Instructor's Guide
This guide is intended to assist instructors in the utilization of the q-NMR learning module. While the module is designed as a stand-alone resource to accommodate self-learners, it can also be used as an active learning resource. This instructor's guide is designed to help

1. Basic Theory Concept Questions. The questions below are also listed on the webpage that links to the Basic Theory section. These questions can be handed out in class or given as a homework assignment. Students should be able to answer these questions using the Basic Theory section of this module as an instructional resource. I would also recommend assigning the excellent web resource created by Joe Hornak, since it contains embedded animations that help clarify many difficult to understand processes in NMR. This site can be accessed directly at http://www.cis.rit.edu/htbooks/nmr/ or students can be directed to this site in ASDL by typing its ID number, 2921, into the search box in the upper right hand corner of the site.

What is spin?
How does absorption of energy generate an NMR spectrum?
Why is NMR less sensitive than UV-visible spectroscopy?
What is chemical shift and how does it relate to resonance frequency?
What is precession?
How does precession produce the macroscopic magnetization (Mo)?
How can the nuclear spins be manipulated to generate the NMR spectrum?
What is the tip angle?
What is a Free Induction Decay?
How do T_1 and T_2 relaxation affect NMR spectra?

2. Answers to Questions in the Basic Theory section. In addition to the conceptual questions given above, the Basic Theory section also contains a series of simple quantitative questions, the answers of which are provided below.

Question 1: How many spin states would you predict for ^2H?

Deuterium has a spin of 1. There for there should be 3 possible spin states: +1, 0 and -1.

Question 2: Given the same magnetic field and temperature, how would the difference in population for ^1H and ^{31}P compare?

For this problem we will use the equation $\frac{N_{upper}}{N_{lower}} = e^{\frac{-\Delta E}{kT}}$

The difference in population for ^1H and ^{31}P will be related to the differences in their ΔE values. Since $\Delta E=\gamma h B_o/2\pi$, for a fixed magnetic field the only differences between ^1H and ^{31}P is in their magnetogyric ratios.

$$\frac{\Delta E(^1H)}{\Delta E(^{31}P)} = \frac{26.752}{10.84} = 2.468$$

The ratio of the N_{upper}/N_{lower} for ^1H is $e^{2.468}$ or =11.80 times larger than the ratio of N_{upper}/N_{lower} for ^{31}P.

Question 3: Calculate the wavelength of electromagnetic radiation corresponding to a frequency of 500 MHz.

The wavelength of electromagnetic radiation corresponding to a frequency of 500 MHz is 0.6 m.

Question 4: What range of frequencies would be excited by a 10 μs rf pulse?

A 10 μs rf pulse would excite a range of frequencies covering 100,000 Hz.

Question 5: What are the resonance line widths of nuclei that have apparent T_2 relaxation times (i.e.T_2* values) of 1 and 2 sec.

$$w_{\frac{1}{2}} = \frac{1}{\pi T_2^*}$$

Therefore, the two resonances have line widths of 0.32 and 0.16 Hz.

3. Practical Aspects Concept Questions. The questions below are also listed on the webpage that links to the Practical Aspects section. These questions can be handed out in class or given as a homework assignment. Students should be able to answer these questions using the Practical Aspects section of this module as an instructional resource.

How do I choose a reference standard for my Q-NMR analysis?

How is the internal standard used to quantify the concentration of my analyte?

What sample considerations are important in Q-NMR analysis?

How do I choose the right acquisition parameters for a quantitative NMR measurement?

What data processing considerations are important for obtaining accurate and precise results?

4. Answers to Questions in the Practical Aspects section.

Question 1. A quantitative NMR experiment is performed to quantify the amount of isopropyl alcohol in a D_2O solution. Sodium maleate (0.01021 M) is used as an internal standard. The integral obtained for the maleate resonance is 46.978. The isopropanol doublet at 1.45 ppm produces an integral of 104.43. What would you

predict for the integral of the isopropanol CH resonance as 3.99 ppm. What is the concentration of isopropanol in this solution?

The isopropanol CH resonance is produced by a single proton whereas the doublet is produced by the 6 methyl protons. Therefore, the CH integral should be 1/6th that of the methyl doublet, or 17.405.

To find the isopropanol concentration we first have to calculate normalized areas for isopropanol and our standard, maleate. The isopropanol(IP) doublet is comprised of 6 protons due to the two equivalent methyl groups of this compound.

$$\text{Normalized Area (IP)} = \frac{104.43}{6} = 17.405$$

Similarly, the normalized area for maleate (MA) is:

$$\text{Normalized Area (MA)} = \frac{46.978}{2} = 23.489$$

The concentration of the isopropanol can be calculated using the known the maleate concentration.

$$[\text{IP}] = \frac{[\text{MA}] \times \text{Normalized Area (IP)}}{\text{Normalized Area (MA)}}$$

$$[\text{IP}] = \frac{0.01021\ \text{M} \times 17.405}{23.489} = 0.007565\ \text{M}$$

Because the accuracy of the determination depends on how well the maleate concentration is know, the standard solution should be prepared with care, using dried sodium maleate of high purity, weighing carefully a mass that is known to an appropriate number of significant figures (in this case 4), transferring the maleate quantitatively to a volumetric flask and finally dilution to the mark. Again, an appropriate solution volume must be selected to produce the desired number of significant figures given the manufacturer specifications for the glassware used.

Question 2: A solution prepared for quantitative analysis using NMR was acquired by coaddition of 8 FIDs produces a spectrum with an S/N of 62.5 for the analyte signals. How many FIDs would have to be coadded to produce a spectrum with an S/N of 250?

S/N increases in NMR experiments as the square root of the number of scans coadded.

$$S/N \propto (n)^{0.5}$$

To increase the S/N from 62.5 to 250 (a factor of 4 increase in S/N) would require coaddition of 16 times as many FIDs as was used to produce a spectrum with S/N of 62.5. The answer is that coaddition of 128 FIDs (8 x 16) would be required to achieve an S/N of 250.

Question 3: A ^{1}H NMR spectrum was measured using a 400.0 MHz instrument by acquisition of 8192 total data points (8192 real points) and a spectral width of 12.00 ppm. What was the acquisition time? Calculate the digital resolution of the resulting spectrum? Is this digital resolution sufficient to accurately define a peak with a width at half height of 0.5 Hz?

We can calculate the acquisition time knowing the spectral width and the total number of data points.

$$AT = \frac{NP}{2\ SW} = \frac{16384}{2 \times 400 \times 12} = 1.707 \text{ sec}$$

$$DR = \frac{SW}{NP(real)} = \frac{2 \times 400 \times 12}{8192} = 1.172 \text{ Hz/pt}$$

This would not be adequate digital resolution to accurately define a peak with a 0.5 Hz width at half height. A longer acquisition time would allow for collection of more points. Also, zero-filling could also be used to help increase the digital resolution.

5. Q-NMR Drylab

KHP T_1 relaxation times. There are two ways of getting the T_1 relaxation times for the KHP resonances. The simplest way is to have students estimate the null times for the two resonances in the inversion-recovery spectra provided. Selected spectra from the dry lab data set were used to make the figure in the Practical Aspects section of the module. Alternatively, students can process the spectra and measure resonance integrals for each peak. These can be plotted vs. the relaxation delay and fit to determine T_1. The integrals we obtained are summarized in the Table below. The fits obtained using Origin 7.5 are also provided. We obtained T_1 values of 4.79s and 3.11s for the KHP resonances at 7.75 and 7.61 ppm, respectively.

The concentration of KHP in the stock solution is determined from the mass of KHP.

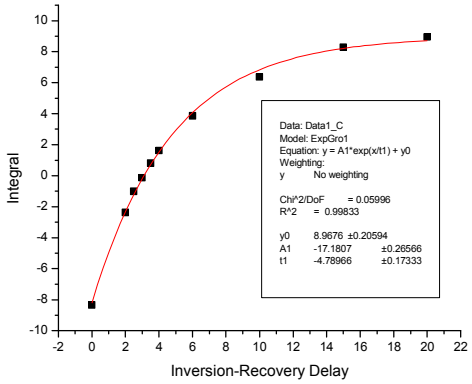
Mass KHP = 0.3533 g – 0.2219 g = 0.1314 g

$$[KHP] = \frac{0.1314g}{204.22g/mol} \times \frac{1}{0.005L} = 0.1287M$$

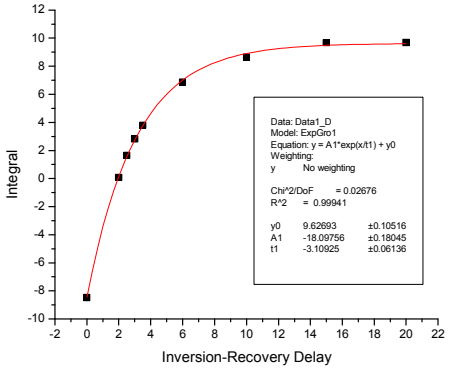
Table 1. Inversion-recovery data for KHP

Delay	Integral R1 (7.75 ppm)	Integral R2 (7.61 ppm)
0 s	-8.34	-8.48
2	-2.38	0.07
2.5	-1.01	1.65
3	-0.14	2.81
3.5	0.8	3.78
4	1.62	4.57
6	3.87	6.86
10	6.37	8.63
15	8.28	9.67
20	8.95	9.65

Inversion-recovery plot for R_1



Inversion-recovery plot for R_2



Malic Acid Standard Solution Determination. From the mass of malic acid weighed and the solution volume, we can calculate the concentration of this standard.

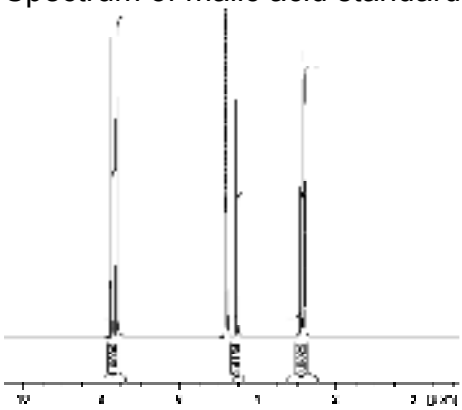
Mass of MA = 0.3324g – 0.1897 g = 0.1427 g

$$[MA] = \frac{0.1427g}{134.09g/mol} \times \frac{1}{0.005L} = 0.2128M$$

Since equal volumes of the KHP and malic acid were mixed to prepare this solution, the dilution factor can be neglected and the concentration of malic acid calculated as shown below. Here we used the integral of the resonances at 2.82 and 2.89 ppm corresponding to the inequivalent malic acid CH_2 protons.

$$[MA] = [KHP] \times \frac{Int_{MA}}{N_{MA}} \times \frac{N_{KHP}}{Int_{KHP}} = 0.1287M \times \frac{0.8322}{2} \times \frac{4}{1.000} = 0.2142M$$

Spectrum of malic acid standard solution containing KHP



Determination of Malic Acid in Apple Juice. The concentration of malic acid can similarly be calculated in an apple juice sample. Here though we will need to take into account the dilutions performed.

The KHP was diluted twice in preparing this solution. First it was diluted by half with D_2O, and subsequently 100 μL of was further diluted by addition to 900 μL of apple juice for a total volume of 1 mL. The KHP concentration in the apple juice sample can be calculated as shown below. Note that the volume added in making the pH adjustment to 1.35 is not important since both the KHP and the malic acid will be diluted by the same amount. This pH adjustment is necessary to resolve the malic acid resonances from those of the other apple juice components. Because of the simplicity of the malic acid standard spectrum, pH adjustment is not needed.

$$[KHP]_{juice} = [KHP]_{stock} \times \frac{1}{2} \times \frac{100}{1000} = 0.1287M \times \frac{1}{2} \times \frac{100}{1000} = 0.00643M$$

The malic acid concentration can then be calculated as before, providing that the dilution resulting from addition of the KHP solution is included in the calculation.

$$[MA]_{juice} = [KHP]_{juice} \times \frac{Int_{MA}}{N_{MA}} \times \frac{N_{KHP}}{Int_{KHP}} \times \frac{1000}{900} = 0.00643M \times \frac{1.000}{2} \times \frac{4}{0.4351} \times \frac{1000}{900} = 0.0328M$$

For purposes of comparison with the table provided in the background section of this laboratory, we can convert this concentration to g/L using the molecular weight of malic acid.

0.0.328 M x 134.09 g/mol = 4.40 g/L malic acid

Spectrum of apple juice sample containing KHP