NMR Newsletter

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NMR FACILITY NEWSLETTER

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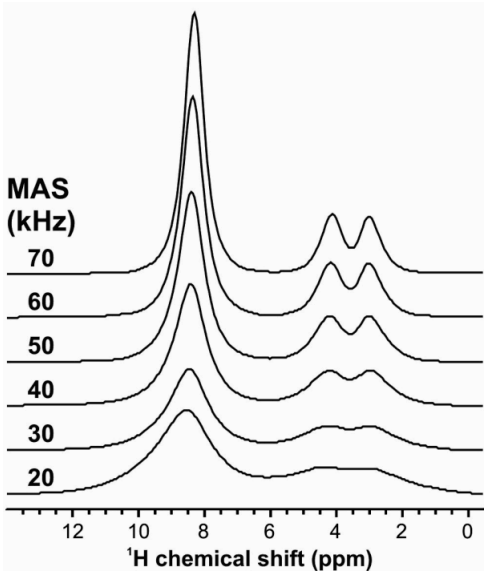
500 MHz NMR upgraded

The 500 MHz NMR has received a $250,000 upgrade/update this month as a result of a $150,000 RTI NSERC grant obtained by Dr. Rob Schurko and a $100,000 contribution from the University of Windsor. This upgrade has resulted in the replacement of the aging DRX console (the 2 door cabinet) and preamplifier stack that was installed in 1996 with a state of the art AvanceIII console and preamps. The computer workstation has also been replaced and the Topspin1 software has been updated to the newest generation, Topspin3. The new console features much faster and more stable electronics that will allow users to obtain better spectra especially when collecting more complex 2D data sets. Users will notice that the BSMS keyboard, the small keyboard used for shimming and ejecting samples, is not a part of this system as all of its functions are now incorporated into the computer software. On this system shimming can be done automatically through the gradient shimming interface, called TOPSHIM. The new system should also allow for much more "up time" for the 500 MHz that has been plagued the last couple of years with several breakdowns and long delays in obtaining replacement parts for the antiquated system. The greatest benefit of the upgrade will be in the area of solid state NMR where the new electronics are essential in order to be able to utilize the full potential of the Ultra Fast Magic Angle Spinning (UFMAS) probe that Dr Schurko received last year. This probe, that permits spinning rates up to 70KHz, will allow the acquisition of ^{1}H spectra of solid samples with close to solution state resolution. (See Figure 1).

A



B

Figure 1 A. Magic angle spinning (MAS) spectra of glycine showing the increase in resolution possible at the high spinning rates of the UFMAS probe . B. The new 500 MHz AVANCEIII console and computer workstation just installed.

Issues in the NMR room

1)During the warm spell in March the NMR instruments were shut down several times due to high temperature in the NMR rooms. I apologize for the inconvenience but the high temperatures can damage the electronics and shorten the life of the NMR instruments. I would ask that if anyone notices temperature extremes in Rm 394-5 or Rm B82 on weekends or after hours to please contact me at mrevingt@uwindsor.ca.

2)The DPX300 MHz instrument (yellow signup book) in Rm 394-5 EH has been the workhorse machine for ^{19}F spectra in this department however the tuning for the probe for that machine has become increasingly sensitive and the tuning rod already has broken once resulting in downtime for that instrument. The 300 MHz should no longer be used for ^{19}F spectra except in emergency situations (ie when the other 19F machines are not working). Users should note that both the 500 MHz and the 300MHz in Rm B82 can also collect ^{19}F spectra with greater sensitivity and those instruments also allow ^{1}H decoupling of ^{19}F spectra which cannot be done on the DPX300.

3) Users are reminded that no one can run variable temperature (VT) without permission of the Facility Manager and no one without training specifically for VT experiments from the Facility Manager will be allowed to run. All users are also reminded that the standard blue spinners should only be used for temperatures from ~ -20° C to +40° C, outside of that range use the white ceramic spinners.

4) New Macro for overnight ^{13}C data collection

When doing overnight experiments such as the ^{13}C collection the data is not "saved" until the experiment finishes unless the user enters the command "tr" to transfer data to the saved file while the experiment continues in the backgound or stops the experiment with the "**halt**" command. When there are power disruptions in the early morning hours many hours of data can therefore be lost. On the 300 MHz instruments I have implemented a macro program that can be used for these long experiments which saves data after every hour of data collection.

Liquid Chromatography Probe For the 500 MHz

The NMR facility has recently received a liquid chromatography-NMR flow probe for the 500 MHz courtesy of the University of Pennsylvania. The probe is equipped with ^{1}H and ^{13}C detection capabilities. Anyone interested in developing uses for this probe should contact me.

NMR Workshops

An introductory NMR workshop will be held in May on the mornings of May 24 and 25. Any users of the NMR facility who have not taken prior workshops should attend. The workshop is intended to supplement the 1 on 1 training that is given in the NMR facility and gives a more complete picture of what is required for getting the best data possible. Topics covered include how to make an NMR sample, how to set up 1D and 13C experiments and how to process data. I am also going to host an additional session prior to the main workshop for students who have not taken a spectroscopy course to introduce the basic concepts of NMR such as spin, chemical shift and scalar coupling. Users can sign up on the NMR facility webpage.

An advanced workshop covering more complex NMR techniques such as DOSY, 2D COSY and NOESY and HMQC experiments will be held later in the summer.

Advanced Technique

Selective 1D Experiments

In the last newletter I discussed frequency selective NMR pulses and their uses in solvent suppression techniques. Selective pulses can also be used to excite a limited spectral range, such as a single peak (spectral widths of 25 Hz are easily selected), to unambiguously see interactions between a selected spin and any other spin in a molecule. Two common experiments that use selective pulses for molecular information are the 1D Gradient Selective NOESY (sometimes called the GOESY) and the 1D Homonuclear decoupling experiment (usually called HOMODEC). Both of these experiments have 2D equivalents (2D NOESY and 2D COSY respectively) that can be used for the same information but for small molecules the 1D experiments are often quicker and simpler to interpret. To work effectively both of these experiments require that the signal of interest be well resolved from all other signals, ie it cannot be overlapped with other peaks or the result will be ambiguous.

Nuclear Overhauser Effect spectroscopy (NOESY) is an NMR technique that allows the identification of nuclei that close to each other in space regardless of the presence or absence of intervening bonds. The internuclear NMR signals (or NOEs) arise from the dipolar coupling interactions between nuclei. The intensity of the NOEs decrease rapidly as the internuclear distance increases resulting in a maximum detectable range of less than 5Å. NOEs can be useful in determining local molecular structure when other techniques give ambiguous results. The NOE signals also arise during exchange processes (EXSY) and can be used to measure the presence of exchange, rates of exchange phenomena and to determine interfaces of intermolecular complexes.

In many cases NOESY experiments are run as 2

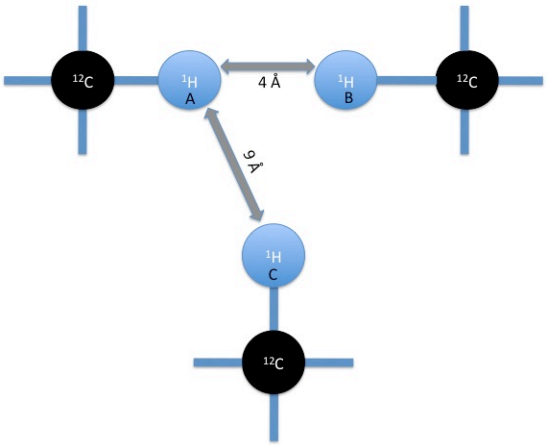


Figure 2: ^{1}H'S A and B will show an inter-nuclear NOE peak since they are separated by only 4Å while A and C will not because they are too far apart.

dimensional NMR spectra where all of the short range interactions in a molecule can be observed simultaneously in 2D plot. However, in the cases where it is only necessary to know the interaction with a single peak and if that peak is resolved from surrounding residues then it is often simpler to run the 1D Gradient Selective NOESY.

The GOESY experiment consists of two main parts, an initial gradient spin echo segment that selects the peak of interest followed by a mixing period where the dipolar coupling between the selected nucleus and any other close nuclei builds up in order to produce an observable NOE during the detection period. The graphic diagram for the pulse sequence is shown in Figure 3. As discussed in a previous newsletter the spin echo, which consists of a 90° pulse

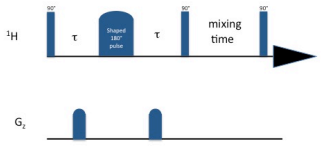


Figure 3: Pulse sequence for GOESY experiment, the selective pulses (180° sel.) refocus only a narrow region while the gradients (Gz) dephase any remaining signals before the NOEs evolve in the final delays, δ.

followed by a delay period τ, a 180° pulse and another equal delay τ, is a standard NMR pulse sequence element that refocuses the signal at the end of the second τ period. The initial pulse is a non-selective high power 90° pulse that excites all nuclei followed by a Gaussian shaped 180°pulse that selects only for a 100 Hz wide region (0.2 ppm on the 500 MHz). Any signal in that 0.2 ppm region will be refocused by the echo while all other signals will not. The pulsed field gradients in each of the τ periods serves to efficiently dephase all of the non-refocused signal so that at the 2τ point only the single selected signal is present. At this point the NOE "mixing time" begins so that the signal from the selected nucleus will undergo dipolar coupling with nearby nuclei and produce signals at their chemical shifts. Mixing times can be on the order of 0.2 seconds to 2.0 seconds and may have to be optimized to get the best signal for individual samples. The experiment usually requires the prior acquisition of a ^{1}H 1D data set so that exact frequency of the peak that is to be selected can be observed. This frequency value is then entered as the **o1** parameter in the GOESY experiment so the selective pulse will be centred on the peak of interest. Figure 4 show the 1D 1H spectrum of ethylbenzene in CDCl3 and a close up of the methylene and methyl peak regions that will be explored by using the GOESY experiment.

This experiment has superseded the older 1D NOE Difference Experiment that was used to get this kind of information previously. The older 1D NOE difference experiment worked on a similar principles except that the older instrument lacked pulse field gradients to efficiently remove the unwanted signals and therefore required the acquisition of an additional control spectrum that had to be subtracted from the NOE spectrum and which

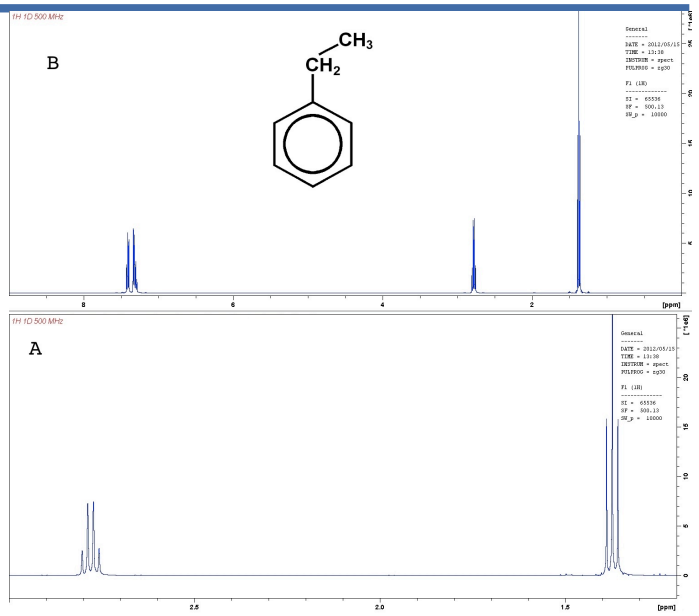


Figure 4: 1D ^{1}H spectrum of ethylbenzene in $CDCl_3$, the methylene group peak at 2.65 ppm selectively pulse in the GOESY and Homodec spectra shown below.

often gave rise to artifacts. The GOESY is quicker and tends to give more unambiguous results than the older experiment.

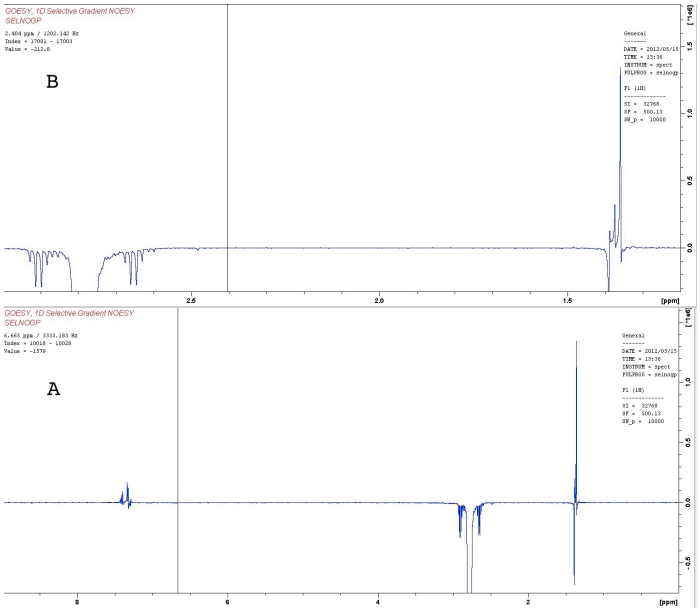


Figure 5: GOESY spectrum of ethylbenzene in $CDCl_3$,.The selectively pulsed methylene group peak at 2.65 ppm shows up as an intense, negative quartet while the methyl group at 1. 2ppm that is close to the methylene is a positive NOE. The more distant aromatic ^{1}Hs produce weaker positive NOE signals.

The second selective 1D experiment is the homonuclear decoupling or HOMODEC experiment. This experiment removes the splitting between a selected ^1H signal and ^1H's scalar coupled to it. Since the splitting due to ^1H scalar coupling can rarely be detected beyond 4 bonds this technique allows quick identification of signals from ^1H's that are close in the molecular structure and aids greatly in assigning the resonances to their source atoms. The 2D COSY family of experiments gives this type of information for all ^1H's in a molecule in a single plot but as with 1D GOESY sometimes this experiment is easier to run, process and interpret.

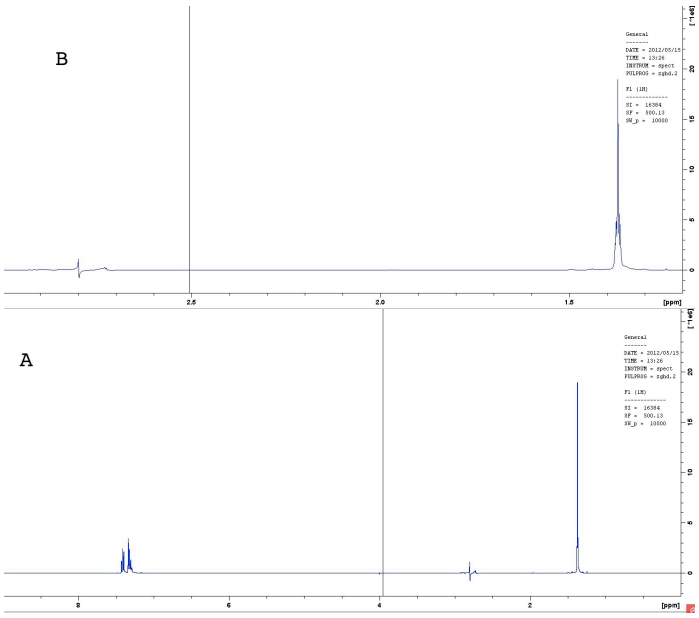


Figure 6: HOMODEC spectrum of ethylbenzene in CDCl3. The selectively decoupled methylene group peak at 2.65 almost disappears while the coupled methyl signal collapses from a triplet to something close to a singlet. The aromatic 1H's show very little difference since the couplings, with at least 4 intervening bonds, are very small.

The selectivity in this case is not accomplished by shaped pulses but by using a very low decoupling power centered on the peak of interest as measured from a standard 1D ^1H experiment. Decoupling refers to a group of specialized NMR techniques that accomplish this removal of splitting due to scalar couplings between nuclei by using series of pulses of varying lengths and phases. In this experiment the decoupler frequency is set by the **o2** parameter. Figure 6 shows the effect of decoupling the methylene peak in ethylbenzene on the methyl peak.