Introduction to NMR Spectroscopy: An Instrumental Analysis

Incorporating instrumentation into undergraduate laboratories increases students’ understanding of fundamental and practical characterization techniques.

The NMReady™ benchtop spectrometer offers a portable and affordable option with a modern, network accessible, easy-to-use interface that can be easily incorporated into teaching laboratories.

**Introduction to NMR Spectroscopy:**

**Nuclear Magnetic Resonance (NMR) Spectroscopy** is one of the premier spectroscopic techniques available for molecular structure identification. Information is obtained in form of a spectrum (plot of absorption frequency vs. intensity) when an analyte is placed in a magnetic field ($B_0$) and subjected to radio frequency (RF) irradiation. The spectrum of a sample is dependent on the nuclei present & the molecular structure of the analyte.

Although most commonly used for organic molecules containing $^{1}H$ & $^{13}C$, any atomic nuclei with a nonzero spin can be exploited to determine chemical structure, relative configuration, etc. without degrading the analyte. Modern NMR spectrometers are programmed with standard parameters, but a basic knowledge of the parameters & instrumentation is vital to obtaining optimal results.

The NMReady™ benchtop spectrometer provides an accessible medium for training both chemistry & chemical technician students about the fundamental parameters of NMR spectroscopy in a guided-discovery laboratory experiment.

**Instrumentation:**

**NMR Spectrometers** are made up of several components, the connectivity of which are illustrated below.

1) Magnet  5) Data acquisition/processing
2) Probe/coil  6) Control Unit
3) RF transmitter  7) Shim System
4) RF receiver

**Theory of Data Collection:**

Nuclear spins tend to align in an external magnetic field $B_0$ (z direction), a slight thermodynamic excess of parallel spins produce a net magnetization in the sample. When an RF pulse (pw) is applied, the nuclei are excited (rotated into the xy plane). The nuclei will precess & relax back to the equilibrium state through lattice ($T_1$) & spin-spin ($T_2$) relaxation processes giving rise to an oscillating, decaying sine wave called a free-induction decay (FID) (plot of emitted RF vs. time).

**Procedure:**

Allow the students to learn about & manipulate critical parameters with the NMReady One-touch software as they work through the procedure and related questions below.

1) **Spectrometer Frequency** - characteristic to nuclei & applied field of spectrometer. For $^1H$ NMR & the 1.41 T field of the NMReady this is 60 MHz.

$$\nu_0 = \left(\frac{\gamma}{2\pi}\right)B_0$$

Where
- $\gamma$ = magnetogyric ratio (property of nuclei that determines precession frequency.
- For $^1H\gamma = 267.5 \times 10^8$ rad/s
- $B_0$ = static magnetic field applied by magnet

2) **Pulse Width (pw)** - described by the angle ($\theta$) the net magnetization is rotated through when RF pulse is applied. Power is applied for a time (tp in us) to rotate to the desired angle, so pw can be discussed in terms of angle or time. The NMReady defaults to a 90° pulse.

$$\theta = 360 \left(\frac{\gamma}{2\pi}\right)B_1tp$$

Where
- $\gamma$ = magnetogyric ratio (property of nuclei that determines precession frequency.
- $B_1$ = RF magnetic field applied by the coil
- $tp$ = time of pulse (us)

(i) What is the $tp$ for a 90° pulse when $B_1 = 290$ uT? (ii) 180°?

3) **Spectral Width (sw)** - (or sweep width) frequency range analyzed by the spectrometer. It can be reported in ppm or Hz. ppm is independent of spectrometer frequency but Hz is not.

(iii) What is the typical range of chemical shifts for protons?
(iv) Given this information, what is a typical sw required for routine analysis of organic compounds?
(v) For a sw = 12 ppm, what is the range in Hz for a 60 MHz spectrometer versus a 400 MHz?
(vi) Changing only the sw determine what happens to the active scan time.

4) **Number of Points (np)** - controls the digital resolution (res) of the measured FID. Nyquist theorem states the analog signal must be sampled at a rate >sw to ensure each peak is properly reproduced in the spectrum.

Generally more points = higher resolution (res)

Typically the resolution must be greater than or equal to 1/2 of the peak line width (in Hz) at 50% ($LW_{50}$).

$$res = \frac{2sw}{np}$$

(vii) For $^1H$ NMR spectra measured with $SW = 12$ ppm & a $LW_{50} = 2.2$ ppm on the NMReady what is the minimum np required?
5) **Acquisition Time (at)** - the time it takes to acquire an FID. For the NMReady this is also called the “active scan time”. There is an optimal value that can be calculated from the np and sw. If the acquisition is too long, noise is acquired but if it is too short the base of each peak is distorted. 

\[
at = \frac{np}{2sw} = \frac{1}{\text{res}}
\]

(xviii) What is a standard at for a typical sw & np you calculated? 
(xix) Does sw or np have a greater effect on active scan time?

6) **Recover Delay (RD)** - is the “down time” or waiting period between scans to ensure that all nuclear spins have returned to equilibrium before pulsing the sample again. Schematically this comes before pw to account for the time it takes nuclear spins to reach equilibrium prior to first measurement.

If this delay is too short the signals are attenuated & the integrations are inaccurate, but if it is too long time is being wasted.

(x) Why is more than one scan acquired? 
(xii) What types of compounds need larger RD’s?

**Applications & Spectral Resolution:**

Now that the parameters governing data collection have been introduced students can experiment with some additional variables that are known to enhance or reduce the observed resolution & effective sensitivity.

1) **Signal Averaging** - repetition of an experiment to increase signal-to-noise ratio (S/N). The signal of the analyte will increase proportionally to the number of scans (ns) whereas random noise will only increase by (ns)^1/2. Reported S/N is a ratio of analyte peak amplitude (A) to root-mean-square (rms) noise.

\[
\frac{S}{N} \propto \sqrt{\text{ns}}
\]

\[
\frac{S}{N} = \frac{A}{\text{rms}_{\text{noise}}}
\]

where \text{rms}_{\text{noise}} = \frac{\text{peak to peak noise}}{2.5}

(xxi) Go Setup → System. Note that there is a default zero filling. Collect two more spectra - one with no zero filling & one with x3.

(xxx) What is the difference between the three spectra? Does the zero filling have an observable effect? Explain.

2) **Exponential Multiplication** - a type of apodization that enhances the apparent S/N by multiplying the FID by an exponential curve to make decay more rapid. As the lines are concurrently broadened, this is often referred to as line broadening (LB).

(xvii) Prepare a 0.5 M sample of 2-propanol in D$_2$O. Predict the ^1H NMR spectrum - how many signals do you expect to see? what are the approximate chemical shifts? What coupling do you expect to observe? 
(xviii) Measure a spectrum. Click the “process” button → manual. This allows you to manipulate the LB as desired. 
(xix) Prepare a new table tabulating LB (Hz), S/N & observed LW$_{90}$ of each peak. 
(xx) What is the optimal LB for this sample? Is it the same for all peaks within the sample?

3) **Zero filling** - a mathematical construct that appears to improve digital resolution without increasing acquisition time & therefore decreasing S/N. Data points of zero intensity are added to the end of the FID.

(xxi) Collect & save 4 spectra for the D$_2$O standard at ns = 1, 4, 16 & 64. Manually calculate the rms$_{\text{noise}}$ and S/N for each. 
(xxii) Compare the S/N values. Does the relationship between S/N and ns hold? 
(xxiii) Make a table reporting ns, S/N, & lw.

Modern spectrometers also have automatic programs to determine the rms noise. On the NMReady go to “Setup” → “Script” → RF self test

(xiv) Record the rms noise value produced automatically.

**References:**

3) Holmes, D., www2.chemistry.msue.edu/facilities/nmr/handouts/DH_NMR_Basics.pdf (viewed April 8/13)

**Data Accessability:**

NMReady outputs to a networked drive and has a print option. Students can process and print in third party software, like Mestrelab™, or use the NMReady directly. An example of data to be incorporated into a lab report processed and printed directly from the NMReady is presented below:

1) **Interpretation of ^1H NMR Spectra**
2) **Synthesis of Aspirin**
3) **Aldol Condensation**