THE
NMR
NEWSLETTER

No. 467
August 1997

PTS
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	Frequency Range	Resolution	Switching Time[1]	Phase-Continuous Switching[2]	Rack-Mount Cabinet Dim.[3]	Remote-Control Interface	Price Example[4]
PTS 040	.1-40 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$5,330.00 (1 Hz resol., OCXO freq. std.)
PTS 120	90-120 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$5,330.00 (1 Hz resol., OCXO freq. std.)
PTS 160	.1-160 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$6,495.00 (1 Hz resol., OCXO freq. std.)
PTS 250	1-250 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$7,440.00 (1 Hz resol., OCXO freq. std.)
Type 1 PTS 310 Type 2	.1-310 MHz	1 Hz	1-20μs	standard	3½"H × 19"W	BCD (std) or GPIB (opt)	1 Hz resol., OCXO: $6,425.00 1 Hz resol., OCXO: $5,850.00
PTS 500	1-500 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$8,720.00 (1 Hz resol., OCXO freq. std.)
PTS 620	1-620 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$9,625.00 (1 Hz resol., OCXO freq. std.)
PTS 1000	0.1-1000 MHz	optional .1 Hz to 100 KHz	5-10μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$11,830.00 (1 Hz resol., OCXO freq. std.)
PTS 3200	1-3200 MHz	1 Hz	1-20μs	optional	5¼"H × 19"W	BCD (std) or GPIB (opt)	$14,850.00 (1 Hz resol., OCXO freq. std.)
PTS x10	user specified 10 MHz decade	1 Hz	1-5μs	standard	3½"H × 19"W	BCD (std) or GPIB (opt)	$3,000.00 (1 Hz resol., OCXO freq. std.)
PTS D310	two channels .1-310 MHz	.1 Hz	1-20μs	standard	5¼"H × 19"W	BCD (std) or GPIB (opt)	$8,560.00 (.1 Hz resol., OCXO freq. std.)
PTS D620	two channels 1-620 MHz	.1 Hz/.2 Hz	1-20 μs	standard	5¼"H × 19"W	BCD (std) or GPIB (opt)	$13,240.00 (.1 Hz/.2 Hz resol., OCXO freq. std.)

1 Switching Time is dependent on digit (decade) switched; see detailed instrument specifications.

2 For applicable digits, see detailed instrument specifications.

3 Bench cabinets are 17" wide.

4 Prices are U.S. only and include Manual and Remote (BCD) Control; PTS 3200 Digital Front Panel.

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FORTHCOMING NMR MEETINGS

Additional listings of meetings, etc., are invited.

mayo

July 22, 1997 (received 7/24/97)

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**Department of Biochemistry
and Molecular Biology**

NOESY in Partially Deuterated Macromolecules: Proton Dilution Improves Build-Up but Obliterates Full Matrix Analysis

Dear Barry:

It is evident that a random partial deuteration of larger macromolecules increases the transparency of their NMR spectra facilitating the structure determination of molecules with ever increasing molecular weights. In that regard, we have analyzed the influence of partial uniform deuteration on the quantitative analysis of cross-relaxation spectra. It is not surprising that full matrix analysis begins to fail at a moderate mixing time even with modest degree of deuteration. This is due to the fact that the experimentally obtained spectrum $<A(\tau_m) A(0)^{-1}>$ is actually the ensemble average of a spectra of molecules with a different distribution of protons, and it does not yield an average cross-relaxation matrix $<L>$, i.e.

$$\left\langle A(\tau_m)A(0)^{-1}\right\rangle = \left\langle e^{L^k \tau_m}\right\rangle \neq e^{\langle L^k\rangle \tau_m} \; . \qquad [1]$$

On the other hand, from the Taylor expansion of the above equality follows

$$\frac{A_{ij}(\tau_m)}{A_{ij}(0)} = \delta_{ij} + \tau_m p_{ij} L_{ij}^0 + \frac{\tau_m^2}{2}\sum_k p_{ikj} L_{ik}^0 L_{kj}^0 + \frac{\tau_m^3}{6}\sum_{k,l} p_{iklj} L_{ik}^0 L_{kl}^0 L_{lj}^0 + \dots \; . \quad [2]$$

where p_{ikj} is the conditional probability that there are protons at sites i and j and k. For a random deuteration $p_{ikj} = p_i \, p_j \, p_k$, where p_i, p_j and p_k are the probabilities of finding a proton at sites i, j and k respectively. As usual, τ_m is the mixing time, L_{ij} the cross-relaxation rate constant, and δ_{ij} the Kronecker delta. With an increasing degree of deuteration, the conditional probabilities decrease faster than the proton concentration (which is proportional to p_i);

hence, the contribution of the higher order terms decreases with the degree of deuteration. In other words, the increased degree of random deuteration improves the build-up rate analysis but obliterates full matrix analysis. This is illustrated in the figure which shows the simulated relative error in cross-relaxation rates as a function of mixing time and degree of deuteration. The cross-relaxations are simulated for a ten spin system, cyclo-(L-Pro-Gly), τ_c = 3.8 ns, with uniform random deuteration from 1% to 90%. The continuous curves show the deviation of FMA calculated cross-relaxation rates from the model values in the range of 0% to 100%. Apparently, the deviation increases with the increase of the mixing time and the degree of deuteration, according to Eq. [2]. The dashed line shows how 20% error limit drifts toward shorter mixing times with an increased degree of deuteration. The simulations are performed by averaging the NOESY spectra of $3 \cdot 10^5$ molecules with a random distribution of deuterons for the given concentration. A detailed analysis of the optimal method for quantifying NOESY spectra in partially deuterated systems will be presented soon.
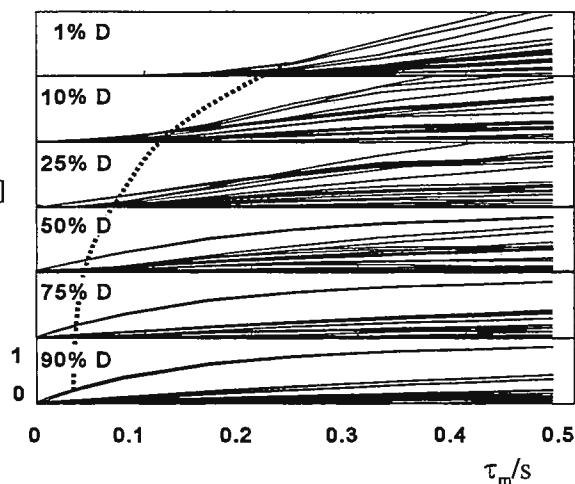
Sincerely yours,

Zsolt Zolnai,

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Nenad Juranić,

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Slobodan Macura

macura@mayo.edu

When you need problem-solving power in an NMR probe,

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Probe Design Factor	Experimental Benefit
• Lineshape	Excellent water suppression. High spectral resolution. High sensitivity – reduced experiment time.
• RF homogeneity	High sensitivity in multiple-pulse, n-D experiements – reduced experiment time.
• Sensitivity	Reduced experiment time.
• Sample size	Highest sensitivity for the available sample volume.
• Pulse width and salt tolerance	Wide excitation bandwidth. Minimal sample heating during decoupling. Minimal losses due to sample salt concentration.
• RF power capacity	Simultaneous multiple-spin lock, multiple-channel 90° pulse, lock and deuterium decoupling.
• Gradient recovery, strength, linearity	Excellent water suppression and quantum coherence selection. Efficient signal dephasing and diffusion-based filtering.
• Variable temperature	Rapid sample temperature equilibration. Reduced sample temperature gradients. Stable sample temperature.

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Using MORASS and GRASP to View NOE Restraints During NMR Structural Refinement

The program **MORASS** (Multispin Overhauser Relaxation AnalysiS and Simulation) analyzes 2D NOESY data to evaluate cross-relaxation rates, σ_{ij}, from which interproton distances can be obtained. MORASS also calculates a NOESY spectrum given a model structure including proton coordinate positions. These calculations are carried out by matrix methods involving the relaxation rate matrix specified by the set of simultaneous Bloch equations. The accuracy of the matrix method in the presence of experimental error for determing distances has been examined. These studies and other details of MORASS have been previously reported (Post, Meadows and Gorenstein, *J. Am. Chem. Soc.*, **112**, 6796 (1990)). MORASS is available free to academic institutions at http://www.nmr.utmb.edu/.

Development of MORASS was motivated largely by a need to analyze NOESY data measured from proteins and oligonucleotides. Although application of MORASS is not limited to these macromolecules, certain features characteristic to them have been adopted. For example, certain standard atom names have been "hardwired" into MORASS to facilitate recognition of specific classes of protons such as deuterium exchangeable protons or methyl protons.

Several new features were recently added to MORASS. One particularly interesting new feature is the ability to produce output for the color-coded display of NOESY restraints by the molecular graphics routine GRASP. This provides GRASP with the necessary information to simultaneously display NOESY distance violations superimposed on a .pdb molecular image using GRASP's "pair-wise interaction" capability. For this purpose, the distance violations are defined as the difference between the NOESY restraint values calculated (simulated) by MORASS from the current iteration of the molecular model (a .pdb file) and the experimental values obtained from the NMR spectra. Using this difference, each violation is assigned a color group and the width of its cylinder is calculated. The NOESY violations are then displayed as color-coded cylinders between the NOE proton pairs with both the color of the cylinder and its diameter are adjusted according to the magnitude of the violation. Cylinder widths are smoothly graduated rather than stepped so that relative differences within the same color group are visible. The user can actually watch the molecule adjust to the NOE restraints after each iteration and see the restraint violations color-coded onto the molecular graphics display. Thus, a fat red cylinder quickly identifies a major violation between the experimentally determined value of the distance calculated from the NOE volume and the distance between the proton pair as shown on the current iteration of the partially equilibrated model. Likewise, skinny green cylinders denote good agreement between the model and the experimental value.

One quickly develops a "feel" for this sort of visualization and we find it very valuable for building intuition during a structural determination. For instance, after several iterations of restrained MD you can visually pick out "problem" protons where most of the violations attached to it are small and well-behaved except for one or two which refuse to equilibrate to their experimental values. This immediately signals some sort of internal inconsistancy and usually sends us back to the spectra to check for anomolous integrations, overlaps, etc. These are vastly easier to detect this way rather than by combing through columns of computer-generated numbers.

Thus we find that the MORASS-GRASP method of visually evaluating our progress during a restrained-MD NOE structure refinement significantly speeds up the refinement process by readily cueing anomolies and inconsistencies. At the same time it greatly enhances the development of our intuition with regard to the emerging structure and facilitates communication with co-workers during the process. It is also just plain more fun than any other way we've found of doing it, too. What more could you ask for?
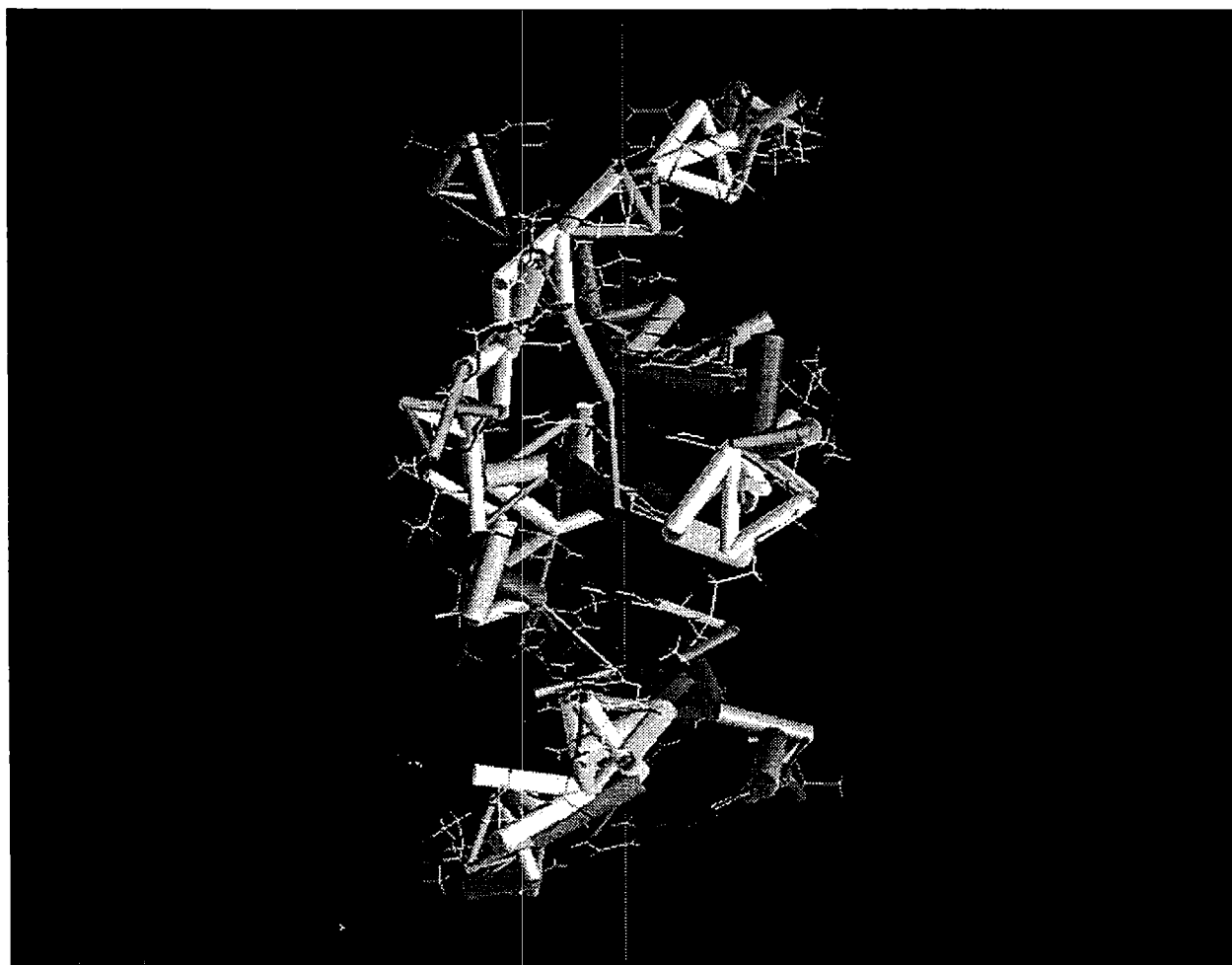
MORASS

Detailed information on how to get MORASS and how to make NOE displays in GRASP are available at our NMR website http://www.nmr.utmb.edu/.

C.B. Post, R. Meadows, B.A. Luxon and D.G. Gorenstein, MORASS 2.3 (1996)

GRASP Graphics Display Package

The GRASP graphics display package was developed by Anthony Nicholls *et al* while in Barry Honig's research group at Columbia in the Department of Biochemistry and Molecular Biophysics:

Anthony Nicholls, Kim Sharp and Barry Honig, *PROTEINS, Structure, Function and Genetics*, **Vol. 11**, No.4, pg. 281ff (1991)

GRASP has a rich variety of capabilities and is especially well-suited for the purpose of examining surface phenomena and electrostatic potentials. GRASP is available from ftp://128.59.96.103/grasp/.

Bruce A. Luxon, Ph.D. and David G. Gorenstein, Ph.D.

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June 23, 1997
(received 7/5/97)

Dr. B. L. Shapiro
The NMR Newsletter
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Dear Barry:

Your *ultimata* have made it to Sweden! However, by the time that this gets printed I will probably be home in Missouri. Thus, I couldn't resist putting both logos on this paper. I have been here since January and can say that it never really gets cold here, but it never really gets hot either. Anyway, we have been studying ^{2}H NMR of adsorbed polymers on surfaces.

Shown in the figure are spectra of poly(methyl acrylate)-d_3, (PMA-d_3) at 75 °C on silica. The polymer was deuterated in the methyl group. The spectra are labelled relative to the amount adsorbed (Am) from a toluene solution. The spectra were taken at the silica-polymer-air interface (i.e. the solvent was removed and the samples were dry).

It can be observed in the figure that the breath of the spectra, hence the mobility of the polymer, varied greatly as a function of the adsorbed amount. Compared to bulk, where all of the polymer molecules had fairly rapid backbone motions resulting in a narrow spectrum, the adsorbed polymers showed multicomponent behavior with many rigid segments showing up as a broad component (residual Pake pattern). The amount of this broad component increased with decreasing adsorbed amounts.

Warmest Regards,

Frank D. Blum
Curators' Professor of Chemistry and Senior Investigator, Materials Research Center

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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July 3, 1997
(received 7/5/97)

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Accounting for incomplete relaxation in NOESY data of nucleic acid samples

Structure determination of most biomacromolecules relies heavily on semiquantitative or quantitative interpretation of NOE crosspeak intensities. For the latter, which involves complete relaxation matix methods, it is important to obtain distances and their error bounds as accurately as possible. This requires that the recycling delay of the NOESY experiment should be long enough that the longitudinal magnetization of the protons is fully recovered. For short DNA duplexes, this can be accomplished by using recycling delays of over 10 sec, which is at least three times the longest T_1-relaxation time. However, for larger RNA samples where T_1-relaxation times are significantly larger at least for certain groups of protons, sufficiently large recycling delays would make 2D NMR virtually impossible; e.g., for a 28mer RNA , our measured T_1-relaxation times ranged from 6-9 sec, which would require delays of over 20 sec between pulsing. Instead, for practical reasons, much shorter relaxation delays (1 - 4 s) are common in RNA NMR, rendering NOE crosspeaks that suffer from seriously incomplete relaxation, which is manifest in different intensities for the NOE crosspeaks for the same poton pair above and below the diagonal.

However, it is possible to correct for the effects of truncated relaxation when the actual T_1-values and the recycle delay are known (1-4). This approach, however, requires that accurate T_1 values are available for individual protons, which might be an obstacle in the case of macromolecules. Another possibility to correct for partial relaxation effects utilizes the ratio between above- and below-diagonal crosspeak intensities. (for details of this approach see (4)). Both correction procedures have been implemented in our program SYMM (4), which we have used for the correction of the NOESY data (mixing times 150, 200, 400ms) of a 28mer RNA, which had been acquired with a typical, short repetition delay of 2.5 sec.

The effect of partial relaxation in NOE intensities was quite obvious when we compared the complete relaxation matrix derived distances, determined with the program MARDIGRAS (5) for the original NOESY data and the SYMM-corrected data. No significant differences were obtained when the two different correction procedures implemented in SYMM were used (measured T_1 values or utilizing intensities ratios for above- and below-diagonal peaks). However, the method with direct T_1-values is limited by the fact that not all values are experimentally available. The other approach works best when the crosspeak intensity difference is large and it is dominated by partial relaxation and not by other sources of error. This, of course, can be a serious limitation for weak peaks where the intensity differences are more due to noise. For the 28mer RNA, up to approximately 500 NOE crosspeaks were integrated, of which 5 -8 % appeared only on one side of the diagonal and 20- 25% had such low intensities such that the correction procedure is not reliable. On a practical level, one can apply both methods together and merge the results, taking care that proper normalization is performed.

The effect of the partial relaxation correction appeared to be quite different for different groups of distances. The most dramatic effect was observed for the distances involving protons with longer T_1 values, namely adenine H2 protons. For example, for the three H2-H2 distances corrected distances were 2 - 3 Å shorter compared to using intensities without correction. In this case, the distance restraints would have been shifted to the unreasonable range of $\approx$ 6 -8 Å. The figure below depicts the distribution of the lower distance bounds for the group of restraints involving H2 (left) and H8 protons (right). For the restraints involving H2 protons, the lower bounds are shifted dramatically toward shorter distances which also led to a small decrease in the restraint widths. Furthermore, during the MARDIGRAS calculations, a number of the very small, original intensities is rejected by the program since the unreasonably small values cause convergence problems. All these distances could be recovered with the partial relaxation correction. Even for a group of restraints involving mostly protons with shorter T_1 values, e.g., the peaks involving H8 protons, the intensity correction has a clear effect although it is less pronounced effect.

Obviously, these effects are large enough to make it mandatory to correct NOE intensities before taking the ensuing complete relaxation matrix-derived distance restraints into structure refinement calculations. Naturally, this step is less important when NOE intensities are used only in a semiquantitative manner, where they are grouped in a few relative categories of, e.g., weak, medium and large. However, in the case of the 28mer, a significant number of peaks involving H2 protons would have been placed in the wrong category. Since distance restraints involving H2 protons are very powerful in structure refinement as they represent sparse cross-strand information, structural artifacts would have been inevitable.
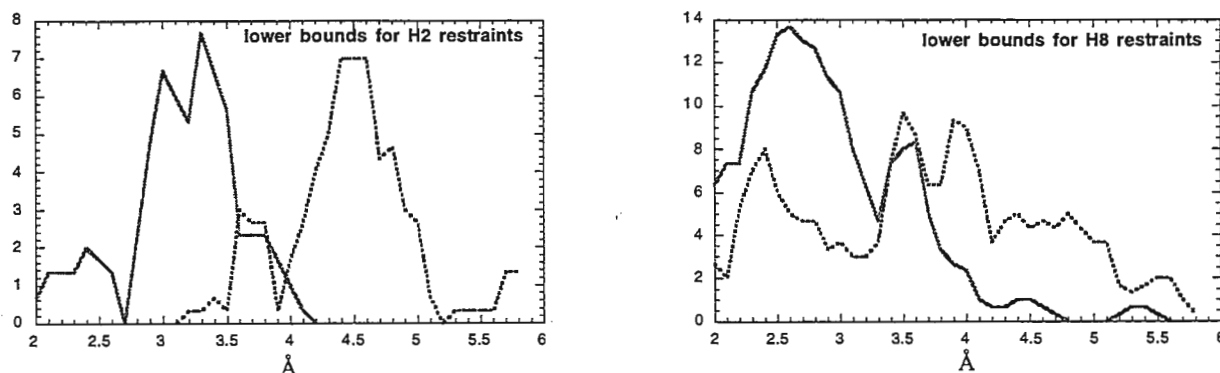
Figure 1. Effect of partial relaxation in 2D NOE spectra on the complete relaxation matrix-derived distances, shown for the lower distance bounds of H2 (left) and H8 (right) involving distances. (Results for corrected intensities, solid; partial relaxation intensities, dotted)

Sincerely,

Uli Schmitz   Peter Lukavsky   Tom James

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2. Kock, M., and C. Griesinger. 1994. *Angew. Chem. -Int. Edt.* 33:332-334.
3. Zhu, L., and B. R. Reid. 1995.*J. Magn. Reson. B.* 106:227-35.
4. Liu, H., M. Tonelli, and T. James. 1996. *J. Magn. Reson. Ser. B.* 111:85-9.
5. Liu, H., B. Borgias, A. Kumar, and T. L. James. 1990, 1994. MARDIGRAS. University of California, San Francisco.

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Dr. B.L. Shapiro
The NMR Newsletter
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June 30, 1997
(received 7/5/97)

What You See in NMRI is Not Always What You Get

Dear Barry,

In a previous letter we talked about some work we are doing at WRI as part of our
FHWA Contract on the use of NMR imaging to study rubber modified asphalts.
This work was initially prompted by the Intermodal Surface Transportation
Efficiency Act (ISTEA), passed by Congress in 1991, which mandated the use of
rubber from scrap tires in Federally funded roadway construction projects. The
rationale for the mandate is easily understood when considering that the United
States discards more than 300 million tires each year and that a large portion of
these end up in stockpiles, which may create environmental problems and health
hazards.

When the mandate was imposed systematic studies concerned with the effects of
such variables as asphalt type, rubber type, particle size, mixing time and
temperature on the asphalt-rubber rheological properties, as well as handling,
safety and health effects of scrap tires had not been carried out. Questions about
what happens when complex materials such as tires and asphalts are blended at
mix temperatures (~170 °C) had not been satisfactorily addressed. For example,
does the rubber dissolve in the asphalt, does it swell in the asphalt, do volatile
components(extender oils, plasticizers) in the rubber migrate into the asphalt, and
how might these reactions influence the rheological properties of the mixture?

The mandate was rescinded in 1995; however, there is still sufficient interest to
more fully understand the compatibility and physicochemical interactions of asphalt
and crumb rubber materials. As part of our FHWA Contract WRI has been
investigating the use of NMR imaging to look into some of these questions.

In one of our experiments we placed pieces of natural and styrene butadiene tire
rubber in 23 mm OD glass vials, and covered them with asphalt. The samples were
then heated at 170 °C (roadway mix temperatures) for varying periods of time, then
imaged while they cooled and after they were at room temperature. The top three
images in Figure 1 are of a piece of natural tire rubber in asphalt, imaged before
heating (left), during cooling (middle) and at room temperature (right) after the
sample was heated for 2 days at 170 °C. The bottom set of three images are of a
piece of styrene butadiene tire rubber in asphalt, heated and imaged under the

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same conditions. In both cases, only the 4th slice of an eight slice image set is shown. This corresponds to a cross section across the center of the sample. Images were acquired at 200 MHZ using a Chemagnetics microimaging probe.

If the images had not been recorded while the sample cooled, one might be tempted to conclude that the sample had dissolved in the asphalt during heating as there is little or no signal remaining at room temperature. However, the middle images show that the tire pieces actually swelled in the presence of the asphalt. We obtain the same results if the room temperature samples are reheated for only 10 minutes and reimaged. The lighter areas of contrast in the middle images are for the asphalt surrounding the rubber pieces. At room temperature asphalt is not imaged because the relaxation times are too short.

Best regards

Fran Miknis

Dan Netzel
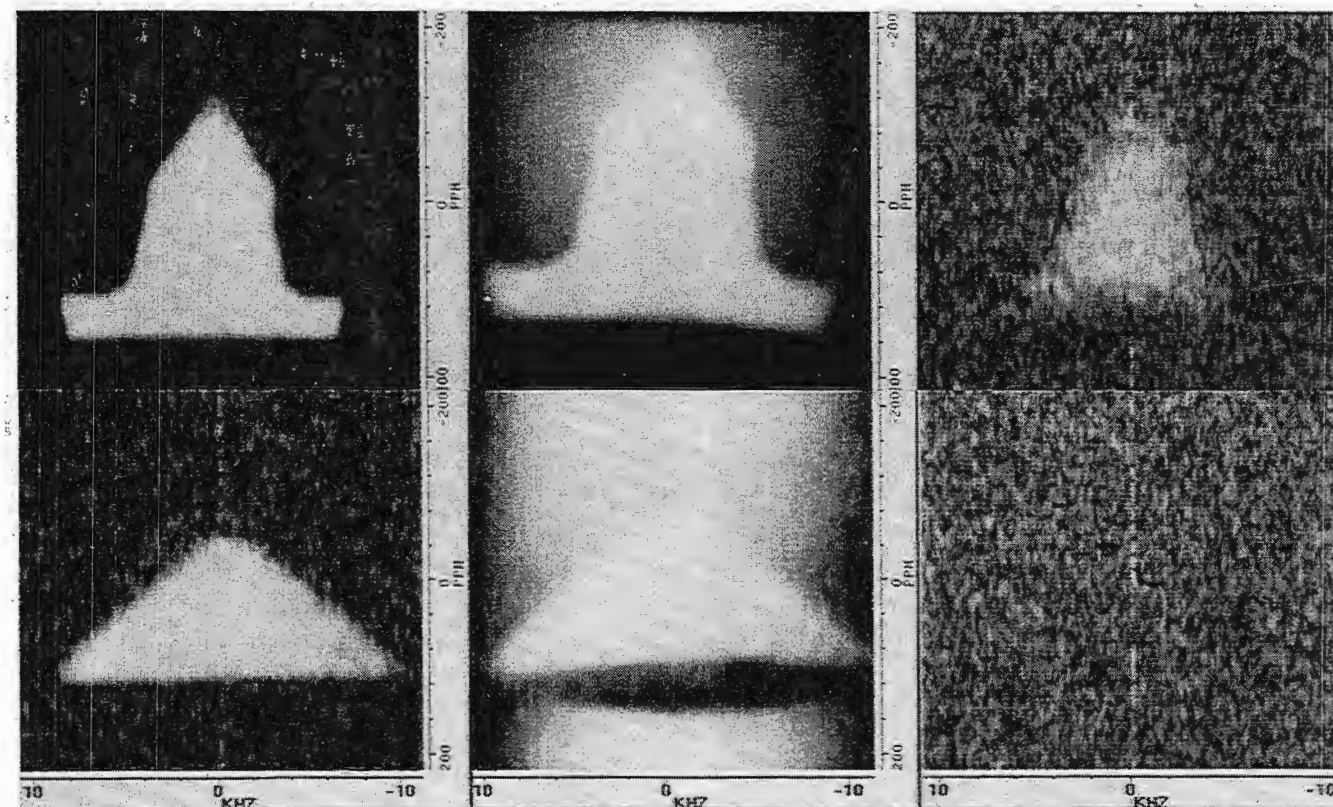
Figure 1. NMR Images of pieces of natural tire rubber (top) and styrene butadiene tire rubber (bottom) in asphalt: (left) before heating, (middle) during cooling and (right) at room temperature after heating for 2 days at 170 °C.

OXFORD

Technical Specifications

200 - 600MHz Narrow Bore NMR Magnet Systems

Oxford Instruments, NMR Instruments are the pioneers of superconducting magnet technology for NMR spectroscopy and are widely regarded by the worlds research community as the foremost manufacturer and supplier. A worldwide base of over 4500 successfully installed magnet systems serves as testament to an innovative magnet design approach and quality manufacturing processes. As an integral part of a high resolution NMR spectrometer, Oxford Instruments magnets are recognized the world over for their superior performance in chemical, pharmaceutical, biological, and materials research applications.

Advanced Design and Manufacture

State of the art superconducting magnet and cryostat designs provide superior NMR performance, as demonstrated in lineshape, resolution, magnetic field stability, and system siting:

- Advanced electromechanical modelling and system design tools optimise magnetic field homogeneity (to at least eighth order, on-axis) while at the same time minimising residual transverse gradients

- Designed for optimal system siting, minimising ceiling height, weight, service access, stray field and system footprints

- High efficiency room temperature shims provide optimal field homogeneity with low heat dissipation

- Rigorous magnet system testing in Oxford Instruments' extensive cryogenic and NMR test facilities ensure that optimal magnet performance is achieved on site

- All systems supplied with a wide range of safety features, providing worry-free operation at all field strengths

- All manufacturing processes certified to the internationally recognised ISO 9001 quality standards

Performance Excellence

Oxford Instruments magnet and room temperature shim systems provide outstanding performance in a complete range of NMR applications. Sample sizes from 37 μl magic-angle-spinning (MAS), 10 mm high-resolution biomolecular to 60 mm microimaging, as well as all other commonly used NMR sample types, are supported:

- Exceptionally low magnetic-field drift rates provide superior stability

- Full range of room temperature shim options for all magnet systems deliver unparalleled lineshape and resolution for both large and small samples

- Minimal cryogen usage provides the lowest possible cost of operation with a range of standard or year hold helium cryostat options available

- All Oxford Instruments magnets feature a virtually unlimited lifetime (with proper maintenance and cryogen service)

See reverse for full technical specifications

Specifications

Specification / Magnet	System Type								
	200/54		**300/54**		**400/54**	**500/51**		**600/51**	
Operating Field (Tesla)	4.7		7		9.4	11.7		14	
NMR Operating Frequency (MHz¹H)	200		300		400	500		600	
Field Stability (Hz/hour ¹H)	<2		<3		<8	<10		<10	
5 Gauss Stray Field Contour						Standard	Shielded	Standard	Shielded
Axial (Metres)	1.75		2.2		2.8	3.5	1.8	4.0	2.5
Radial (Metres)	1.5		1.7		2.2	2.8	1.3	3.2	1.75
Cryostat	Standard	Compact	Standard	Compact					
Standard Cryostat Minimum Helium Refill Interval (Days)	235	80	235	80	183	150		150	
Standard Cryostat Helium Refill Volume (Litres)	79	26	79	26	62	52		80	
Year Hold Cryostat Option Available	✓	X	✓	X	✓	X		X	
Nitrogen Refill Interval (Days)	14	14	14	14	14	17		18	
Nitrogen Refill Volume (Litres)	61	32	61	32	61	84		131	
Nominal Room Temperature Bore Diameter (mm)	54	54	54	54	54	51		51	
Minimum Operational Ceiling Height (Metres)	2.9	2.5	2.9	2.5	2.9	3.1		3.4	
System Weight (kg) Including Cryogen's	315	120	325	133	380	533		1100	

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NMR with Supercricital Fluids

July 21, 1997
(received 7/24/97)

Dear Dr. Shapiro:

I have started a series of high pressure NMR experiments, with particular emphasis on supercritical fluids comprised of Xe or CO_2. A series of experimental setups have been published previously, each emphasizing very different capabilities; a nice review can be found in [1]. In brief, for very high pressures a hydraulic piston system can be used, as described by Merbach et al.[2]; while pressures of 3 kbar are attainable, inert gas atmospheres can not be utilized. Another approach uses fused silica capillaries that are pressurized up to 1kbar [3]. This setup is very safe because of the low volume ($100\mu m$ ID) of fluid. Unfortunately, I require much larger volumes, and am dealing with heterogeneous mixtures that are unlikely to pass through a capillary. I have decided to follow the example of Christopher Roe from DuPont [4], but have made a few minor modifications which some of your readers may find interesting.
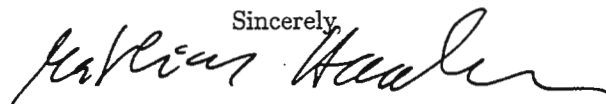
Rather than use a titanium valve, I have constructed a valve using a special alloy of BeCu. It is glued with epoxy (Aremco568) to thick wall pyrex tubes, or large volume sapphire tubes. At first I had great difficulties when sealing, re-opening, and re-sealing my samples because the front seal formed a little grove with the inlet hole, and did not open when I unscrewed the top. The use of Vespel instead of other materials (Teflon, Viton) was a perfect solution to these problems; additionally the Vespel withstands many different chemicals. The valve is double-sealed with a Viton O-ring to establish inert gas conditions while filling the tube. I also added a little inlet screw inside of the BeCu-valve; this enables the transfer of bulky polymer pellets or catalyst support materials which is otherwise not possible (or a real pain). The whole system sits then at our copper transfer line as it is being filled at liquid nitrogen temperature with the gas/liquid mixture of choice. Before and while using a sapphire/pyrex tube, I execute frequent hydraulic pressure checks up to 5000 psi. The worst case scenario that I encountered was the leaking of the Vespel seal, and leaking of the epoxy glue.

To demonstrate my apparatus I show the limitation of the use of Xenon as a probe of morphology in semicrystalline liquid crystalline polymers. Because of the high glass transition temperature (115C) and the low solubility of Xe in Vectra^{TM}, I decided to use supercritical Xenon(SCF) at $\approx$100 bar and 120C (P_c=80 bar, T_c=16C) as an NMR probe of polymer morphology. The figure shows the ^{129}Xe NMR spectra that have been observed at 83MHz. The liquid phase Xenon at -65C shows the characteristic chemical shift of 215 ppm. I detected a volume expansion of the (still) liquid Xenon around 0C, and this was accompanied by a large shift, as shown in the figure. After heating the sample to 50C, I found the relatively broad Xe signal at 39 ppm, which is due to SCF Xenon. Additionally, I observed a very "funky" phase transition behavior, i.e., I detected a very broad lineshape for the transition from the liquid phase to the supercritical phase (not shown). I assume that this broad peak is the result

of "clusterification" in the semi-liquid state; that has not been detected before because other pressure dependent investigations so far were all executed at higher temperatures, i.e., in the gas phase. Anyway, beside these spectra, and the fact that our setup is working very nicely, I was not successful in detecting any other chemical shift value that could be assigned to Xenon incorporated inside the polymer, even though I left the system for a total of 48 hours in SCF Xenon at high temperature, cooled it down later on, always monitoring the xenon shifts in the range from 500 to -500 ppm. I feel that this method has a very limited applicability for polymer systems that are of engineering interest; the setup is, however, well suited to probe catalysis in SCF Xenon or CO_2, and I will continue to investigate those (often heterogeneous) reactions. Additionally, the superior transparency of the sapphire tube in the full UV through IR range of the electromagnetic spectrum facilitates side-by-side investigations of NMR and IR analysis of photochemical processes at variable temperatures and SCF-phases.

Please credit this contribution to the Raychem account.

Sincerely,

Dr. Mathias Haake

Postdoctoral Fellow with Professor Jeffrey A. Reimer

[1] Horvath, I.T.; Millar, J.M. *Chem. Rev.* 91, (1991) 1339-1351.

[2] Frey, U; Helm, L.; Merbach, A. *High Pressure Research* 2, (1990) 237-245.

[3] Yonker, C.R.; Zemanian, T.S.; Wallen, S.L.; Linehan, J.C.; Franz, J.A. *J.Magn. Res.* 113 (1995) 102-107.

[4] Roe, D.C. *J. Magn. Res.* 63, (1985) 388-391.
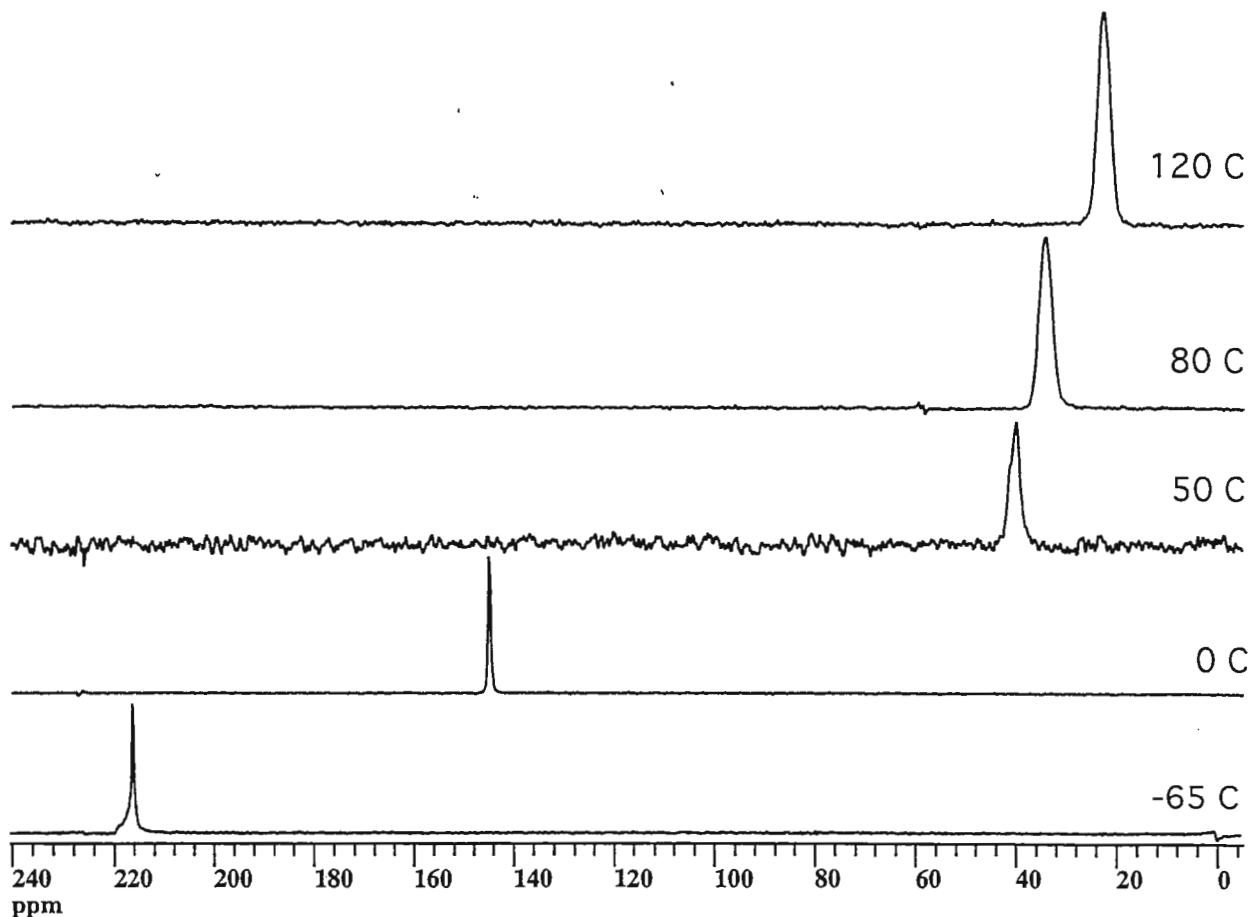
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Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

July 4, 1997
(received 7/8/97)

SPECIFICALLY LABELLED HUMAN TRANSFERRIN

Dear Dr. Shapiro,

As promised, here is our "not-so-timely" latest contribution.

In a collaboration with Prof. Robert Woodworth we have been studying by NMR spectroscopy the 38 kDa N-terminal lobe of an iron transport protein, human serum transferrin. Dr. Woodworth's laboratory specifically labels amino acids believed to be involved in conformational events surrounding iron binding and release in this protein. By supplying specifically labeled essential amino acids to baby hamster kidney cells labels are incorporated at the desired points in the protein. Using this method ^{15}N and/or ^{13}C labeled Met, Phe, Lys, His, Trp, etc. have been introduced into the protein with considerable success.

One such sample, with all Met and Trp residues labeled at the ε-[^{13}C] carbon of Met and the ring-2-[^{13}C] carbon of Trp gives an interesting direct observe ^{13}C spectrum. As well as the expected resonances at approx. 125 and 15 ppm due to the labeled Trp and Met residues, the spectrum reveals a background envelope of ^{13}C resonances. These resonances are most noticeable in the carbonyl and methylene regions of the spectrum (Fig. 1-2). After some discussion, we have concluded these resonances likely arise from catabolism of the ring-2-[^{13}C]Trp, in which the labeled carbon enters the C-1 pool and finds its way into glycine, serine, and other amino acids. Incorporation of ^{13}C labeled Met alone, and of many other specifically labeled amino acids does not result in this occurrence.

Sincerely,

Dr. Barbara A. Lyons

Dr. Robert C. Woodworth

Dr. Anne B. Mason

Dr. Qing-Yu He
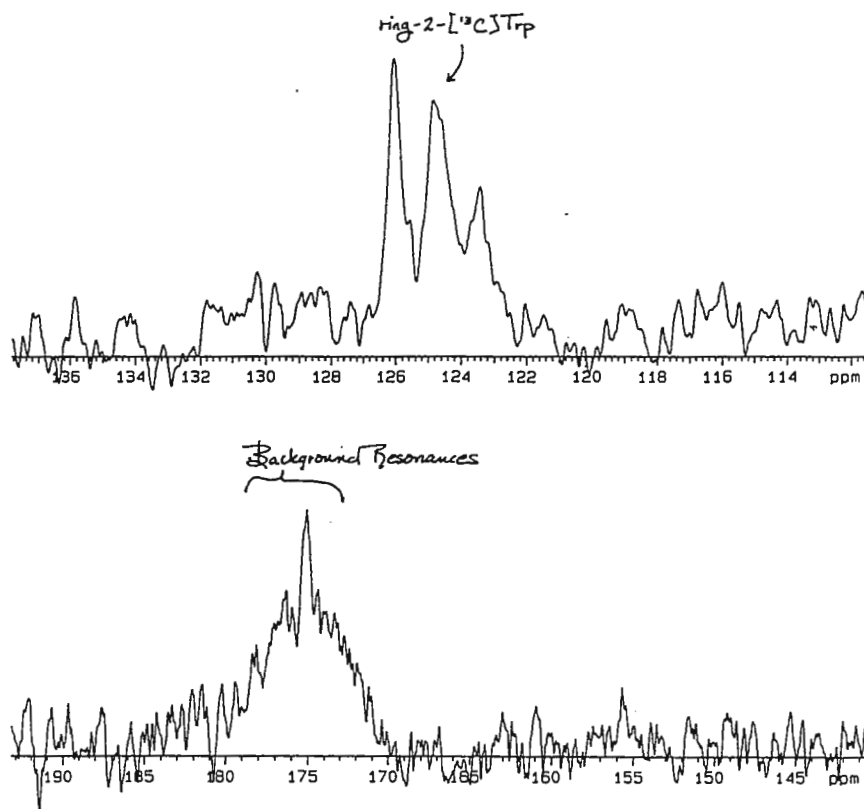
Fig. 1. N-lobe human transferrin
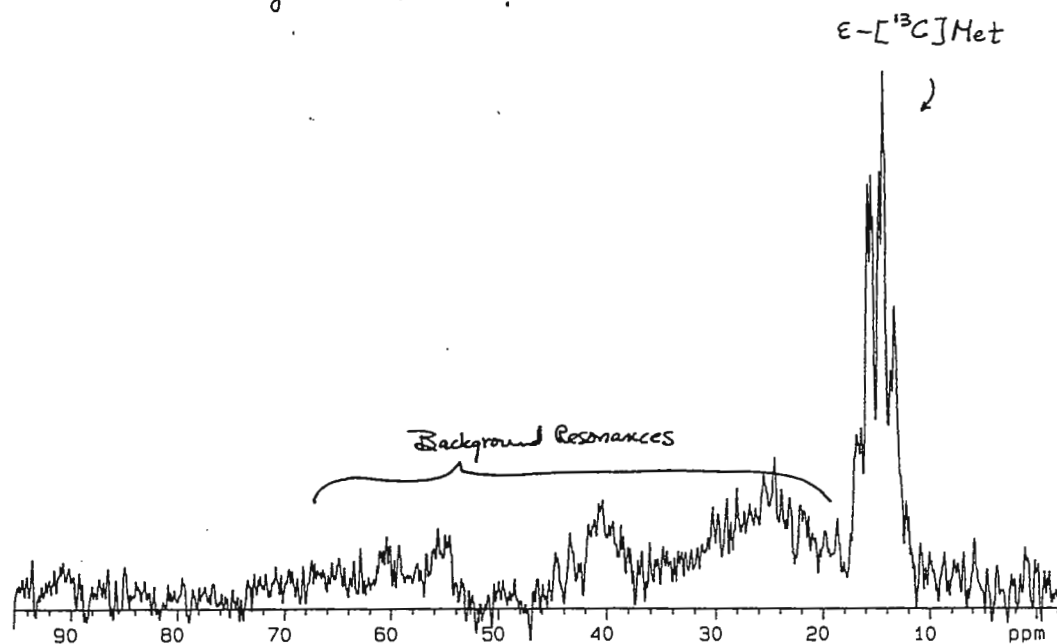
Fig. 2. N-lobe human transferrin

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Linearity (±1 dB to 30 dB		
down from rated power)	1500 W	800 W
Pulse width	20 ms	20 ms
Duty cycle	Up to 10%	Up to 10%
Amplitude droop	5% to 20 ms typ.	5% to 20 ms typ.
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	Third: -24 dBc max.	

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(received 7/23/97)

Dr. B. L. Shapiro, Publisher
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

The Young's Modulus of Aged Organic Materials

Dear Barry,

We have an interest in predicting the performance of organic materials as a function of aging history. While NMR spectroscopy is regularly used to detect chemical changes in a material, the end user of the material is often more interested in the effect of aging on the material's mechanical properties. The Young's modulus of a viscoelastic material is an important parameter used to describe the material's mechanical properties. Unfortunately, in many instances, the size or physical form of a sample prohibits the measurement of its modulus by traditional methods. Moreover, many materials consist of two or more microscopic phases or components, and conventional approaches only measure the overall modulus. We are using cross-polarization experiments to measure the modulus of a variety of organic materials.

A description of the relationship between the Young's modulus and the NMR cross polarization time has been reported by Parker *et al.* [1]. The reported correlation successfully described polymers with various backbone structures, with and without plasticizers, with both single and multiphase components, and at two observation temperatures. The NMR cross polarization experiment is unique in that it measures the individual responses of chemically distinct molecular components as well as the individual responses of distinct phases such as the crystalline and amorphous phases of partially crystalline polymers. This selectivity has the potential to increase our understanding of the relationship between molecular structure and the mechanical behavior of the material.

Figure 1 shows the response of the ^{13}C magnetization for a butadiene/natural rubber copolymer during a cross-polarization experiment. The aged sample had been stored for 44 days in an ambient atmosphere at 110°C. The spectra were recorded on a Bruker AMX-400 spectrometer with a 7mm CPMAS probe. The initial portions of the response curves were fit with a simple exponential function with time constants, T_{cp}, of 0.417 and 0.116 ms for the unaged and aged samples respectively.

Parker *et al* have shown that the Young's modulus is proportional to $(1/T_{cp})^2$, therefore the NMR measurements predict that the modulus of the aged sample is 13 times greater than that of the unaged sample. Measurements using a modulus profiler [2] give a factor of 12. Although this quantitative agreement may be somewhat fortuitous considering the nature of the experiment, the results clearly demonstrate the sensitivity of the cross-polarization experiment to material aging. No signs of chemical changes were visible in the spectrum of the aged sample.

These measurements are having application for two broad classes of viscoelastic materials. First, organic materials are often composed of multiple phases. Examples of such materials are partially

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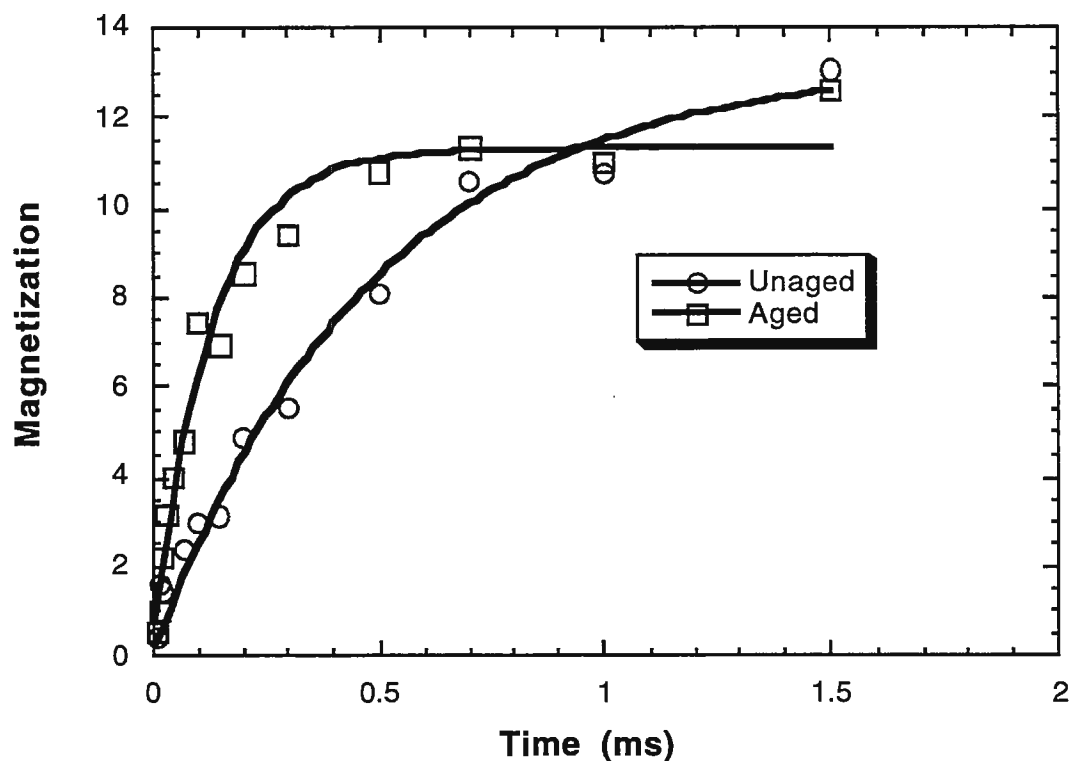
Figure 1. The ^{13}C magnetization response for an unaged and aged sample of butadiene/natural rubber copolymer during a cross-polarization experiment.

crystalline polymers, hard and soft phase polyurethanes, carbon and silica filled elastomers, and glass filled composites. The ability to probe the behavior of each phase individually will improve our understanding of the relationship between a material's mechanical properties and its degradation mechanism. Second, many materials do not have a physical form conducive to traditional modulus measurements. These materials include foams, thin films, coatings and chars which are the result of destructive testing. The cross polarization technique requires only 50 to 100 mg of a powdered sample and measures its intrinsic modulus.

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[2] K. T. Gillen, R. L. Clough and C. A. Quintana, Polym. Deg. Stab., **17**, 31 (1987).

Please credit this contribution to the account of Paul A. Cahill

Sincerely,

Roger A. Assink and Ken T. Gillen
Materials Aging and Reliability: Bulk Properties

A new "twist" on Magic Angle Spinning from Bruker!

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Rotor diameter	4mm	Outer diameter
Rotor Volume	70 uL	Full
Rotor Volume	20 uL	With spacers
Resolution	1.5 Hz	^1H, $CHCl_3$ sample, FWHH
^1H 90° pulse	5 us	100 W
^{13}C 90° pulse	5.5 us	300 W
Gradient Strength	30 G/cm	at 10 A
VT range	-20 to +70 °C	with ceramic rotor cap
Max. Spin Rate	10 kHz	With ZrO rotors

Gradient MAS Heteronuclear Correlation Experiment
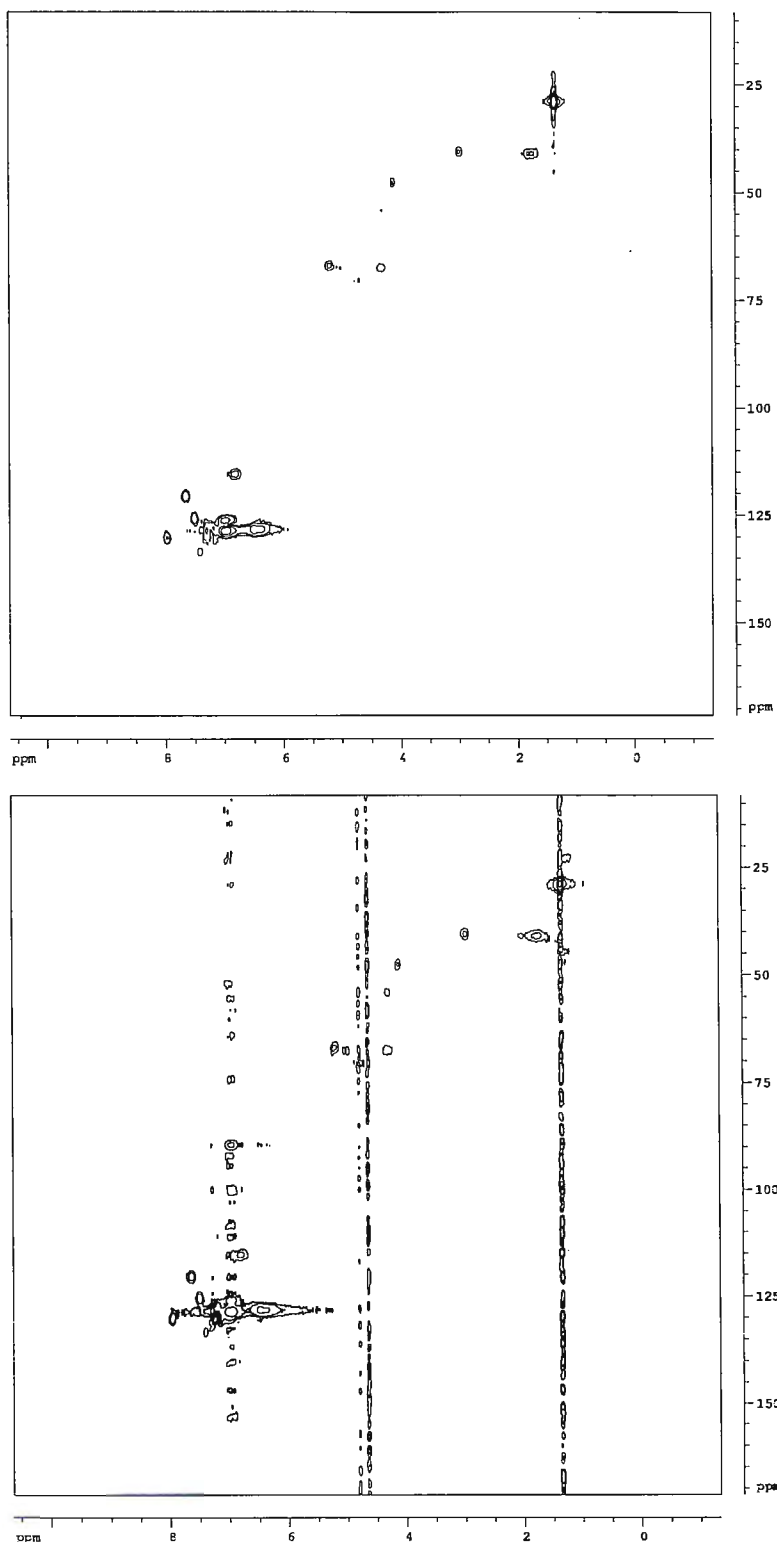
A. ^1H-^{13}C HMQC spectrum of an N-FMOC-N-Boc-L-Lysine derivatized Wang resin swollen with $CDCl_3$, obtained at a proton frequency of 400 MHz and at a spinner frequency of 5 kHz. 1 ms pulsed field gradients were used (with strengths of 10, 10 and 5 G/cm) to select magnetization only from those protons coupled to a ^{13}C. The lower spectrum (B) is a phase cycled version, acquired under identical conditions as the spectrum of figure A. Note the excellent suppression of t_1-noise in the gradient spectrum versus the phase cycled version.

Yong Pan
email: pany@pg.com
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June 30, 1997
(received 7/25/97)

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
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NMR Microimaging, a Useful Tool to Study the Dissolution of Solids

Dear Barry:

Dissolution is an important issue to many consumer products. Factors that affect dissolution include the compound's intrinsic solubility, particle size, porosity, and molecular interactions. The dissolution rate of a solid tablet is normally controlled by one or more of the following steps: 1) water penetration to the tablet dry core, 2) disintegration of the tablet into small fragments or particles, 3) solubilization of the disintegrated particles, and 4) diffusion of the solubilized molecules into the homogeneous dissolution medium. The identification of the rate-controlling step is the key to controlling the release of actives.

Conventional methods for measuring water penetration require physical manipulation of samples, which may introduce considerable errors in measurement. NMR imaging provides a non-invasive method to examine the spatial distribution of mobile spins (protons) in a sample. The intensity in an image voxel reflects intrinsic NMR properties, namely, spin density, spin-lattice relaxation, and spin-spin relaxation. NMR imaging is an ideal tool to monitor a process like dissolution in real time. We have used NMR imaging to study the dissolution of a newly formulated pain reliever tablet.

The new pain reliever contains a sleep-aid in addition to an analgesic as active ingredients. The sleep-aid/analgesic tablets had a much slower release of both actives than the analgesic-only tablets in a standard dissolution test. We performed NMR imaging experiments on both of the tablets using a Bruker MSL-300 spectrometer. The tablet was placed in a 15 mm tube filled with a regular or deuterated dissolution medium at various pH. The images of a transverse plane were recorded every two minutes for four hours using a spin-echo imaging pulse sequence. Figure 1 shows the images of the sleep-aid/analgesic and analgesic-only tablets after 30 minutes dissolution in a regular dissolution medium. The dry core area over time was measured using a macro in the imaging analysis program, Optimas, and the results are plotted in Figure 2.

The results clearly show the water penetration rate of the sleep-aid/analgesic tablets was much faster than that of the analgesic-only tablets. The images in the deuterated medium show that 1) the drug actives in the sleep-aid/analgesic tablet were quickly solubilized; however, the release to the medium was very slow, and 2) the drug active in the analgesic-only tablet was solubilized and then quickly released to the media. A gel-like material appeared in the image of the sleep-aid/analgesic tablet, which might be due to the formation of an ion pair complex. The complex was isolated as a precipitate in a mixture of solution with both actives and identified by GC, IR, and solution NMR. The NMR imaging results suggest that water penetration is not the rate-controlling step in the dissolution of the sleep-aid/analgesic tablets. Rather the drug release rate is controlled by the diffusion of drug actives through the gel matrix.

NMR imaging is an ideal tool to study water penetration and active release in general. In our studies of several other systems, we have identified dissolution-controlling processes as water penetration, gel formation, individual particle solubilization, or disintegration, depending upon the nature and composition of the systems.

Sincerely,

Nicole Black

Todd Vienneau

Yong Pan

Figure 1 NMR Images at 30 Minutes

Analgesic-only   **Sleep-aid/analgesic**
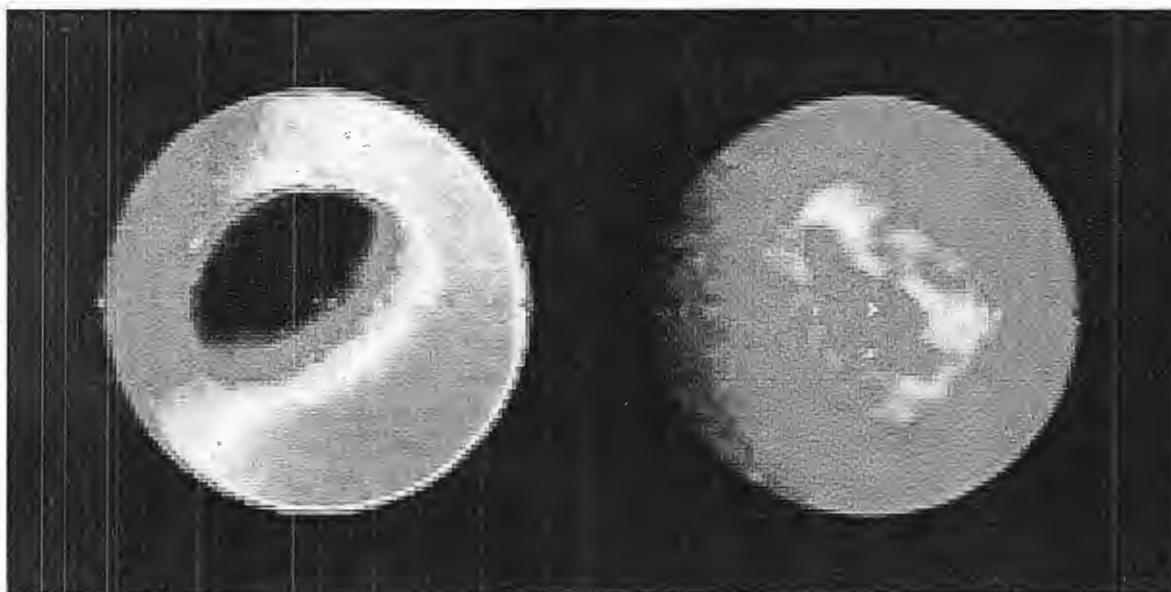
Figure 2 NMRI Dissolution Measurements
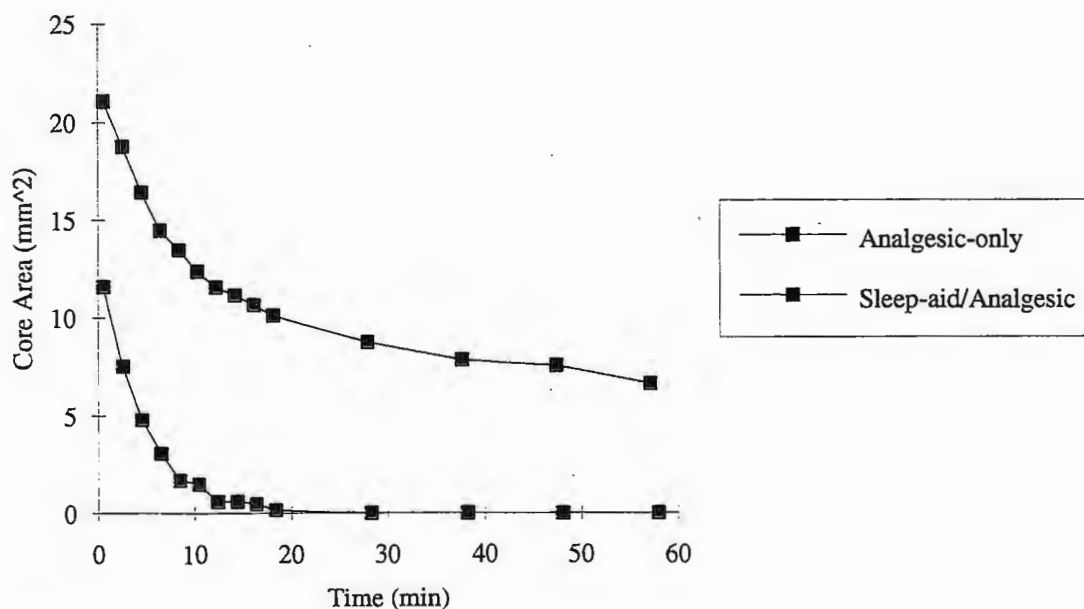
Please credit this contribution to Charlie Eads' account.

Brandeis University

Graduate Department ±15 South Street. MS 009
of Biochemistry   Waltham, Massachusetts
             02254-9110

July 11, 1997 (received 7/16/97)
Dr. B. L. Shapiro:

Win a 4th Channel Free (for deuteron Decoupling)!

Actually its not free, it will cost $1-3K depending on what RF amplifier you have lying around, and the element of chance is whether you can actually make it work.

The only thing that is new is that we generate and switch a Walz-16 modulated deuteron frequency very inexpensively. (See figure.) We use a commercial direct-digital-synthesis (DDS) module to generate a low frequency TTL square wave whose Kth harmonic (where K=9) is picked out by a 76 MHz narrow-band filter, after WALZ modulation. This is passed through a manual rotary attenuator, amplified by an ENI amplifier having 50 DB gain and 10 W output (we use only $\lesssim 2W$) and connected transiently to the lock input of the probe of our AMX 500 after passing through the Bruker notch filters that cut out noise at the other NMR frequencies. Connection is via two "coax" relays (Pasternak Enterprises (phone (714)261-1920), actually they are reed relays in a box), one of which switches the probe to our 4th channel, and the other of which switches the Bruker's lock system to a dummy load. There is a 10 millisec delay in these relays, so the decoupling has to be strobed on 10 ms before it is needed in the pulse sequence. The strobe is a low level produced by our lock "track and hold" circuit provided by Bruker. If you don't have this you have to figure out how to disable your lock system with a flip-flop and a Fet switch, and generate on/off strobes from your timer to control the flip-flop, as we did for years on our home-built system.
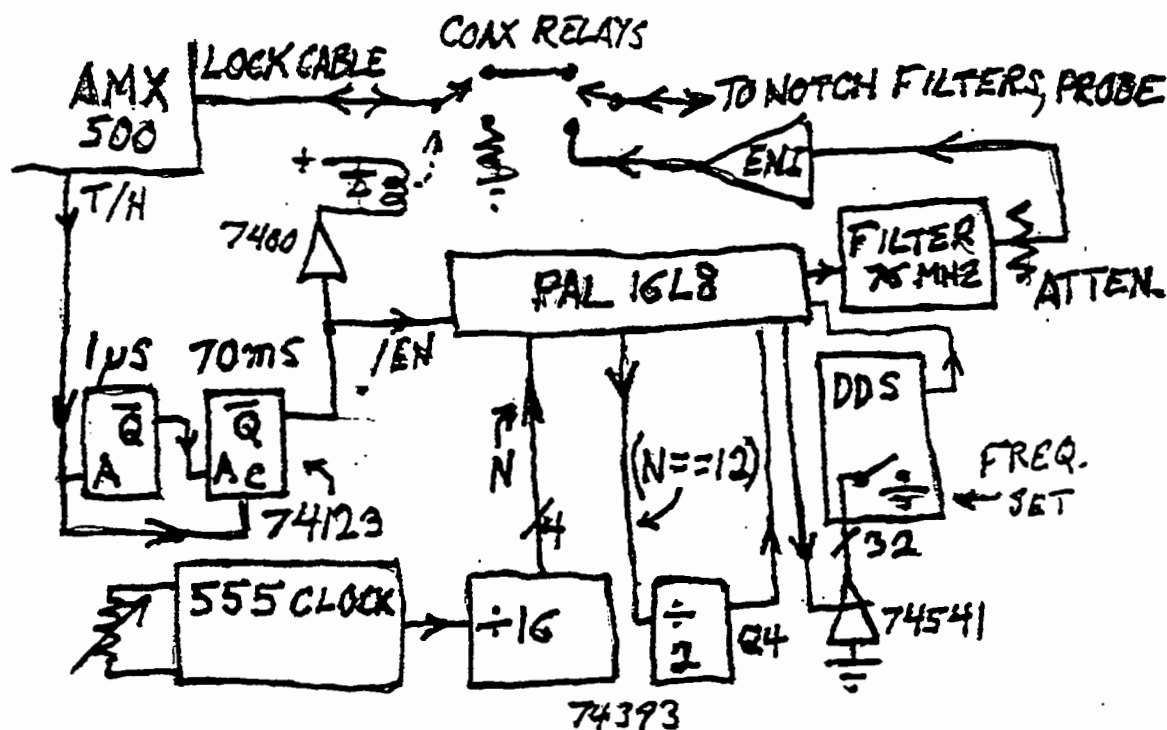
The DDS generator is a Qualcom model Q0320-2. (Their phone is (619)597-5005) costing ~$300, and the filter is a fancy commercial one but a homemade one would probably be fine (see the ARRL Handbook). The DDS generator has its own built-in crystal oscillator, which is probably good enough for ~1 PPM stability, and 2 Hz resolution. We use its on-board DIP switches to set the frequency semipermanently. We learned the proton frequency by asking Tom Pochapsky, divided it by K, and used a low-class counter to set up the board, temporarily disabling the WALZ modulator. Then we leaked the frequency into the lock channel and looked for a strong audio beat. (Pick the strongest; typically there are weak ones too.) Then we did CW decoupling of the ^{13}C spectrum of a deuterated compound such as methanol to verify the frequency, which is set ~1 PPM upfield of solvent.

Besides what I have mentioned the system consists of a chassis containing all the parts and a power supply; a transistor to drive the relays from a TTL line; a rotary attenuator and a Vector circuit board connected with a ribbon to the DDS generator, to access the 32 lines that determine its frequency. The circuit board contains an adjustable 555 generator whose period is set at the 90° nominal pulse length period of 0.5 millisec, feeding one section of a 74393 binary counter, generating a 4 bit number N. A 16L8 PAL generates a clear CL for this counter when N=12(or OXC hex) which also runs a second one-bit section of the 74393 whose output is Q4. The same PAL generates the output to the filter, in two stages, which is out = (in∧Q4∧Nx), where

$$Nx = (N==0)\| (N==1)\| (N==6)\| (N==7)\|(N==Oxb).$$

Here "in" is the output from the DDS, and this output is also enabled within the PAL by the strobe from the AMX500. Theoretically the harmonic K should be an odd number, otherwise the WALZ phase shifting does not propagate to the harmonic. In practice, however, the "in" from the DDS is not symmetric, and the 8th harmonic exists and works also; further, the supposed phase shift also has an amplitude shift probably for the same reason, but who cares? When the strobe is inactive its complement enables 3 74541 gates whose inputs are grounded and whose outputs then ground the 24 frequency-determining lines of the DDS generator. This sets it to zero frequency, essentially disabling it.

We run this between 1 and 2 watts out and suspect that 1 watt would be enough but don't have enough experience to know, and we don't know (or care) whether this gives a 0.5 millisec 90° pulse as long as it decouples. The strobe mentioned above is not actually the input from the AMX; it is a 74123 one shot pair that is arranged to fire on the negative edge of the strobe and turns itself off either after 70 msec, no matter what, or whenever the strobe is inactive (high), whichever is earlier. This protects the probe in case the strobe remains low. The only control is the rotary attenuator, and the system is disabled by turning off its AC power and, in case of paranoia, bypassing the coax relays. The latter have to be a few feet from the magnet. We built the box with some attention to RF shielding but that does not seem to be needed. It has been used for several 3D runs without incident.

Last month's newsletter had a contribution from the University of Pennsylvania aimed toward the same thing. We think that ours is simpler.

Sincerely

Cathy Moore and Alfred Redfield

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Reagents for Protein Chemistry

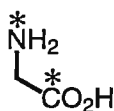Isotec also offers detergents, protein stabilizers and chelating agents.

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82-202-20-1	**Dodecylphosphocholine-d$_{38}$**	98
82-022-01-3	**Ethylenediaminetetraacetic-d$_{12}$ Acid**	98
82-008-24-4	**2-Mercaptoethanol-d$_6$**	96
82-008-04-6	**Sodium Dodecyl-d$_{25}$ Sulfate**	98

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July 7, 1997
(received 7/9/97)

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

The Intraresidue H_N-H_α Distance: A Reference for NOEs in Proteins

Dear Dr. Shapiro:

Precision and accuracy of molecular structures determined using NMR spectroscopy are limited by the number of distance and torsion angle restraints and also by the precision and accuracy of each individual restraint [1]. Torsion angle restraints are normally derived from scalar coupling constants using the Karplus relationship. For the conversion of NOE intensities into distance restraints, however, a distance reference is needed. The backbone interproton distances in regular secondary structure elements have been commonly used as references [2]. The disadvantage of this practice is that a secondary structure element has to be identified before a reference is available. In order to find a simple distance reference, we have analyzed the intraresidue H_N-H_α distance and found that the H_N-H_α distance is highly correlated with ϕ for protein structures deposited in the Brookhaven Protein Data Bank [3].

Figure 1 shows the intraresidue H_N-H_α distance as a function of the backbone ϕ torsion angle. The solid line is the calculated value based on the following geometrical parameters: [H_N-N] = 1.020 Å, bond [C_α-H_α] = 1.100 Å, bond [N-C_α] = 1.458 Å, angle [H_N-N-C_α] = 119.0°, angle [N-C_α-H_α] = 108.3°. The circles represent measured values for 1141 amino acid residues from 13 NMR structures in the Brookhaven Protein Data Bank (ID codes are 1BTA, 1CRE, 1BBN,

1HWA, 2PNB, 1PNJ, 2PLD, 2GB1, 1CRR, 1COO, 1HCS, 1FRC, 1SRL). It is well known that the torsion angle ϕ is related to the homonuclear three-bond coupling constant, $^3J[H_N-H_\alpha]$, through a Karplus equation [2]:

$$^3J[H_N-H_\alpha] = 6.4 \cos^2(\phi - 60) - 1.4 \cos(\phi - 60) + 1.9 \tag{1}$$

After the measured value of ϕ was converted to $^3J[H_N-H_\alpha]$ using the Karplus equation, a relationship between the intraresidue H_N-H_α distance and $^3J[H_N-H_\alpha]$ can be obtained as shown in Figure 2. Estimation of the H_N-H_α distance from the scalar coupling constant, $^3J[H_N-H_\alpha]$, is therefore a straightforward exercise. The distance obtained in this manner can be used as a reference for other NOEs in proteins. In this manner, attenuation of NOE intensities due to amide proton exchange is, to a first approximation, accounted for in the estimation of distances between the amide proton and carbon-attached protons. Our solution structures for the Hck SH2 domain were determined using distance constraints determined in this manner [3] and these structures closely resemble those for other SH2 structures solved using either NMR spectroscopy or X-ray crystallography.

References:

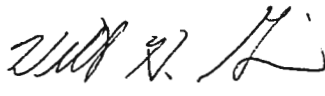1. Clore, G. M. and Gronenborn, A. M. (1993) in *NMR of Proteins* (Eds, Clore, G.M. and Gronenborn, A.M.) CRC Press Inc., Boca Raton, FL, pp. 1-32.
2. Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, John Wiley and Sons, New York.
3. Zhang, W., Smithgall, T. E. and Gmeiner, W. H. (1997) *J. Biomol. NMR*, in press.

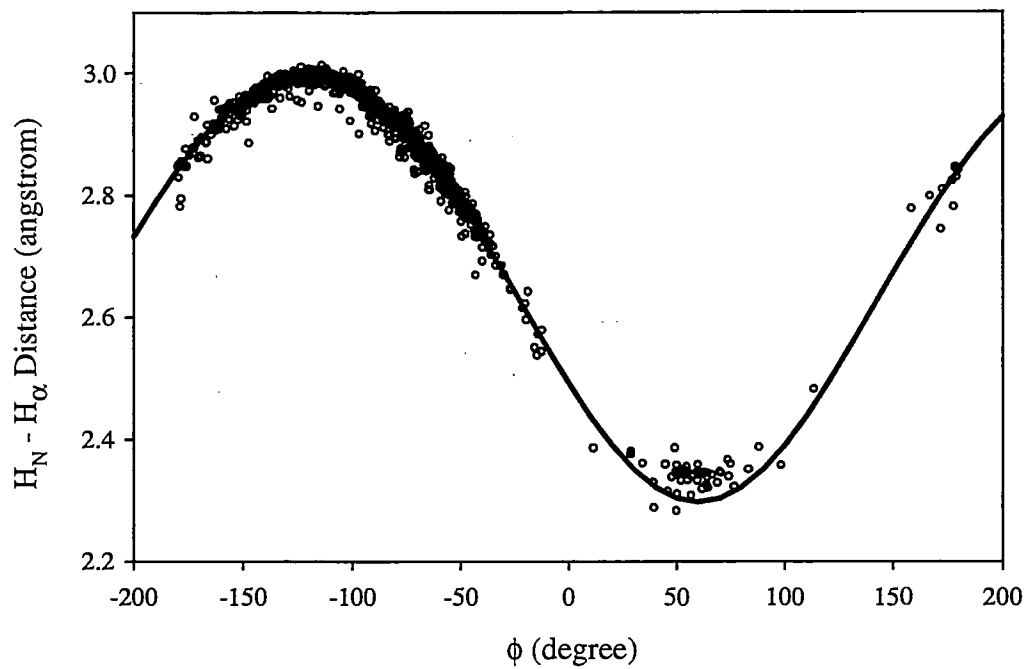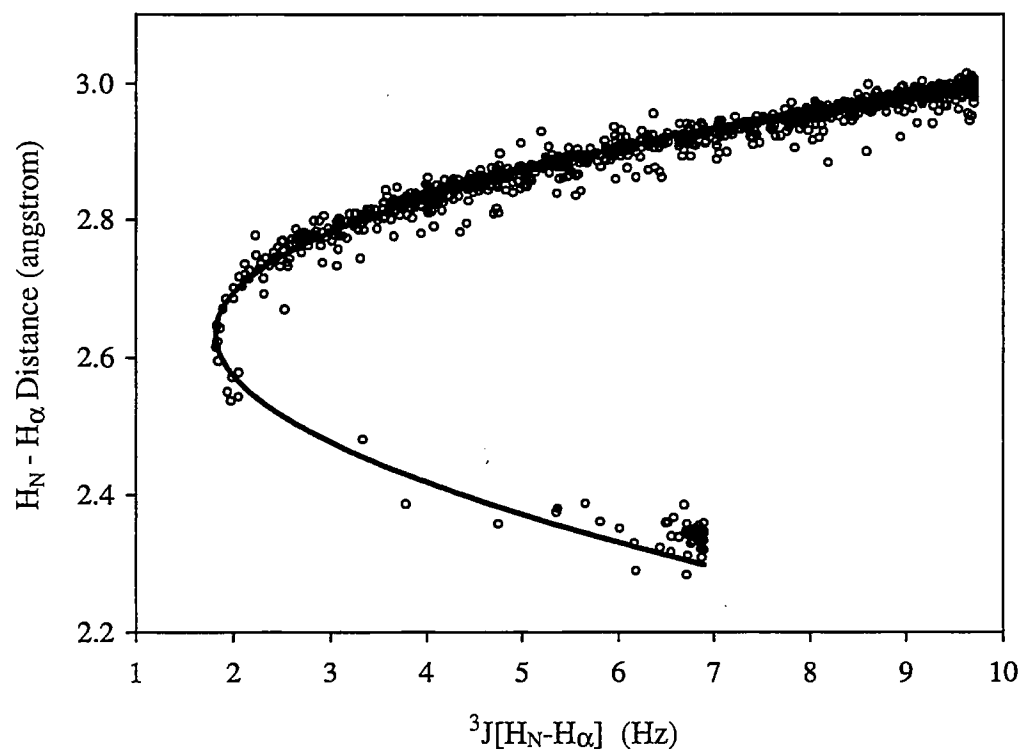Yours sincerely,

Weixing Zhang

William H. Gmeiner

Figure 1

Figure 2

Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065

Dr. Bernard L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

MERCK
Research Laboratories

July 9, 1997 (received 7/12/97)

Spiking Methanol with TFA - A Cautionary Note

Dear Barry,

Most of us who deal with a variety of structure problems in our every day work have recognized that poor quality spectra of nitrogen - containing compounds can often be improved by a change in pH. We recently obtained a spectrum of a metabolite in CD_3OD that was an obvious candidate for improvement. After spiking the solution with TFA and allowing 3-5 minutes to reestablish thermal equilibrium, an overnight acquisition was started with presaturation of the solvent OH. The resulting spectrum showed only minimal suppression of the solvent peak and the reason was apparent: the OH had shifted almost 0.2 ppm upfield! (For the benefit of skeptics, temperature was regulated at 25°C.) Common sense tells us that a small metabolite sample could not be responsible for the result, and this consequently triggered a study using CD_3OD and TFA. The result of the time study over three days is shown in figure 1. Clearly TFA has been neutralized since the chemical shift of the OH after 65 hours is typical for neat CD_3OD. The initial explanation, that TFA reacted with alkaline constituents in the glass, was a reasonable extension of a colleague's claim that the resolution of CD_3OD in a sealed tube deteriorates over time. This hypothesis had to be abandoned when analogous experiments with DCl and acetic acid showed no chemical shift change of the OH after several days.[a]

A more plausible explanation is that TFA was slowly converted to the methyl ester. When the experiment was carried out with CH_3OH in chloroform, a new, slightly broadened peak appeared at 3.96 ppm which grew over time (figure 2). The chemical shift is consistent with an acylated methoxy. The suspicion that unresolved coupling with fluorine was responsible for the broadening was verified by reacquiring a spectrum using an acquisition time of five seconds. The quartet in the spectrum shown in figure 3 confirmed a connectivity between the 3.96 ppm peak and the trifluoromethyl group thus providing convincing evidence for the proposed CF_3COOCH_3.

As an exercise in overkill, the reaction between TFA and CD_3OD was followed by [19]F. Note that the rate of growth of the new peak at 245.1 ppm in figure 4 roughly parallels that in figure 2. It should come as no surprise that the ms on the 24 hour solution detected a molecular ion of 128.

While the esterification of TFA is not a problem for short term experiments it is a factor to be aware of when a long term acquisition in methanol is planned.

Regards,

Byron

---

[a] A third experiment with trichloroacetic acid did in fact reveal small incremental upfield displacements of the OH which became evident only after 24 hours.

CHANCE IN CHEMICAL SHIFT OF OH WITH TIME
AFTER TFA SPIKE
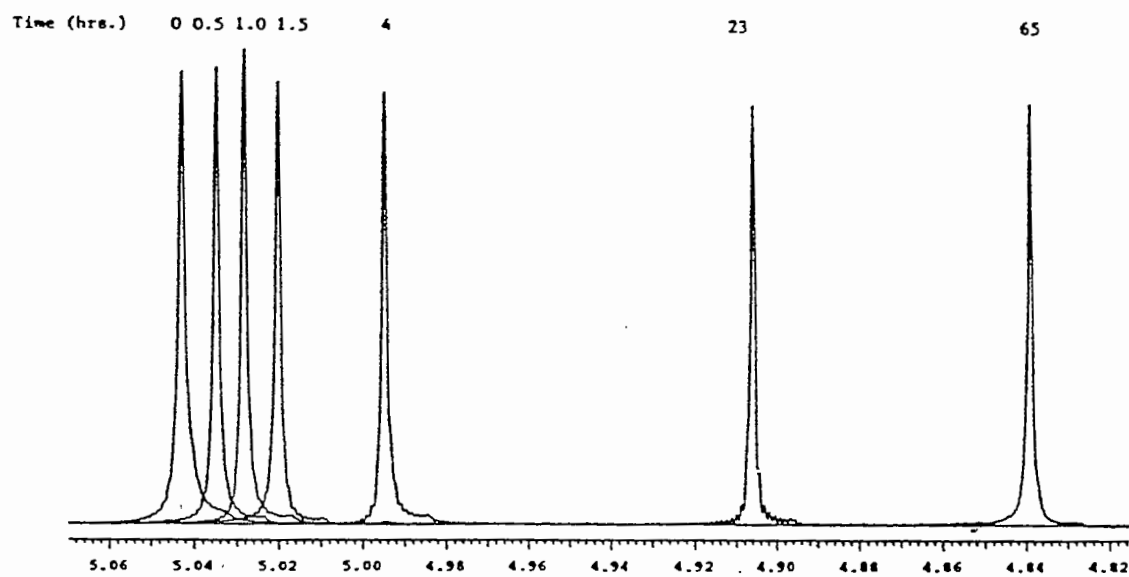
Figure 1
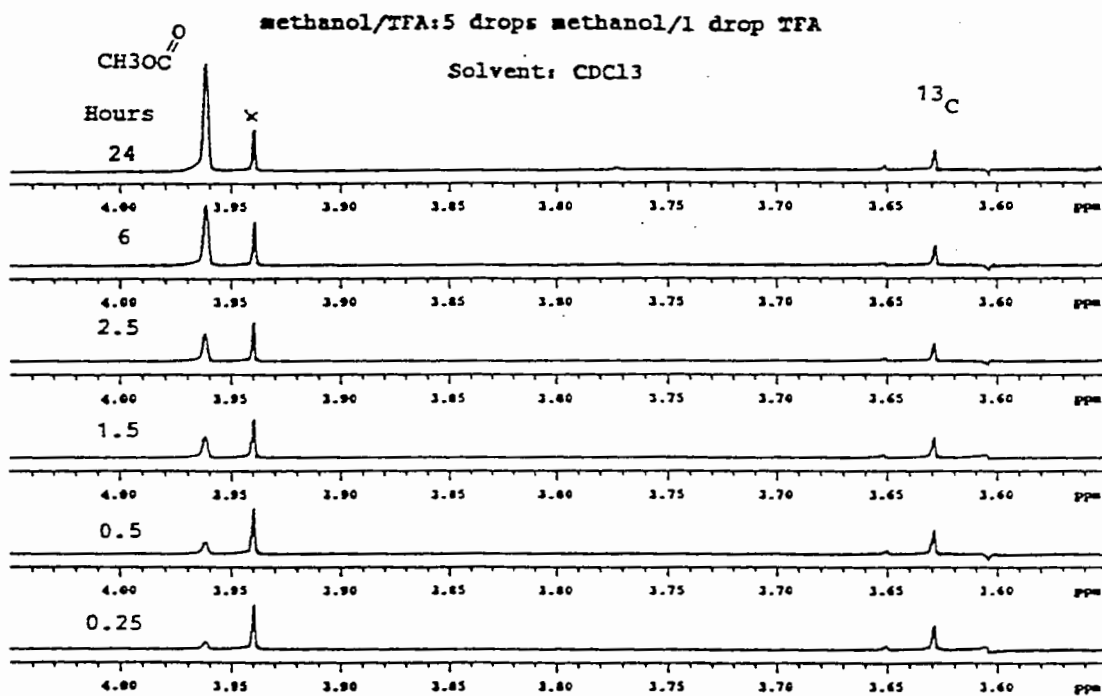
Figure 2

methanol/TFA
5 drops methanol/1 drop TFA
time:8:10 [24 hrs after soln makeup]

Solvent: CDCl3

0.33 Hz

3.970   3.965

13
C

4.00   3.9   3.90   3.85   3.80   3.75   3.70   3.65   3.60   ppm
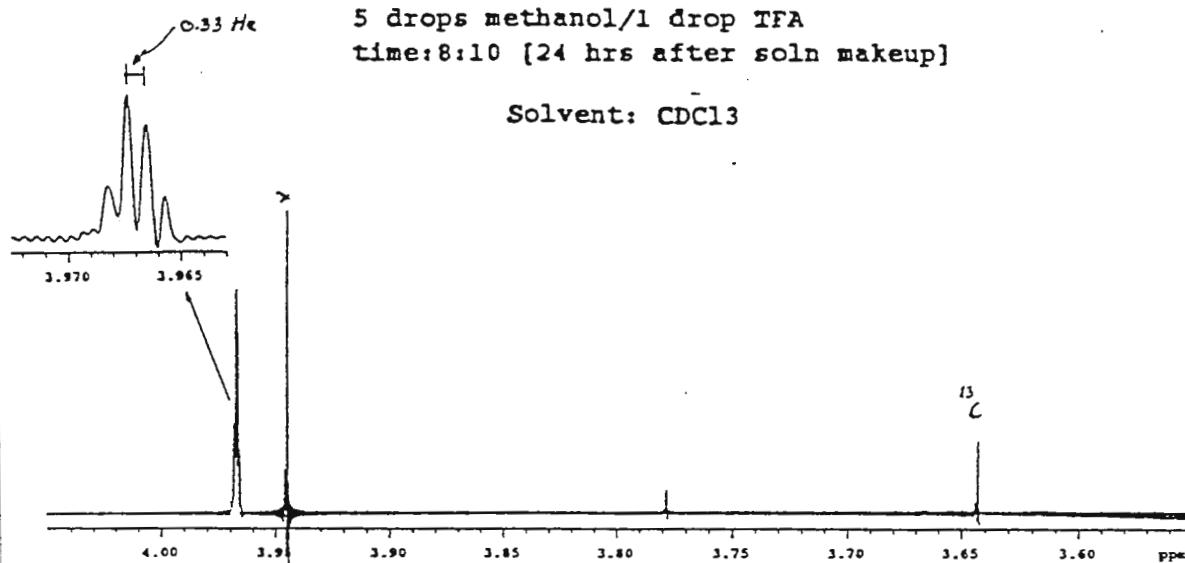
Figure 3

TFA
methyl ester

TFA in CD3OD
10 drops CD3OD/2 drops TFA

F19

Hours   TFA

28

      245.1   245.0   244.9   244.8   244.7   244.6   244.5   244.4   244.3   244.2   244.1   ppm
6

      245.1   245.0   244.9   244.8   244.7   244.6   244.5   244.4   244.3   244.2   244.1   ppm
4

      245.1   245.0   244.9   244.8   244.7   244.6   244.5   244.4   244.3   244.2   244.1   ppm
2

      245.1   245.0   244.9   244.8   244.7   244.6   244.5   244.4   244.3   244.2   244.1   ppm
0.75

      245.1   245.0   244.9   244.8   244.7   244.6   244.5   244.4   244.3   244.2   244.1   ppm
0.3

      245.1   245.0   244.9   244.8   244.7   244.6   244.5   244.4   244.3   244.2   244.1   ppm
0.1

      245.1   245.0   244.9   244.8   244.7   244.6   244.5   244.4   244.3   244.2   244.1   ppm

Figure 4

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MMRS-VII
Missouri
Magnetic
Resonance
Symposium

This year, the 7th Missouri Magnetic Resonance Symposium (MMRS-VII) will be held jointly with the 32nd Midwest Regional American Chemical Society Meeting from Oct.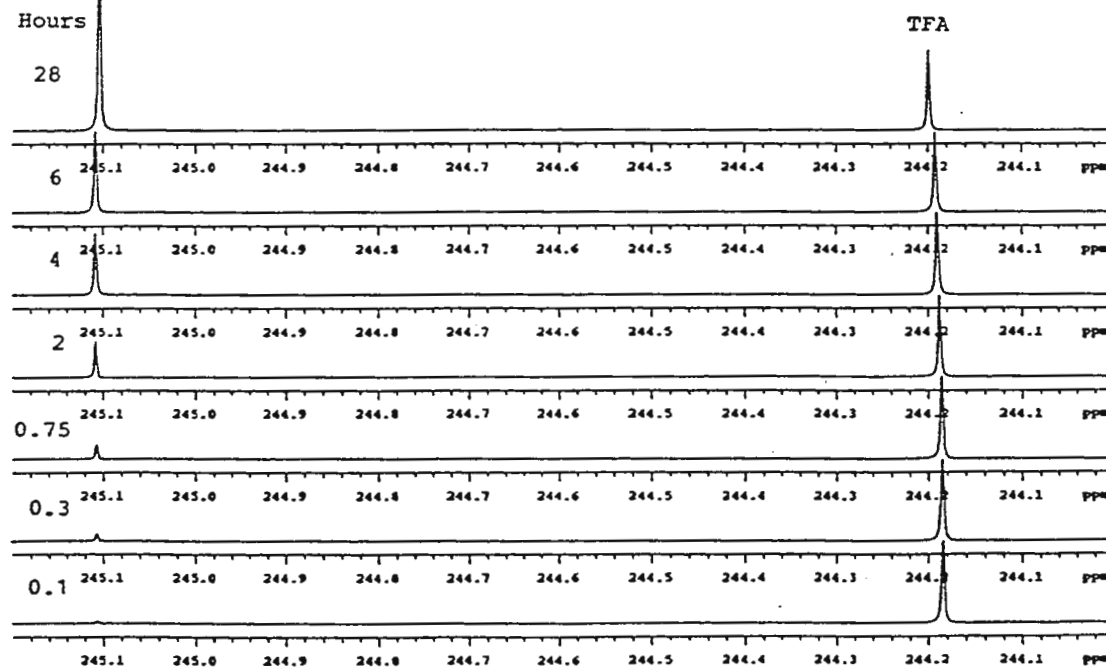 29 - Nov. 1, 1997 in Osage Beach, MO, USA at the beautiful Tan-Tar-A Lodge. The MMRS oral sessions are tentatively scheduled on Friday (AM and PM) with posters either Thursday or Friday PM.

Contributed papers from MMRS participants are being solicited for this symposium in all areas of magnetic resonance. **Full time students giving papers in the MMRS sessions are eligible to apply for a stipend to cover registration costs.** To apply, a copy of the abstract of the poster presentation should be sent to: Frank D. Blum, Department of Chemistry, University of Missouri-Rolla, Rolla, MO 65409-0010, 573-341-4415, fblum@umr.edu. There are a limited number, so apply soon.

Registration for the meeting will be handled through the 32nd Midwest Meeting. For further information see the meeting website:

http://www.chem.umr.edu/midwest32.html

A preliminary list of speakers can also be found there.

UMEÅ UNIVERSITET
Avd. för Organisk Kemi
Dan Johnels

UMEÅ UNIVERSITY
Dept. of Organic Chemistry

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
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U.S.A.

Umeå 970721
(received 7/28/97)

^{6}Li-^{13}C REDOR Measurements of the TMEDA Complex of Indenyl Lithium.

Dear Barry,

We have in a recent paper decribed the use of the ^{6}Li-^{13}C REDOR technique to determine the Li-C dipolar coupling, and thus the Li-C distances, in the TMEDA complex of fluorenyl lithium[1]. The complex has, based on the obtained distances, a symmetric structure where the lithium cation is located above the central five-membered ring. There is no X-ray data for this TMEDA complex to compare with, so in order to get more information regarding the reliability of the REDOR method, we have now studied the TMEDA complex of indenyl lithium, of known X-ray structure[2]. In the Figure a stereo plot is presented indicating the position of the litium cation as determined by REDOR and X-ray in light and dark grey, respectively. The match is not exact, but similar deviations have been observed earlier and are supposed to be due to dynamic effects that reduce the measured dipolar coupling[3]. The difference between the Li positions is ca 0.2 Å, similar to the experimental error in the REDOR experiment.

As a conclusion, the REDOR methodology is applicable to the field of organolithium chemistry as an additional aid in investigating the solid state structures of this such organometallics.
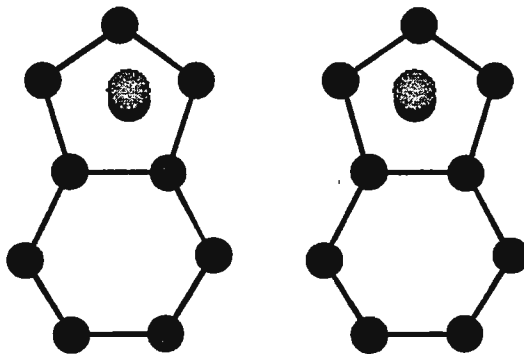
Sincerely,

Dan Johnels

Ulf Edlund

1. P.-O. Quist, H. Förster and D. Johnels, J. Am. Chem. Soc., 119, 5390, 1997.
2. W.E. Rhine and G.D. Stucky, J. Am. Chem. Soc., 97, 737, 1975.
3. A. Schmidt, R.A. McKay and J. Schaefer, J. Magn. Reson. 96, 644, 1992.

OSU

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FAX 405-744-6007

July 8, 1997 (received 7/12/97)

Dr. Bernard Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

NMR Studies on the Mechanisms of Separation of Chiral Species by Capillary Electrophoresis

Dear Barry,

In order to understand the mechanisms of separation of chiral species by capillary electrophoresis, 1D and 2D NMR methods were used to assign the ^{1}H and ^{13}C resonances of an electrophoretic system composed of octyl β-D-glucopyranoside (OG) and silvex, a toxic herbicide. The OG-OG interactions as well as the OG-silvex interactions were determined by the proton chemical shifts and by 2D NOE experiments.

The chiral OG micelle, which was formed by increasing the concentration of OG, exhibited different interactions with the R and S enantiomers of silvex. Figure 1 shows proton spectra of OG and OG-silvex mixtures above and below the critical micelle concentration of OG. Comparing the spectra of 10 mM OG and 40 mM OG, we can see that all of the resonances are moved, especially -CH$_2$OH, which resolves into two multiplets, because of the formation of the OG micelle. In the 10 mM silvex/40 mM OG spectrum, the -CH$_2$OH signals even moved further. This indicates that -CH$_2$OH strongly interacts with silvex. We also can see no changes in chemical shifts for 10 mM OG when adding 10 mM silvex. The 3' resonance of silvex moved to a higher field when the OG concentration was increased from 10 to 40 mM. Even more interesting is the splitting into two peaks of the 3' resonance, due to different orientation of the R and S isomers of silvex in the micelle. The 2D NOE experiment showed clearly intermolecular NOE interactions between OG and silvex. Figure 2 is a NOESY spectrum of 10 mM silvex with 40 mM OG. The crosspeaks clearly show the NOE interactions between 3',6' protons of silvex and -(CH$_2$)$_5$- group of OG. It is interesting to know which -CH$_2$- of -(CH$_2$)$_5$- is interacting with 3' and which is interacting with 6'. Then we will have good understanding of how the silvex orients itself in the micelle. This is what we plan to do next.

Sincerely yours,

Feng Qiu   Z. El Rassi
Department of Chemistry, Oklahoma State University

Figure 1: 400 MHz ^{1}H NMR spectra for octyl β-glucoside (OG)/silvex interaction
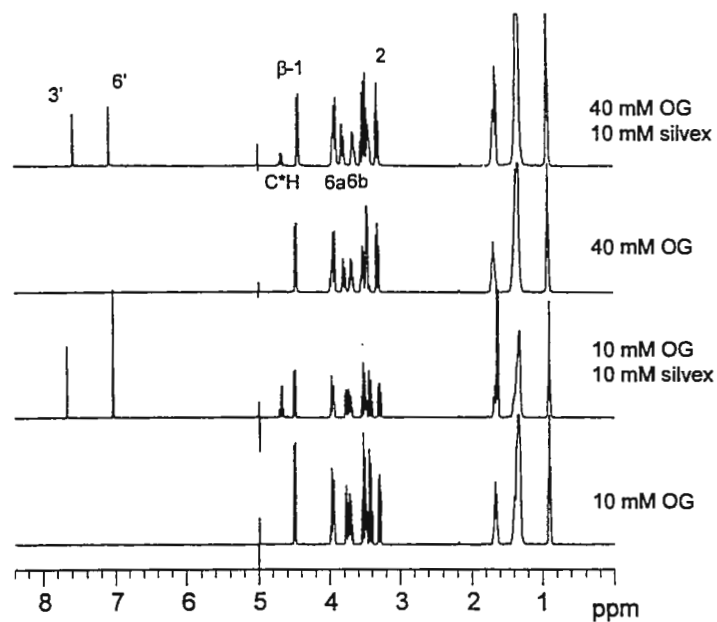
All solutions are in 200 mM phosphate buffer, pH 6.5, D_2O as solvent at 10 °C.

Figure 2: NOESY spectrum of 40 mM OG/10 mM silvex at 10 °C

Octyl-β-D-glucopyranoside (OG)

Silvex

Pittsburgh

NMR
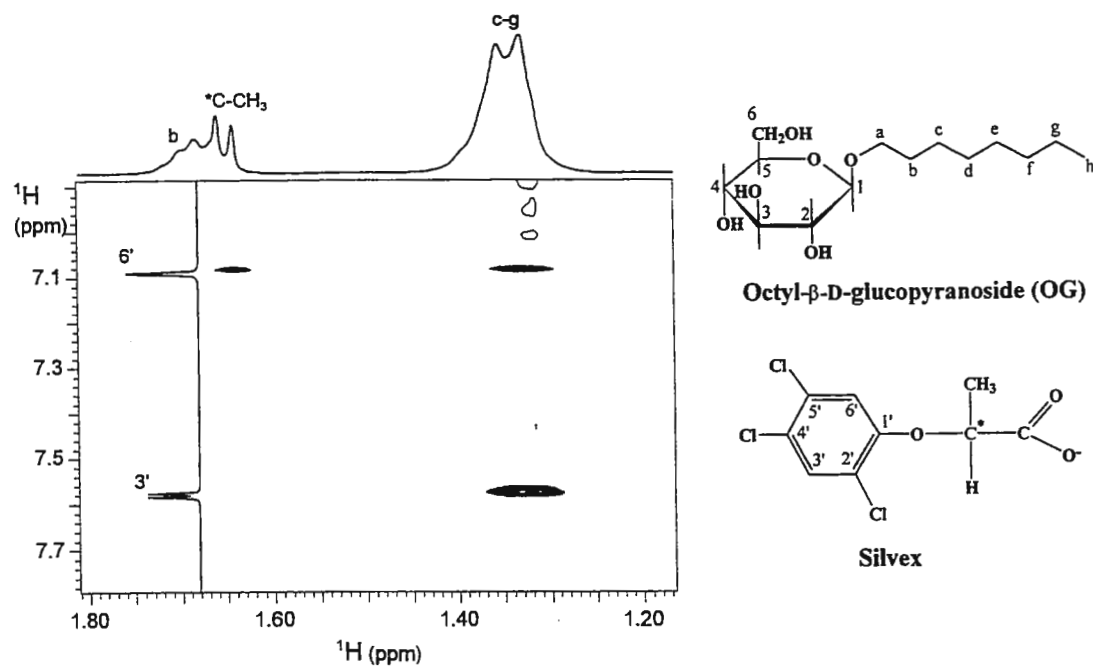
Center for Biomedical Research

Carnegie Mellon University
University of Pittsburgh

Pittsburgh NMR Center for Biomedical Research
Mellon Institute
Carnegie Mellon University
4400 Fifth Avenue
Pittsburgh, Pennsylvania 15213-2683
412-268-6336

July 25, 1997

Dear Colleagues:

We are pleased to invite you to attend a special symposium honoring Aksel A. Bothner-By, Josef Dadok, Irving J. Lowe, and Robert T. Shumacher; the pioneers of NMR in Pittsburgh. Enclosed you will find an announcement with information describing the symposium. The symposium is entitled, "New Directions in NMR: A Symposium Honoring Aksel A. Bothner-by, Josef Dadok, Irving J. Lowe, and Robert T. Shumacher". As you can see, we have an all-star list of invited speakers. Please share the symposium information with interested colleagues.

The symposium will be in Pittsburgh at the Mellon Institute at Carnegie Mellon University on September 20, 1997 beginning at 8:30 AM. We have reserved a block of rooms at the Shadyside Inn near the Mellon Institute. Reservations can be made by calling (800) 767-8483. When placing your reservation, tell the Reservation Department that you are with the NMR Center, Code #1225mw to get the special rate. (Shadyside Inn web address: http://pittsburgh.net/ShadysideInn). We will host a banquet the evening of the symposium on September 20, 1997. You are invited to attend this banquet. The cost will be $35 per person.

Please RSVP to Ms. Michelle Waters at (412) 268-6336 [fax: (412) 268-7083; e-mail: waters@andrew.cmu.edu] by August 20, 1997 to inform us whether you will be attending the symposium and the banquet. Ms. Waters can also help if you have trouble getting room reservations in Pittsburgh, or with advice about your travel plans.

We are looking forward to an exciting symposium and the chance to honor these Pittsburgh pioneers that made seminal contributions to NMR. We hope you can join us.

The Organizing Committee: Chien Ho, Alan P. Koretsky, Miguel Llinas, Gordon S. Rule

New Directions in NMR: A Symposium Honoring

Aksel A. Bothner-By
Department of Chemistry
Carnegie Mellon University

Irving J. Lowe
Department of Physics and Astronomy
and Pittsburgh NMR Center
University of Pittsburgh

Josef Dadok
Department of Chemistry
Carnegie Mellon University

Robert T. Schumacher
Department of Physics
Carnegie Mellon University

■ ■ ■ September 20, 1997 ■ ■ ■

8:30 AM - 5:00 PM
Mellon Institute Auditorium, Carnegie Mellon University
4400 Fifth Avenue, Pittsburgh, Pennsylvania

Guest Speakers:

Ad Bax
NIH
Bethesda, MD

Richard R. Ernst
ETH Zentrum
Zurich, Switzerland

Ray Freeman
University of Cambridge
Cambridge, UK

Lila M. Gierasch
University of Massachusetts
Amherst, MA

Erwin L. Hahn
University of California
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Paul C. Lauterbur
University of Illinois
Urbana-Champaign, IL

John L. Markley
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Address all Newsletter correspondence to:

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303.
☎ 415-493-5971* - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

</td><td>

Deadline Dates

No. 468 (Sept.)	22 Aug. 1997
No. 469 (Oct.)	26 Sept. 1997
No. 470 (Nov.)	24 Oct. 1997
No. 471 (Dec.)	21 Nov. 1997
No. 472 (Jan.)	19 Dec. 1997

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* Fax: ☎ 415-493-1348, at any hour. Do not use fax for technical contributions to the Newsletter, for the received fax quality is very inadequate.

E-mail: shapiro@nmrnewsletter.com

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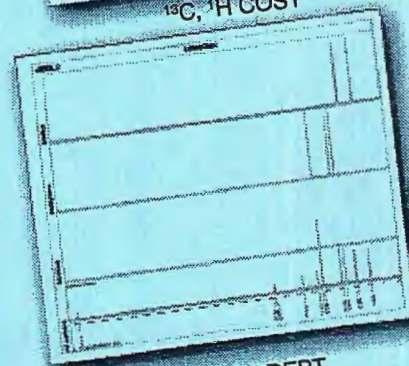
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¹H

¹³C

¹³C, ¹H COSY

¹H, ¹H COSY

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