want the highest resolution possible. If this is the first time shimming the gradient, you may want to perform over 100 iterations total and increase rx recovery delay. In the text box at the bottom of the screen, the program gives results from every iteration, including best and worst peak heights. The PicoSpin will have the highest resolution not when the peak is highest, but when the difference between best and worst are smallest. • Once you feel that the magnetic gradient is adequately shimmed, fill a syringe with ethyl acetate and fill the cartridge using the same procedure as with water. Don't worry about getting all of the water out, the incoming liquid will displace most, and anything left won't be in concentration large enough to affect the experiment. • Now, select the "OnePulse" script under the script menu and take a scan. • Make sure your "tx frequency" hasn't changed. The result you should get is a group of spikes, a single large one and one group on either side. If the result dips below the zero line, you can adjust the "phase correction" to account for this. By running multiple scans, the software will take a fourier transform and eliminate most noise. Your result should look similar to figure 2, below.

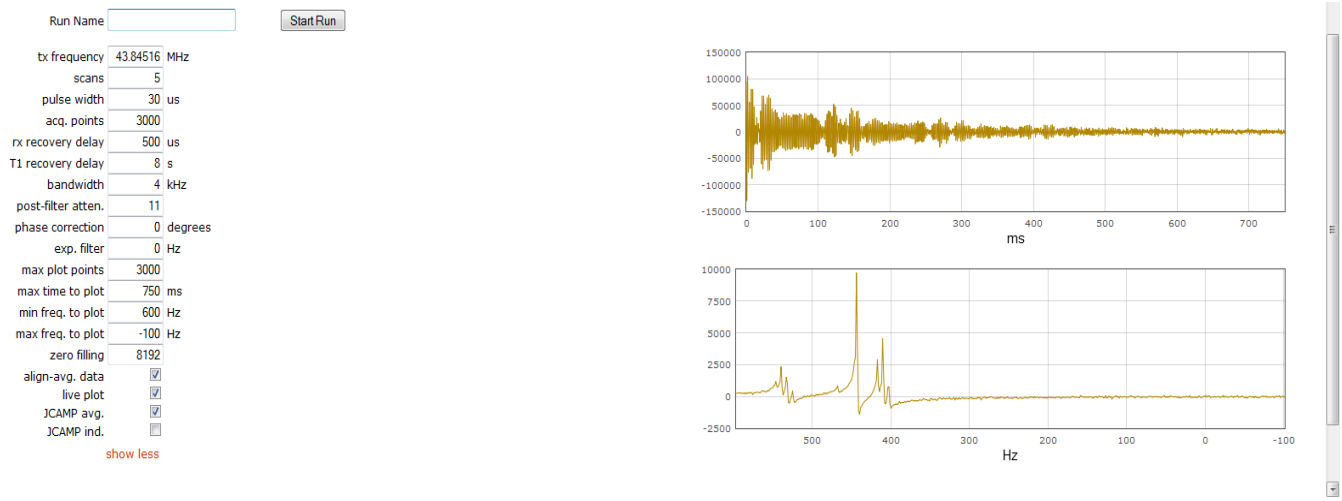

Figure 2

FINAL ANALYSIS

Once you have your completed scan, go to files. The program remembers the most recent run, as well as any named runs. If you open your run, scroll down and find your run data, it's a .jdx file (don't download all data, this gives each individual run, and they are packaged as .tar). You can open this file in MestReNova.

MestReNova is a very useful analytic tool. It allows you to see frequency changes in Parts Per Million, relative weights of groups attached to the molecule, and much more. Spend some time with the software and try to understand exactly what you are looking at.

WEEK 2: FURTHER UNDERSTANDING AND APPLYING WHAT YOU HAVE LEARNED

GOALS

Now that you are familiar with the idea of Nuclear Magnetic Resonance Spectroscopy and the idea behind it, and have a working knowledge of how the equipment works, it is time to put this knowledge to good use! This week, you get to design your own experiment.

Designing your own	There are several ways you can design your own experiment. Try starting with what you know the NMR Spectroscopy is capable of doing and expand from there. Here are some ideas to help get you started. a) Try taking a scan of ethanol and water. Take another with the ethanol at a different concentration. Is the separation at the same ppm? This is because the OH group is "labile", which means it moves back and forth from ethanol to water. Concentration will affect this. Research this effect and try to figure the "fast exchange" and "slow exchange" limits. b) Research what a "satellite peak" is. To begin, it is useful to know this is observed due to carbon-13. Can you get a spectrum that illustrates this? What is the occurrence of C-13 to C-12? c) NMR sample thermometry.

For further reading and other project ideas, go to http://www.thermoscientific.com/en/products/nuclear-magnetic-resonance-nmr.html or visit your local library.

WHAT IS NUCLEAR MAGNETIC RESONANCE GOOD FOR?

Nuclear Magnetic Resonance has a wide range of applications. For chemistry, it allows chemists to determine the structure and composition of many different compounds, saving countless hours of work. By unambiguously identifying a molecule, it allows the field to move forward faster than it ever had. This is the most common use of NMR Spectroscopy, as you may have already surmised.

In the medical field, you may already be acquainted with the use of NMR. An MRI, Magnetic Resonance Imaging, is a device that relies almost exclusively on these principles. The name was changed due to a public fear of the word "nuclear". This technology allows doctors and scientists to make great strides in research as well as diagnosing patients without having to perform invasive biopsies.

Other applications of an NMR are to determine purity in a sample, perform non-destructive testing, acquiring data (in the petroleum industry, for example), and many more. All of these have come from the idea and theory of nuclear magnetic resonance. In this lab, the student frequently goes on to experiment with NMR spectroscopy to further understand and grasp the concepts of nuclear magnetic resonance.

REFERENCES

- NMR Spectrometer Experiment Price, John, University of Colorado