NMR FACILITY NEWSLETTER

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Workshop Announcement : Advanced Topics in NMR

There will be an Advanced NMR Training Workshop held later this spring. Topics that could be covered in this workshop include: advanced 1D techniques like, Solvent Supression, HOMODEC, Selective NOESY, DEPT, DOSY and various 2D experiments such as COSY, TOCSY, NOESY and HMQC. Dr. Schurko is also willing to teach a section on solid state NMR.

To put together a final list of topics I am looking for feedback from faculty, postdocs and students for experiments that you are interested in trying and that would be of value to you in your research.

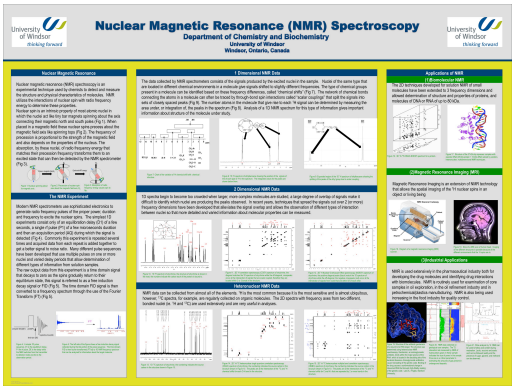
As with the introductory workshop there will be a combination of lecture and hands on portions. I would prefer, where possible, that students bring actual research samples and we will focus on doing the experiments that are of use to them. Time constraints will make it necessary for most of the 2D data collection to be done before the workshop. Processing for 2D data sets will be also covered during the hands on session. This workshop will be open to postdocs, grad students and to interested undergrads that have taken the Introductory Workshop and Chem-330. Enrolment will be limited and preference will be given to individuals who have research samples that are amenable to NMR analysis.For more information on the workshop or to suggest topics, or to just discuss which NMR techniques might be of use to you, stop by the NMR Facility or email me at: mrevingt@uwindsor.ca.

NMR Facility Updates

Topspin V1.3 was installed on the 500 MHz last summer, after a little initial resistance most users seems happy with the program. The upgrade was done in part to allow the instrument to take full advantage of the ^{19}F capabilities of the BBFO probe that was installed in the fall of 2008. ^{19}F data acquisition on the BBFO probe in the 500 MHz is available, however at this point ^{1}H decoupling during ^{19}F acquisition, ^{19}F decoupling during ^{1}H and ^{1}H -^{19}F 2D experiments are still not available. Bruker service personnel are presently consulting with their engineers for a fix.

The 500 MHz was down for 3 weeks due to a failure of the power supply for the AQX portion of the console. Due to the age of the system the replacement part had to be shipped from a secondary supplier in Europe. It has been replaced and is now working well.

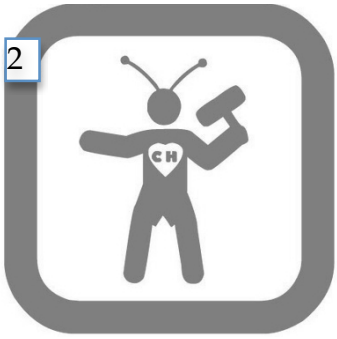
A series of posters explaining NMR, the workings of NMR spectrometers and important advances in the history of NMR are being put up in and around Rm 394-5 EH.



NMR Geek Alert-Contest

Safety in the vicinity of the strong magnetic fields of the superconducting NMR magnets is a serious concern. There are serious health implications for those with pacemakers, magnetic implants and prosthetics. Magnetic storage media and devices (credit cards/cell phones etc) are vulnerable to damage and erasure. In addition, damage to the very expensive magnet vessel itself can occur from the impact of sharp objects drawn by the field. Finally large metal objects (ie gas cylinders, carts) that "interact" with the magnet can disrupt the superconducting conditions for the magnets that results in rapid warming and boiling off of the liquid nitrogen and helium that surrounds the magnet (termed a quench) causing possible asphyxiation of individuals in the room and irreparable damage to the magnet.

Bruker, the maker of the solution NMR spectrometers in our department, in an effort to alert users to these potential problems provides a series of signs to be posted in NMR facilities. Since Bruker NMR's are marketed worldwide these signs usually do not contain text, just somewhat cryptic symbols. I have shown examples of some of their signs below. The contest will be to assign meanings to their symbols. There are two parts to this contest, one prize will be given for the correct answers and one for the most creative. Answers should be emailed to me by 9 am Feb 15, 2010. I am the sole judge of any entries. (Note1: some of these symbols may not be actual Bruker symbols, bonus points for identifying it/them)(Note2: Aaron Rossinni of the Schurko lab won a Toblerone Bar for being the first to provide the correct answers for the NMR crossword puzzle in the last newsletter.)



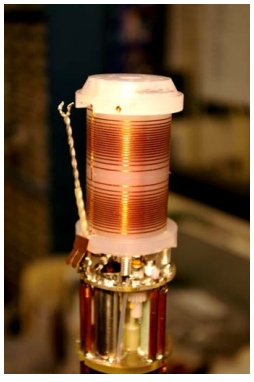
Technical Comment: Pulsed Field Gradients

One of the most useful technical developments for NMR spectrometers in the 1990's was the inclusion of pulsed field gradients (pfgs) in NMR probes and gradient amplifiers in the consoles. These have allowed for selective elimination of solvent signals, elimination of artifacts, clean 2D spectra without the formerly common t1 noise streaks and for the common use of diffusion ordered spectroscopy (DOSY). Pulsed field gradients transiently change the magnetic field strength along the z axis (co-linear with the overall magnetic field of the superconducting magnet) over the length of the sample in a linear, controlled manner. Before I explain how this is helpful for collecting NMR data I will briefly describe how the coil causes this change in the magnetic field experienced by the sample.

Pulsed field gradients are created by an additional coil in the probe outside of the standard coils used for ^{1}H and X nuclei excitation and detection. The pfg coil consists of windings of wire over the entire NMR sample length. This coil is a small electromagnet so that current passing through the coil generates a magnetic field that alters the magnetic field strength encountered in the sample. The maximum density of the windings is at the ends of the sample volume and gradually decreases to zero at the middle (See Fig 1). This configuration of the windings results in a field of greater intensity produced at the ends of the pfg coil at the top and bottom of the sample length whereas the centre of the sample feels only the magnetic field produced by the main NMR magnet. The direction of the winding is reversed above the center of the sample volume from that below the center. This reversal of direction results in a reversal in the orientation of the magnetic field produced by the coil in the two regions so that there is a smooth gradient in field strength (as shown in Fig 2) from the top (in this case positive) to the middle of the sample where it is zero to the bottom of the sample (in this case negative). Gradient pulses produce a characteristic rapid shift in the lock signal observed in the lock window(Fig 3). When gradients are being used in a pulse sequence the lock is put on "hold" during the pulsing and then turned back on during the relaxation delay to prevent loss of the lock.

A simple demonstration of the effects of gradient pulses can be seen using the basic 1D pulse sequence in Figures 4 a, b and c where ability of gradients to defocus and refocus NMR coherences is illustrated.

1A



1B

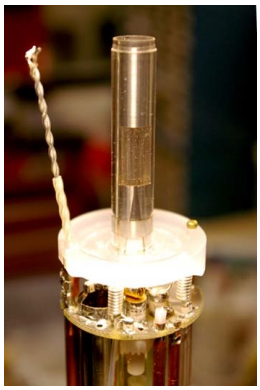


Figure 1. (A)A partially disassembled NMR probe showing the gradient coil. Note the distribution of the windings. (B) Probe with the gradient coil removed showing the inner ^{1}H Helmholtz coil. (From the University of Colorado, Chemistry Department website)

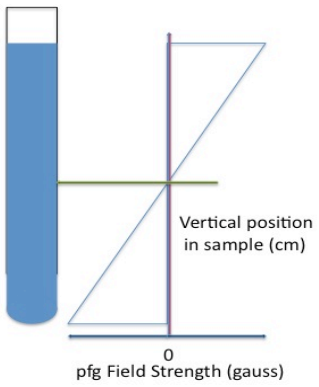


Figure 2. A diagram of the distribution of the pulsed magnetic field strength over an NMR sample produced by a gradient coil like that shown in Fig 1.

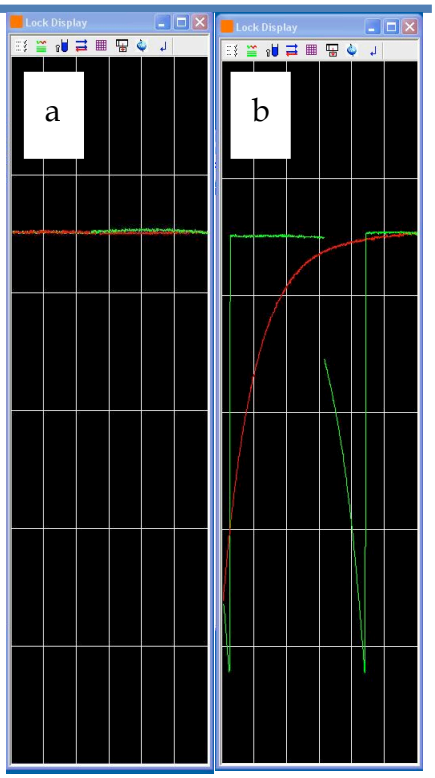


Figure 3. (a) An image of the lock window under normal conditions where the lock signal sweeps back and forth as a horizontal line. (b) An image of the lock window during a gradient experiment, the pronounced dips in the lock signal are periods when the gradients are turned on and field homogeneity is transiently reduced.

A somewhat more practical example is shown in Figure 4 d,e and f where the placement of gradients on either side of a 180° pulse can be used to select for or eliminate desired coherences and to reduce the need for extensive phase cycles to remove artifacts.

Gradient pulses are usually not rectangular, unlike most of the other pulses used in NMR, because the sharp rise and fall of the magnetic field in the rectangular pulses causes eddy currents in the probe that result in distortions in the spectra. Most commonly sine shaped or "smoothed" square pulses are used.

Beyond their role in improving the quality of spectra the pfgs are essential for DOSY experiments. This will be explained in more detail in the following Advanced Techniques section.

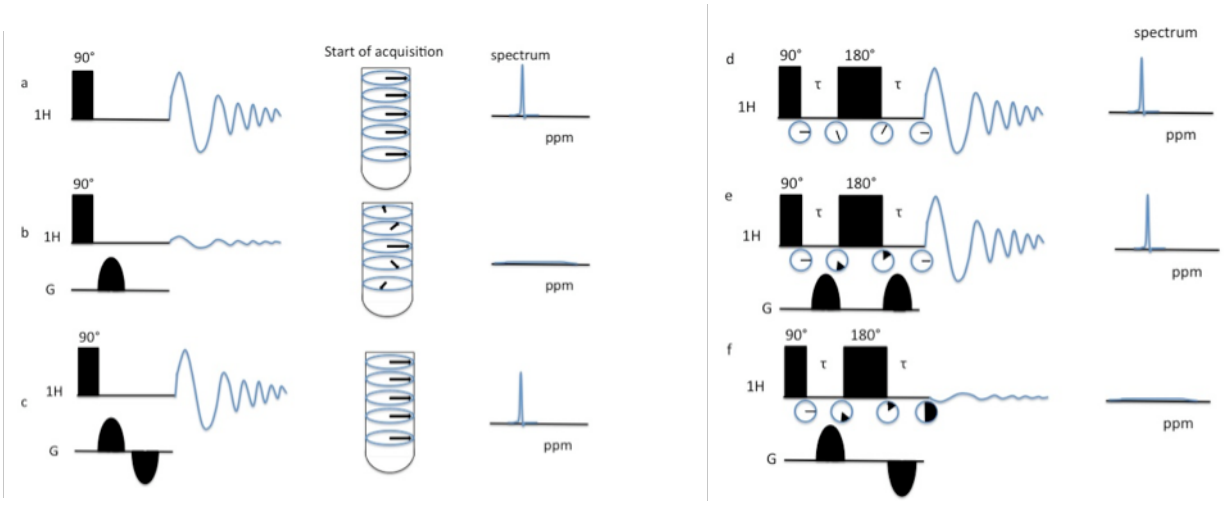


Figure 4. (a) The basic 1D NMR pulse sequence. A 90° pulse flips the magnetization from the z axis into the xy plane where it is initially in phase at the start of acquisition (neglecting evolution that may occur in the period between the pulse and the start of acquisition). The arrows in the diagram of the NMR tube are meant to show the orientation of the spins at the star of the acquisition period. Aligned arrows indicate the spins are rotating together throughout the sample and add together to produce a sharp peak. (b) Insertion of a sine shaped gradient pulse in the period between the 90° pulse and acquisition dephases the spins to a degree that depends on their position in the sample, this spreads out the signal over a big frequency range that produces a very broad peak with a low signal to noise ratio. (c) Application of an additional gradient of equal strength and duration but opposite sign to the first rephases the spins to produce a signal similar to that in (a). (d) The basic spin (or Hahn) echo sequence, the circles beneath pulses illustrate the evolution of a signal from a single resonance, the initial 90° pulse puts the equilibrium z magnetization into the xy plane where it evolves at its chemical shift frequency, after period τ a 180° pulse flips that signal in the plane so that in the second τ it refocuses back to its start position. Because pulse values are never exact the actual output of a spin echo will contain artifacts unless the experiment is repeated 4 times with different pulse and receiver phases (called an EXORCYCLE) and added to cancel artifacts. (e) Introduction of gradient pulses to either side of the 180° pulse results in a initial dephasing as seen in part (b), the 180° pulse works as in (d) and since the spins have been flipped the gradient pulse with the same sign refocuses them and they evolve back to the staring point before acquisition. The difference between (d) and (e) is that the gradients only refocus the desired signals and any artifacts produced by pulse issues are defocused, this eliminates the need to run the 4 step EXORCYCLE to cancel artifacts and actually does a better job than EXORCYLE does. (f) This pulse sequence will selectively eliminate anything that is refocused by the 180° pulse and preserve other signals, often used with a frequency selective 180° to suppress solvent signals.

Advanced Techniques: DOSY

Diffusion ordered spectroscopy, or DOSY, is a NMR technique that has shown an explosive growth in usage over the last few years. DOSY allows the measurement of molecular diffusion rates in the NMR tube, estimation of the hydrodynamic radii of molecules and differentiation between components in a mixture depending on the individual diffusion rates.

DOSY experiments require an instrument equipped with a pulsed field gradient (pfg) probe, in the case of the University of Windsor NMR Facility only the 500 MHz instrument and the 300 MHz Ultrashield have gradients. For an introduction to gradients and some of their other uses see the Technical Comment section of this newsletter.

Particles in solution diffuse due to Brownian motion at rates that are dependent on their mass, volume, the solvent viscosity and temperature. The net rate of translational motion, D, (for a spherical molecule) is described by the simplest form of the Stokes-Einstein equation shown in equation 1.

$$D = \frac{kT}{6\pi\eta R} \qquad [1]$$

Where T is the temperature, k is the Boltzman constant, η is the viscosity of the solvent and R is the radius of the molecule.

The usefulness of pfg's in DOSY experiments arises from their ability to create reproducible, magnetic fields that vary with their position along the NMR tube as shown in Figure 2 in the Technical Comment section. The different magnetic field strengths along that field gradient dephase the spins to different degrees. The basic DOSY experiment is similar to the spin echo pulse sequence shown in Figure 4e in the Technical Comment section. After the spins are moved into the xy plane by the initial 90° pulse they are dephased by a gradient pulse as shown in Figure 4b by a degree that is a function of their position in the NMR tube. The spins are then refocused by 180° pulse and a second identical gradient. If the molecule of interest has not moved during the time between the gradient pulses it will be exactly refocused by the gradient and the intensity of the signal in the spectrum will be maximal, if it has moved the refocusing will be less efficient and the resulting signal will be smaller. The more a molecule moves betweed the gradients the less well it will be refocused. A DOSY data collection consists of a series of 1D experiments with the strength of the gradient increasing for each spectrum. The stronger the gradient pulses the greater the attenuation of the signal for the same amount of translational motion. In theory simply lengthening the time between gradients to allow a greater amount of diffusion could also be done however the spectra would have to be corrected for the greater amount of relaxation that occurred in the delay so in practice it is the gradient strengths that are varied. Figure 5 shows a series of DOSY 1D's with decreasing amplitudes due to increasing gradient strength.

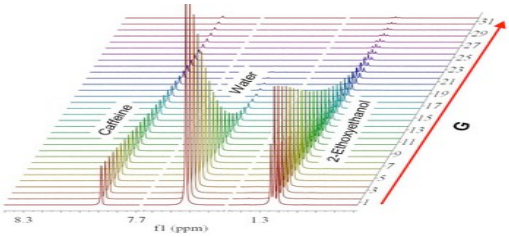
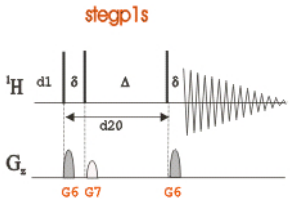


Figure 5. A section of a series of 1D DOSY spectra of a mixture of caffeine, water and 2-ethoxyethanol selected to show 1 peak from each compound. The y axis labeled G is the strength in gauss of the gradient pulses. The peaks show an exponential decrease with increasing gradient strength and each compound shows a different rate of reduction, the smallest molecule, water, decreases fastest because it diffuses at the highest rate while the largest molecule, caffeine decays most slowly due to more restricted diffusion.

A



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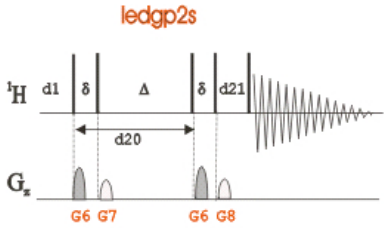

Figure 6.(A) The stimulated echo (STE) DOSY pulse sequence differs from the gradient spin echo in that the 180 ° pulse has been replaced by 2 90° pulses, the result is that magnetization if moved back to the z axis after first gradient pulse where it undergoes T1 relaxation during the long delay Δ that is slower than the T2 relaxation experienced by magnetization in the xy plane. This results in a greater signal to noise ratio for this experiment. (B) The longitudinal eddy current delay (LED) pulse sequence. This sequence is a further modification of the STE sequence that has an extra delay (d21) inserted between the last gradient and the beginning of the signal acquisition to allow any eddy currents developed by the gradients pulses to decay before acquisition begins.

The pulse sequences used in modern DOSY experiments have been improved from the original gradient spin echo sequence to enhance the signal to noise ratio and to eliminate artifacts arising from eddy currents produced by the gradient coils. Figure 6 shows the 2 most common pulse sequences, the stimulated echo (STE) sequence and the longitudinal eddy current delay (LED) sequence.

To get the best DOSY data the user needs to first find the range of gradient power levels that provide a wide range of intensities of the peaks of interest. Generally ranges of 5 to 95% or 10 to 90% are sought after (it is best to stay away from possible non-linear values at the upper and lower power limits). If the signal intensity has been reduced by a factor of 10 between the highest and lowest level then sufficient decay will be present to provide a good estimate of the diffusion rates. If it has not has decreased to this extent then the length of the delay Δ and/or the lengths of the gradient pulses can be increased in steps until the proper level of attenuation is achieved.

Using the Bruker TOPSPIN or XWINNMR programs there are a series of macros that guide the user through the set up of the pseudo 2D DOSY experiment. The user inputs the upper and lower gradient power level and the number of increments between those levels and then starts the data collection for the multiple experiments automatically.

Once the data collection is complete the ^{1}H dimension of all of the 1D FIDs are fourier transformed with the command xf2 and a spectrum like that in Figure 5 is produced.

The diffusion rates are determined by fitting a curve to the intensities of the peaks using equation 2

$$I = I_0 \, e^{-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)} \qquad [2]$$

where I is the observed intensity, I_0 the reference intensity (initial signal intensity), D the diffusion coefficient, γ the gyromagnetic ratio of the observed nucleus, g the gradient strength, δ the length of the gradient, and Δ the diffusion time. This fitting is done by another macro and the final output is a spectrum that looks like Figure 7 with the ^{1}H chemical shift on the x axis and the log of the diffusion coefficient (in m^2/s) on the y axis.

If quantitative, reproducible values are desired then the DOSY experiments should be set up with long interscan delays (ie d1= 10 sec or more) and with the temperature controlled.

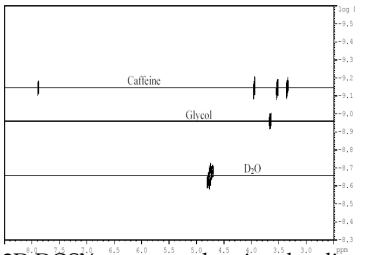

Figure 7. A pseudo 2D DOSY spectrum showing the alignment of the 1H peaks on x axis with the $\log_{10}$ in m^2/sec of the diffusion coefficients on the y axis for a series of components.