THE NMR NEWSLETTER

No. 463

April 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.) |
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| **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<br>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.) |
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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

International Society for Magnetic Resonance in Medicine, Fifth Scientific Meeting and Exhibition, Vancouver, BC, Canada, **April 12-18, 1997**; Contact: ISMRM, 2118 Milvia St., Suite 201, Berkeley, CA 94704, USA; (510) 841-1899; Fax (510) 841-2340; Email: info@ismrm.org.

Symposium on NMR Spectroscopy of Synthetic Macromolcules, ACS National Meeting, San Francisco, **April 13-17,** 1997; Contact: H. N. Cheng or English, A. D. See Newsletter 456, 20.

International School of Structural Biology and Magnetic Resonance, 3rd Course: Protein Dynamics, Function and Design; Erice, Sicily, Italy; **April 18-28, 1997**; Contact: Ms. Robin Holbrook, Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, CA 94305-5055; (415) 723-6270; Fax: (415) 723-2253; Email: holbrook@smi.stanford.edu. See Newsletter 462, 54.

Symposium and Training: $^{13}C$ in Metabolic Research, Dallas, TX, **May 8, 1997;** Contact: J. Cody: (214) 648-5886; fax: (214) 648-5881; email: jcody1@mednet.swmed.edu, or N. Bonsal: (214) 648-5887. See Newsletter 462, 22.

6th Meeting of AUREMN (NMR Users Association of Brazil), Rio de Janeiro, Brazil, **12 - 16 May, 1977**; Contact: Snia Maria C. de Menezes, Petrobás/Cenpes/Diquim/Radial 2, Quadra 07 - Ilha do Fundão, 21949-900 Rio de Janeiro, Brazil; Tel. +55 21 598-6171 and 598-6914; Fax. +55 21 598-6296; Email; sonia@cenpes.petrobas.com.br.

39th Rocky Mountain Conference on Analytical Chemistry, Denver, Colorado; NMR Symposium, **August 4-7, 1997**: Contact: J. P. Yesinowski, Code 6120, Naval Research Laboratory, Washington, DC 20375-5342; 202-767-0415; fax 202-767-0594; email yesinowski@nrl.navy.mil. See Newsletter 458, 8.

Fourth International Meeting on Recent Advances in Magnetic Resonance Applications to Porous Media, Trondheim, Norway, **Aug. 31 - Sep. 3, 1997**; Contact: John J. Attard, SINTEF Unimed MR-Center, N-7034 Trondheim, Norway. Tel: +47 73 59 89 25; Fax: +47 73 99 77 08; Email:john.attard@unimed.sintef.no;

Additional listings of meetings, etc., are invited.

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**Labor für NMR-Spektroskopie**
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CH - 3012 Bern

*PD Dr. Peter Bigler*

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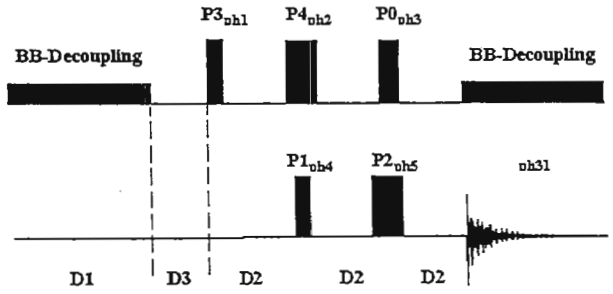
Dr. B. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303 USA

february 25th 1997
(received 3/7/97)

# $^{13}$C DEPT: A Revision

Dear Dr. Shapiro

$^{13}$C DEPT is one of the most popular techniques when using NMR on a routine basis. Most textbooks dealing with DEPT point to its „inherent handicap", i.e. the missing signals of quaternary carbons in the corresponding spectra and for this reason recommend to apply either an additional one-pulse $^{13}$C experiment or alternatives such as the recently proposed PENDANT experiment. A closer inspection, however, reveals that the above statement is not true, that quaternary carbons can indeed be measured with DEPT, but are simply cancelled in the course of the usual phase cycle, acting as double quantum filter. If the basic DEPT sequence is decomposed in two sub-experiments DEPT A and DEPT B, solely differing in their phase cycle (see below), FIDs obtained with these two experiments may be combined in two different ways. Addition yields the usual DEPT spectrum, with no quaternary carbons visible; subtraction and - after FT - adding a zero-order phase correction of +/- 90° to the value used before, yields a spectrum with all carbon multiplicities, including quaternaries, visible. Whereas the $^{13}$C sensitivity with the first DEPT spectrum is obviously the same as for the normal DEPT experiment, reduced $^{13}$C sensitivities are measured for the protonated carbons, compared to the normal DEPT experiment, but also for the quaternary carbons - with almost no NOE enhancement - compared to the basic one-pulse experiment. Nevertheless all carbon multiplicities may be obtained within the measuring time used for the DEPT experiment and no additional one-pulse experiment need to be mesaured in most cases. Furthermore all the benefits of DEPT (spectral editing, distorsionless, minor J dependence) survive and probably make the proposed variant superior to PENDANT. With the aim to get the signals of all carbon multiplicities within a measuring time as short as possible, BB decoupling may be introduced in the first part of the preparation period (see below), thereby enhancing the signal intensities of quaternary carbons (partial NOE) at the expense of the signal instensities of the protonated carbons. This allows to adjust D1 and D3 to the actual sample and to „tune" the experiment to the spectroscopist's needs.

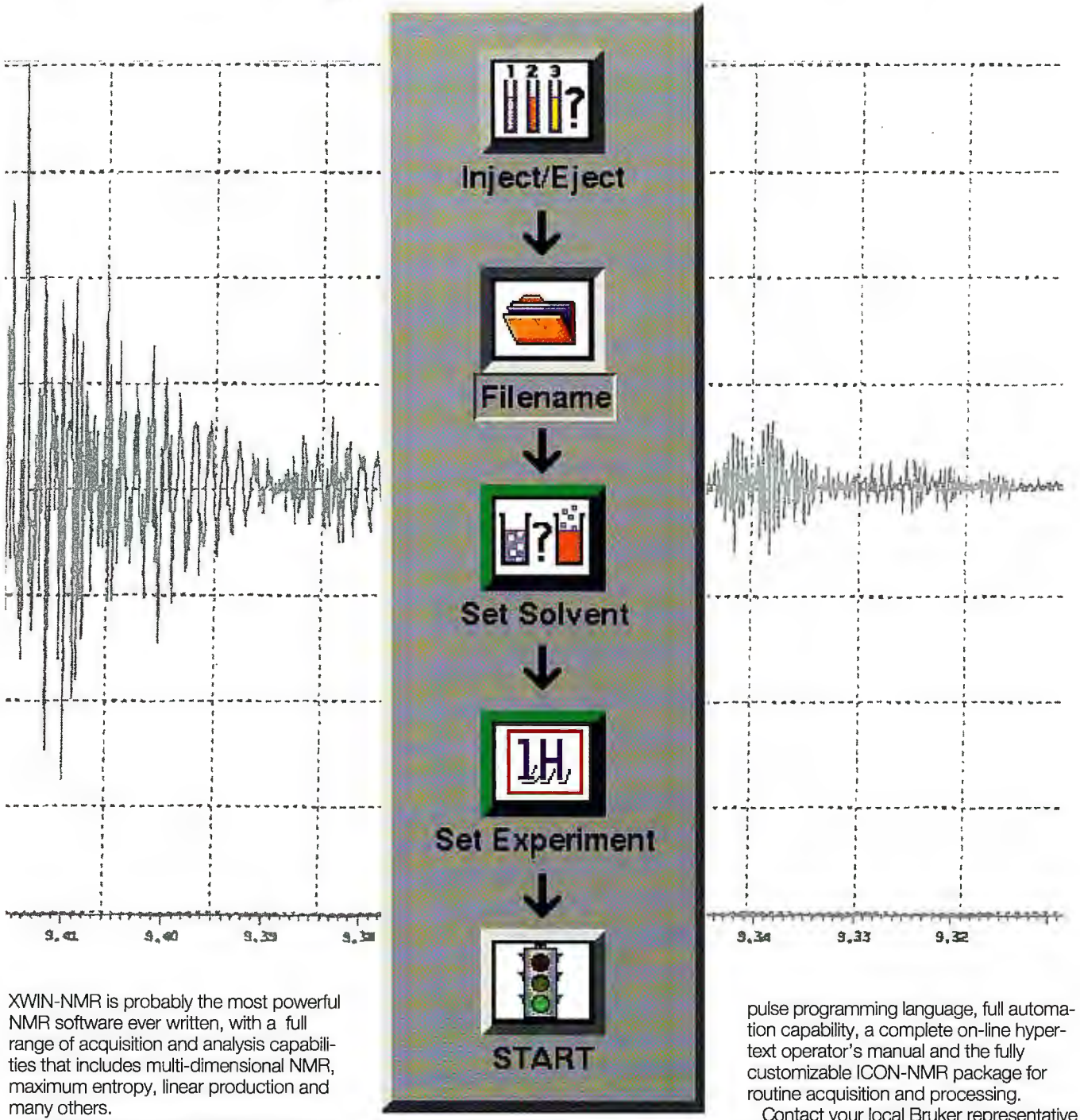



| | DEPT A | DEPT B |
|---|---|---|
| ph1 | 0 | 0 |
| ph2 | **0 2 1 3** | **1 3 0 2** |
| ph3 | 1 1 3 3 | 1 1 3 3 |
| ph4 | 0 0 0 0 1 1 1 1 | 0 0 0 0 1 1 1 1 |
| | 2 2 2 2 3 3 3 3 | 2 2 2 2 3 3 3 3 |
| ph5 | 0 2 0 2 1 3 1 3 | 0 2 0 2 1 3 1 3 |
| ph31 | **1 1 1 1 2 2 2 2** | **3 3 3 3 0 0 0 0** |
| (Rec.) | **3 3 3 3 0 0 0 0** | **1 1 1 1 2 2 2 2** |

Yours sincerely

Peter Bigler

# It doesn't get any easier than this:

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KUNGL TEKNISKA HÖGSKOLAN

Department of Chemistry, Physical Chemistry
Professor Peter Stilbs

Stockholm February 27, 1997
(received 3/7/97)


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

Re: Global least-squares analysis of kinetic and relaxation-type spectral data sets by CORE-processing

Dear Barry - thank you for the yellow ultimatum. For some time we have been working with data processing of large spectral data sets. Our original problem concerned overlapping bandhapes in FT-PGSE NMR self-diffusion measurements. We found a method that seems very powerful and general, and named it CORE-processing. It has been described in two papers (J.Phys.Chem. 100 (1996) 8180-89 and Rev.Sci.Instrum. 67 (1996) 4380-4386). The first one describes the general idea, as applied in FT-PGSE, and the second one gives a more clear description of it, and how the approach can be applied to 'kinetic' spectroscopic data sets. The notation 'Kinetic type' includes spin relaxation data and FT-PGSE data as well. A prerequisite for CORE processing is that all bandshapes stay constant with 'the time parameter' of the experiment, however - only the amplitudes may change. CORE is thus not applicable if the signal(s) shift during the experiment, or if different parts of a component bandshape have different relaxation rates. More info is found in the publications, and on my WWW-page.

As an illustration, the Figure shows proton NMR kinetic data on glucose mutarotation in heavy water, over a period of 330 minutes ((a) original data plotted on the spectrometer;(b) the same data displayed from another angle with a Matlab routine;(c) the fitted data (omitting the water band) and (d) the residuals map). The first trace was recorded about 10 min after dissolving the glucose in water. No assumptions whatsoever are made with regard to component bandshapes. The form of the kinetic equations is assumed or tested, and the parameters iterated upon are the rate constants only. The complete CORE-processing on the 32*8K data set was finished in about 10 min on a 64-bit DEC-station Alpha AXP 3000/700, running OpenVMS. Of course, the programming was done in standard good old FORTRAN, and a minimization routine from the late 1960-ies was used as well. The results include the rate constant, the deviation from time '0' and the two anomer component bandshapes.

I have not had time to clean up the code, and make it portable to other platforms - there are some problems to handle and read the huge binary spectrometer data files - and the mode that this should be done seems to differ between 64-bit and 32-bit machines.

Yours Sincerely

Pelle

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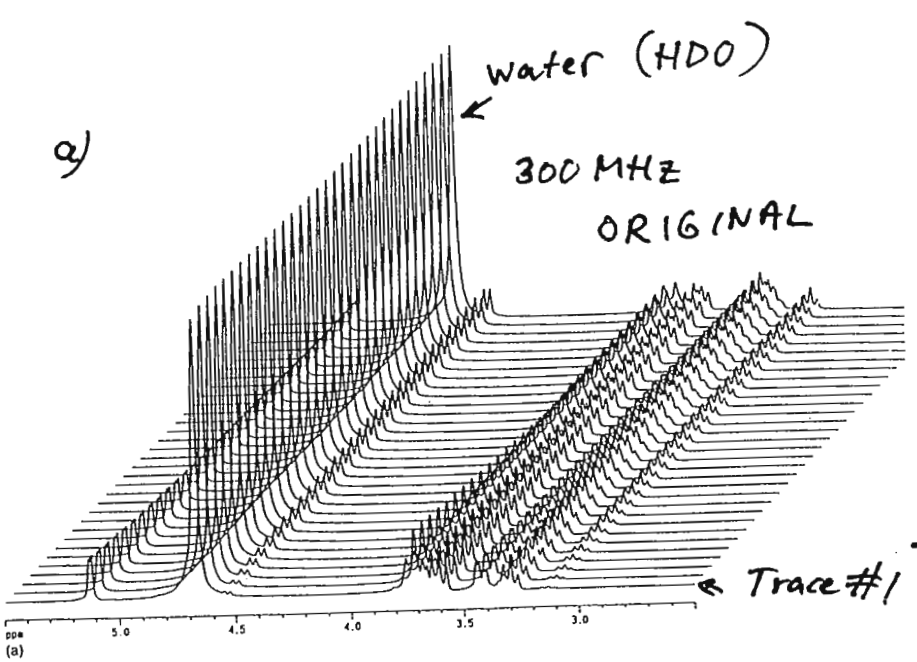
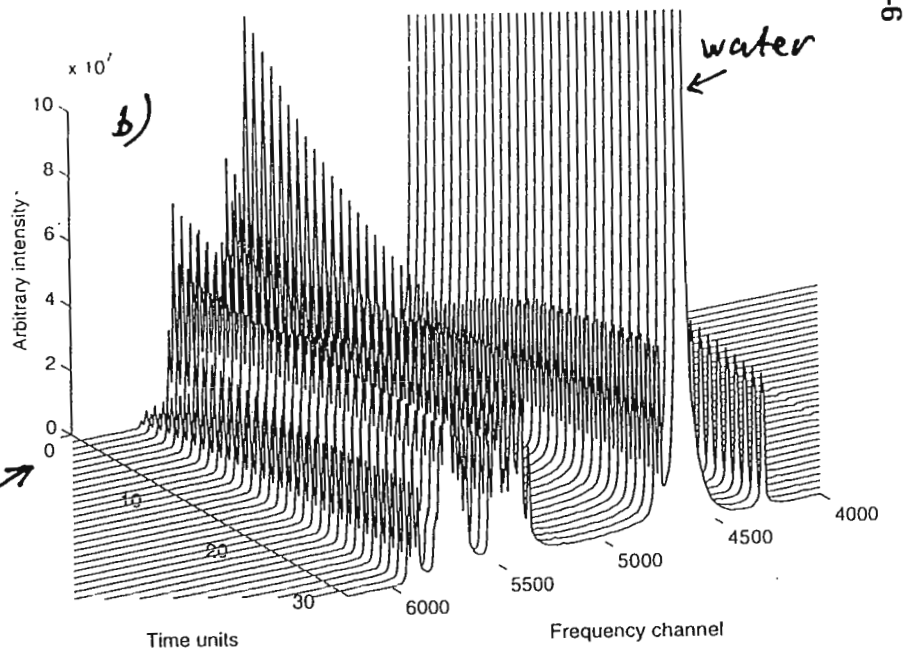
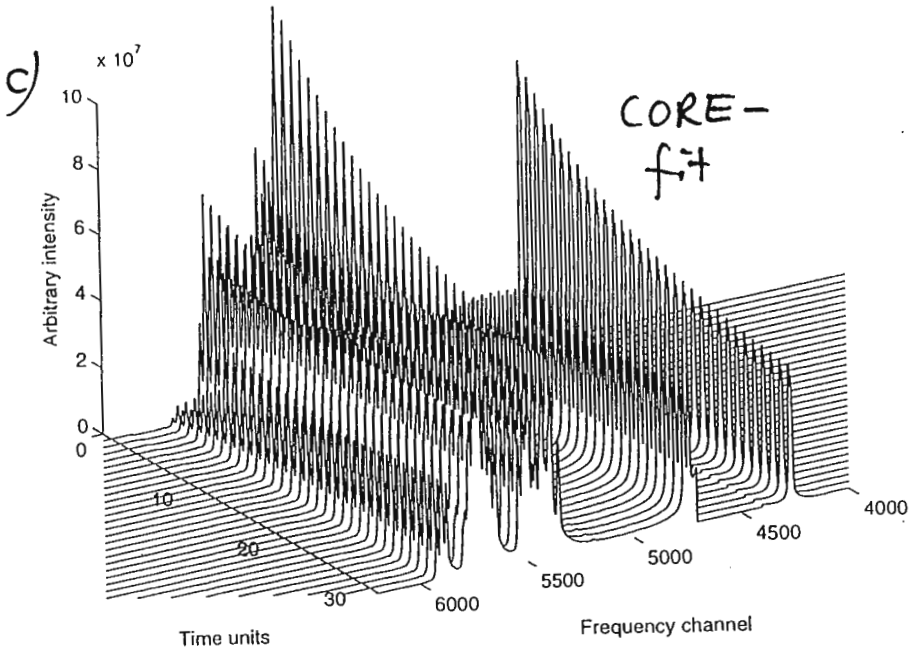
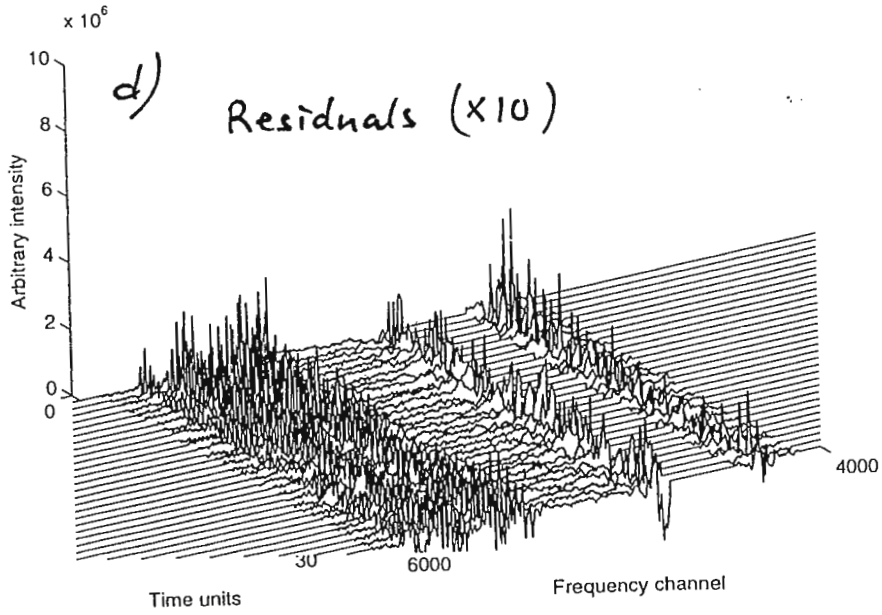
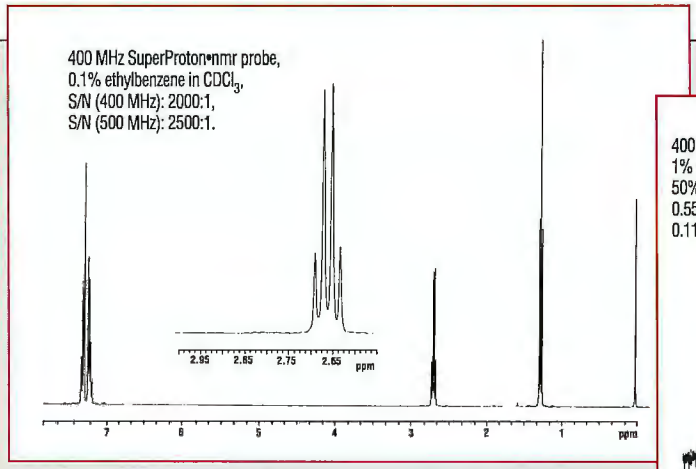

a)
water (HDO)
300 MHz
ORIGINAL
Trace #1
ppm
(a)
5.0
4.5
4.0
3.5
3.0

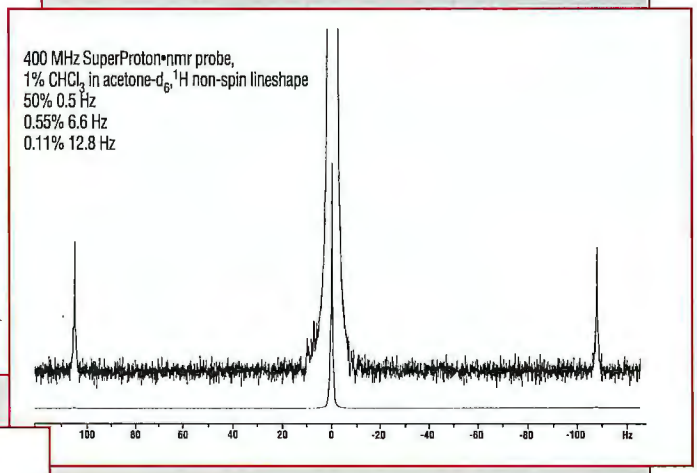

b)
water
x 10⁷
Arbitrary intensity
10
8
6
4
2
0
0
10
20
30
6000
5500
5000
4500
4000
Time units
Frequency channel

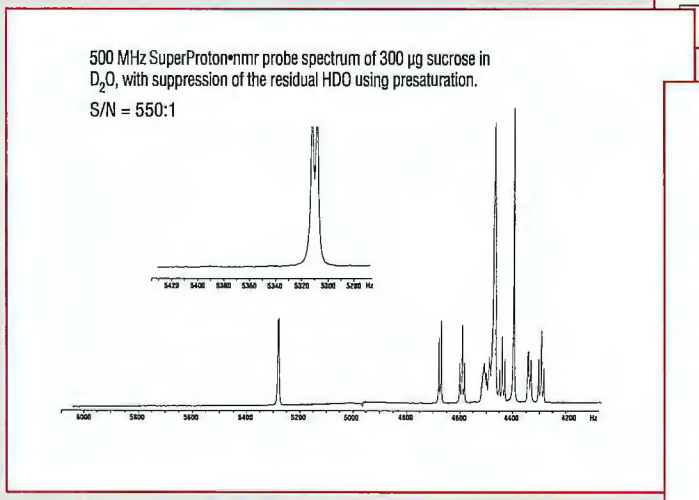

c)
CORE-
fit
x 10⁷
Arbitrary intensity
10
8
6
4
2
0
0
10
20
30
6000
5500
5000
4500
4000
Time units
Frequency channel

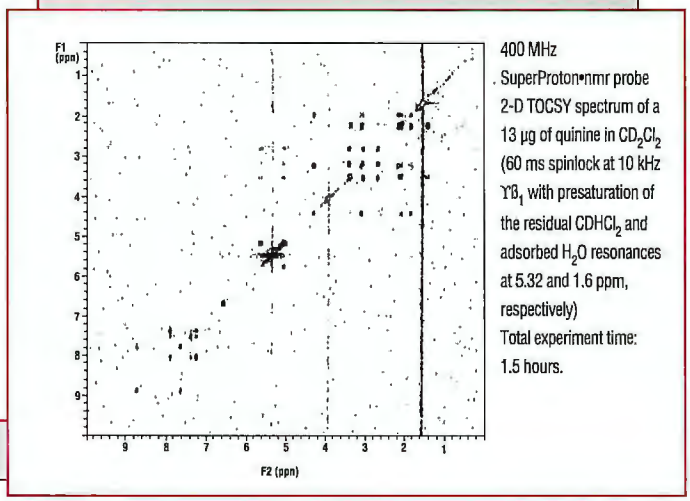

d)
Residuals (x10)
x 10⁶
Arbitrary intensity
10
8
6
4
2
0
0
30
6000
4000
Time units
Frequency channel

# Break Through the NMR Detection Limit Barrier




400 MHz SuperProton•nmr probe 2-D TOCSY spectrum of a 13 µg of quinine in $CD_2Cl_2$ (60 ms spinlock at 10 kHz $\gamma B_1$ with presaturation of the residual $CDHCl_2$ and adsorbed $H_2O$ resonances at 5.32 and 1.6 ppm, respectively) Total experiment time: 1.5 hours.

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- Available for 400 and 500 MHz systems

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## Extending the $^{1}H$ and $^{19}F$ NMR Detection Limits

| Features | Benefits |
|---|---|
| Sensitivity advantage of a factor of 4 relative to a standard 5 mm $^{1}H$ or $^{19}F$ probe | Factor of 16 reduction in data-collection time; ability to obtain high sensitivity 1-D and 2-D spectra of samples in very low concentrations (e.g. 60 µg of MW 600 in 600 µl) |
| Excellent pulse width performance | Wide excitation bandwidth, supporting the wide range of $^{1}H$ and $^{19}F$ spectral dispersion |
| Excellent RF homogeneity | High sensitivity for homonuclear experiments involving multiple pulses and pulse trains |
| Excellent spinlock performance | High sensitivity for homonuclear experiments utilizing high-power spinlock |
| Excellent lineshape performance | High spectral resolution and sensitivity; excellent solvent suppression |
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# Dim Sum--Direct Image Simulation by Summation Over Isochromats

Dear Barry,

March 10, 1997 (received 3/18/97)

We have been having a lot of fun with an imaging simulator that we have developed here. It's based on summation of the signal from individual isochromats, but the design turned out to be rather tricky with many subtleties that were not apparent at the beginning. Some of these were presented in [1]. The time-domain signal is calculated for the entire experiment, instead of treating phase encode steps on a one-by-one basis. The time domain data resulting from the imaging sequence is pasted into a standard **k**-space matrix, and yields an image after two dimensional Fourier transformation. Because there are no assumptions about transverse dephasing between pulses, unlike other simulations we are aware of, all direct and stimulated echoes are correctly modeled in terms of their amplitude, phase, timing, and shape.

One of our computer phantoms is a genetically-altered (to keep up with the times) rat [Fig. 1]. Each of the labeled regions is assigned values for $T_1$, $T_2$, and $\rho$ (arbitrary units). Local linewidth and motion can also be incorporated. The simplest results for a spin-warp sequence are shown in Fig. 2. Region assignments for ($T_1$, $T_2$, $\rho$) are: 2a: A: (5 s, 1 s, 1), B: (2 s, 250 ms, 1), C: (3 s, 300 ms, 1), D: (4 s, 500 ms, 1); TR=100 s, TE =15 ms, resulting in a density weighted image. 2b: Same region assignments as in 2a, with TR=100 s, TE=500 ms, resulting in a $T_2$ weighted image. 2c: A: (5 s, 1 s, 1), B: (2 s, 1 s, 1), C: (3 s, 1 s, 1), D: (4 s, 1 s, 1); TR=100 s, TE =15 ms, resulting in a $T_1$ weighted image.

We can simulate in a natural fashion many MRI artifacts, including susceptibility effects in gradient-recalled imaging, aliasing, errors due to TR ~ $T_2$, and pulse angle imperfections. We can demonstrate removal of these artifacts by inserting appropriate homospoil pulses or other fixes. Since we see the entire time-domain signal, we can establish whether artifacts are due to FID intrusion into the observation window, stimulated echo formation, or other effects. One example is shown in Fig. 3. Region parameters as in 2c; TR=1 s, TE =15 ms. When TR ~ $T_2$, a zipper artifact appears [3a]. A homospoil pulse placed after acquisition removes the artifact [3b].

The sequences we have simulated in detail, to date, are spin-warp, gradient-recalled echo, Burst, QUEST, and EPI. An example of a fast imaging sequence is shown in Fig. 4.

Results from a Burst sequence with increasing excitation pulse are shown in Fig. 4a. Region assignments for ($T_1$, $T_2$, $\rho$) are: A: (5 s, 2 s, 5), B: (2 s, 2 s, 2), C: (3 s, 2 s, 3), D: (4 s, 2 s, 1). Acquisition period is 204.8 ms. The simulation fully accounts for short pulse spacing, and keeps track of the magnetization components of each sample isochromat subject to pulses and free precession. So, all echoes are precisely modeled. An excitation pulse angle that is too large results in an echo train which is not flat in the absence of phase gradients, resulting in image distortions, as shown.

Burst works well for $T_2$ >> duration of the pulse sequence. Fig. 4b shows the blurring resulting from $T_2$ (100 ms, all other parameters as in 4a) being only half the acquisition period of 204.8 ms.

The computer code is undergoing constant development but at some point will be stable enough to distribute to anyone who might be interested.

**Reference:** Shkarin and Spencer. Concepts in Magnetic Resonance 8(4):253, 1996.

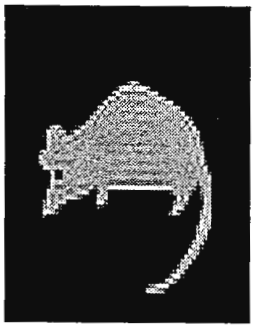
Best regards,

Pavel Shkarin

Rick Spencer
NMR Unit/NIH/National Institute on Aging
spencer@helix.nih.gov

Fig. 1


Fig. 2 a



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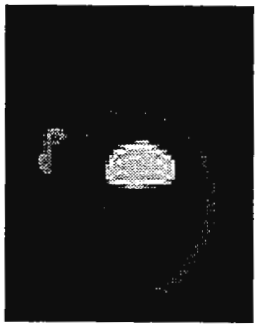

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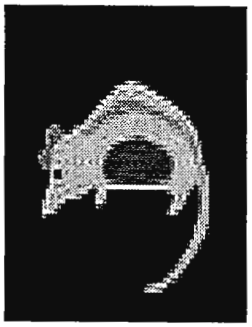
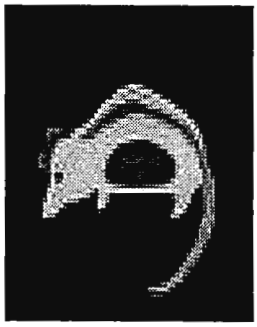


Fig. 3 a

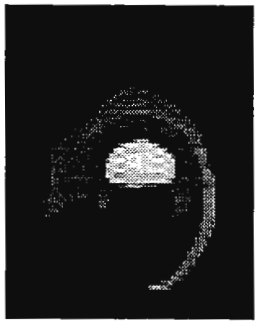
b



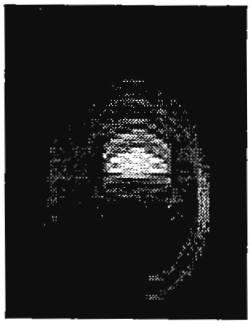
$1^0$

Fig. 4 a

$4^0$



$7^0$

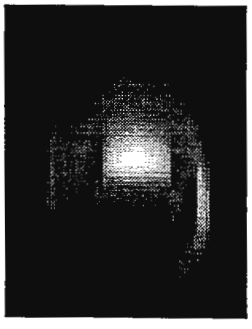


$1^0$

Fig. 4 b

$4^0$

$7^0$



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| Duty cycle | Up to 10% | Up to 10% |
| Amplitude droop | 5% to 20 ms typ. | 5% to 20 ms typ. |
| Harmonics | Second: -25 dBc max.<br>Third: -24 dBc max. | |
| Phase change/output power | 10° to rated power, typ. | |
| Phase error overpulse | 4° to 20 ms duration, typ. | |
| Output noise (blanked) | <10 dB over thermal | |
| Blanking delay | <1 µ s on/off, TTL signal | |
| Blanking duty cycle | Up to 100% | |
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|---|---|---|---|
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| System monitors | 1. Forward/Reflected RF power<br>2. Over pulse width/duty cycle | 3. DC power supply fault | 4. Thermal fault |
| Front panel controls | 1. AC power | 2. Forward/Reflected power | |
| AC line voltage | 208/230 VAC, 10%, 1Ø, 47-63 Hz | | |

| | 3445 | 3446 |
|---|---|---|
| AC power requirements | 1400 VA | 700 VA |
| Size (HWL, inches) | 8.75 x 19 x 24 | 8.75 x 19 x 24 |
| Net weight | 110 lbs. | 75 lbs. |

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

CSIRO
AUSTRALIA

CSIRO Division of
Chemicals & Polymers
Private Bag 10,
Victoria 3168
Australia.
4 March 1997
(received 3/12/97)

Dear Dr. Shapiro,

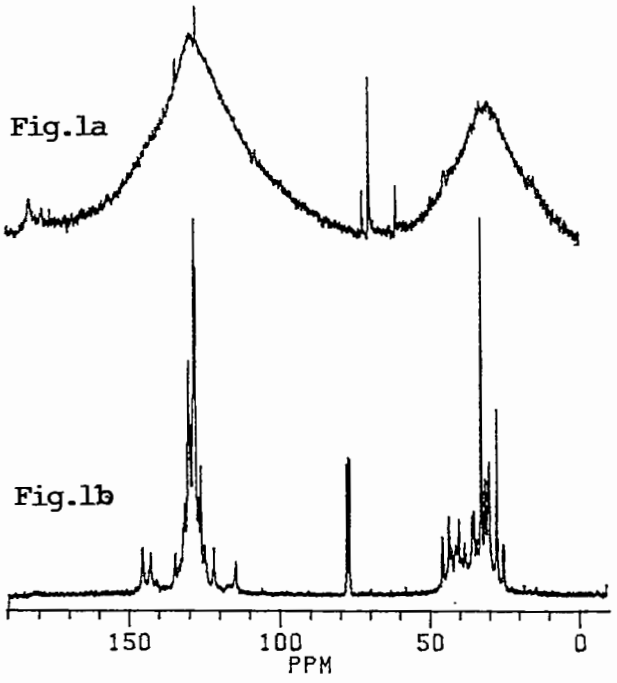
**13C Polymer sample techniques.**

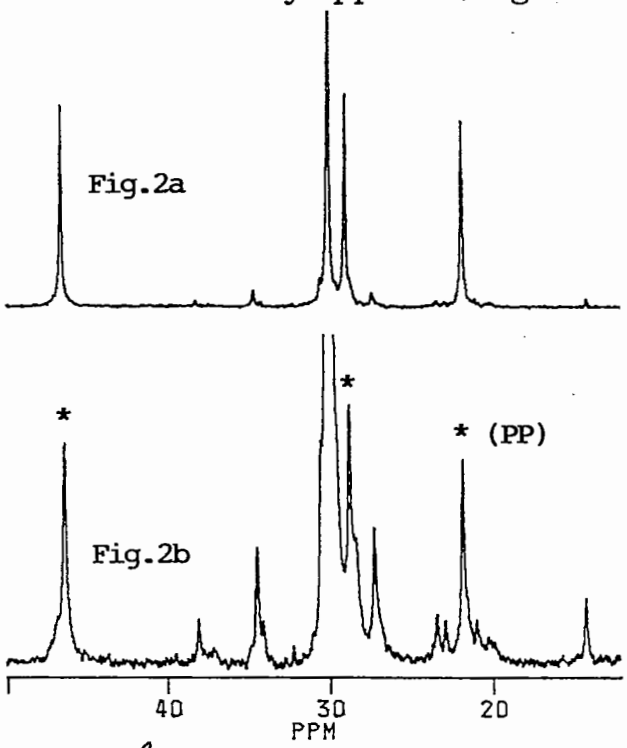
High resolution spectra of small molecules are readily obtainable but polymers can be a different matter. We use a number of sample handling techniques that can help overcome some initially intractable sample problems. Two examples are illustrated.

A sample of a Latex (the result of a polymerization reaction performed in water in the presence of a surfactant) gives a typical uninformative 13C spectrum as shown in Fig.1a. However after the addition of an equal volume of CDCl3 and gentle shaking, a swollen gel is produced. The CDCl3 has "loosened" the hydrophobic polymer so producing a quite respectable spectrum Fig.1b.

Likewise partially crosslinked polymers will also give good 13C spectra if a suitable solvent can be found to produce at least a sixfold volume expansion gel. However for best results the sample should be finely ground to minimise sample inhomogeneity.

Fig. 2a. is a 13C spectrum of polypropylene (PP) contaminated with polyethylene in chlorobenzene at 110 C. The polyethylene is visible but may be accentuated by simply lowering the temperature to 90 C so causing the polypropylene to partially solidify. The polyethylene chain branching signals are now readily apparent, Fig. 2b.




Sincerely,

Jonathan Hodgkin

R. Ian Willing

Please credit this contribution to Dr. Jo Weigold of Monash University.

# NMR Spectroscopist

SmithKline Beecham is an international leader in research and development, built on the excellence and commitment of our employees. An opportunity currently exists for an NMR Spectroscopist to join our team.

The selected candidate will perform NMR spectroscopy experiments, including liquid magic angle spinning (MAS) NMR, LC-NMR and NMR Imaging experiments to develop new applications of NMR to drug discovery and to execute program related research in NMR spectroscopy and imaging. Our NMR group has a state-of-the-art facility with two 500 MHz spectrometers and one 400 MHz NMR spectrometer with micro-imaging accessories and a 200 MHz/40 cm imaging instrument, with well-equipped data analysis and electronics laboratory. The data analysis laboratory consists of a series of UNIX, SGI and Sparc workstations.

Qualified candidates will have an MS in Physics, Chemistry or Biochemistry and a strong experimental background in 1D and 2D NMR techniques, including familiarity with home-built probes, and their application to chemical and biological problems. Familiarity with NMR imaging techniques is a definite plus.

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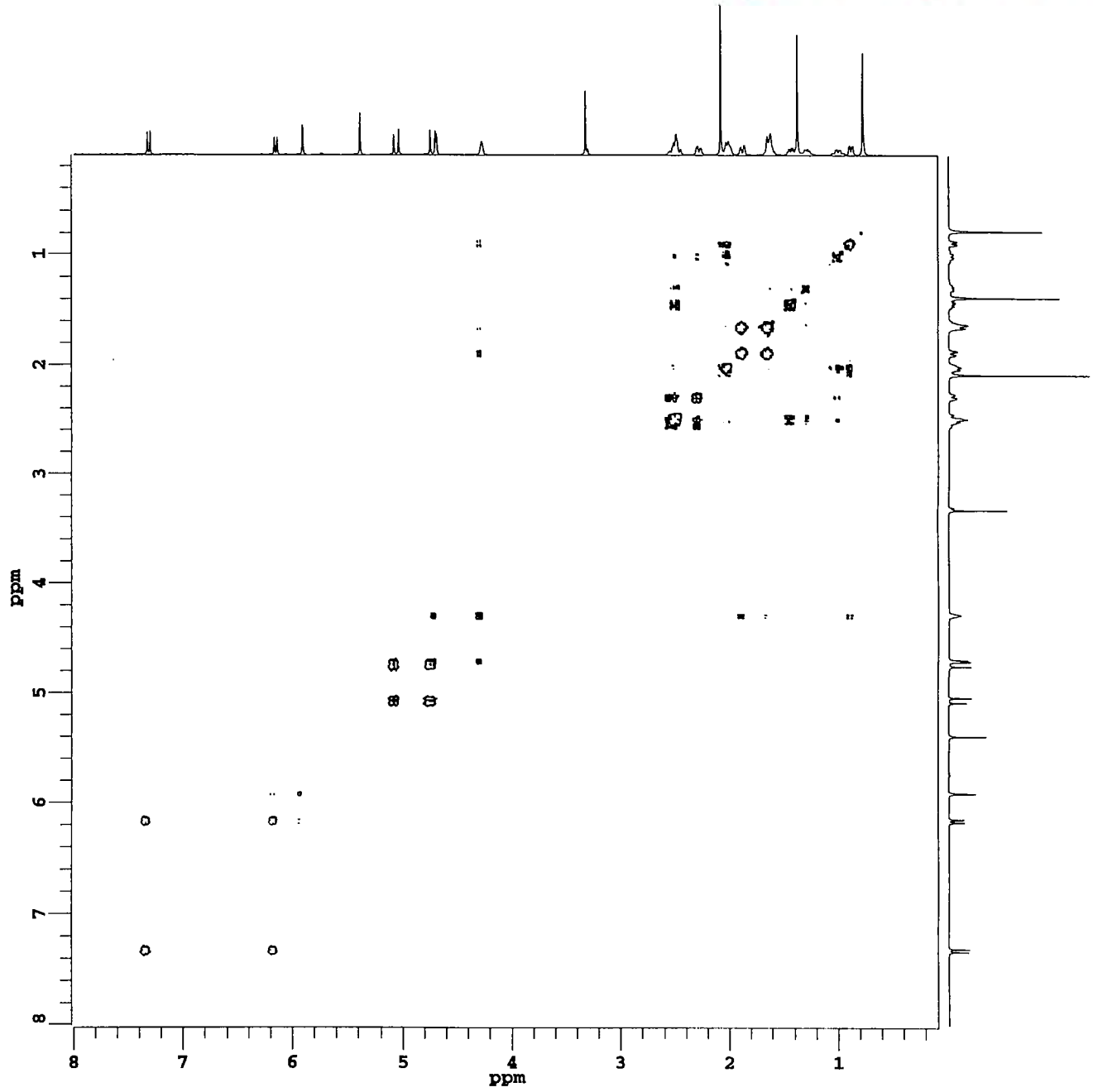


# $^{1}H$-$^{1}H$ PHASE-SENSITIVE PFG DOUBLE QUANTUM FILTERED COSY OF PREDNISOLONE 21-ACETATE

A Division of Otsuka Electronics USA Inc.
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# Shown here is the $^1H$-$^1H$ PFG DQFCOSY spectrum of prednisolone 21-acetate in DMSO-$d_6$ collected on the *Chemagnetics*™ 400 MHz CMX Infinity Spectrometer.

- Excellent signal-to-noise and resolution are obtained in this spectrum. Only two scans per row were necessary for the signal-to-noise seen here.
- Spinsight™ software features multiple viewports which can contain acquisition data and processed data of multiple dimensions. Parameters can be exchanged between the viewports for easy experiment setup.
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- Compared to the traditional phase-sensitive COSY experiment, the double quantum filtration version suppresses single quantum coherences.
- This filtration simplifies the spectrum along the diagnonal thereby allowing for more straightforward structural analyses. Because we are observing higher order coherences, greater sensitivity is necessary to detect these weak couplings

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U.S.A.

(received 2/24/97)
Nijmegen, February 18, 1997

# $T_{1\rho}$ or standing waves ?

Dear Dr. Shapiro,

A spectrometer that works perfect most of the time, but produces a spurious signal once in a while, destroying the experiment, is a nightmare for both the spectroscopist and the responsible technician. It is extremely difficult to trace such a problem as it "almost never" occurs. To be armed for such occasions we recently developed a device we call the "Stabilog". As the name suggests, it can monitor the long- and/or short-term stability of a spectrometer. The "Stabilog" consists of a piece of home-built hardware and a PC running a C-program to sample the data. Long-term results are displayed using a little Matlab application.

The hardware consists of three analog and four digital channels. The channels are activated by TTL pulses. The analog channels sample their input voltages every 16 µsec and accumulate as long as they are activated. The digital channels are measuring at a rate of 10 nsec. The PC runs a C-program that graphically shows the statistics per channel during the experiment. To process the data at sufficient speed, the data are handled by an interrupt service routine. As different channels are available, different parameters can be monitored making it possible to correlate timing, rf-amplitude, temperature, line voltage etc. After the PC has taken a predefined number of measurements, the accumulated data are stored on disk, and the program starts over again. In this way it is possible to directly monitor the short term stability on screen whereas the long term stability can be inspected later by studying the stored values.

The first variable we studied with this device was rf-stability. We tested the output voltages of two rf-amplifiers "on the bench", by pulsing into a dummy load. The tests were performed at approximately 30% of the maximum rf-outputlevel, halfway the frequency range of an AMT 3200 and a Bruker BLAX 300RS amplifier. The results are listed in table 1. The most important column is column 3. It shows the thermal stability of the output voltages as a function of ambient temperature. In both cases we measured a dependency of -0.5% per degree centigrade from 22 to 30°Celsius. The first column gives a good indication of how long one has to wait before starting a measurement after switching the transmitter on (1 hr.). Notice that the AMT3200 has a "standby/operate" switch, whereas the BLAX has not. It looks a bit strange, that it would be better to totally switch off the AMT than to just disable it, but this may be caused by compensating temperature coefficients in different parts of the transmitter. We

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FOR MOLECULAR STRUCTURE, DESIGN AND SYNTHESIS

did not find any change in output voltage upon varying the duty cycle (0.2%-2%) or the line voltage (-15%).

Table 1: Percentage change of transmitter output voltage. 1) during warm up after switching the amplifier on 2) during warm up after switching from standby to operate and 3) as a function of temperature.

| | 1. warm up | 2. Standby/operate | 3. Temperature |
|---|---|---|---|
| AMT3200 | -2%(1 hr) | -3%(0.5 hr) | -0.5%/degr |
| BLAX300RS | -10%(1 hr) | - | -0.5%/degr |

Armed with this knowledge and with plans for stabilization of the RF-amplifiers during operation in the back of our minds, we went to look at the real thing, a straightforward $^{13}C$ $T_{1\rho}$-measurement that did not produce straightforward exponential-curves. The experiment consists of a normal Hartmann-Hahn cross-polarization followed by the (variable length) lock pulse and the ($^1H$ decoupled) acquisition. The different lines in the spectrum behaved rather strange. After an initial decay the signal intensity started to build up again. We hooked up the Stabilog to find out whether there was any noticeable drift on one of the transmitters. We were astonished to find out that the output voltage of the proton amplifier increased 4% and the X-transmitter 12% during the measurement. This increase in output voltage could not be attributed to a temperature change of the transmitter. Moreover, we know that the stability is much better when the amplifiers pulse into a dummy load. Thus the problems must be attributed to a standing wave in the transmitter cables. This can only be caused by a detuning and/or a change of the matching of the probe head. This means, that the variation in locktime (10 µsec-50msec) seriously changes the temperature of the electronics in the probe head. The end result is that the Hartmann-Hahn condition changes, and thus the cross-polarization gets more or less effective, depending upon the initial setting. The problems were circumvented by applying the remainder of the lockpulse after the acquisition-time, making the overall duty cycle and therefore probe-heating effects constant.
A more elegant solution is to stabilize the actual rf-field strength in the probe head rather than the amplifier output. Therefore we need an rf-field measurement in the probe head and regulate the amplifiers to keep this constant. Regarding the color of the ultimatum we hope to report on this at some later date. Please credit this contribution to the account of Prof. Dr. E. de Boer.

Sincerely yours,

Jan van Os

Hans Janssen

Arno Kentgens

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All of the pulse techniques typically used to analyze dissolved samples may be applied in HRMAS, including 1D proton and $^{13}C$, HMQC, HMBC, TOCSY, and many others. Now gradients can be used to accelerate these methods and eliminate artifacts and $t_1$ noise in exactly the same way as for conventional high resolution NMR.

Other unique features of the Bruker HRMAS accessory include:

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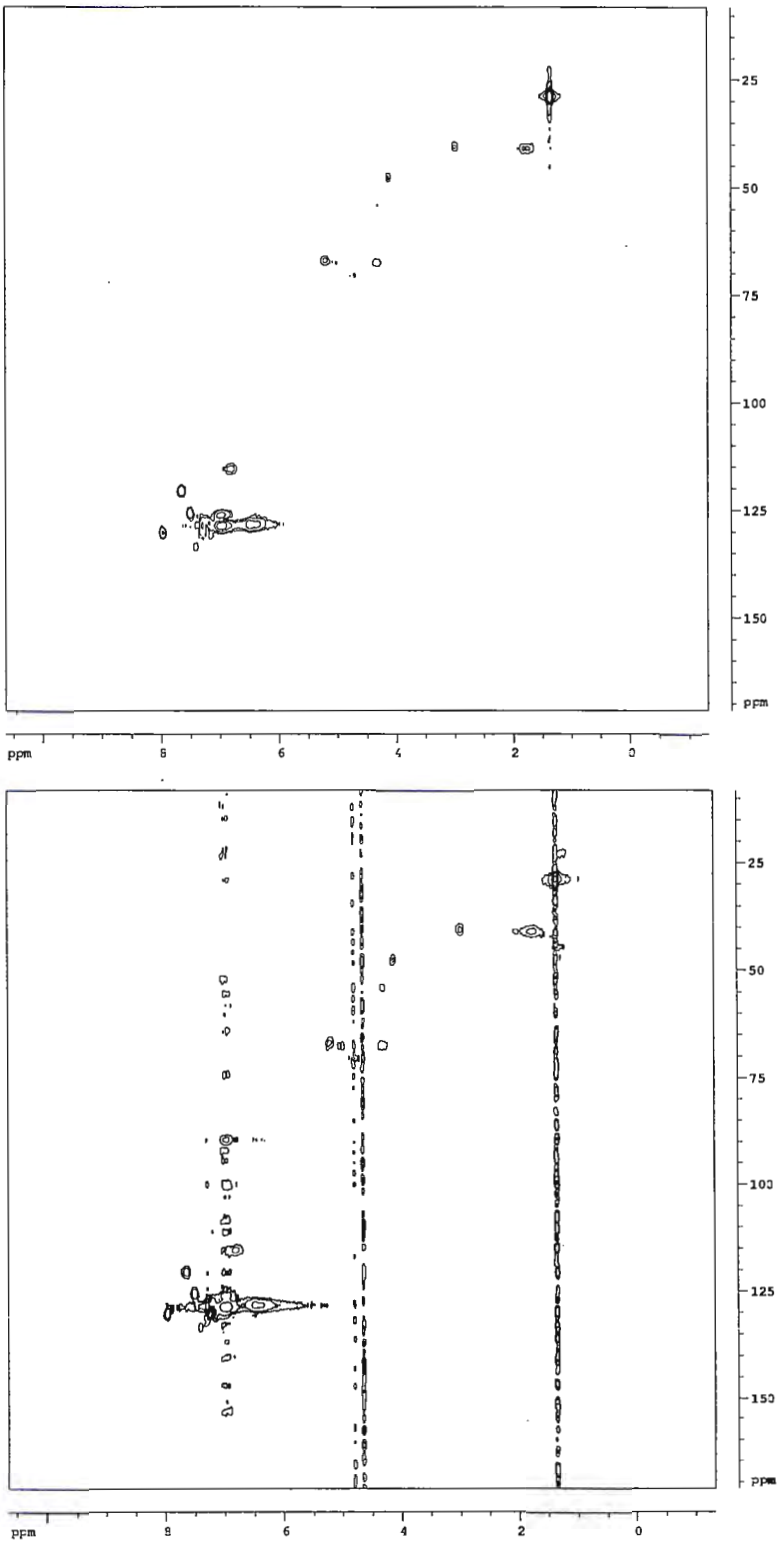
- Automated sample changer, for unattended analysis of up to 40 samples

Gradient HRMAS is compatible with any Bruker gradient accessory that includes pre-emphasis. Call your local Bruker office, and ask for more details.

### SPECIFICATIONS FOR THE GRADIENT HRMAS PROBE

| Category | Specification | Comment |
|---|---|---|
| Rotor diameter | 4mm | Outer diameter |
| Rotor Volume | 70 uL | Full |
| Rotor Volume | 20 uL | With spacers |
| Resolution | 1.5 Hz | $^{1}H$, $CHCl_3$ sample, FWHH |
| $^{1}H$ 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. $^{1}H$-$^{13}C$ 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.*

**SEARLE**

Chemical Sciences Analytical
4901 Searle Parkway
Skokie, IL 60077

March 11, 1997
(received 3/18/97)

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

**A Deceptively Simple Spectrum of the Second Kind**

Dear Barry,

It is clear that two of the most obvious indicators of how fast time moves are (1) the rate at which other people, especially children, seem to age, and (2) the shorter and shorter intervals between your warnings that it is time for another submission to the NMR Newsletter!

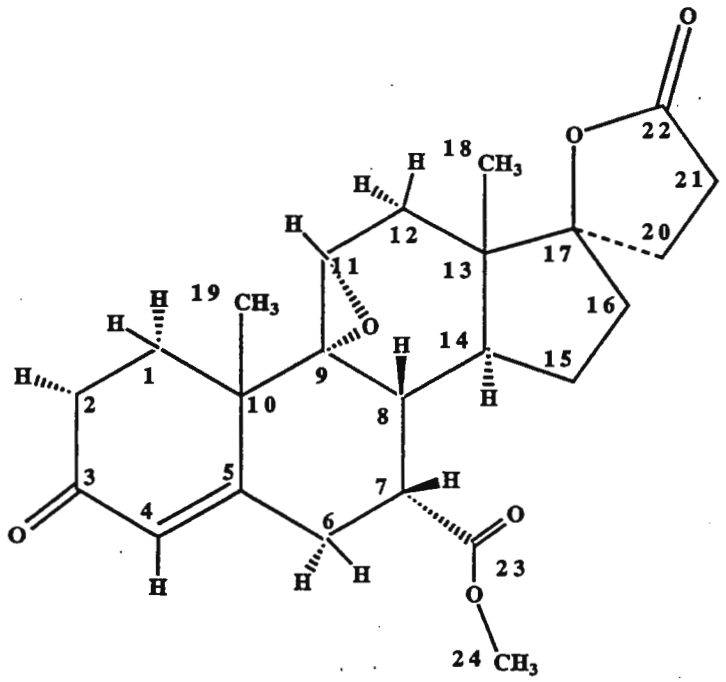
Although we, like others, have not found proton J-resolved spectra to be particularly helpful in our work, we occasionally run one to make sure we have not lost our touch. One which we ran recently is shown in Figure 1. We were very pleased to see the splitting of the C-11 proton signal into a nice doublet of quartets (inset in Figure 1). The larger coupling was clearly due to coupling to the C-12 beta ("equatorial") proton, and we naturally assumed that the smaller coupling (0.5 Hz) into quartets must obviously be to the three protons in one of the two angular methyl groups. The only question was whether the coupling was to the C-18 or C–19 protons. Coupling in either direction would be over five bonds, and why the coupling would be in one direction and not the other was rather puzzling.

Our puzzlement led us to do the long-range COSY, which is shown in Figure 2. We had already assigned all of the proton chemical shifts using a variety of 1- and 2-D techniques. The long-range COSY shows that the smaller couplings are not to the protons in either of the two angular methyl groups, but rather to the C-12 alpha (axial) (three bonds), the C-7 beta (equatorial; five bonds), and C-1 beta (equatorial; five bonds) protons. This is not something which we would have predicted.

Sincerely,

Roy H. Bible, Jr.

Elisabeth Hajdu



SC-66110

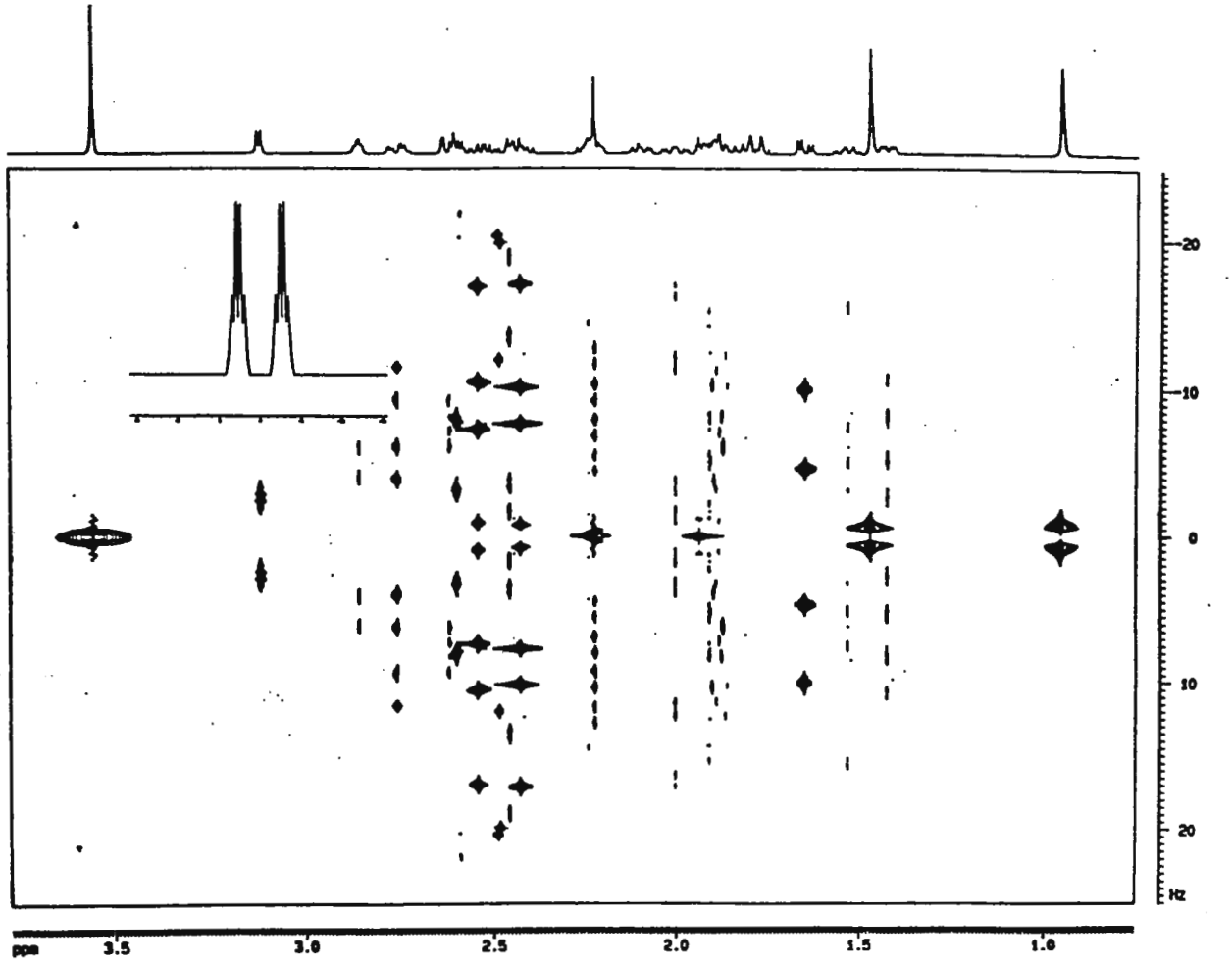



Figure 1. The proton J-resolved spectrum of SC-66110 determined on an AMX-500 insrument using a deuterated acetonitrile solution. The spectrum was symmetrized along the center of F1 after substraction of partial projections from both domains.

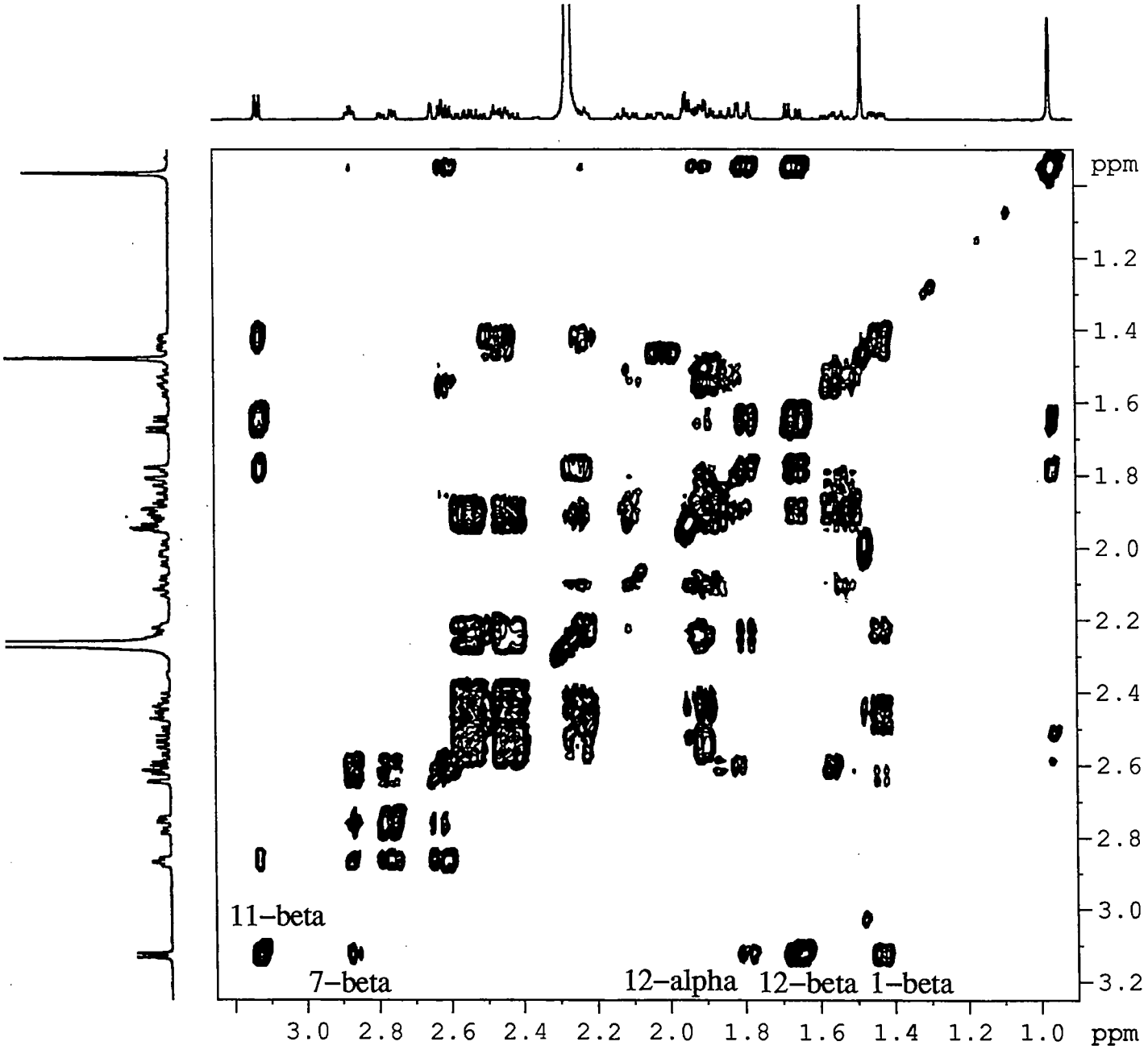



Figure 2. The 500 MHz long-range COSY spectrum of a deuterated acetonitrile solution of SC-66110 obtained using a 600 millisecond delay before and after the second 90 degree pulse.

## Position Available

BRUKER Instruments Inc. has an immediate opening in the Billerica MA applications laboratory for an

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Tel: (415) 221 - 4810, ext. 3644
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E-mail: jerrym@itsa.edu.ucsf

February 26, 1997
(received 3/1/97)

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

Dear Dr. Shapiro:

In your last NMR Newsletter (#461) I inadvertently inserted in extra "i" in the internet address from which my MATPULSE program can be found. The correct address is:

http://nmrsg.biophys.upenn.edu/

I apologize for that error. Mark Elliott at that site can help with any installation problems.

Sincerely,

Gerald B. Matson
Adjunct Professor, Pharm. Chem., UCSF
Facilities Manager, MR Unit, DVAMC

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Dr. Bernard L. Shapiro
The NMR News Letter
966 Elsinore Court
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March 10, 1997
(received 3/15/97)

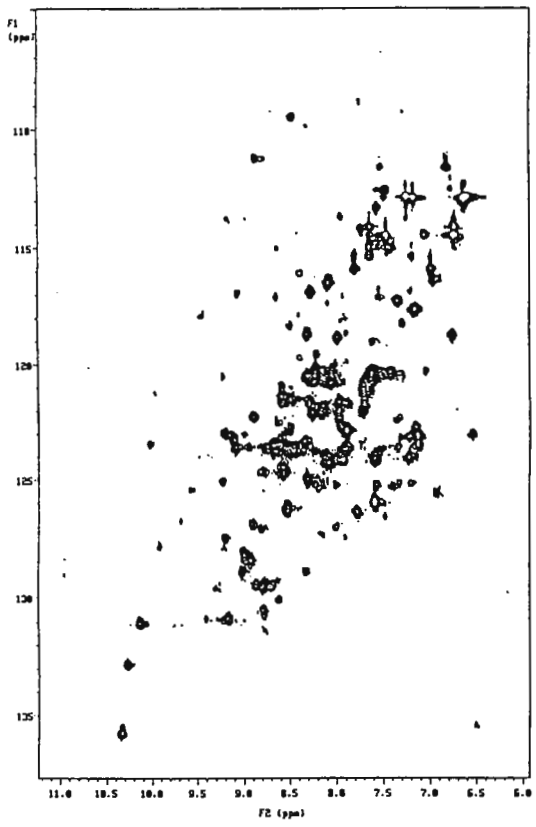
# Are Dilute Protein Samples Better?

Recent advances in heteronuclear multidimensional NMR methods have enabled detailed structural studies of proteins with molecular weights greater than 30 kDa.[1] Higher molecular weight proteins often suffer from low solubility or aggregation at millimolar concentrations. We have been evaluating conditions which give good $^{1}H$-$^{15}N$ HSQC spectra[2] for a 247 amino acid (27 kDa) protein. We expect the quality of heteronuclear NMR spectra of this protein to improve upon deuteration of the non-labile protons. However, before expressing the protein in $D_2O$, we need to be certain that the protein will yield high-quality NMR data. Using a $^{15}N$-enriched protonated sample, we have explored solvent conditions such as pH, salt, buffer, and temperature in order to establish conditions under which the protein is stable and gives the best NMR spectra. Other important parameters in the optimization of sample conditions are the concentration and volume of the sample. Here we describe many of the technical details associated with choosing the concentration and sample volume to be used for NMR studies of larger proteins. Emphasis is given to methods which enable optimal probe selection for proteins which exhibit aggregation or viscosity effects on their NMR spectral quality.

We have both 5mm and 8mm $^{1}H/^{13}C/^{15}N$ probes equipped with Z-axis gradients (Gz) available in the laboratory. We have been given 15.3 mg of the 27 kDa $^{15}N$-enriched protein which is enough to make either a sample of 0.9 mM concentration in a 5mm tube, or 0.45 mM concentration in an 8 mm tube (susceptibility matching plugs were not used in this comparison, the sample volumes of 630 µl and 1260 µl were determined to be optimal for giving good lineshape in the 5mm and 8mm probes, respectively). The spectra in Figure 1A and 1B were collected with the same amount of $^{15}N$-enriched protein sample. The spectrum in Figure 1B, obtained using the 0.45 mM sample in the 8mm probe, is clearly superior. The empirical fact that a fixed amount of protein, diluted by a factor of 2, gives a significantly better HSQC spectrum is a somewhat counterintuitive result. This advantageous "dilution effect" is likely to come from reductions in aggregation and viscosity in the more dilute sample. The combination of volume and concentration that gives optimal spectral quality is a function of relative probe performance and the dilution effect. We have investigated these parameters in a quantitative manner and established a protocol for optimizing them for a given sample.

**Figure 1. Comparison of Gradient, Sensitivity-enhanced $^{1}H$-$^{15}N$ HSQC Spectra for a 27 kDa Protein. (Buffer: 37 mM Tris-$d_{11}$, 50 mM NaCl, 1.5 mM $NaN_3$, 1.0 mM DTT, 8% $D_2O$, pH = 7.2) Note: This protein exhibits a "dilution effect" of 2.0.**

**A. 0.9 mM Protein Sample**
(5mm $^{1}H/^{13}C/^{15}N$/Gz Probe,
630 µl sample volume)



**B. 0.45 mM Protein Sample**
(8mm $^{1}H/^{13}C/^{15}N$/Gz Probe,
1260 µl sample volume)

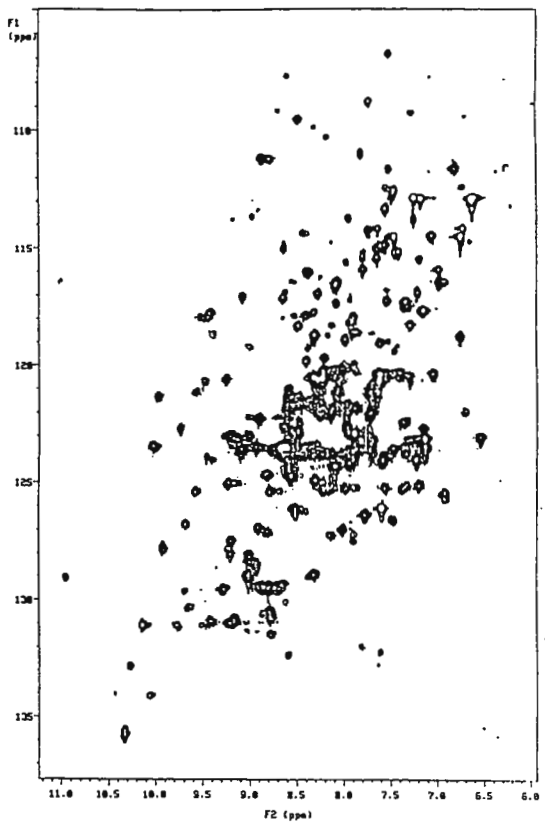


**Table 1. Parameters for 5mm vs. 8mm Probe Comparison and "Dilution Effect" Calculations.**

| Probe | Varian 5mm $^{1}H/^{13}C/^{15}N$/Gz | Nalorac 8mm $^{1}H/^{13}C/^{15}N$/Gz |
|---|---|---|
| Probe Vintage | 1994 | 1996 |
| NMR Tube Diameter | 5mm(outer)/4.20(inner) | 8mm(outer)/6.99mm(inner) |
| Total Sample Volume | 630 µl | 1260 µl |
| Coil Length (assumed) | 1.6 cm | 1.5 cm |
| Active Volume (calculated) | 222 µl | 576 µl |
| Integrated Signal Intensities: | | |
| N-acetyl-alanine in $D_2O$ | 1.00 (10.9 mM) | 2.30 (10.9 mM) |
| $^{15}N$-gly-peptide in salty buffer | 1.00 (1.70 mM) | 0.84 (0.85 mM) |
| $^{15}N$-protein in salty buffer | 1.00 (0.9 mM) | median value 1.70 (0.45 mM) |
| Relative Molar Probe Performance: | | |
| $^{1}H$/no salt | 100% | 88% |
| $^{1}H/^{15}N$/Gz/salt | 100% | 64% |

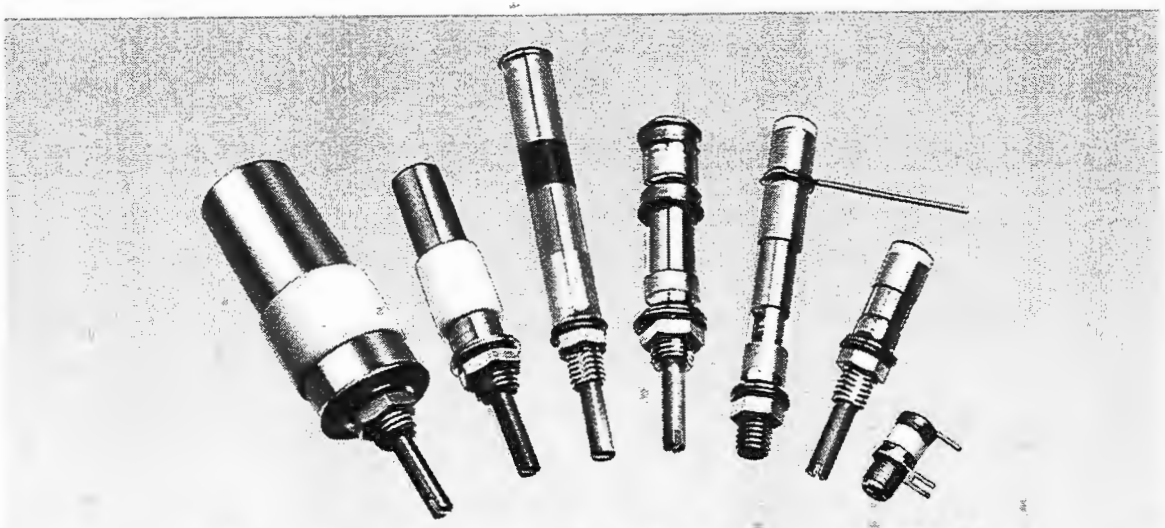


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**Figure 2. Comparison of $^{1}H$ Sensitivity for the Acetyl-$CH_3$ Resonance of N-Acetyl-Alanine in $D_2O$. (No Buffer or Salt)**

**A. 10.9 mM N-Ac-Ala** (5mm $^{1}H/^{13}C/^{15}N/Gz$ Probe, 630 µl volume)

**B. 10.9 mM N-Ac-Ala** (8mm $^{1}H/^{13}C/^{15}N/Gz$ Probe, 1260 µl volume)

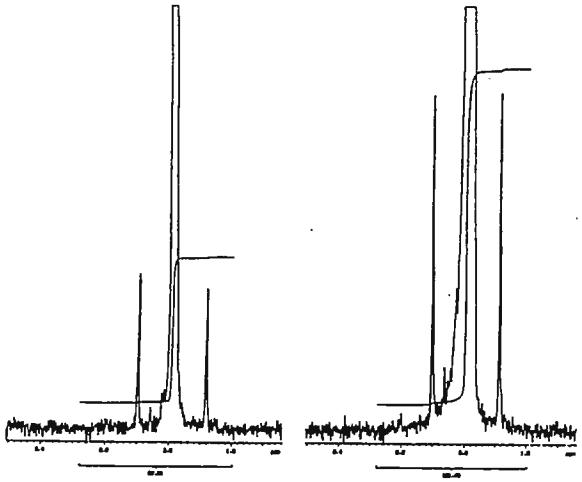
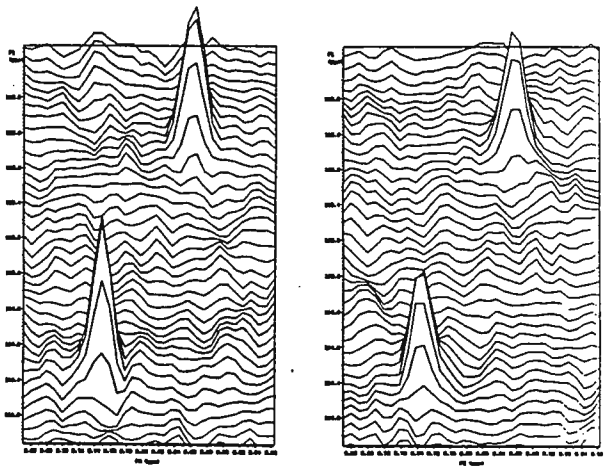


**Figure 3. Comparison of $^{1}H$-$^{15}N$ HSQC Sensitivity for the 1.13 kDa $^{15}N$-Gly-Peptide. Note: "Dilution Effect" assumed to be 1.0.**

**A. 1.7 mM $^{15}N$-Gly-Peptd** (5mm $^{1}H/^{13}C/^{15}N/Gz$ Probe, 630 µl volume)

**B. 0.85 mM $^{15}N$-Gly-Peptd** (8mm $^{1}H/^{13}C/^{15}N/Gz$ Probe, 1260 µl volume)



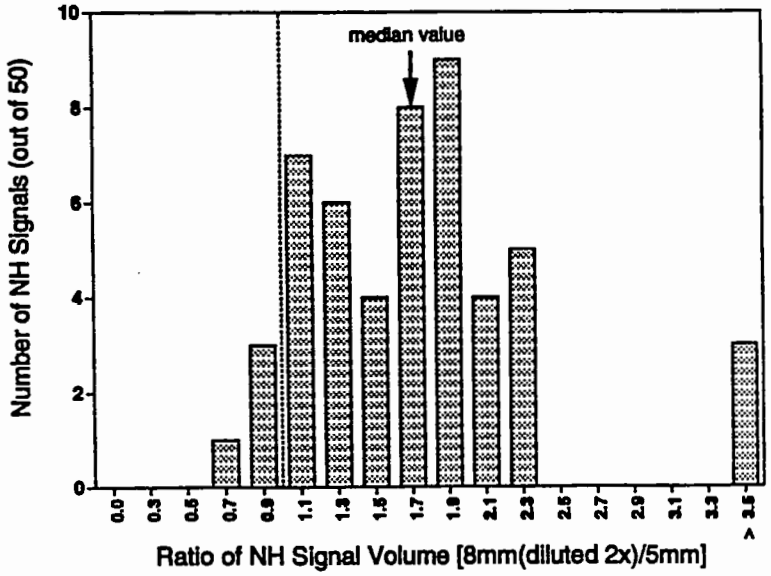
We have evaluated probe performance using three types of samples: 1) N-acetyl-alanine in $D_2O$, representing a non-aggregating molecule in a salt-free buffer; 2) a non-aggregating 1.13 kDa cyclic peptide containing two $^{15}N$-glycines, in a salty buffer; 3) a $^{15}N$-enriched 27 kDa protein, in a salty buffer. The first pair of spectra (Figure 2) compares integrated signal intensities for 5mm and 8mm samples of N-acetyl-alanine in $D_2O$ at the fixed concentration of 10.9 mM. The expansions in Figure 2 show the integrated signal intensities for the acetyl methyl peak whose relative integrals are reported in Table 1 (normalized such that the 5mm intensity is set to 1.00). In Figure 2, the methyl peak is expanded to show the $^{13}C$ satellites and the noise level. To calculate the expected signal improvement for a sample of fixed concentration, one must estimate the ratio of the active volumes for the two probes. The estimated ratio of the 8mm/5mm active volumes is 2.6 (576 µl/222 µl). If the assumptions of coil lengths are correct within 10%, then the observed ratio of 2.3 for the $^{1}H$ signals must reflect a small drop in probe performance per unit volume in the 8mm probe. We can express this performance on a molar basis to get a more intuitive feel for the relative signal expected per molecule in the active volume. That is, since there are 2.6 times more molecules in the active volume of the 8mm probe but we only get a 2.3 fold signal enhancement, we can say that the relative molar $^{1}H$ performance of the 8mm probe with respect to the 5mm probe is 88%.

The relative molar performance calculated for the $^{1}H$ spectrum in $D_2O$ is not useful in calculating the dilution effect on signal performance of the HSQC spectrum because it does not include effects of salt, $^{15}N$-coil $B_1$ inhomogeneity, and Gz-pulse effects. Figure 3 illustrates a pair of gradient, sensitivity-enhanced HSQC spectra[2] taken for a 1.13 kDa cyclic peptide. This peptide has two $^{15}N$-glycine residues which account for the two observed NH resonances. The spectra in Figure 3 are collected in the same buffer as the

protein spectra illustrated in Figure 1. The buffer used for both the peptide and the protein spectra is: 37 mM Tris-$d_{11}$, 50 mM NaCl, 1.5 mM $NaN_3$, 1.0 mM DTT, 8% $D_2O$, pH = 3.0 for the peptide, and 7.2 for the protein. If we assume that this small peptide does not exhibit a dilution effect below 1.7 mM concentrations, we can use its $^{15}N$-glycine resonances to calculate the molar probe performance which includes the $^{1}H$-, $^{15}N$-, and Gz-coil effects as well as the salt effects. The observed signal intensity of the 8mm probe in this case must be scaled up by a factor of 2 to compensate for the dilution of the peptide in the 8mm sample. Using the data from Table 1 we calculate the molar $^{1}H/^{15}N/Gz$/salt performance of the 8mm probe to be 64% with respect to the 5mm probe.

Now that we know how much relative signal to expect from each molecule in the 8mm protein sample vs. the 5mm protein sample, we can calculate the dilution effect contribution by comparing the signal intensities in the spectra of Figure 1A and 1B. Figure 4 shows a histogram of 50 randomly-selected, resolved signals in the HSQC spectra of Figure 1. There is a wide distribution of enhancements seen for various NH signals. The median enhancement obtained by diluting the protein sample by a factor of 2 and running it in the 8mm probe is 1.7. Comparing this enhancement with the .84 enhancement seen for the $^{15}N$-gly peptide suggests that the protein exhibits a beneficial dilution effect which results in a median signal enhancement of 2.0 times the expected enhancement.

**Figure 4. Histogram of Relative NH Signal Intensities Obtained in HSQC Spectra Upon Two-Fold Dilution of a 27 kDa Protein.**




The median enhancement (ME) expected upon dilution of a sample is a function of: the dilution effect (DE), the dilution factor (DF), the relative active volumes of the probes used (RAV), and the relative molar probe performance (MPP):

$$ME = (DE * RAV * MPP) / DF \qquad \text{eq. 1}$$

Equation 1 predicts the improvements in spectral quality (expressed as median signal enhancement) expected for a given set of conditions. Interestingly, the dilution effect of 2.0 measered here suggests that, if one were to compare the 0.9 mM protein spectrum to a 0.45 mM protein spectrum in the same 5 mm probe, the median enhancement would be

1.0 (DE=2.0, RAV=1.0, MPP=1.0, DF=2.0). This indicates that a spectrum of comparable quality to that shown in Figure 1A, would be obtained by diluting the sample 2 fold and running half of the diluted sample in the same 5mm probe. Another interesting question to ask is: at what magnitude of dilution effect does one expect to break even (ME=1.0) upon diluting two-fold to go from the 5mm probe to the 8mm probe? If we rearrange equation 1 to solve for the dilution effect:

$$DE = (ME * DF) / (RAV * MPP) \qquad \text{eq. 2}$$

and set ME=1.0, equation 2 predicts the dilution effect to be 1.2 [(1.0 * 2.0) / (2.6 * .64)]. Thus, for the case of a salty protein with a dilution effect of 1.2, the quality of the gradient, sensitivity-enhanced HSQC spectrum would be similar for the 5mm and diluted 8mm samples. For a protein with this dilution behavior we would expect the histogram of enhacements to shift so that the median enhancement occurs at the dotted line in Figure 4.

A dilution effect of 1.2 is the threshold below which it is best to use the 5mm probe and above which it is best to use a sample diluted 2 fold in an 8mm probe. One can quantitate this dilution effect using a single probe. Start with a concentration of protein which is stable for the protein of interest, collect an HSQC spectrum, then dilute by a factor of 2.0 and collect another HSQC on the same probe. Measure the median enhancement in the two spectra and plug it into equation 2 with RAV=1.0, MPP=1.0, and DF=2.0.

In the case of $^1$H-only spectra where the relative molar probe performance is 88%, the break-even dilution effect value is 0.9. A dilution effect less than 1.0 is unlikely to occur because it implies that the protein gives less signal per molecule in the diluted form. Therefore, barring any problems with water suppression or gain levels, $^1$H spectra would always be better in the 8mm probe.

Please credit this contribution to the account of David C. Fry.

1. Yamazaki, T.; Lee, W.; Arrowsmith, C. H.; Muhandiram, D. R.; Kay, L. E. *J. Am. Chem. Soc.* **1994**, *116*, 11655-11666.
2. Kay, L. E.; Keiffer, P.; Saarinen, T. *J. Am. Chem. Soc.* **1992**, *114*, 10663.

Sincerely,

Steven Donald Emerson
Principal Scientist, Biomolecular NMR
Physical Chemistry Department

David C. Fry
Research Leader, Biomolecular NMR
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THE UNIVERSITY OF UTAH

David M. Grant
Distinguished Professor

5 March 1997
(received 3/13/97)

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

## A High Resolution 3D $^{13}C$ CSA-CSA-CSA Spectrum

Dear Barry,

It has been shown [1-3] that the CSA-CSA 2D correlation spectrum is useful in measuring chemical shift tensor principal values and is especially attractive in extracting the detailed orientation parameters for the molecules in an oriented sample. A limitation of the CSA-CSA technique is that the broad powder patterns from different types of the carbons may be severely overlapped and are often difficult to be distinguished from one another when there are a number of non-equivalent carbons in the compound. This complexity is demonstrated in Figure 1 with results obtained from a powdered 1,2,3-trimethoxybenzene (1,2,3-TMB), where seven inequivalent carbons with distinct differently principal values [4] are superimposed.

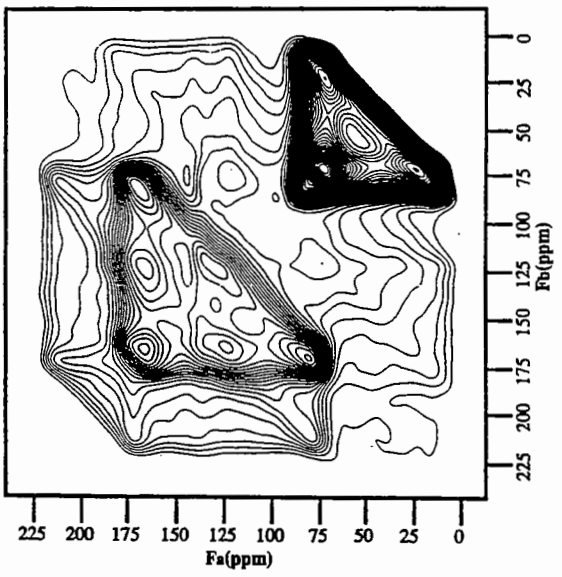


Figure 1. Contour plot of $^{13}C$ CSA-CSA spectrum of 1,2,3-TMB.

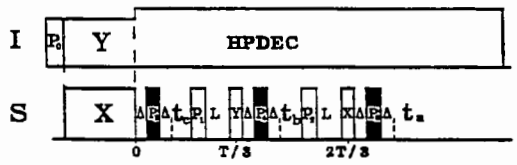


Figure 2. Pulse sequence for the 3D MAT CSA-CSA-CSA spectrum. T is the rotor period. Δ is the echo time required to suppress the probe ring-down and receiver recovery. The shaded pulses are π pulses, the rest of the pulses after the CP pulse are π/2 pulses. The pulses phases and experimental details will be reported elsewhere.

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In order to simplify the measurement, ideally one needs a 3D experiment, in which the patterns can be separated by isotropic chemical shifts. This goal can be achieved by the pulse sequence depicted in Figure 2. As in the MAT experiment [5], the read pulses are synchronized to 1/3 of the rotor period and the sample is slowly rotated about the magic angle axis. $t_a$, $t_b$, and $t_c$ are acquisition (a), evolution (b) and evolution (c) time variables, respectively. Since the initial sample orientations for a, b and c dimensions are mutually perpendicular in space, a 3D spectrum which correlates three mutually perpendicular magnetic field directions results. When a very slow sample spinning rate, i.e., 20-50 Hz, is used, the spinning side-bands are not distinguishable from each other, the spectrum obtained from a slowly rotating sample approaches that obtained from a stationary sample.

In the 3D CSA-CSA-CSA experiment, the spectrum for each carbon is separately displaced in a plane perpendicular to the diagonal of the cubic spectrum by its isotropic chemical shift value. The projection to the diagonal of the cubic spectrum resembles the conventional high speed MAS spectrum, while the projection to one of the three 2D spectral planes, i.e., $F_a$-$F_b$, $F_a$-$F_c$ and $F_b$-$F_c$, is a conventional CSA-CSA 2D correlation spectrum. A selected projection at the isotropic chemical shift position of the specific carbon to the $F_a$-$F_b$ 2D spectral plane produces the conventional 2D CSA-CSA spectrum for the specific carbon.

The application of the 3D CSA-CSA-CSA on 1,2,3-TMB is shown in Figure 3, where the 2D projection for each inequivalent carbon in the molecule is given. Clearly, the superimposed powder patterns in Figure 1 are successfully separated. Though $M_1$ and $M_3$ are not resolved in the isotropic projection spectrum due to the truncation of the data sets, the 2D patterns for $M_1$ and $M_3$, which exhibit a difference of about 4 ppm in the $\delta_{33}$ values are successfully obtained by choosing the projections at both sides of the superimposed isotropic peak.

**References**


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2. C. D. Hughes, M. H. Sherwood, D. W. alderman and D. M. Grant, **J. Magn. Reson., A 102,** 58 (1993).
3. B. F. Chmelka, K. Schmidt_rohr and H. W. Spiess, **Macromolecules, 26 (9),** 2226 (1993).
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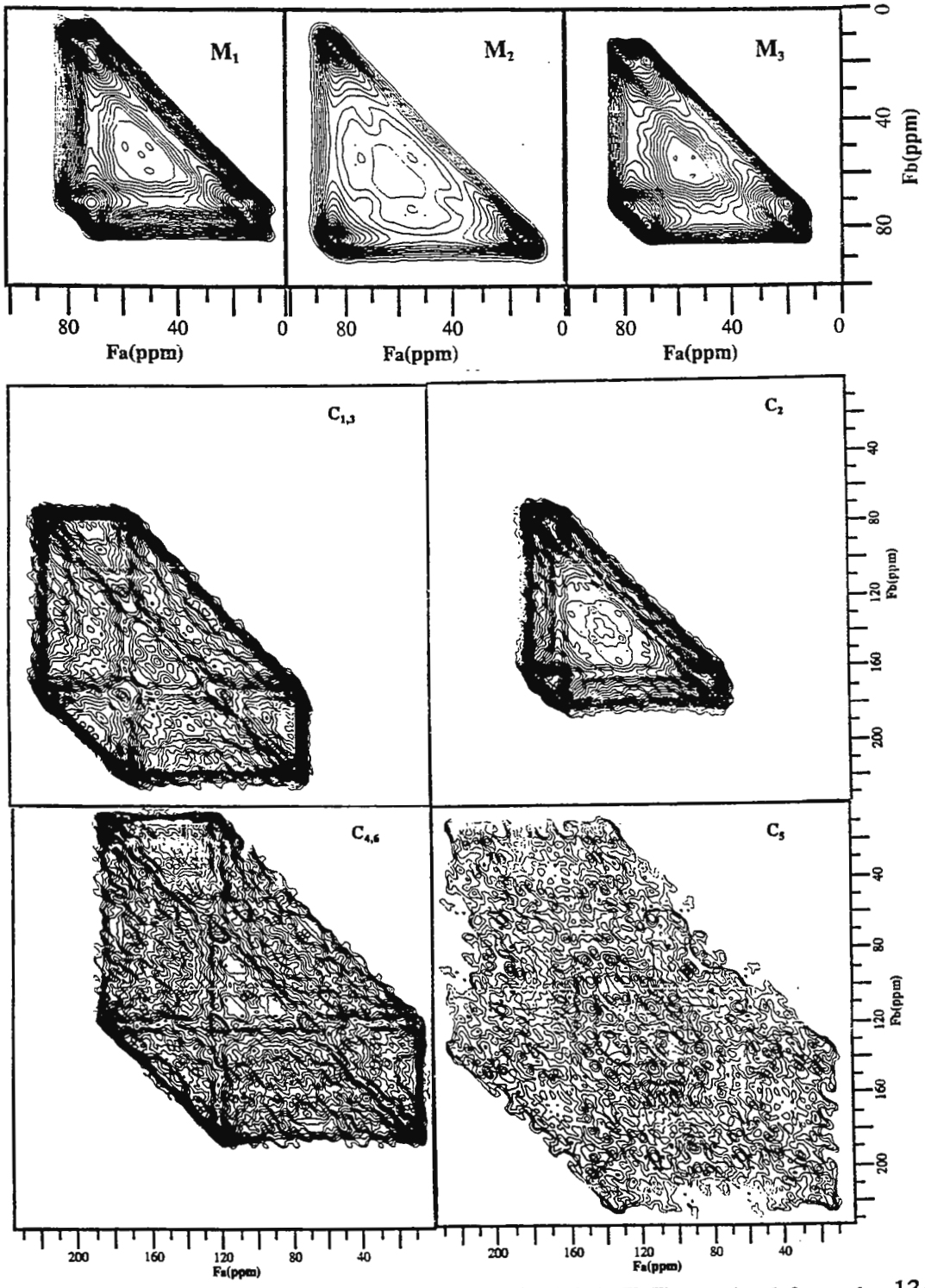


Figure 3. 2D projection for each inequivalent carbon in 1,2,3-TMB obtained from the $^{13}C$ 3D MAT CSA-CSA-CSA spectrum in Figure 3.

Sincerely yours,

Jian Zhi Hu D. W. Alderman Ronald J. Pugmire David M. Grant

## Symposium and Training:

# $^{13}$C in Metabolic Research

### THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER AT DALLAS
### Thursday, May 8, 1997

This program is aimed at faculty, fellows and students using or considering $^{13}$C NMR or $^{13}$C mass spectrometry for metabolic studies. The morning session is an introduction to $^{13}$C NMR isotopomer analysis, metabolic questions which can be answered, factors in experimental design and interpretation, and analysis of $^{13}$C NMR spectra. Software needed for both experimental analysis and simulation will be demonstrated. In the afternoon session, the guest faculty will review current applications of $^{13}$C for metabolic research.

## PROGRAM SCHEDULE

7:45 **On Site Registration**

**TRAINING: INTRODUCTION TO $^{13}$C NMR ISOTOPOMER ANALYSIS FOR METABOLIC STUDIES**

8:15 **Designing the Question and the Experiment** *Craig R. Malloy, M.D.*

9:00 **Cardiac Metabolism by $^{13}$C NMR: Kinetics and the Non-Steady State Experiment** *A. Dean Sherry, Ph.D.*

9:45 **Hepatic Metabolism and Complex Pathways by $^{13}$C NMR: the Steady-State Experiment** *F. Mark Jeffrey, D.Phil.*

10:30 **Break**

10:45 **Participants' Presentations and Discussion**

12:00 **Adjourn**

**SYMPOSIUM: $^{13}$C NMR and $^{13}$C MASS SPECTROMETRY IN METABOLIC RESEARCH**

1:00 **Magnesium Regulation in Erythrocytes Studied by $^{13}$C NMR** *Maren Laughlin, Ph.D.*

2:00 **$^{13}$C Mass Spectrometry for Metabolic Analysis *In Vivo*** *Robert Wolfe, Ph.D.*

3:00 **Break**

3:30 ***In Vivo* $^{13}$C MRS in Clinical Research** *Rolf Gruetter, Ph.D.*

4:30 **Quantitative Analyses of High Resolution NMR Spectra** *Paul A. Keifer, Ph.D.*

5:30 **Wine and Cheese Reception at the A. W. Harris Faculty Club**

6:30 **Buffet Dinner at the A. W. Harris Faculty Club**

7:15 **The Human Radiation Experiments: the Future for Radioisotopes in Clinical Investigations** *Bernard Landau, M.D., Ph.D.*

## TRAVEL AWARDS

Limited funds are available for students, fellows and young faculty with strong interest in biological $^{13}$C NMR. Awardees must actively participate in the morning training session. For more information, please contact Navin Bansal, Ph.D., at (214) 648-5887.

## REGISTRATION

The regular advance registration fee is $80. Advance registration for students, fellows and residents is $35. In order to facilitate planning, the last day for advance registration is May 1, 1997. Late and on-site registration fee is $95 ($50 for students and fellows). The coffee break, reception and buffet dinner are included in the registration fee. No money will be refunded if registration is canceled after May 5, 1997. For more information, please contact Ms. Jean Cody tel.: (214) 648-5886, fax: (214) 648-5881, email: jcody1@mednet.swmed.edu, or visit our WWW homepage: http://www.swmed.edu/home_pages/rogersmr or call Ms. Dolly Christensen at (214) 648-8013.

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March 13, 1997 (received 3/21/97)

# COUPLED EXCHANGE SOFTWARE

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

Dear Barry,

We were all taught (from time-dependent perturbation theory) that the transition probability in NMR is given by equation 1.

$$Intensity \propto \left| \langle \Phi_1 | I_x | \Phi_2 \rangle \right|^2$$

With a bit of algebra, you can show that this intensity can also be interpreted as the product of how much a line in the spectrum receives from the total Z magnetization, times how much it overlaps with the receiver. We have recently (1) shown that this works for exchanging systems as well. There you get a *complex* transition probability, with the real and imaginary parts giving, for instance, the width and position of the line. This means that there is no fundamental difference between exchange and ordinary lineshapes.

To implement this, we have written a program called MEXICO that calculates chemical exchange lineshapes, to compete with the very durable DNMR3. The program is written in C, using functions from a nice package of numerical routines in C called "meschach". The program works by calculating the spectrum and putting it into a dummy file for the spectrometer software (Bruker's XWINNMR in our case, but that's not critical) for comparison, display and plotting. You can vary the parameters until the calculated and observed match.

As a test of the program we tried fitting some spectra of a series of oxadiazolines (2) made by Phil Couture in John Warkentin's lab. They wanted them to make carbenes, but they are lovely examples of two-site unequal-population exchange. We can cheat and analyze the methyl signals to get the rates, but we can also look at the isolated $CH_2$-$CH_2$ group at the top of the ring. The major and the minor site each are ABCD spin systems, and the minor site is particularly fun, since all four protons are within a range of 25 Hz. Moreover, the shifts are all temperature dependent. Figure 1 shows the story so far. The simulations took about 20 seconds on an R4400-based SGI INDY (10 seconds to diagonalize the matrix and 10 to put the 112 detected lines into the spectrum). The minor site still needs some work, but the program seems to work quite well. A similar two-site, unequally populated, five strongly coupled spins calculation took about 7 minutes (420 lines this time).

So the program is ready for beta test, I think. Source code and SGI executables are available. If anyone wants to play with it, please send me an email.

Yours truly,

Alex D. Bain
Professor of Chemistry
bain@mcmaster.ca

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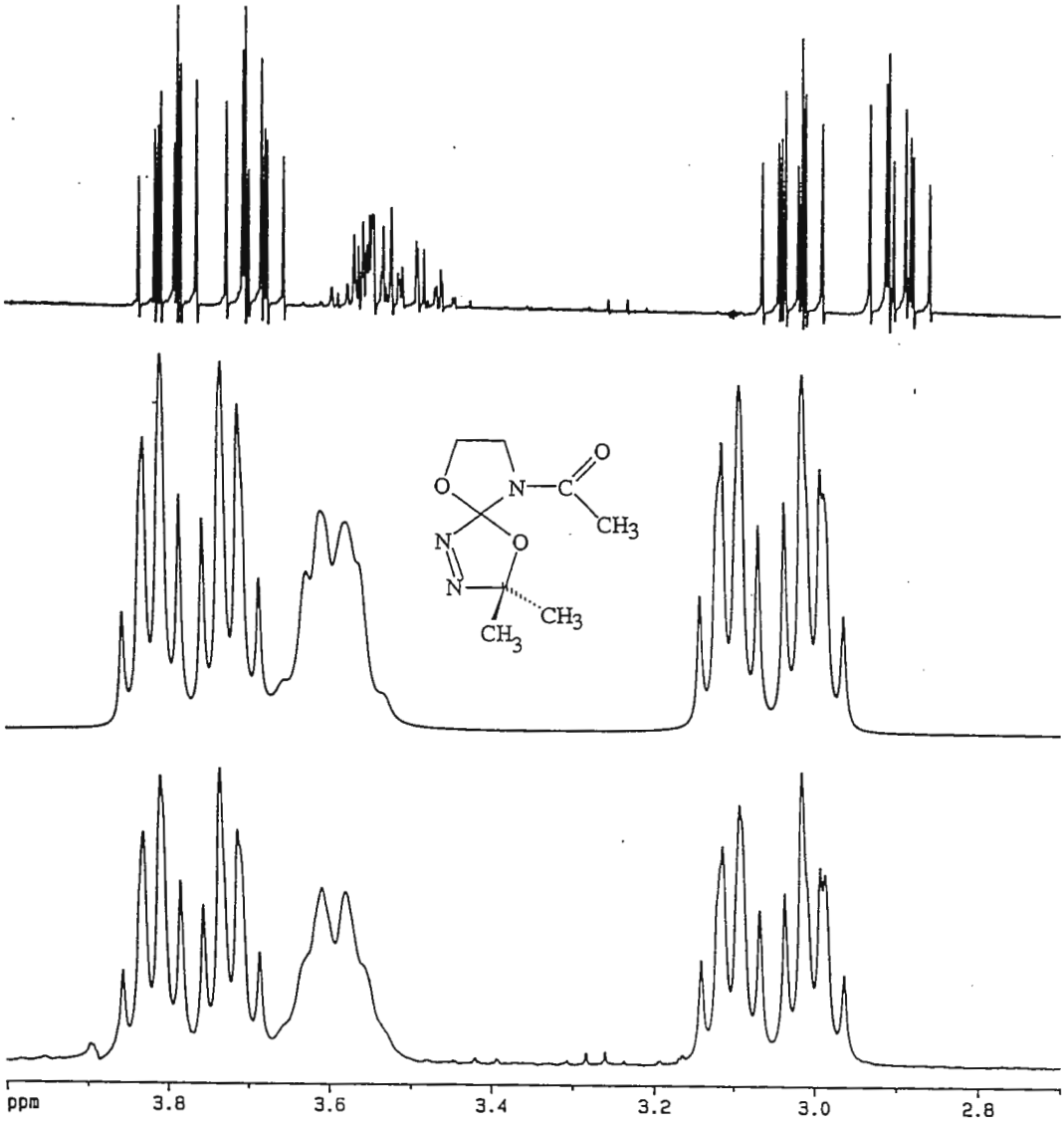
2. P. Couture, J.K. Terlouw and J. Warkentin. 2-alkoxy-2-amino-$\Delta^3$-1,3,4-oxadiazolines as novel sources of alkoxyaminocarbenes. *J. Am. Chem. Soc.* 118, 4214-4215, (1996).

Figure 1: Top. Spectrum of the $CH_2$-$CH_2$ region of the methyl oxadiazoline derivative at 260 K (resolution enhanced)
Middle. Spectrum simulated with MEXICO
Bottom. Experimental spectrum at 295 K



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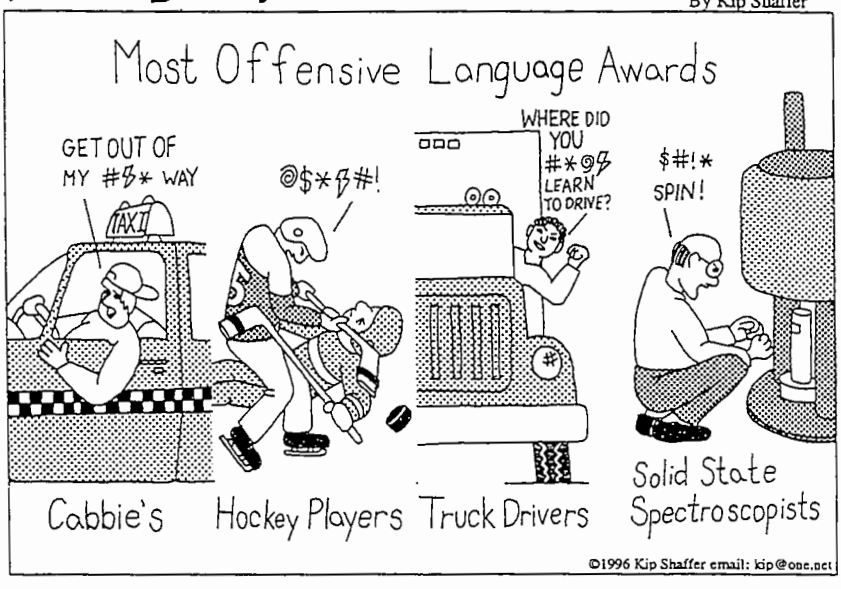

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*The NMR Newsletter*
966 Elsinore Court
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(415) 493-5971* - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

**Deadline Dates**

| | |
|---|---|
| No. 464 (May) | 25 Apr. 1997 |
| No. 465 (June) | 23 May 1997 |
| No. 466 (July) | 27 June 1997 |
| No. 467 (Aug.) | 25 July 1997 |
| No. 468 (Sept.) | 22 Aug. 1997 |

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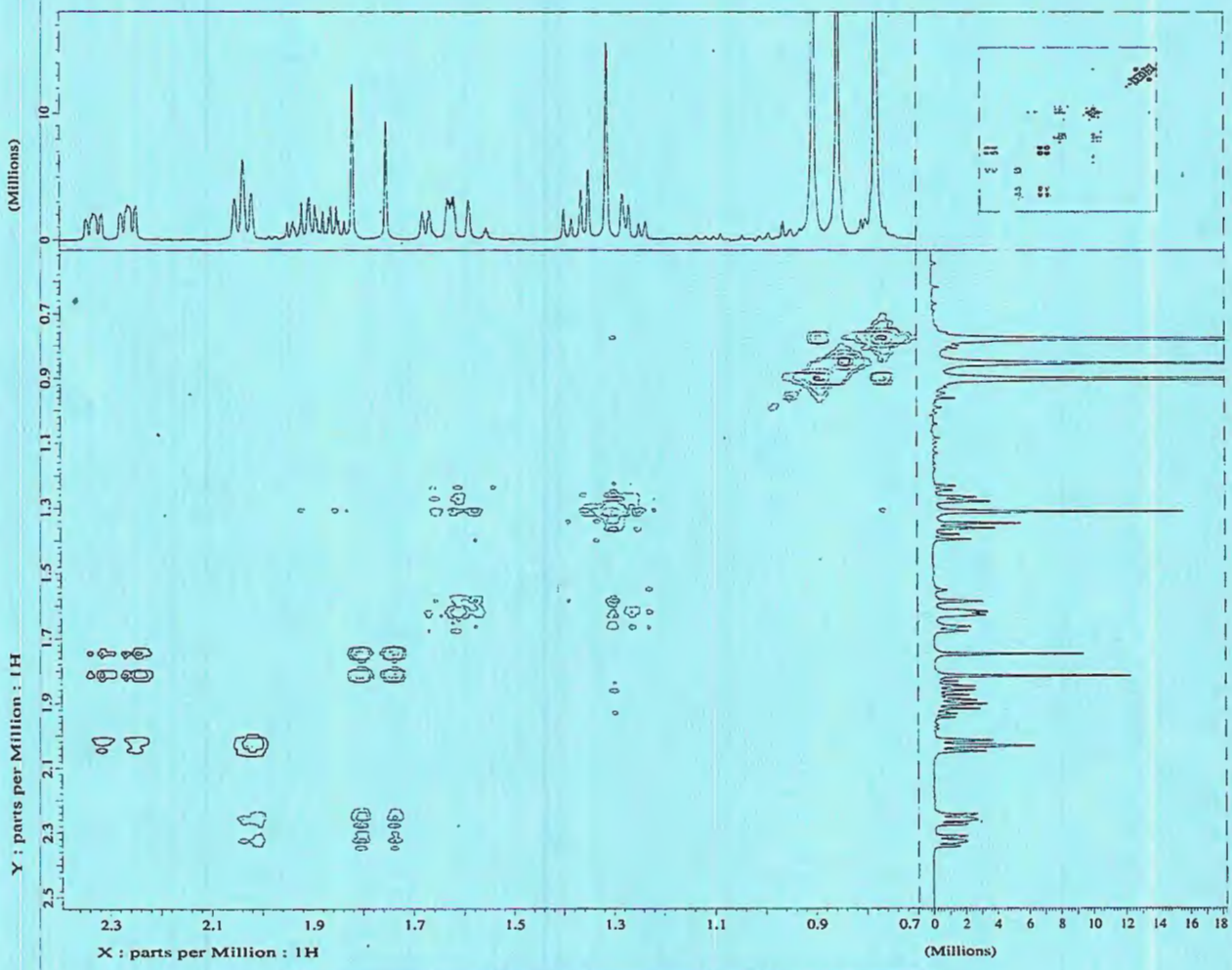


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