NMR FACILITY NEWSLETTER

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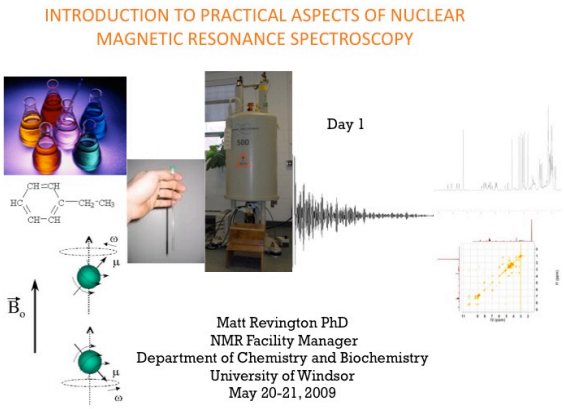
Inaugural University of Windsor NMR Training Workshop



A NMR workshop entitled "Introduction to Practical Aspects of NMR Spectroscopy"was held on the mornings of May 20 and 21. This was the first workshop offered by the NMR Facility and was aimed at giving relatively inexperienced users more information and insight into the steps required to obtain a good NMR spectrum. On Day 1 topics included NMR sample preparation and how to set up 1D ^{1}H or ^{13}C NMR experiments. After the lecture portion and a coffee break the students had the opportunity to prepare samples and then set up experiments on the spectrometers in the NMR facility. On Day 2 the focus was data processing and formatting the data. Lectures and computer processing sessions were held in the Student Resource Centre, the sample preparation session was held in Student Lab F and the experimental set up session was located in the NMR facility. We had a good turn out with the 11-12 students in attendance both days. I would like to thank Aaron Rossini and Hiyam Hamaed for the great job they did in running the lab demonstrations and also to thank Rob Schurko for all of his efforts in helping set up the workshop.

This workshop will be offered again in the fall for incoming students and is intended to become a standard part of the training for students who make use of the NMR facilities

A more advanced workshop covering 2D NMR and other more complex experiments is planned for later in the year. I welcome input as to the content of any of the workshops and in particular advanced techniques that users may want presented.

NMR Facility Updates

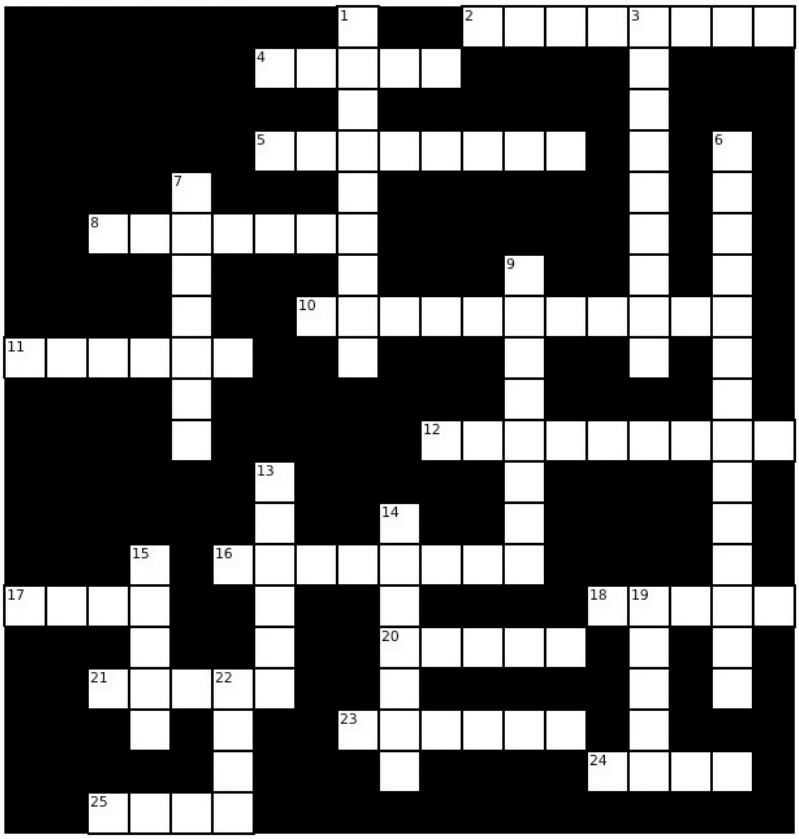
The DPX300 in Rm 394-5 was down for a couple of weeks in April due to overheating problems. The problem was eventually traced to several burnt out fans in the console. The fans have been replaced and it is currently working well.

During the first week of June all of the probes and shim stacks were removed from the instruments and cleaned. This should remove some background in spectra from accumulated grime. It also appears to have helped the spinners to work much better.

Over the next few weeks I will be making up a list of "good" parameter sets for each spectrometer for as many kinds of experiments as possible. The list and description of how to set up the experiments will be left in a binder at each spectrometer. If there are specific experiments that you would like to see implemented please contact me.

NMR Geek Alert

The first student to bring me a correctly filled out puzzle wins a valuable prize.



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Technical Comment: Shimming

In the first issue of the newsletter I wrote an article on Tuning the probe for optimal sensitivity. In this issue I am going to discuss the other hardware adjustment that needs to be done for every sample, shimming. Shimming a NMR magnet is the procedure that optimizes the homogeneity of the field. Inhomogeneity of the magnetic field experienced by the spins in an NMR sample results in faster relaxation, lower spectral resolution and an overall lower signal to noise ratio, therefore, optimal shimming of the magnet is a key part of the set up of any NMR experiment.

The term shimming comes from the early days of NMR where small pieces of metal were used to physically adjust (or shim) the position of the electromagnets to give the most uniform, homogeneous, magnetic field. The room temperature shims in a modern NMR system are metal coils that can generate small variable magnetic fields that can compensate for imperfections in the in the field of the large superconducting magnet. The shim coils line the central bore of the superconducting magnet immediately outside of the probe. When current flows through these coils a magnetic field is produced. The user can vary the current flowing through each individual "shim coil" to change the magnitude of the magnetic field produced by that coil. In a modern NMR system there are 20 or more of these coils that are configured to produce fields of distinct shapes. For example there are 6 Z shims on the Bruker instruments, Z indicates that field produced by these shims are symmetric about the z axis of the magnetic field which is co-linear with the long axis of the sample tube. These Z shims are often referred to as the "spinning shims" since their axial symmetry allows them to be adjusted while the tube is spinning while shims that contain X or Y terms cannot be shimmed unless the sample rotation is stopped. The shape of fields is described by their names Z^1 is a linear gradient across the sample, Z^2 is parabolic, Z^3 is cubic and so on as shown in Figure 1a . Some combination shims of the Z and X or Y can be represented as surfaces as for the XZ^2 shim in Figure 1b while higher dimensional shims cannot be represented visually. Shimming can be viewed as combining all 20 (or more) of these shim profiles and adjusting their amplitude to produce a field that counters the deviations from homogeneity in the magnetic field around the sample. Figure 2 shows graphically how the Z^1 and Z^2 shims can be used to optimize the field.

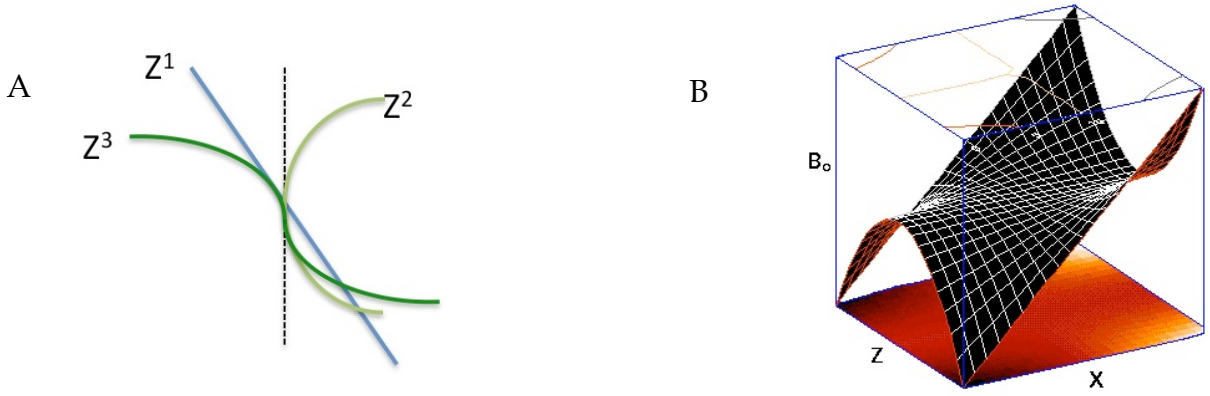

Figure 1. (A)Profiles of the magnetic fields produced by Z^1, Z^2 and Z^3. (B) Surface representing a XZ^2 shim profile.(From "The Basics of NMR", Joseph P.Hornak, online textbook)

Shimming and judging the homogeneity of the field can be done in several ways on the Bruker spectrometers. For samples using a H_2O/D_2O solvent system we can run the gradshim program (on the instruments that have pulsed field gradients, the 500 MHz and 300 US) that maps the magnetic field and attempts to use combinations of the shim profiles to minimize the field inhomgeneities much as I described in Figure 2. In most other cases selecting individual shims

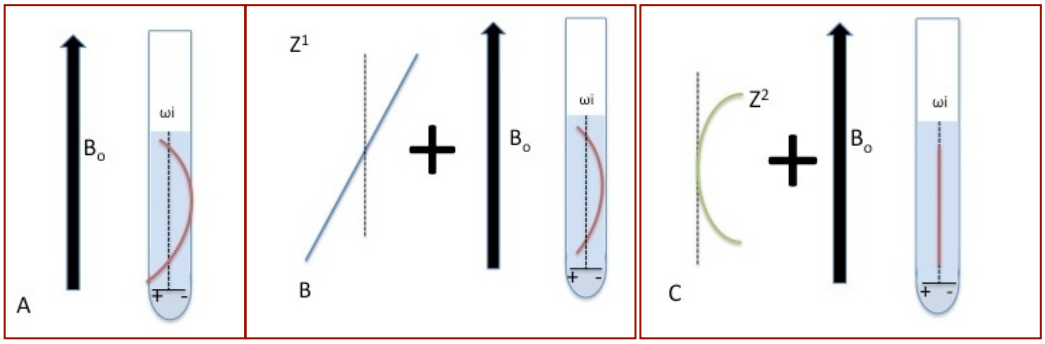

Figure 2. (A) A NMR sample in an inhomogeneous magnetic field, Bo, the red line traces a contour that has the same magnetic field strength. (B) Application of the linear Z^1 shim improves homogeneity and in (C) the Z2 shim brings the field line to a linear homogeneous state.

and adjusting the current using the knob on the BSMS keyboard until the lock signal reaches a maximal value optimizes the shimming. Because there is some interdependence between shims the process usually requires several iterations of optimization for each shim in the set of shims being adjusted. The initial manual shimming of a new magnet requires the iterative optimization of 20 shims and may take a couple days even with an experienced technician. Once a good basic shim set has been established it needs to be updated regularly by the facility manager. The quality of the shims can be judged by resolution in a measure spectrum usually as the linewidth at half height of an isolated peak in a standard sample.

In practice for the multiple users and short experiments in our NMR facility the most efficient method of shimming has been for the facility manager to provide a good shim file (aa_best) that can be loaded by every user and the individual users need only adjust the Z^1 and Z^2 shims. The effects of badly set shims can be seen in the spectra of ethyl benzene shown in Figure 3.

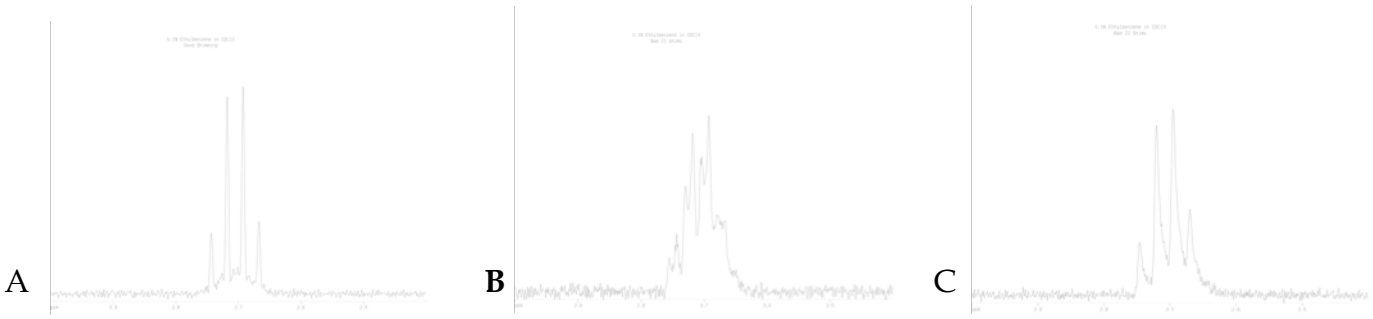

Figure 3. (A) The methylene quartet of ethylbenzene in a well shimmed spectrum.(B) The same spectral region in a spectrum where the Z^1 shim was miss-set. (C) The quartet when the Z^2 shim was incorrectly set.

Advanced Techniques: HSQC and HMQC

In January's newsletter I discussed the use of the DEPTQ experiment as a more useful alternative to the overnight ^{13}C 1D experiments. A second alternative is to record a 2D ^{1}H -^{13}C single bond correlation experiments, either the heteronuclear multiple quantum correlation (HMQC) or the heteronuclear single quantum correlation (HSQC) experiments. These experiments produce almost identical looking spectra with peaks at the intersection of the ^{1}H chemical shift and the ^{13}C chemical shifts for each C-H in the spectrum. On the positive side you will obtain a set of unambiguous ^{1}H -^{13}C correlations making assignment of the source nuclei easier than the case when you have a pair of standard 1D ^{1}H and ^{13}C spectra. On the negative side the quaternary C's will not produce a peak in these spectra. In Figure 1 ^{1}H -^{13}C HSQC, 1D ^{1}H and^{13}C spectra of 0.1% Ethylbenzene in CDCl3 collected in the same total amount of experimental time (~8 hours each) are shown.

Resistance to running 2D experiment seems partly to be due to a perceived greater difficulty in setting up and processing the 2D experiments and a feeling that they are less sensitive than the 1D experiments. The sensitivity is dealt with below. The first time a 2D experiment is set up it does take somewhat longer than a 1D, I strongly suggest that for all samples a 1D ^{1}H spectrum be collected to ascertain the quality of the sample and to determine the spectral width necessary for the ^{1}H dimension. It is also important to have a good estimate of the ^{13}C spectral width, if you have a pre-existing 1D ^{13}C spectrum that can be used or if you know the types of carbon nuclei in the sample a good estimate can be made. The spectral width values must be entered in the "eda" menu. If you use one the parameter sets that end in "_mr" then they are sets that I have optimized recently and the pulse lengths and delays should be set correctly. I will be posting details of 2D processing methods on the NMR Facility web page in the near future. As with all FT-NMR experiments the HMQC and HSQC are run as computer programs, called pulse sequences, which specify a series of pulses and delays. The standard pulse sequences for these two spectra are shown in Figure 2. There is large gain in sensitivity for these type of transfers over the 1D ^{13}C pulse sequence because of the 4 times greater magnetogyric ratio of ^{1}H over ^{13}C. Both of these experiments start with pulses on the ^{1}H's thereby allowing a greater initial excitation that can then be transferred to the ^{13}C nuclei for chemical shift evolution in the middle part of the sequence then back to the more sensitive ^{1}H for detection.

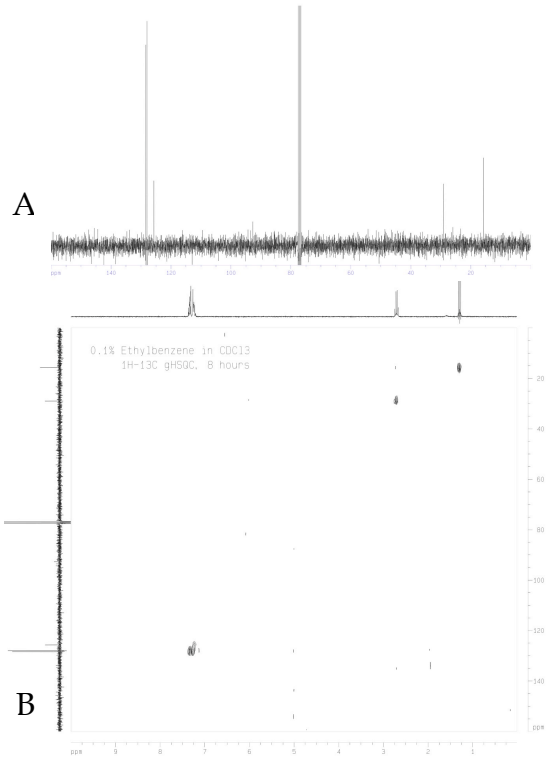

Figure 1. (A) 1D ^{13}C spectrum (with proton decoupling/NOE} of 0.1% ethylbenzene in $CDCl_3$, collected for 8 hours (20,000 scans). (B) ^{1}H -^{13}C HSQC of the same sample collected in 8 hours (96 scans/128 increments).

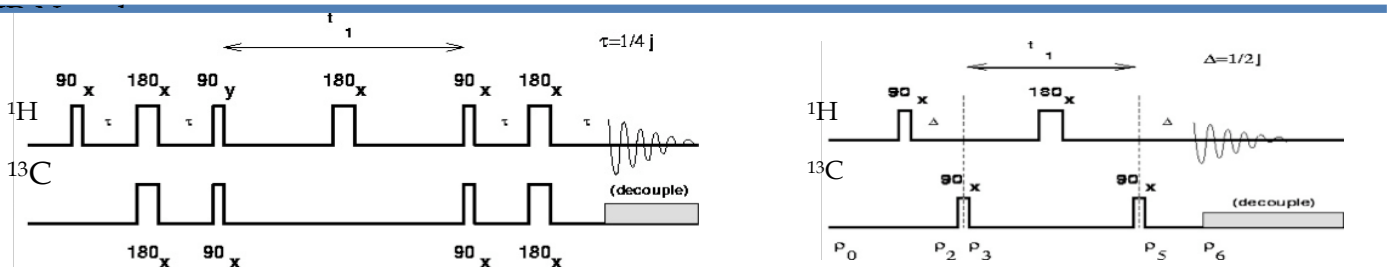

Figure 2.(A) HSQC pulse sequence, narrow rectangles denote 90° pulses, wider rectangles are 180° pulses, maximal coherence transfer occurs when the delay τ is 1/(4J), where J is the one bond ^{1}H – ^{13}C coupling constant. (B) HMQC pulse sequence, Δ is a delay optimally set to 1/(2J).

For a ^{1}H -^{13}C group these types of experiments allow a theoretical 32 fold increase in sensitivity over the 1D ^{13}C experiment (for the ^{31}P the gain is 10 times and for ^{15}N the gain is 300 times) . The actual increases are usually somewhat less due to losses from imprecisely set pulse lengths and relaxation losses during the longer, more complex, pulse sequences but there is still a considerable increase in sensitivity. In practice the 2D experiment still requires a similar amount of total instrument time as the 1D ^{13}C experiment because there is a need to collect many increments to get digital resolution in the second (or indirect) frequency dimension.

Since the HSQC and HMQC produce similar looking spectra there is often some question about which one is preferable to run. A good summary of the differences and relative advantages is found at

http://u-of-o-nmr-facility.blogspot.com/2009/01/hmqc-vs-hsqc.html

To summarize the HSQC has a slightly higher inherent resolution however the HSQC, as can be seen from the pulse sequences in Figure 2, has many more pulses and delays and therefore requires more effort and knowledge to set up optimally. The bottom line is that if you need a high resolution spectrum then it is worth the effort to calibrate pulses for a HSQC but for everyday use the HMQC will be more robust.

Since the 1D ^{13}C is a standard spectrum that is required by many journals and most supervisors I have written a macro that will allow researchers to record a 1D ^{13}C spectrum of predetermined signal to noise ratio and then to follow that with either a 1D DEPTQ-135 or an ^{1}H -^{13}C HSQC in the time left in an overnight data collection. At present these macros are only on the 300US instrument. The user sets up a standard 1D ^{13}C in experiment #1 a signal to noise ratio value (S/N) is set and the range that the signal to be measured is in is specified by the user. It is best to select a signal region that excludes the ^{13}C resonance of the solvent and that the researcher is confident will contain peaks, the noise region by default is set to the farthest downfield (higher ppm value) sixteenth of the spectrum. When the macro is run the 1D ^{13}C spectrum is automatically processed after every 1024 scans and signal to noise ratio is calculated. When the preset S/N is exceeded the experiment is terminated and a new experiment is created with the parameters for the DEPTQ or HSQC. Since the time available for the second experiment is not known the macro is set up to collect the 2D HSQC in the interleave mode, this mode collects all of the increments for a complete spectrum (with good digital resolution in the indirect dimension) with a limited number of scans in about an hour and then collects another identical spectrum and adds it to the first. In this macro the spectrometer collects 8 successive, added 2D's but it can be halted at any point after the first hour and still have a complete spectrum. If you are interested in running this macro please speak to me.