TEXAS A&M UNIVERSITY

NMR NEWSLETTER

No. 418

July 1993

Continued on inside back cover

TABLE 1 DEUTERATED SOLVENTS

Cat. No.	Description	Formula	Min. Iso. (% D)	Density (g/mL)	MP (°C)	BP (°C)	$-\chi_v \times 10^6$ @ (°C)
D-11	Acetone-d_6	CD_3COCD_3	99.9%	1.12	-94	56	0.551 (32)
D-120	Acetone-d_6 + 0.1% TMS	CD_3COCD_3	99.9%	1.12	-94	56	0.551 (32)
D-13	Acetone-d_6	CD_3COCD_3	99.8%	0.87	-94	56	0.460 (20)
D-121	Aceonte-d_6 + 1% TMS		99.8%				
D-129							
D-14							543 (20)
D-21							0.611
D-122							
D-130							
D-28	Chloroform-d	$CDCl_3$	99.8%	1.50	-64	62	0.740 (20)
D-31	Chloroform-d + 1% TMS		99.8%				

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VARIAN

BOX/500 SHEETS

Cat. No.	Size	Instrument Model	Type
WCV-100 (S-100)	11" X 26"	XL-100, VXR-100A, VXR-100D	Gridded-Two Color
WCV-60 (S-60)	11" X 26"	XL-50, XL-60, VXR-60A-60D	Gridded-Two Color
WCV-XL (XL-200)	11" X 26"	XL-200 (CF and FT)	Gridded-Two Color
WCV-XLFT-200	11" X 26"	XL-200 (CF and FT)	Gridded-Two Color
WCV-XL-200	11" X 26"	XL-200, XL-300, XL-400	Gridded-Two Color
WCV-20 (CFT-20)	11" X 16 3/4" X 17"	CFT-20, FT-80, FT-80A All Models 16 1/2" or 16	Gridded-Two Color Blank

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TRIPOS Associates/New Methods Research, Inc.
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Union Carbide Corporation
The Upjohn Company
Varian, Analytical Instrument Division

FORTHCOMING NMR MEETINGS

Science Innovation '93, Boston, Mass., **August 6 - 10, 1993**; "NMR Determination of Protein Structure", Discussion Leader - Ad Bax, *NIH*; : For information contact AAAS Meetings, 1333 H Street, NW, Washington, DC 20005; Phone: (202) 326-6450; Fax: (202) 289-4021; See *Science*, 260, 557-565 (23 April 1993).

12th Annual Scientific Meeting and Exhibition of the Society of Magnetic Resonance in Medicine, New York, NY, **August 14-20, 1993**; Contact: SMRM, 1918 University Ave., Suite 3C, Berkeley, CA 94704; Phone: (510) 841-1899; Fax: (510) 841-2340.

NMR of Biological Macromolecules, Orthodox Academy of Crete, Kolympari, Crete, Greece, **August 23 - September 2, 1993**; Contact: Ph. Dais, Chemistry Dept., Univ. of Crete, Heraklion 71409, Greece; Phone: (30) 81-238400, ex. 292; Fax: (30) 81-233669.

Second International Conference on Magnetic Resonance Spectroscopy, Heidelberg, Germany, **September 6-10, 1993**; Contact: Dr. Winifried Kuhn, Fraunhofer Inst., Ensheimer Str. 48, DW-6670 St. Ingbert, Germany; Phone: (49) 6894-89738; Fax: (49) 6894-89750, or Dr. Bernhard Bluemich, Max-Planck-Inst. for Polymer Research, Postfach 3148, D-6500 Mainz, Germany; Phone: (49) 6131-379125; Fax: (49) 6131-379100.

1993 FACSS Meeting, Detroit, Michigan, **October 17-22, 1993**; Contact: H. N. Cheng, Hercules, Inc., Research Center, 500 Hercules Road, Wilmington, DE 19808; Phone: (302) 995-3505; Fax:. (302) 995-4117. See TAMU NMR Newsletter 411, 10.

Pacific Conference, Pasadena, California, **October 19-23, 1993**; Contact: Ms. B. Belmont, Pacific Conference, 14934 S. Figueroa St., Gardena, CA 90248;Phone: (310) 538-9709.

1993 CABM Fall Symposium on "Macromolecular Recognition", Piscataway, NJ, **October 21-22, 1993**; Program Organizer: G. T. Montelione; Contact: Linda VanDerveer, Symposium Coordinator, Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, NJ 08854; Phone: (908) 235-5309; Fax: (908) 235-4850.

35th ENC (Experimental NMR Conference), Asilomar Conference Center, Pacific Grove, California, **April 10 - 15, 1994**; Contact: ENC, 815 Don Gaspar, Santa Fe, NM 87501; (505) 989-4573; Fax: (505) 989-1073.

12th International Meeting on NMR Spectroscopy, Sponsored by the Royal Society of Chemistry, Manchester, England, **July 2 - 7, 1995** [sic]; Contact: Dr. J. F. Gibson or Ms. G. B. Howlett - See TAMU NMR Newsletter 415, 5; Phone: (44-71) 437-8656; Fax: (44-71) 437-8883.

ISMAR 1995, Sydney, NSW, Australia, **July 16-21, 1995** [sic]; Contact: Dr. Wm. A. Bubb, Secretary, Univ. of Sydney, Dept. of Biochemistry, Sydney, NSW 2006, Australia. See TAMU NMR Newsletter 414, 8.

Additional listings of meetings, etc., are invited.

ANORGANISCH-CHEMISCHES INSTITUT
DER TECHNISCHEN UNIVERSITÄT MÜNCHEN
Dr. Janet Blümel

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Lichtenbergstraße 4
Tel.: (089)3209-3118

May 26, 1993
(received 6/1/93)

Dr. B. L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, California 94303
USA

^{93}Nb NMR: Magic Dimensions for Piezoelectric Ringing

Dear Dr. Shapiro,

In a recent collaboration with the mineralogists of our facility I have investigated diverse $LiNbO_3$ single crystals in the form of thin plates in order to find out by ^{93}Nb solid state NMR how many different Nb sites there are in the crystals. Usually this works quite well in spite of the quadrupolar nature of the ^{93}Nb nucleus (I = 9/2) and line widths as small as 17 kHz (see Figure 1) can be observed for the -1/2 → +1/2 transition even in wideline NMR when the crystals are carefully oriented with respect to the external magnetic field.

However, all $LiNbO_3$ species are strongly piezoelectric and therefore the naked crystals display quite copious piezoelectric ringing (Figure 2). Fortunately there are good old remedies for this: The crystals can be wrapped with adhesive tape, or immersed in oil or in H_2O. In this case the best results are usually obtained with the latter (the glassy $LiNbO_3$ crystals do not dissolve in H_2O) and no electrostatic shield and not even a quadrupolar echo technique is needed for the measurement (Figure 1).

Recently I got the usual kind of material but this time the thin plates were cut precisely to the dimensions shown in Figure 3, in order to keep me happy and to make life easier for me. Previously I had cut the plates myself, not caring too much about rough edges and lost corners. However, it turned out that these manufactured plates displayed record ringing that could neither be removed by the remedies above nor by changing the orientation of the crystals. Finally, cutting off one corner (see Figure 3) made the mysterious ringing disappear and a spectrum similar to Figure 1 was obtained.

My only explanation for this is, that the amplitude of piezoelectric ringing depends also on the geometry of the single crystal. The more regular the form, the more persistent the piezoelectric ringing. Other suggestions from readers of these newsletters are welcome. More experiments are definitely required, but how can I tell the mineralogists that I desperately need little cubes and balls and cylinders and pyramids and long plates and broad plates... all made of their $LiNbO_3$ material?

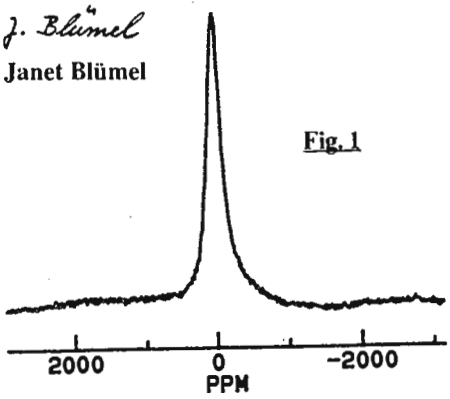
Please credit this contribution to Prof. Köhler's account.

With best regards

J. Blümel

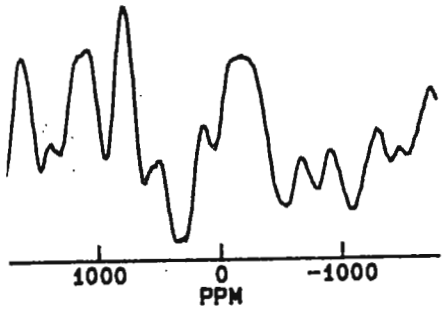
Janet Blümel

Fig. 1



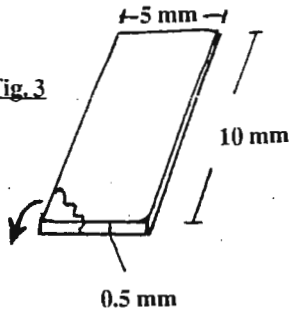
73.5 MHz ^{93}Nb wideline NMR spectrum of a $LiNbO_3$ single crystal wrapped with adhesive tape. 90° pulse 2 µs, 14000 scans, pulse delay 200 ms.

Fig. 2



"Ringing", parameters see Fig.1

Fig. 3



MICROSAMPLE PROTON PROBES

In the past various attempts have been made in the NMR industry to design probes for milli-, micro- and even high nanogram amounts of sample. However, these probes typically suffered from a number of drawbacks which rendered them not very useful in practice: some designs use solenoidal coils which make it impossible to load/eject the sample at the top of the magnet; instead the probe has to be removed from the magnet to change a sample. Moreover, these probe designs are primarily useful for 1H-only coils, but cannot be readily built as broadband inverse or inverse triple-resonance probes. Typically, lineshapes for older microsample probes were not satisfactory for biological NMR experiments which require water suppression.

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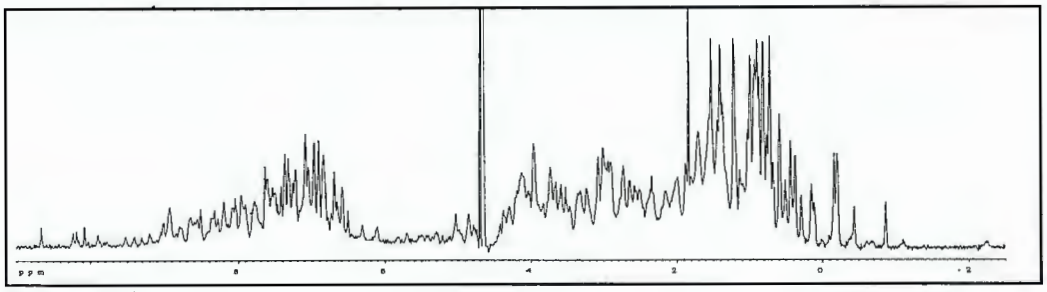
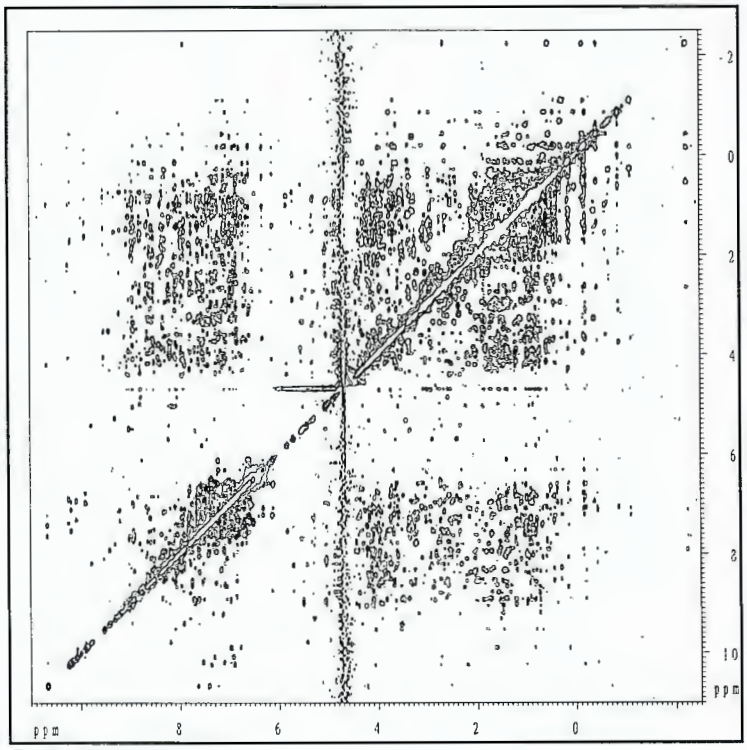


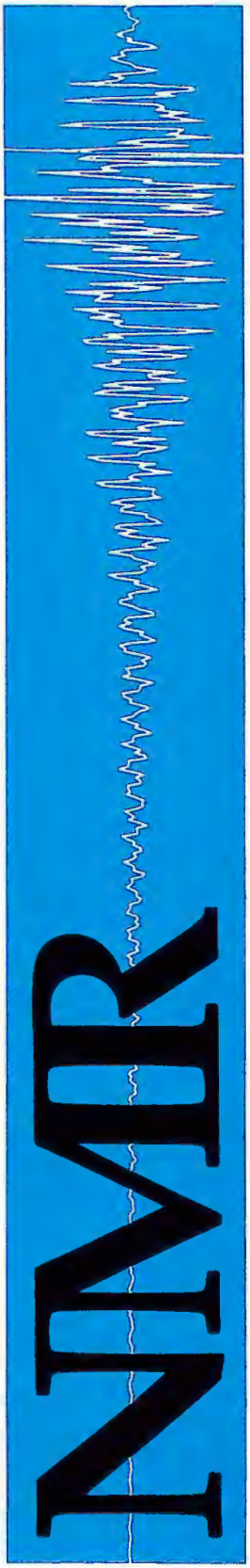
Spectrum 1



Spectrum 2

Spectrum 1: 64 scans of 1 milligram of lysozyme in 0.1 milliliter of 90% H_2O / 10% D_2O. The first increment of a NOESY experiment demonstrates the excellent lineshape and water-suppression capability.

Spectrum 2: 2D NOESY with 150 msec mixing time at 12 1/4 hours acquisition time.



BRUKER

NMR

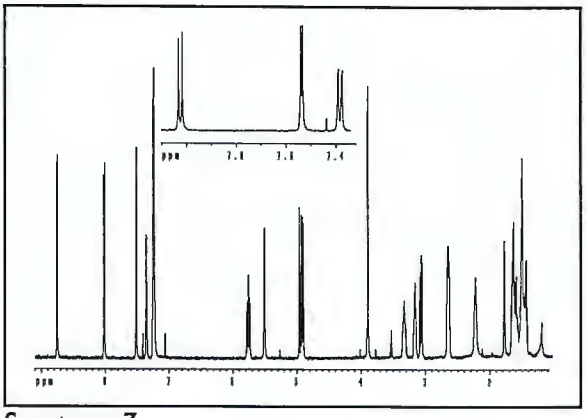
Spectrum 3: A 10 minute acquisition on 10 micrograms of quinine in $CDCL_3$. Note the excellent resolution for the aromatic peaks in the inset.

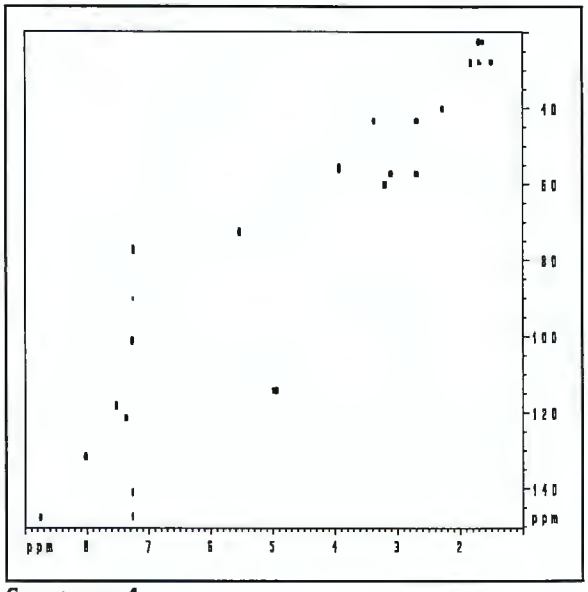
Spectrum 4: 2D HMQC at 12 hours. Same sample as spectrum 3.

Spectrum 5: A 2D TOCSY with 3 hour acquisition time, same sample.

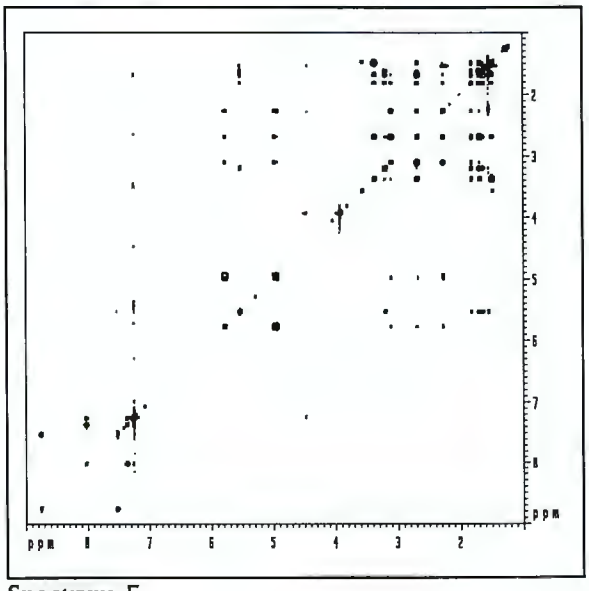
Experimental details:
All spectra shown here were acquired on an **AMX 600** equipped with the high-dynamic range **SE 451™** receiver, which is essential for measuring small sample amounts with good sensitivity. A **BOSS2™** (Bruker Orthogonal Shim System) was used for optimal lineshape, and the probe was a 2.5 mm inverse triple-resonance probe [$^1H\{^{13}C,^{15}N\}$]. All spectra were acquired non-spinning, and 90° pulse widths were <5 microseconds for ^1H, <12 microseconds for ^{13}C, and <40 microseconds for ^{15}N.

This novel generation of Bruker 2.5 mm proton probes ideally complement the standard 5 mm and 8 mm probes (for solubility-limited cases) in biological NMR applications.


Spectrum 3



Spectrum 4



Spectrum 5

DEPARTMENT OF HEALTH & HUMAN SERVICES
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National Institute of Diabetes and
Digestive and Kidney Diseases
Bethesda, Maryland 20892

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

June 23, 1993
(received 6/25/93)

Dear Barry:

Watch your temperature

Many of the experiments we use for the study of proteins require pulse sequences that utilize substantial RF power for relatively long durations. For example, 2D HOHAHA (TOCSY) experiments are sometimes recorded with mixing times of up to 100 ms, using several Watts of ^{1}H RF power. Many of the heteronuclear triple resonance experiments require high power ^{13}C and/or ^{15}N decoupling, for durations of up to ~100 ms. The D_2O (and H_2O) frequency depends strongly on temperature and shifts upfield by ~0.01 ppm per °C. A temperature gradient across the sample, caused by the applied RF power, results in the commonly observed decrease in lock signal intensity. Note that *reshimming* by maximizing the lock signal during the pulse sequence actually results in *broader* line widths for the solute of interest.

For many of our studies, line widths are not all that critical, as the natural line widths of many of the protein resonances are frequently more than 20 Hz. However, the assignment procedure for the protein relies on the simultaneous analysis of a substantial number of 3D spectra which supposedly are all recorded under the same experimental conditions and at the same temperature. Success in automating the assignment procedure largely depends on how reproducible chemical shifts are from one experiment to another, i.e. it depends strongly on being able to reproduce the temperature *inside* the sample. Note that most spectrometers only measure and regulate the temperature of the air that is blown over the sample. In the absence of RF power this will result in a sample temperature very close to the set value. In the presence of RF power, the sample can become substantially warmer than the set temperature, and the spectrum will shift downfield because the D_2O lock frequency moves upfield. If the spectral frequencies, relative to TSP, do not vary with temperature the downfield shift is uniform and is rather easily recognized and corrected for in practice. However, many of the protein resonances also have temperature dependent chemical shifts and can shift either upfield or downfield relative to TSP. Indeed, the temperature dependence of amide proton shifts is commonly used in peptides to establish the presence of hydrogen bonding.

As it is desirable to have the sample at the same temperature during the various multi-dimensional experiments, we have chosen to solve the heating problem by analysing how much warmer the sample becomes for a given amount of ^{1}H (or $^{13}C/^{15}N$) power at a given airflow (300 liter per hour), and setting the "spectrometer control temperature" by this amount below the desired temperature.

The change in temperature depends strongly on the salt concentration in the sample, particularly when considering irradiation at ^{1}H frequencies, and on the RF coil geometry. For example, on our AMX-600 spectrometer we observed the following: In the absence of salt, we get an increase in sample temperature of 6.7 °C/Watt, giving rise to a 0.4 °C increase for a HOHAHA

experiment with a mixing time of 50 ms, a repetition rate of 1 s^{-1}, and a ^1H pulse width of 25 μs during mixing. In the presence of 200 mM NaCl, for the same experiment the temperature goes up by 3.1 °C. For ^{13}C, heating is 1.6 °C/W for low salt and 3.0 °C/W for high salt, increasing the sample temperature by 1°C and 1.8°C, respectively, during a HCCH-TOCSY experiment with 24 ms mixing with a 7 kHz RF field (15 W) and 50 ms of 5 W ^{13}C decoupling during data acquisition, again for a repetition rate of 1 s^{-1}. For ^{15}N we find the heating effects to be almost negligible. A somewhat more detailed account of the RF heating and ways to reduce its effect has been submitted to the Journal of Biomolecular NMR.

People have long been aware of the effects of RF sample heating in NMR (1). It actually was one of the primary reasons for the development of improved (composite pulse based) decoupling schemes. The heating problem increases somewhere between linearly and quadratically with RF frequency. Moreover, the problem is aggravated by the quadratic increase in the required RF power for covering a given bandwidth, causing the heating problem to scale with a power of between 3 and 4 of the static magnetic field strength. This may therefore pose some technical problems for applying some of these power-hungry experiments at 750 MHz and above.

Kindest regards,

Andy C. Wang Ad Bax

(1) J.J. Led and S.B. Petersen, *J. Magn. Reson.* **32**, 1-17 (1978).

Abbott Laboratories
D-47G, AP9
Abbott Park, IL 60064

June 11, 1993 (received 6/21/93)

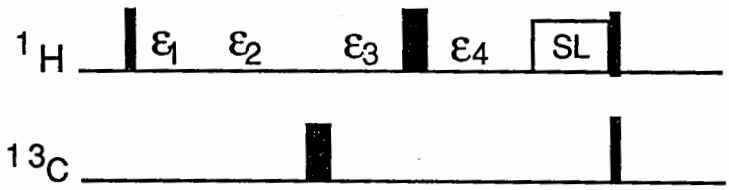
Dr. B. L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, California 94303

RE: SHARED INCREMENTATION TIMES

Dear Barry,

In an effort to facilitate the resonance assignments of proteins, we[1] and others[2,3] have developed an experiment called HC(CO)NH-TOCSY in which the ^{1}H and ^{13}C chemical shifts of residue i are correlated to the ^{1}H and ^{15}N amide chemical shifts of residue i+1. We've recently described[4] an improved version of this experiment in which the chemical shift evolution and scalar transfer periods are combined into a single period referred to as a shared incrementation time. This modification has the effect of shortening the overall duration of the pulse sequence, resulting in an increase in sensitivity, especially when applied to the study of molecules with short relaxation times.

The idea, which has also been reported by Bax and coworkers[3], is illustrated in the HC(CO)NH-TOCSY experiment. As shown below, the experiment begins with an INEPT sequence to transfer magnetization from ^{1}H to ^{13}C combined with a ^{1}H evolution period.



The ^{1}H chemical shifts evolve during ($\varepsilon_1+\varepsilon_2+\varepsilon_3-\varepsilon_4$); whereas, the ^{1}H-^{13}C scalar couplings evolve during ($\varepsilon_1+\varepsilon_2-\varepsilon_3+\varepsilon_4$). For the initial t_1 increment, ε_1 and ε_4 are set to $1/4J_{CH}$ (ca. 1.7ms). In subsequent t_1 increments, ε_1 is incremented and ε_4 is decremented by (ε_4(initial)/k_H) while ε_2 and ε_3 are incremented by (dwell/2-ε_4(initial)/k_H) where k_H is one more than the number of points collected in the ^{1}H dimension. By sharing the ^{1}H chemical shift evolution and ^{1}H-^{13}C scalar transfer times in this way, this overall period is shortened, resulting in a greater sensitivity.

This scheme is robust and easily adapted to other chemical shift evolution periods and other NMR experiments. In fact, we have modified our entire arsenal of 3D and 4D NMR experiments to take advantage of this trick. Now, **ALL** of our NMR pulse sequences are full of **SH**ared **I**ncrementation **T**imes.

Sincerely,

Tim Logan Ed Olejniczak Andrew Petros Heng Liang Andrew Hansen

Dave Nettesheim Robert Xu Yves Theriault Liping Yu Steve Fesik

1. Logan, T.M., Xu, R.X., Olejniczak, E.T. & Fesik, S.W. FEBS Lett., 314, 413 (1992).
2. Montelione, G.T., Lyons, B.A., Emerson, S.D., & Tashiro, M. J. Am. Chem. Soc., 114, 10974 (1992).
3. Grzesiek, S., Anglister, J. & Bax, A. J. Magn. Reson. 101B, 114 (1993)
4. Logan, T.M., Olejniczak, E.T., Xu, R.X., and Fesik, S.W. J. Biomol. NMR, 3, 225 (1993).

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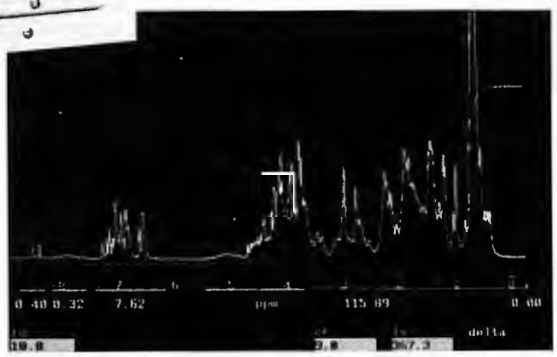

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Pulse width	20 ms
Duty cycle	Up to 10%
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Amplitude droop	5% to 20 ms typ.
Phase change/output power	10° to rated power, typ.
Phase error overpulse	4° to 20 ms duration, typ.
Noise figure	11 dB typ.
Output noise (blanked)	< 20 dB over thermal
Blanking delay	< 2 μs on/off, TTL signal
Protection	1. VSWR: infinite VSWR 2. Input overdrive: up to 10 dB 3. Over duty cycle/pulse width 4. Over temperature

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Connectors, rear panel	1. RF input: BNC (F) 2. RF output: Type N (F) 3. Noise blanking: BNC (F) 4. Interface: 25 pin D(F), EMI filtered
Indicators, front panel	1. AC power on 2. Peak power meter 3. Over pulse width 4. Over duty cycle 5. Over temperature 6. Over drive 7. CW mode
System monitors	1. Forward/Reflected RF power 2. Over pulse width/duty cycle 3. DC power supply fault 4. Thermal fault
Front panel controls	1. AC power 2. Pulse width 3. Duty cycle
Cooling	Internal forced air
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Lehrstuhl für Makromolekulare Chemie, RWTH Aachen
Sammelbau Chemie, Worringerweg 1, 5100 Aachen

Dr. Barry Shapiro
TAMO NMR Newsletter
966 Elsinore Court
Palo Alto, Cal 943 63

Aachen, May 27th, 1993/bl/sz
(received 6/21/93)

Dear Barry,

as of March 31st, 1993, I have accepted the chair of Makromolecular Chemistry at the Aachen University of Technology. Our research will be centered on NMR of polymers, in particular solid-state NMR spectroscopy and materials oriented NMR imaging.

I am interested in receiving your Newsletter and would like to know the rules. Until the new equipment arrives, our present work still continues largely in Mainz at the Max-Planck-Institute for Polymer Research in the Department of Prof. Spiess.

My activities are focussed on applications and development of materials imaging methods for solids, for example aging and deterioration studies of elastomers, multi-solid echo imaging, MAS imaging, the use of surface coils for materials, and imaging with noise excitation.

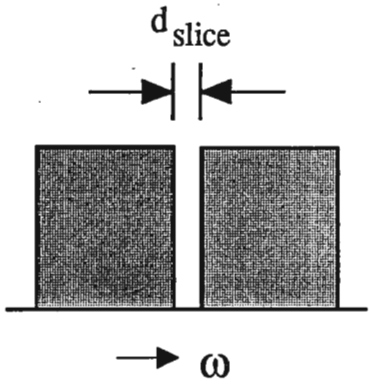
In the context of the last effort, Helge Nilgens, Peter Blümler, Jürgen Paff, and myself have developed a new class of low power "Saturation Pulses with REduced Amplitude Distribution: SPREAD. Here, the applied energy is distributed over the whole saturation interval, leading to a substantial decrease in rf power requirements. As compared to the DIGGER pulse of Doddrell et al (J.Magn.Reson. 29 (1987) 433) the rf amplitude is reduced by about three orders of magnitude and correspondingly the saturation bandwidth can be increased substantially. This is illustrated in the enclosed figure.

With kind regards,

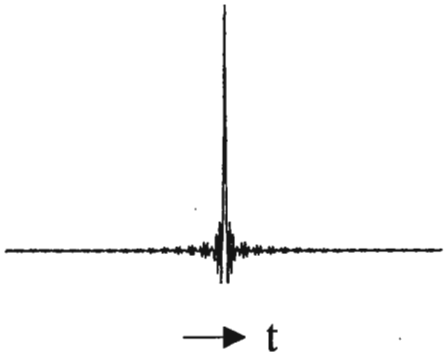
Prof. Dr. B. Blümich

saturation profiles

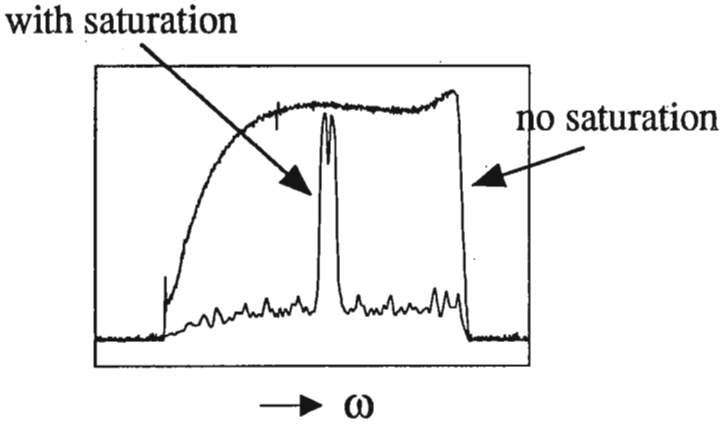
excitation profiles

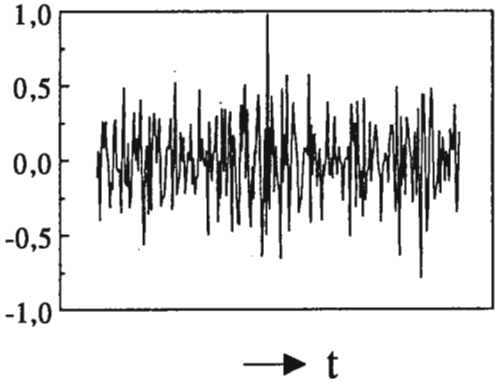


FT

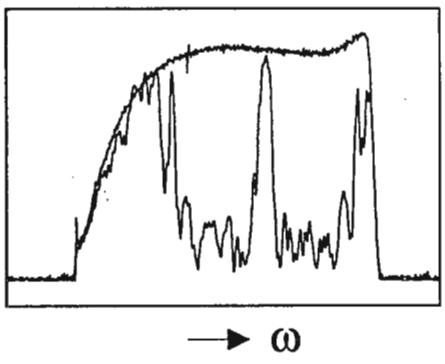


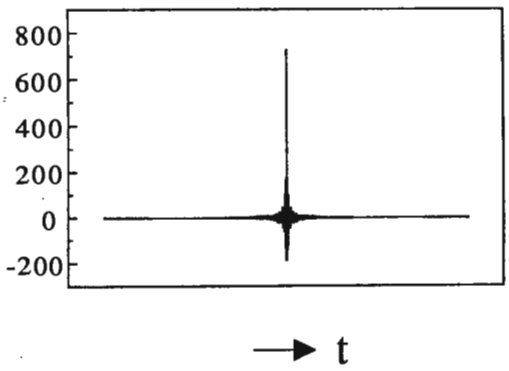
SPREAD





DIGGER





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

June 23, 1993
(received 6/24/93)

DMSO Is Better Sometimes than Other Times.

Dear Barry:

This is unforgivably late, but I hope to be reinstated with minimal loss of issues and face. Actually, I'm out of phase with this leter, having completed little of import for the last several months, and having just undertaken some major studies which I cannot yet discuss. Still, some recent events have me musing over the mixed blessings of DMSO as an NMR solvent.

I have recently used it in analyzing for glycerin and water in some development products. A sample is slurried in DMSO-d_6, solids allowed to settle out, and ^{1}H NMR conducted on the supernate; integration relative to an internal $CHCl_3$ standard yields the amount of each analyte. I was surprised to see three sets of ^{1}H resonances: glycerin -OH, water, and -CH_2-/-CH- at 4.5, 3.7 and 3.5 ppm, respectively. The water peak was confirmed by adding more water and seeing the peak grow and move downfield. In normal solvents like D_2O or methanol, of course, rapid exchange yields a single -OH/water resonance. DMSO apparently solvates hydroxyl groups strongly, inhibiting dissociation of O-H bonds and proton exchange with water, which apparently also is strongly solvated.

This anti-dissociation property, beneficial in the above example, backfired in my fledgling study of esterification of cellobiose by alkenylsuccinic anhydride (ASA). DMSO was selected as solvent because, except for water and methanol, it seems to be the only one that dissolves cellobiose at appreciable concentration; water and alcohols were avoided since they could solvolyze the ASA. The upshot of this study is that, *sans* catalyst, esterification with cellobiose does not occur; prolonged heating causes only slow hydrolysis to the succinic acid.

The backfire occurs in the carboxyl carbon chemical shift difference between the anhydride and acid forms of ASA: (δ_{acid} - δ_{anhyd}) is 7.5 - 8.0 ppm in $CDCl_3$, but only 1.5 - 2.0 ppm in DMSO-d_6. Since ester carboxyls have very similar chemical shifts to those of the analogous anhydrides, my working frequency range in DMSO will be very narrow indeed. Why this chemical shift narrowing? In normal solvents, carboxylic acids partially dissociate at the O-H bond, form neat resonance structures with partial positive charge on the carbon, and resonate at appreciably lower field than esters or anhydrides. DMSO appears to prevent this useful diagnostic event.

Respectfully,

Larry

Larry W. Amos
Analysis & Testing

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Instrumentation and Biophysics Branch
Food and Drug Administration (HFS-717)
200 'C' Street, SW, Washington, DC 20204
phone: 202-205-4409; FAX: 202-205-4758

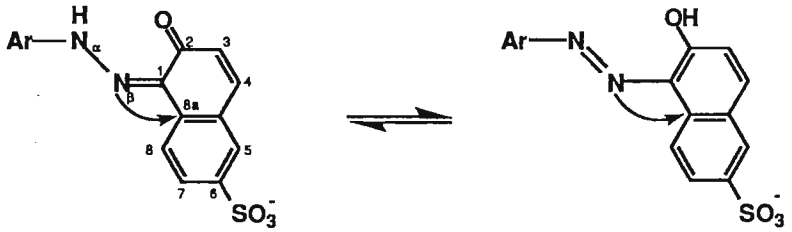
May 25, 1993
(received 5/28/93)

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

Azo-Hydrazone/Acid-Base Equilibria in Yellow No. 6

Dear Barry,

Yellow No. 6 (Y6) exists in different "forms" over the pH range 7-14. It occurs almost exclusively as a hydrazone (**1**) at pH 7 and predominantly in the azo form (**2**) at pH 14. Approximately equal concentrations of these two species are present at pH 12. Their ^{13}C nmr



1 **2**

spectra exhibit considerable line broadening (with a maximum at pH 12) and shifting with pH of most resonances arising from these two forms.

These dynamic effects could be due to slow tautomeric interconversion of **1** and **2** or a combination of *syn-anti* hydrazone interconversion (predominantly at pH values below 12) and *trans*-azo rotational isomerism (principally at pH values above 12). However, 2-bond N_β-C_{8a} couplings range from 8.6 Hz at pH 7 to 3 Hz at pH 14. If signal broadening were due solely to the appearance of higher-energy isomers, *viz. anti*-hydrazone and *trans*'-azo species, then the observed $N_\beta C_{8a}$ coupling constants should decrease from either 8.6 or 3 Hz as the hydrazone-azo equilibrium point (pH 12) is approached from either pH extreme. We, therefore, favor hydrazone-azo tautomerism as an explanation for the observed line broadening. Crucial to this argument, however, is the pK_a value of the hydrazone-NH of Y6. Approximate determinations have established it at 11.5. Sufficient concentrations of the protonated hydrazone and azo forms of Y6 should exist at pH 12 to account for the proposed tautomerism.

Sincerely,

E.G.Mouchahoir E.P. Mazzola

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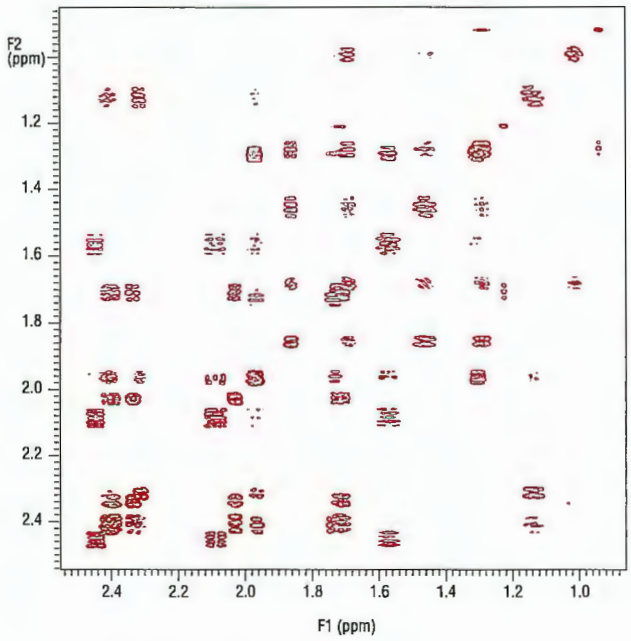

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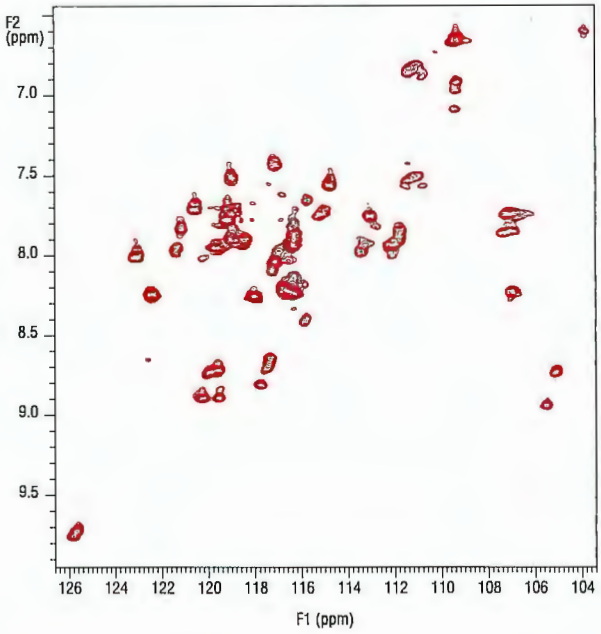

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Dr. Bernard L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

June 16, 1993
HvH 93-A-152
(received 6/17/93)

Measuring long-range ^{1}H-^{13}C couplings by ^{13}C-filtered exclusive 1D ^{1}H TOCSY

Dear Dr. Shapiro:

The NMR community has a growing interest in determining long-range ^{1}H-^{13}C coupling constants for biomolecules in general and for carbohydrates in particular. Researchers can choose from a vast array of ^{1}H-detected multiple-pulse experiments to measure these couplings in molecules with natural ^{13}C abundance. Particularly attractive are methods that involve the detection of "exclusive-type" correlations, with spectra being recorded in 2D or 3D format.

In this letter we present the selective variant of the ^{13}C-filtered exclusive 2D [^{1}H,^{1}H] TOCSY experiment (which we dubbed CFE-TOCSY) to measure long-range ^{1}H,^{13}C couplings in a carbohydrate molecule. The pulse sequence shown in Figure 1 (top) was applied to panose (a linear triglucoside) in D_2O at 600 MHz. Trace A shows the normal 1D TOCSY subspectrum of the "internal" glucoside moiety of the trisaccharide. Trace B shows the 1D CFE-TOCSY spectrum with selective excitation of the H4 proton in the internal glucoside residue. The corresponding proton multiplets in both traces are shifted by $0.5x\ {}^{n}J_{C4H}$. Most interesting to us were the shifts observed for the H6 multiplets, from which we deduced values for the $^{3}J_{C4H6R}$ and $^{3}J_{C4H6S}$ couplings. The values of these long-range couplings are crucial in the stereospecific assignment of the prochiral protons H6R and H6S. Performing the CFE-TOCSY experiment in the 1D mode achieves the high spectral digitization required to measure these couplings with sufficient accuracy.

Sincerely yours,

Leszek Poppe Shuqun Sheng Herman van Halbeek

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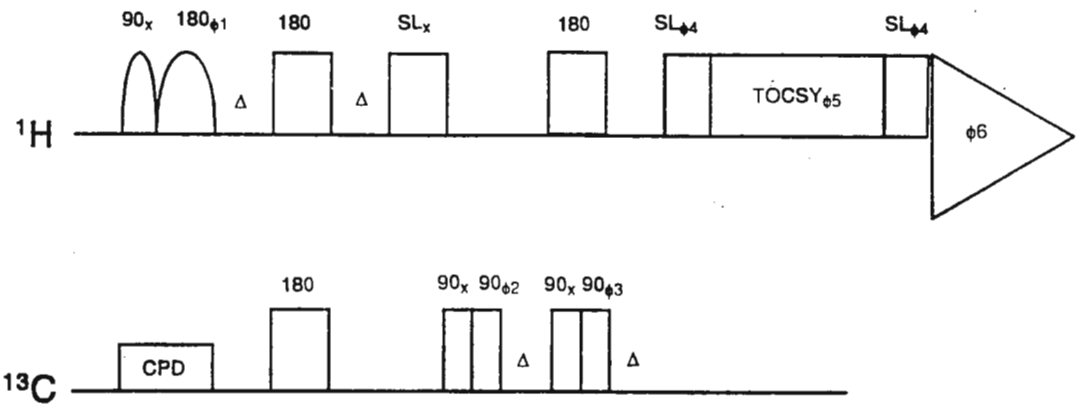
CCRC Telephone: 706-542-4401 Facsimile: 706-542-4412
*Located in Life Sciences Bldg.: c/o Biochemistry Dept., Life Sciences Bldg., Athens, Georgia 30602-7229 USA



The pulse phases are cycled as follows: φ_1=4(x), 4(y), 4(-y), 4(-x); φ_2= x, -x; φ_3= x, x, -x, -x; φ_4= y, y, x, x; φ_5=x,x,y,y; φ_6=x, -x, y, -y, -x, x, -y, y. $\Delta=(4\,{}^1J_{CH})^{-1}$. SL stands for spin-lock.

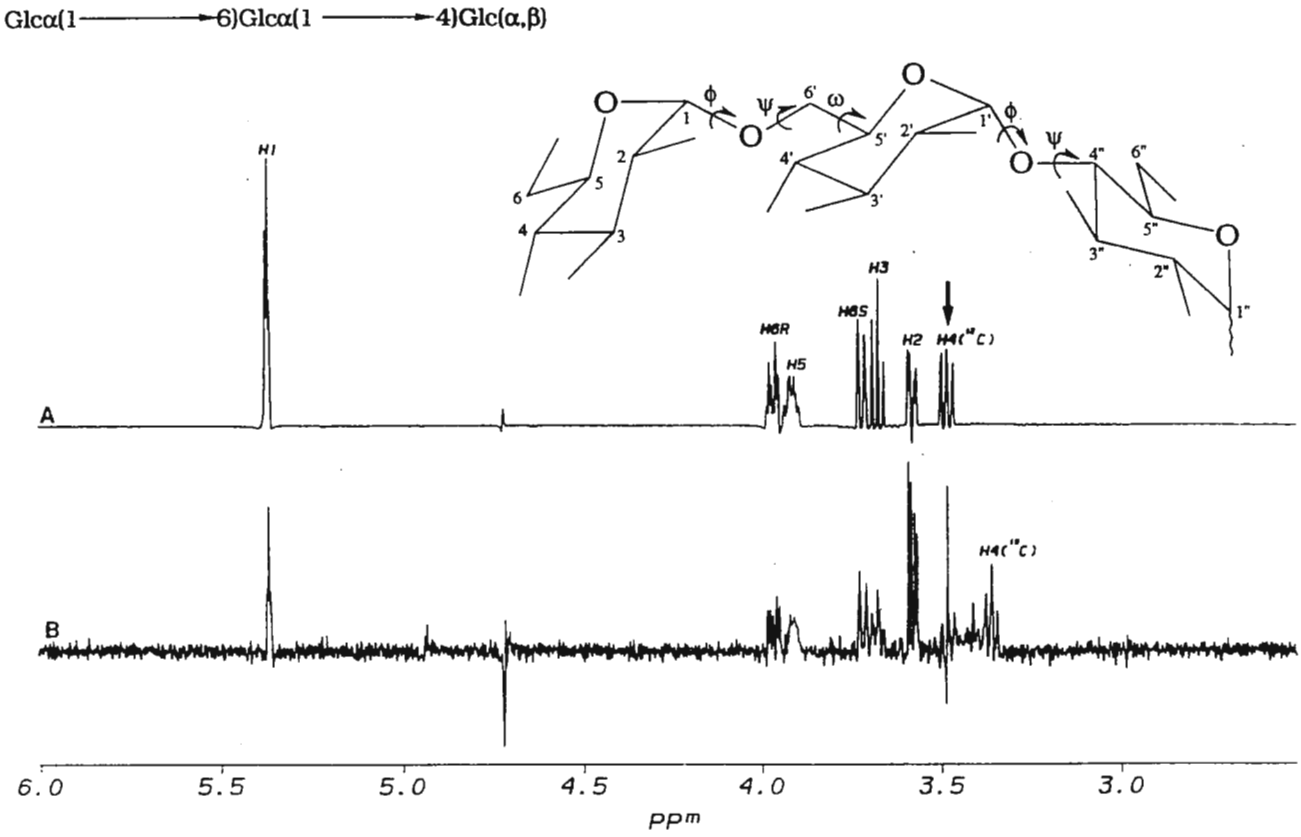


Figure 1

Dalhousie University

Department of Chemistry
Halifax, Nova Scotia
Canada B3H 4J3
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Fax: (902) 494-1310

Re: **Analysis of NMR dipolar-chemical shift powder patterns**

Dear Barry, (received 6/17/93) 10 June 1993

For some time now we have been interested in characterizing chemical shielding (CS) tensors[1]. While it is generally straightforward to measure the principal components of a CS tensor, it is much more difficult to determine the orientation of shielding tensors. Many years ago, VanderHart and Gutowsky[2], and Zilm and Grant[3] and others[4] demonstrated that valuable orientational information can be obtained from powder patterns arising from "isolated" spin-pairs. In principle, the orientation of each of the two CS tensors with respect to dipolar vector (*i.e.*, the internuclear axis) can be obtained. To create an "isolated" spin-pair several groups have introduced an isotopically labelled spin-pair (*e.g.*, ^{13}C-^{15}N) into the system of interest by synthetic procedures[1,4].

A typical "A"-spin ($I=½$) dipolar-chemical shift powder pattern arising from an "AX" spin-pair is shown in Fig. 1a. Provided the anisotropic CS interaction is much greater than the dipolar interaction, the dipolar splittings at δ_{11}, δ_{22}, and δ_{33} will generally be given by the following equations: $\Delta\nu_{11}=R_{eff}(1-3\cos^2\alpha\sin^2\beta)$, $\Delta\nu_{22}=R_{eff}(1-3\sin^2\alpha\sin^2\beta)$, and $\Delta\nu_{33}=R_{eff}(1-3\cos^2\beta)$, respectively, where α and β describe the orientation of the dipolar vector with respect to the CS tensor, and R_{eff} is the effective dipolar coupling constant. Although it might appear simple to obtain α, β and R_{eff} from the three splittings, $\Delta\nu_{ii}$, it is important recognize that because the dipolar tensor is traceless, the sum of the three splittings is zero and hence the three equations are not independent. In fact, in the absence of symmetry there will often be numerous combinations of α, β and R_{eff} consistent with the observed splittings. Unfortunately, this fact is often not recognized or clearly stated in the literature.

We have developed a procedure for analyzing dipolar-CS powder patterns which will yield all possible solutions consistent with the experimental spectrum. The method involves comparing ratios of observed dipolar splittings (*e.g.*, $\Delta\nu_{33}/\Delta\nu_{11}$) with theoretical values. We have found it convenient to represent the theoretical ratios as contour curves on triangular plots (*e.g.*, see Fig. 1b). A user-friendly, menu driven computer program for IBM compatible computers has been prepared for this purpose and is available on request. The advantage of the graphical dipolar splitting ratio method is that all combinations of α and β compatible with the experimental spectrum are immediately obvious, thus many of the subtleties involved in analyzing dipolar-CS powder patterns become apparent. We now routinely use this procedure in our laboratory and hope that others will consider using it as well.

Yours sincerely,

Rod Klaus

Roderick E. Wasylishen Klaus Eichele

1. R.E. Wasylishen *et al.* in "Nuclear Magnetic Shieldings and Molecular Structure", edited by J.A. Tossell, NATO ASI Series, Series C, Vol. 386, Kluwer Publishers, p. 297, 1993.
2. D.L. VanderHart and H.S. Gutowsky, J. Chem. Phys. **49**, 261 (1968).
3. K.W. Zilm and D.M. Grant, J. Am. Chem. Soc. **103**, 2913 (1981).
4. see: W.P. Power and R.E. Wasylishen, Ann. Rep. NMR Spectrosc. **23**, 1, 1991 for original references.

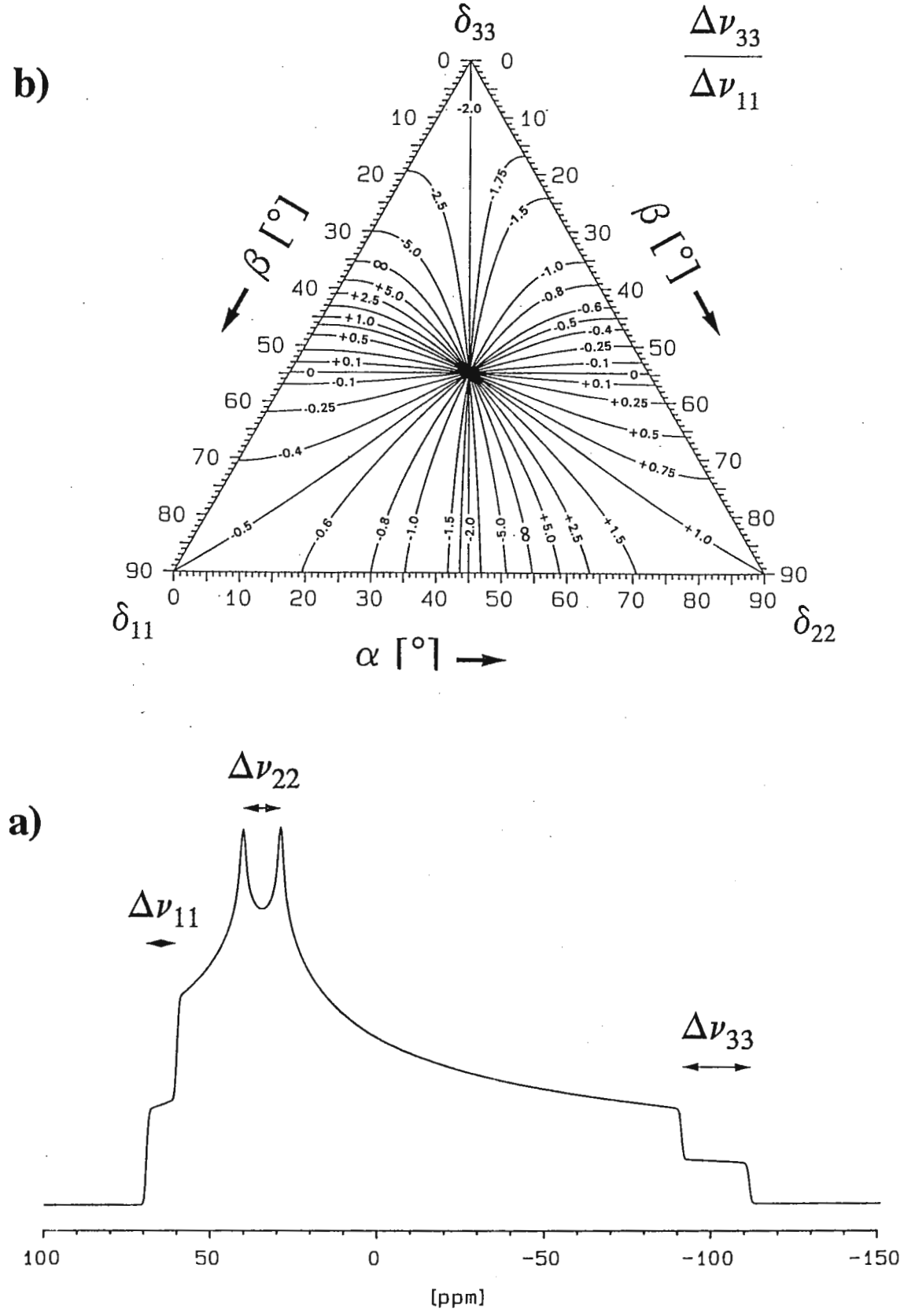

Fig. 1 (a). "A"-spin dipolar-chemical shift powder pattern arising from an "AX" spin-pair.
(b). Theoretical contour plot for $\Delta\nu_{33}/\Delta\nu_{11}$.

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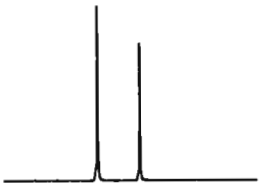
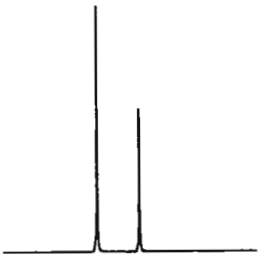
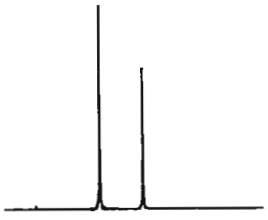


Variable amplitude cross polarization (VACP) *under* –2.6 KHz *mismatch condition*

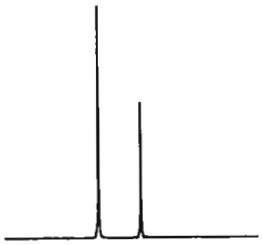
Deliberate "mismatch" of –1.9 KHz



VACP *under normal match condition*



Normal "ideal" matched conditions



VACP *under* +2.0 KHz *mismatch condition*

Deliberate "mismatch" of +1.4 KHz

Chemagnetics would like to thank Professor Steve Smith for suggestion of this work and useful discussions during its implementation.

SCHOOL OF NATURAL
SCIENCES AND MATHEMATICS

Department of Chemistry

June 14, 1993
(received 6/18/93)

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

Multinuclear NMR Studies on Aminoalane Dimers

Dear Barry:

Recently, we have analyzed the ^{13}C, ^{1}H, and ^{27}Al NMR spectra of three series of aminoalane dimers, resulting from our Group 13-Group 15 reaction chemistry studies. The ^{1}H and ^{13}C assignments for the three series $[Me_2AlR']_2$ (*Polyhedron*, 12, 389, 1993), $[Bu^i_2AlR']_2$, and $[R_2AlNMe_2]_2$ (*Polyhedron*, 12, 89, 1993) were obtained using standard 1-D and 2-D NMR techniques. The ^{1}H and ^{13}C NMR chemical shifts for R' are typical for those of the parent secondary amines (R' = NMe_2, NEt_2, NPr_2, NPr^i_2, NBu^n_2, NBu^i_2, NC_4H_8, NC_5H_{10}, NC_6H_{12}, $NC_4H_8NCH_3$, NPh_2, and $NBzl_2$). The ^{1}H and ^{13}C NMR chemical shifts and linewidths for R (R = Me, Et, Pr^n, Bu^n, and Bu^i) are influenced by the electronegativity and quadrupole moment of the ^{27}Al, particularly in the C-1 position. For example, δ_C = -1.5 ppm for the CH_2 and 9.40 ppm for the CH_3 group in $[(CH_3CH_2)_2AlNMe_2]_2$.

The ^{27}Al chemical shifts fall in a narrow range of 150-180 ppm, indicative of tetracoordinate aluminum {$[Al(H_2O)_6^{3+}$, ext. ref.}. However, there is a large variation in $\nu_{1/2}$, the value of the half-height linewidth increasing in each series as the R or R' groups become more sterically demanding. For $[R_2AlNMEt_2]_2$, R = Me, 625 Hz; Et, 1150 Hz; Pr^n, 2450 Hz; Bu^n, 4000 Hz; Bu^i, 3400 Hz. We are presently conducting x-ray structural determinations to possibly relate steric effects with lowering of the electric field gradient symmetry about the aluminum. The ^{27}Al linewidth may also be expected to increase with increasing size of the dimers due to a change in the correlation time (A. Abragam, "The Principles of Nuclear Magnetism," 1961).

Best regards.

Charlie

Charles L. Watkins
Professor

Larry K. Krannich
Professor and Chairman

The University of Alabama at Birmingham
219 Physical Sciences Building • 901 South 14th Street
Birmingham, Alabama 35294-1240 • (205) 934-4747 • FAX (205) 934-2543

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The Dow Chemical Company
Midland, Michigan 48667

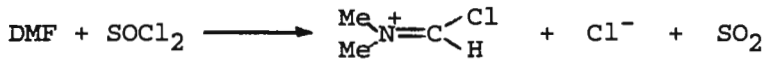
5 June 93
(received 6/15/93)

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

COUNTER ION OF VILSMEIER-HAACK REAGENT: Cl^- or SO_2Cl^-?

Dear Dr. Shapiro,

The Vilsmeier-Hacck reagent can be generated by the addition of thionyl chloride to dimethylformamide (DMF). Based on the reported evolution of SO_2 the counter ion associated with the formylation reagent is believed to be Cl^-[1].



When the reaction is conducted in the absent of solvent and with excess DMF, removal of SO_2 is accomplished only under vacuum. However, over a period of a few days SO_2 does evolve. The presence of dissolved SO_2 can be confirmed by ^{17}O NMR: 1.5M SO_2 in DMF produces a resonance at 474.6 ppm ($\Delta\nu_{1/2} \approx 30$ Hz). The ^{17}O NMR spectrum of the mixture with initial concentration 1.5M $SOCl_2$, contains two resonances at 328.7 and 432.7 ppm, $\Delta\nu_{1/2} \approx 100$ and 30 Hz, respectively. While, the more intense upfield resonance is readily assigned to DMF, the other resonance does not appear to be dissolved SO_2. Integration of the spectrum indicates that two equivalent oxygens are responsible for the resonance at 432.7 ppm. Adding aliquots of the 1.5M SO_2 solution to the $SOCl_2$/DMF mixture does not yield a separate SO_2 resonance; however, the resonance attributed to the oxygen containing product shifts downfield. The dependence of the chemical shift on mole fraction SO_2, $X(SO_2)$, is linear, δ(observed) = 432.7 + $X(SO_2)$41.9. This behavior indicates that SO_2 is undergoing rapid exchange with the oxygen containing product. In the $POCl_3$/DMF system, NQR measurements have suggested that the counter ion of the Vilsmeier-Haack reagent is $PO_2Cl_2^-$[2]. We propose a similar sulfur based ion, SO_2Cl^-, is formed. Disassociation of the proposed SO_2Cl^- ion into SO_2 and Cl^- would account for the required use of a vacuum for SO_2 removal as well as the behavior of the ^{17}O chemical shift. An alternative explanation could be association of SO_2 with the positively charged nitrogen of the Vilsmeier-Haack reagent. In order to test this idea, tetramethylammonium chloride (TMA) was added to the SO_2/DMF mixture. Unfortunately, the ^{17}O resonances shifted upfield a few tenths of a ppm before broadening beyond detectability. The loss of the SO_2 ^{17}O resonance occur at approximately a mole ratio of SO_2 to TMA equal to 0.5:1. At this level of TMA the ^{17}O resonance associated with DMF broadened significantly. It appears the question is still open for debate.

Dennis L. Hasha

Dennis L. Hasha

[1] G. J. Matin and S. Poignant, J. C. S. Perkin II, 1964 (1972).
[2] G. Jurgie, J. S. A. Smith and G. J. Martin, J. C. S. Perkin II, 925 (1975).

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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May 28, 1993
(received 6/15/93)

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

Automated Analysis of ^{31}P *in Vivo* Spectra

Dear Barry,

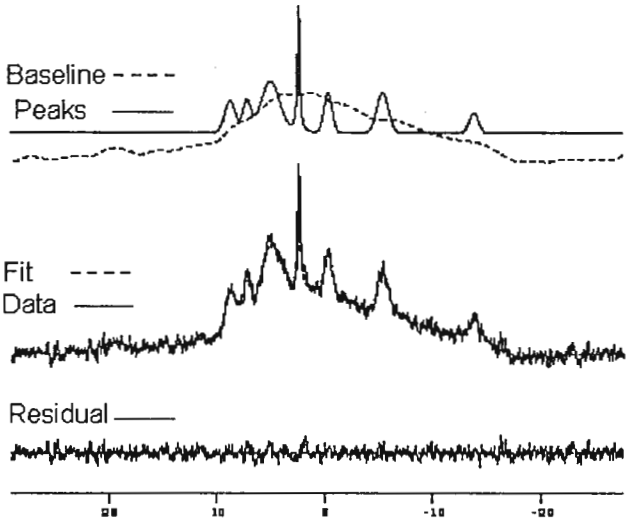
The analysis of *in vivo* ^{31}P NMR data from spectroscopic imaging studies of brain presents a challenge. Firstly, the acquisition of spectra from multiple regions over a 3D volume means that we can have hundreds of spectra to examine with each study, and totally automated analysis methods are therefore required. For automated processing, the advantages of parametric modeling and optimization approaches has previously been demonstrated in a number of publications. However, for ^{31}P brain spectra there is a broad baseline hump which is known to come from phospholipid resonances, but is difficult to parameterize. Previously we have removed this 'unknown' baseline using convolution difference, but have recognized that the choice of CD values is subjective and operator dependent, especially with the low S/N data frequently obtained with *in vivo* studies, and that it can also remove some signal from the other resonances. Furthermore this broad baseline resonance may contain valuable information which we would like to keep.

For automated analysis of ^{31}P spectra we have developed a semiparametric approach which operates on the frequency domain data, and simultaneously fits the spectrum with a nonparametric baseline together with a parametric model of the known resonances. This uses a statistical formulation of the ^{31}P spectral model, with the peak positions, linewidths and integrals each represented as probability distributions. This approach allows resonance positions to shift, e.g. with changing pH, while still using prior information on peak positions. For the lineshape model we have found that the best fit is obtained using a Lorentzian line for PCr and a Gaussian for the other resonances.

The spectrum is modeled as the output of a hidden Markov model (1) and the baseline is fitted non-parametrically with splines. Since the family of possible baseline and peak combinations is enormous, a local optimization search method would undoubtedly fail. Instead we have used dynamic programming methods, which in conjunction with the hidden Markov model gives an efficient scheme for performing a near global search of all possibilities. The procedure operates on the data moving from left to right, maintaining a list of possible peak and

baseline combinations, and for each combination evaluating the difference between the fit and the real data. The dynamic programming approach then allows us to keep only those most likely combinations, keeping the list of possibilities within a manageable range. A full description of the program has been submitted for publication (2).

This procedure is currently operational on SUN computers, and takes approximately 1 minute on a SPARK 1, to analyze a spectrum with 256 data points. Shown below is a fit using data obtained from the human brain at 2.0 T, using an ISIS acquisition.



We have tested this program with a number of human brain spectra obtained using ISIS and SI, and have found it to be very reliable. We are continuing to develop this program for totally automated analysis of multidimensional ^{31}P spectroscopic images to create metabolite images from fitted peak integrals. This presents additional challenges. For our current ^{31}P MRSI studies we typically obtain data in a 16x16 array for 8 planes. Even assuming that only half of these points contain data, then processing times will be at least 17 hours for each study.

Yours sincerely,

Andrew A. Maudsley Chris Raphael Michael W. Weiner

1. L.E. Baum, "Inequalities III". Edited by: O. Shisha, Academic Press, New York, 1972.
2. C. Raphael. Analysis of magnetic resonance phosphorus spectra using hidden Markov models. Submitted 1993.

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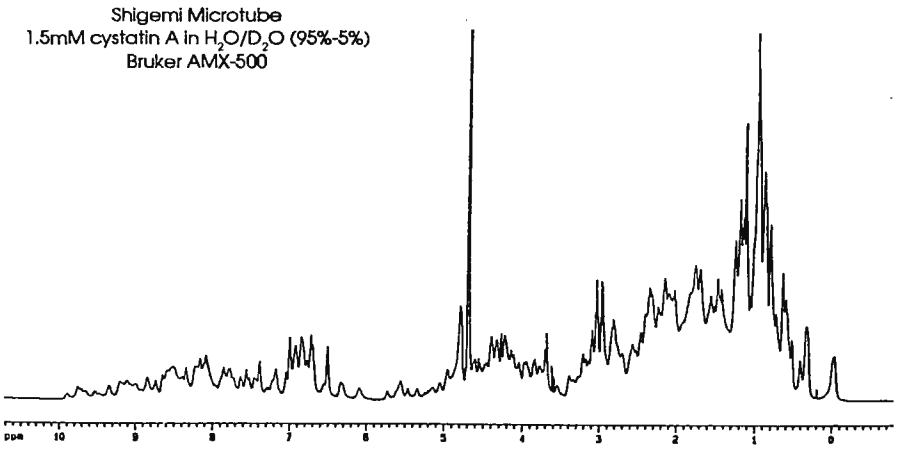

Figure 1: 1D spectrum of 1.5 mM protein dissolved in 250µl H_2O solution.

A protein, 11kDa, was dissolved in 250µl H_2O solution (pH 3.8) containing 5% D_2O for frequency lock and packed into the Shigemi Microtube. This spectrum was measured at 37°C on a Bruker AMX500 spectrometer with scan times 64. The solvent signal was suppressed by using low power RF irradiation.

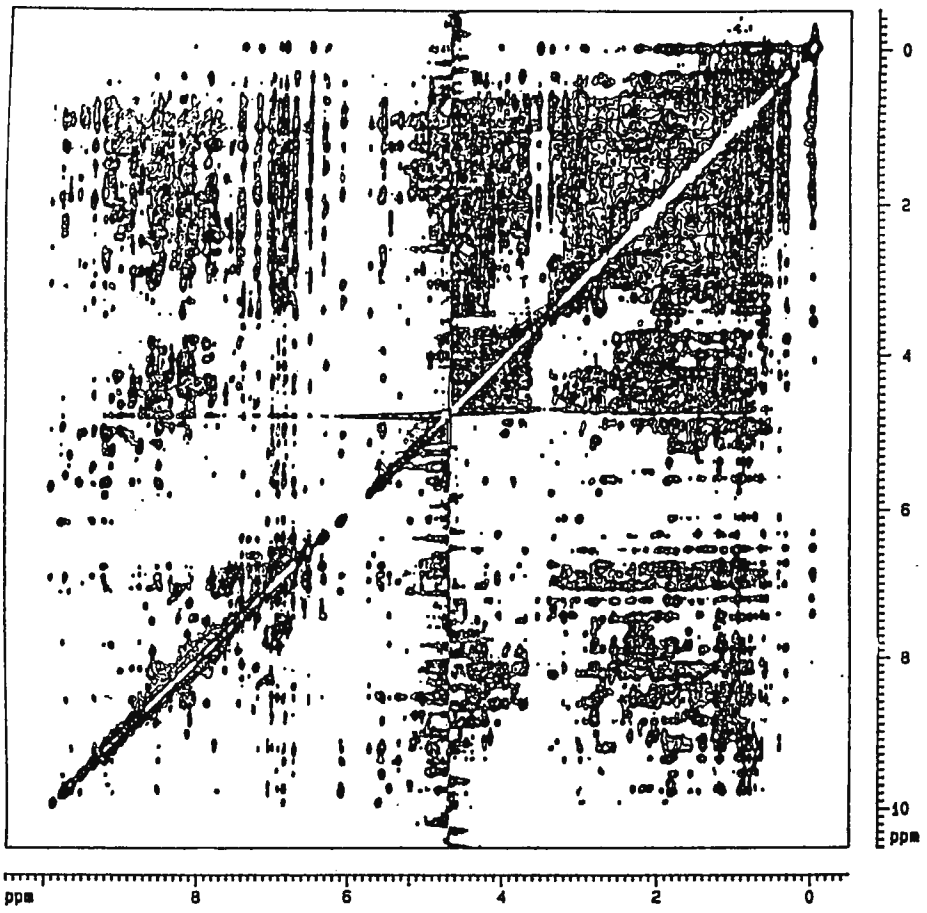

Figure 2: 2D NOESY spectrum of the same sample in Shigemi Microtube.

This 2D NOESY spectrum (τm=150 msec) of the protein in 250µl H_2O solution was measured with scan times 32 on a Bruker AMX500 spectrometer. The observed data matrix size was 1024 (t_2) x 200 (t_1) complex points. This matrix was processed with zero filling along t_2 dimension and resulted in a final data matrix of 1024 (F2) x 512 (F1) real points. It should be noted that baseline correction and digital processing were **not** applied to remove the water signal.

TOKYO METROPOLITAN UNIVERSITY
Department of Chemistry, Faculty of Science
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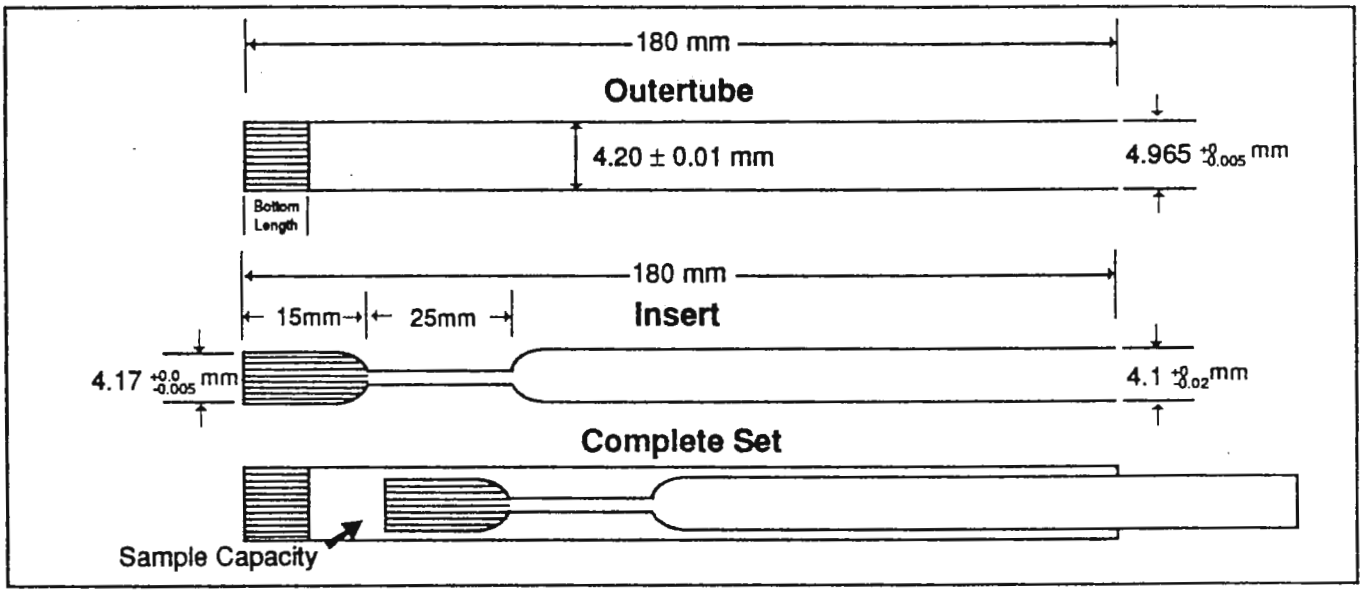
Japan



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Prof. B.L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto CA 94303, USA

(received 6/1/93)

Your reference and date	Our reference	Office telephone	Date
			24-May-93.
Subject		Sub-division	

Optimal Signal Strength for Glutamate and Glutamine in the PRESS and STEAM sequence.

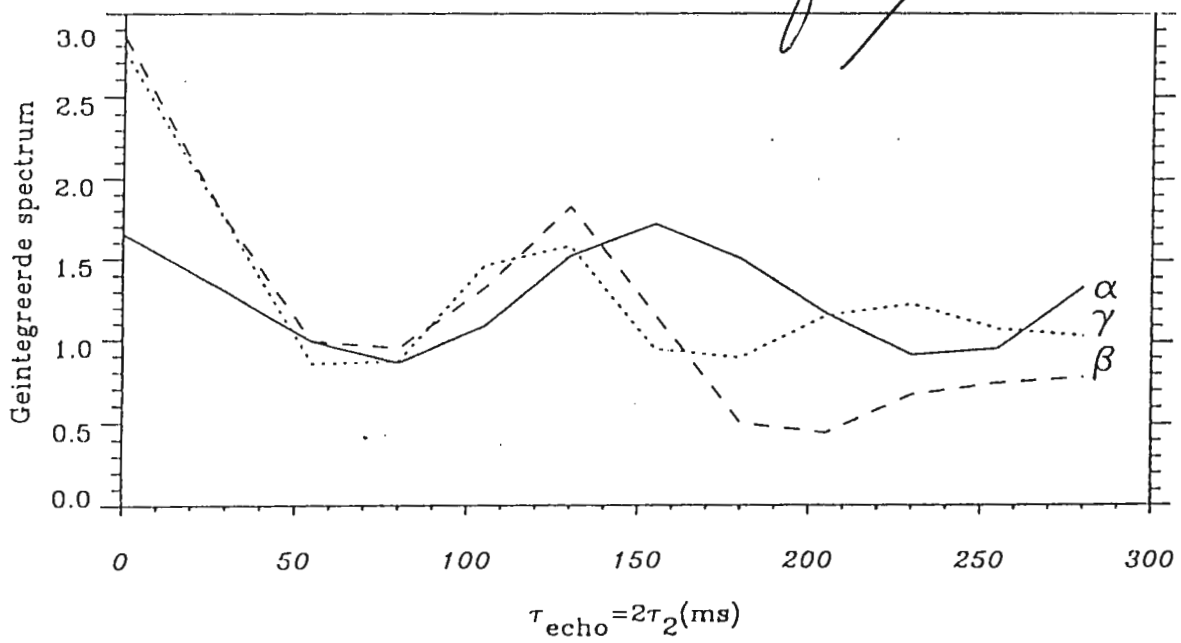
In vivo MRS spectra are usually obtained by applying multipulse (localisation and spectral editing) NMR experiments. Compared to the FID experiment, these sequences may lead to signal loss and distorted intensity ratio's by J-modulation effects in the spectra of coupled spins. Especially for strongly coupled spin systems like the protons in the metabolites glutamine (Gln) and glutamate (Glu), an unacceptable loss in signal may result, depending on the kind of pulse sequence, the pulse delays, and the line width. To determine the optimal experimental conditions for the detection of these metabolites, the dependence of the signal strength on pulse delays and line widths was simulated and measured for the PRESS and STEAM sequences, and a pH value of 7. The echo time T_E for both sequences was varied between 5 and 280 ms. For PRESS the delay τ_1 between the excitation and first refocussing pulse was varied between 2.5 and 77.5 ms, whereas the effect of the delay τ_m between the second and third 90° pulse for STEAM was studied as well.

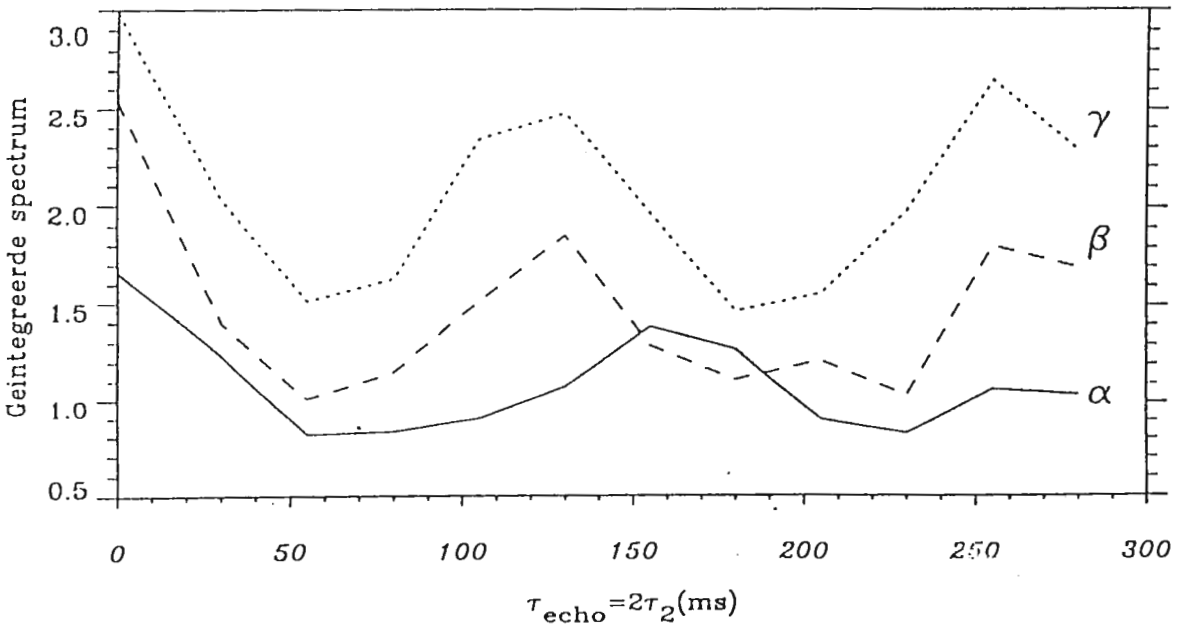
The figure shows the integrated intensities of the simulated absolute value mode signals at 7T of the α, β and γ protons of Gln (top) and Glu in the PRESS sequence, τ_1=15 ms. Further simulations for the **PRESS** sequence showed a striking difference in modulation patterns at 1.5 and 7 T. In general the strongest signal is obtained at very short echo times. However, at 7 T the γ signal shows a maximum at 130 ms as well, while Glu has another maximum at 250 ms. At 1.5 T such maxima are not observed. The multiplet signals are only preserved at the shortest echo times (<30 ms). Another difference is the shortest echo time for which the modulation patterns become really destructive. At 1.5 T this is at echo times above 30 ms, at 7 T already above 10 ms. Also at 7 T Glu shows a stronger signal than Gln, which is reversed at 1.5 T. The τ_1 dependence is small. In the **STEAM** experiment there is hardly any dependence on the distance between the second and third 90° pulse, and the modulation effects are less pronounced than for PRESS. The signal strength gradually decreases with T_E, so very short echo times are favourable; only for Glu at 7T there is still a maximum at T_E~250

ms. Line broadening leads for both experiments in all cases to extra signal loss because more multiplet components with different phases cancel each other. An important observation was that signal loss with STEAM as compared to PRESS for both Glu and Gln was more than the well known factor 2 at both field strengths.

W.Q. Both

W.M.M.J. Bovée





EASTMAN

Research Laboratories
Kingsport, Tennessee 37662

May 20, 1993 (received 6/5/93)

Prof. Bernard L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Ordered Melt Phase in PPS/DS

Dear Prof. Shapiro:

Poly(p-phenylene sulfide/disulfide), PPS/DS, is a high temperature thermoplastic made from the reaction of p-diiodobenzene (DIB) and sulfur. Based on DSC analysis, the crystalline melting point, T_m, of this semicrytalline polymer is typically around 280°C. One would expect that the solid-state carbon-13 NMR spectrum of this polymer would exhibit broad resonances below 280°C that sharpen to appear more liquid-like above 280°C. In fact, this does not occur. In stead, the solid-state carbon-13 NMR spectra of this polymer remain broad well above T_m. A series of solid-state carbon-13 NMR spectra of this polymer over the temperature range of 250 to 340°C are shown in the attached figure. Clearly a transition to liquid-like character occurs between 315 and 320°C. Above 320°C, the polymer was noted to flow from pores of the sample rotor, but not to flow below this temperature.

Our explanation of this observation is that PPS/DS exhibits an ordered structure above the crystalline melting point that persists to a temperature approaching the estimated equilibrium melting point of 320°C.

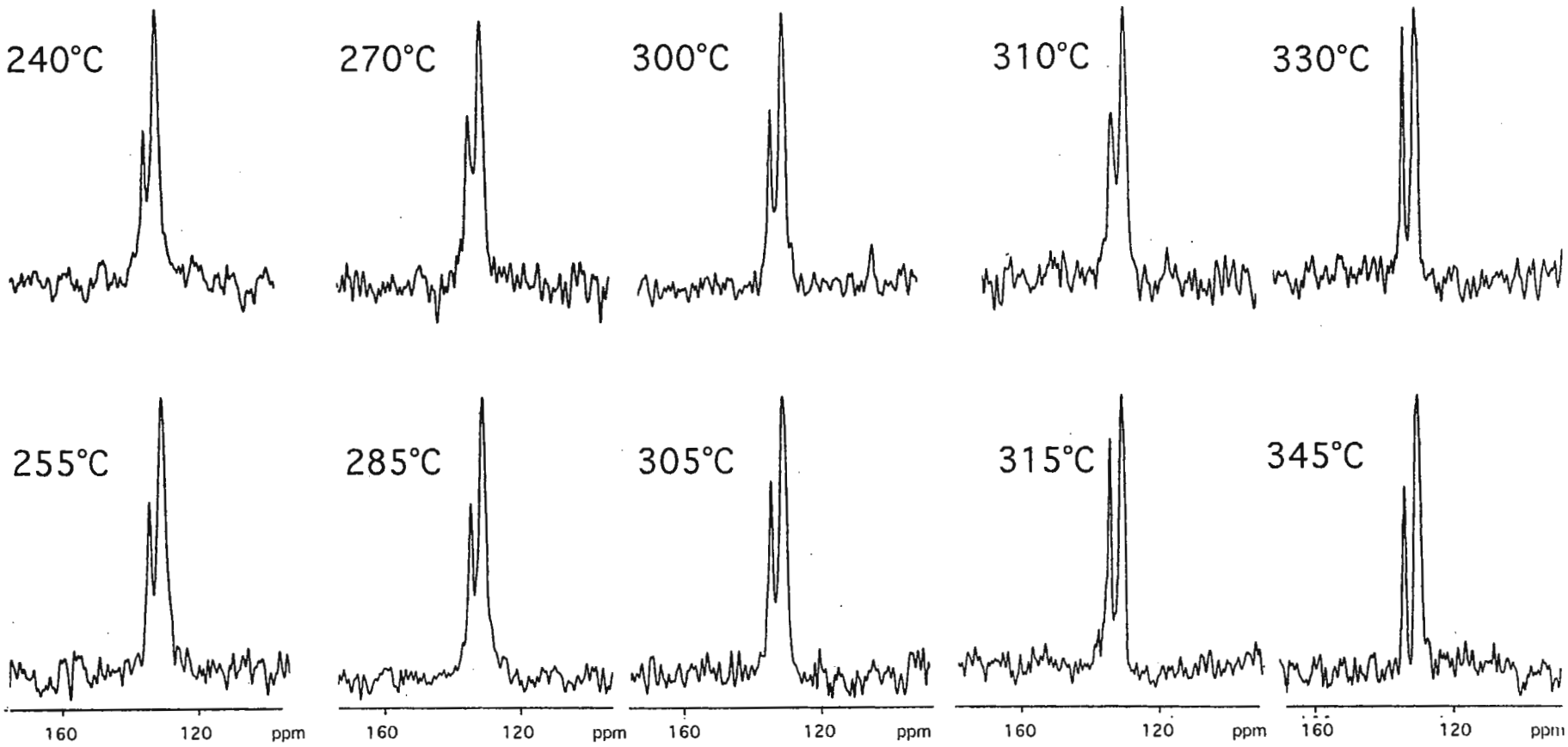
This result and other interesting observations on phase heterogeneity of the amorphous phase of PPS/DS based on epr experiments have been submitted to Macromolecules.

Sincerely yours,

Douglas W. Lowman
Principal Research Chemist
(615) 229-4728
Research Laboratories
Eastman Chemical Company
P. O. Box 1972

David R. Fagerburg
Research Associate
(615) 229-4727

Solid-state carbon-13 nmr spectra of DP 150 perdeuterated PPS/DS as a function of sample temperature.



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Bethesda, Maryland 20892

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

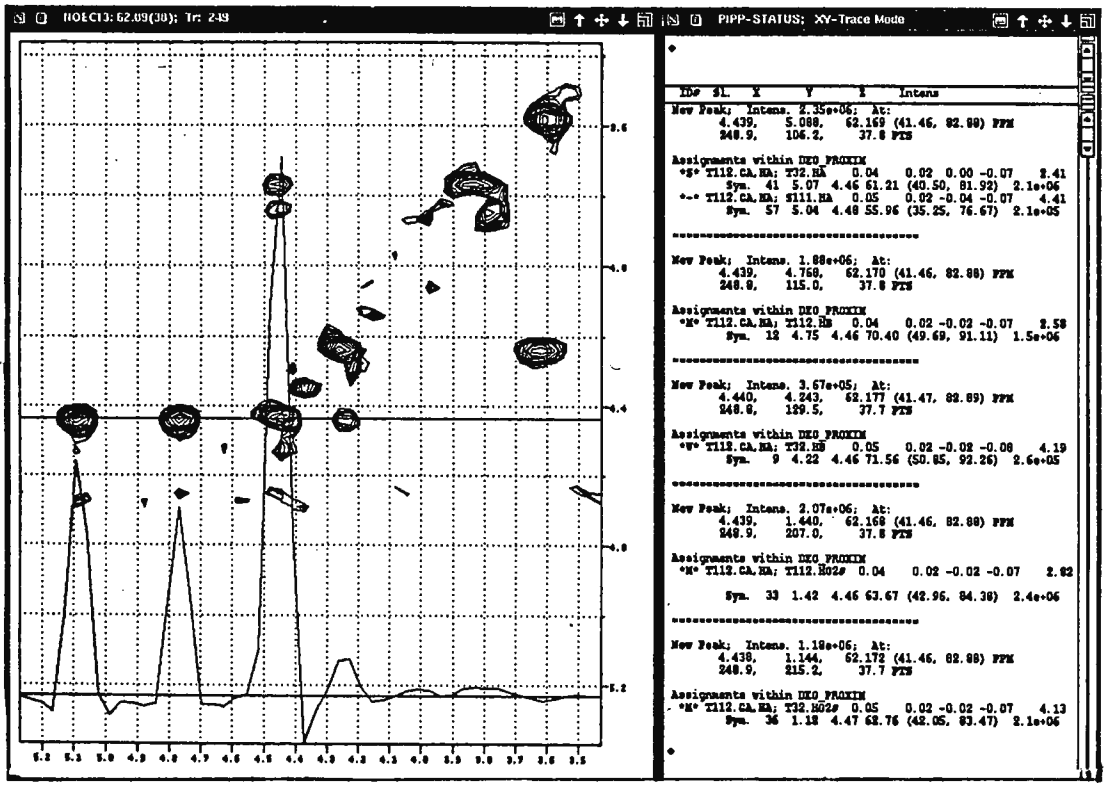
(received 5/24/93)

May 18, 1993

Straightforward Analysis of 3D and 4D NOESY Data with PDB Structures Using PIPP

Dear Barry,

We are frequently asked what programs we use to analyze 3D and 4D NOESY data. In this letter we illustrate the program PIPP, primitive interactive peak picker, which has become our principle tool for analyzing multidimensional NMR data sets. The figure below shows a screen dump from a Sun 1+ workstation with a Data Window and a Status Window created by PIPP. The Data Window shows the contours from plane 38 (at 62.09 PPM) and the 1D trace from point 249 (at 4.44 PPM) of the 3D ^{13}C-edited NOESY of IL-4. The Status Window shows potential assignments for five cross peaks (the two upfield peaks are outside the current display) along the 1D trace with valuable information about each peak and assignment. The information for each peak begins with the chemical shift in PPM and points followed by possible assignments. The *S* indicates that this NOE was previously known as a strong NOE. The first number after the assignment is the RMS deviation (in PPM) between the shift in this experiment and the values in the assignment table. The next three numbers are the individual deviations along each axis (in PPM) between this experiment and the assignment table. The last number is the minimum distance in Å between the proton pairs of this assignment found in the curent set of structures that were read in. The line immediately following the assignment which is labeled "Sym." shows the plane, position and intensity of the 3D symmetry related peak.



Sincerely,

Dan Garrett Angela Gronenborn Marius Clore

Texas A&M University NMR Newsletter - Book Reviews

Book Review Editor:

William B. Smith, Texas Christian University, Fort Worth, TX 76129

" Structure Elucidation by NMR in Organic Chemistry - A Practical Guide "

by **Eberhard Breitmaier**

John Wiley & Sons, 605 Third Avenue, New York, NY.10158-0012;
paperback: ISBN 0-471-93381-3, $35.00; hardback: ISBN 0-471-93745-2, $63.95;
261 pages with index.

This book first saw life in a German edition, and was subsequently updated and translated to form the present volume, which is exactly described by the title. It is not about the details of modern pulse techniques or about how to extract spectral parameters. It is about how to use these parameters to deduce the structures of organic molecules.

NMR parameters, covered in the first eleven pages, include chemical shifts, coupling constants, signal multiplicity, CW and FT-NMR, spin decoupling, NOEs and relaxation. Needless to say, you had better know these subjects in advance as they are only given a once-over-lightly here. Chapter Two deals with the type of thinking required to recognize structural fragments. The use of 2D spectral analysis falls here as do the affects of relative and absolute configuration, and molecular dynamics. A summary ends on page 68, and the text is ready to launch itself on to its main thrust.

What follows is a set of fifty NMR structure problems. While I make no pretense at having read them all, the several I did consult seemed very well constructed. The value of this volume rests primarily on these problems. When coupled with another text on spectral techniques and interpretation, one has the makings of a very thorough course in the application of NMR to organic chemistry. After presenting each of the problems, the volume concludes with a detailed analysis of each. This is followed by a brief formula index to problem solutions.

W. B. S.

University of Arkansas for Medical Sciences
Departments of Radiology and Pathology
Biomedical NMR Center
Little Rock, AR 72205

(received 6/7/93)

Dr. B. L. Shapiro
TAMU NMR Newsletter
966 Elsinor Court
Palo Alto, CA 94303

^{31}P NMR Analysis of Solubilized Phospholipids in Human Amniotic Fluid

Dear Barry:

In our previous letter we described the ability to resolve the ^{31}P NMR resonances of various molecular species (acyl side chain unsaturation and to some extent chain length) of phospholipids (PLs) by direct detergent (sodium cholate) solubilization. In this method we typically see resolved resonances for glycero-PLs containing 0, 1, or 2 unsaturated acyl side chains. In collaboration with J. T. Krone and A. A. Pappas, we have now applied this direct-solubilization method to the analysis of human amniotic fluid.

Figure 1A shows a typical ^{31}P spectrum (121 MHz) of a solubilized amniotic fluid. Trimethylphosphite (TMP) is an internal chemical shift and quantitation standard, P_i is inorganic phosphate, SM is sphingomyelin, PI is phosphatidyl-inositol, and PC is phosphatidylcholine. The expansion (Figure 1B) shows the typical 3-peak structure of the PC peak for 0, 1, and 2 unsaturated acyl chains going from lower to higher field. Figure 1C shows an expansion of the organic-extract spectrum, where the additional structure of the PC peak is not observed.

Our interest has been to develop an NMR method for measuring fetal lung maturity, and hence we initially have attempted to correlate NMR-derived parameters with estimated gestational age (EGA). Although the readily measured PC/P_i ratio weakly correlates with EGA($r = 0.52$, $p = 0.022$), the best correlation is obtained for disaturated PC (dsPC), which is represented by the most downfield PC peak. This is not surprising since dsPC is a major component of mature lung surfactant, and increases as a percentage of total PC during gestation. Figure 2 shows a plot of %dsPC vs. EGA for 16 patients. Two patients (represented by the filled and open triangles, respectively) were run on more than one occasion. A 1st order correlation coefficient of 0.85 ($p = 0.000002$) was found for dsPC vs. EGA.

Sincerely,

Richard A. Komoroski

John M. Pearce

Figure 1

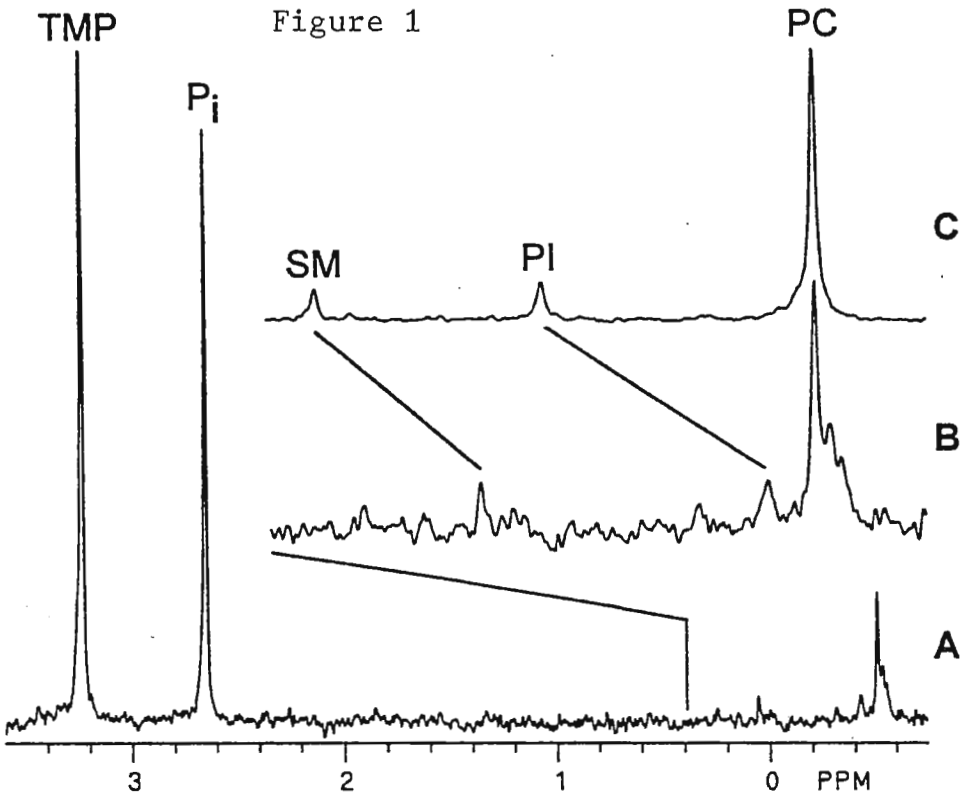
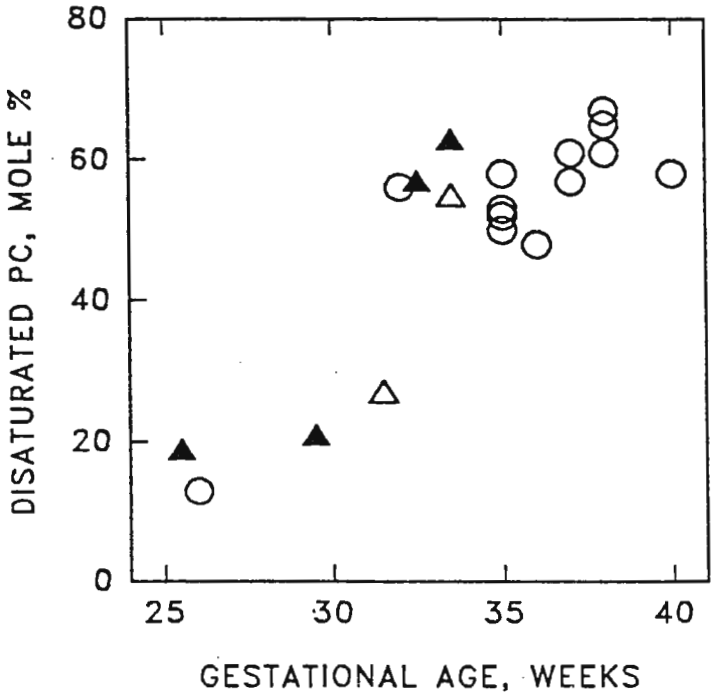


Figure 2



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83-50001-7	Cholesterol-3,4-$^{13}C_2$	99 atom%
82-52001-6	Cholic-2,2,4,4-d_4 Acid	98 atom%
82-84030-7	Chrysene-d_{12}	98 atom%
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82-70041-0	Deuterium Oxide ULTRA-D	99.999 atom%
82-00815-2	DL-1,4-Dithiothreitol-d_{10} (Cleland's Reagent)	98 atom%
82-82200-8	Ethyl-d_5 Carbamate (Urethane)	98 atom%
83-30200-0	D-Glucosamine-1-^{13}C HCl	99 atom%
83-42007-5	Glyceryl Tri(oleate-1-^{13}C)	99 atom%
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82-04059-3	Poly(ethylene-d_4)	98 atom%
83-30022-8	D-Ribose-1-^{13}C	99 atom%
81-12213-7	L-Serine-2-^{13}C,^{15}N	99 atom% ^{13}C,^{15}N
83-30040-0	D-Sucrose-$^{13}C_{12}$	≈99 atom%
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UCD NMR FACILITY DAVIS, CALIFORNIA 95616

June 1, 1993
(received 6/2/93)

SAVE THE BEARS

Dear Dr. Shapiro:

We would like to report on some (occasionally) on-going "forensic NMR" work in our lab. The original report on this appeared in Wildlife Society Bulletin, a journal not likely read by NMR spectroscopists!

The poaching of bears in California is a serious problem. Various parts of the bear are sold illegally for supposed value as folk medicines. Chief among these transactions is sale of the gall bladder. Pig and cow gall bladders are sometimes substituted because of their similar size, appearance, and easier accessibility. Once the gall bladder is removed and the bile dried to a marketable state, it is not possible to identify the species of origin by immunological methods. However, the predominant bile salts differ in these species (independent of diet or environmental factors).

We embarked upon a program to see if NMR could reliably distinguish between bile samples from different species based on the different molecular structures of the species specific bile salts. NMR has the advantage for forensic work of minimal handling and preparation of the sample and the non-invasive nature of the sampling, i.e., we can't convert the sample to anything else. Approximately 90 known samples of bear, pig, and cow biles were examined and showed reproducible spectra as shown in Figure 1. Thus encouraged, we examined nearly 100 samples of the three species' biles sent to us by Calif. Dept. of Fish & Game as unknowns, and were able to identify every one correctly. This laid the groundwork for Operation Ursus, a F&G sting operation that netted 22 sellers of bear gall bladders. Samples from the sting operation were identified in our lab and charges filed. Faced with the evidence, 20 of 22 people accused pleaded guilty to reduced (misdemeanor) charges, 1 jumped bail, and 1 case went to court. In the latter case our evidence was presented and a felony conviction obtained.

At present we continue to handle bear poaching samples, and would be interested in hearing from others using NMR in forensics. We thank Sue Toto for her work on this project; please credit this contribution to G.N. La Mar's subscription.

Sincerely,

Jeff de Ropp

Jerry Theis
Med. Microbiology

Jeff de Ropp
NMR Facility

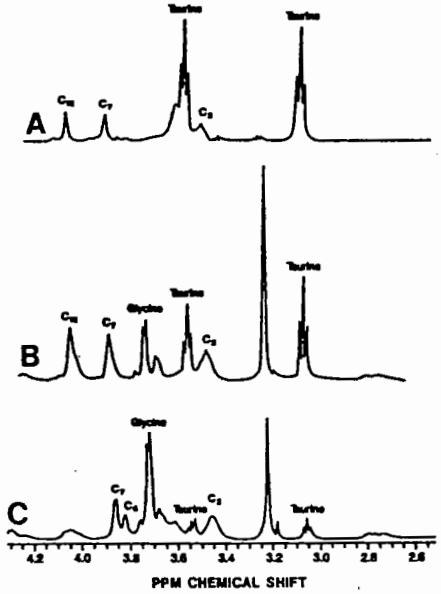

Figure 1: Portions of the 500 MHz NMR spectra of biles from (A) Bear, (B) Cow, and (C) Pig. Bear bile is predominantly taurine conjugated to cholic acid (taurocholic acid); pig bile is predominantly a trihydroxy cholic acid conjugated with glycine, plus a small amount conjugated with taurine; while cow bile consists of a trihydroxy cholic acid conjugated to taurine and glycine in approximately equal amounts. Adapted from Wildlife Society Bulletin 16:430-433 (1988).

418-42

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June 20, 1993
(received 6/25/93)

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

Subject: Laser outputs on the JEOL and BRUKER

Dear Dr. Shapiro:

JEOL and BRUKER users out there who would like to obtain laserjet plots without the added expense of purchasing a "black box" from BRUKER, or upgrading to Multiplexus 3.1 from JEOL, can do it for approximately $250.00 by obtaining a HPGL emulation cartridge which is compatible with HP Laserjet IIP, IID and III printers with 2.0 Mb memory (Plotter in a Cartridge™, Pacific Data Products, San Diego CA.). BRUKER systems must have the HP license to obtain the plots.

BRUKER hardware setup: RS-232 communication between a free channel on the ASPECT-3000 and the laser printer. Pinouts are similar to those for the RS-232 connected to the existing 7550A plotter. Set the laser printer to emulate HP 7475A (setting it to HP 7550A messes things up), correct baud rate and other communication parameters. To redirect plots to the laser printer calibrate (command=CA in DISNMR) the printer to HP7550A and correct port.

JEOL hardware setup: RS-232 communication between any open channel on the data system (in this case TT5:) and the printer (straight through cable). In the [1,2]setup.cmd file search for ASN TT4:=HP0:/gbl. Add ASN TT5:=HP1:/gbl on the next line. Reboot the system, set PLTFL to HP1:, set laser printer to landscape mode to plot. Additionally TT5: can be setup similar to the TT2: print queue in the startup.cmd file to serve as a printer for peak printouts etc. Once the laser printer is on the queue escape sequences can be sent to software switch many of the features of the printer, i.e. portrait to landscape, font sizes etc. (refer to the Laserjet manual).

Both systems have been checked and no bugs have been found. The only misplot on the BRUKER is that the ellipses for the logo are skewed which is a minor eyesore.

Yours sincerely,

Ashok Krishnaswami

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* * * * *

All Newsletter Correspondence

Should Be Addressed To:

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

(415) 493-5971

DEADLINE DATES

No. 420 (September) -------- 20 August 1993
No. 421 (October) -------24 September 1993
No. 422 (November) -------22 October 1993
No. 423 (December)-----19 November 1993

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Mailing Label Adornment: Is Your Dot Red ?

If the mailing label on your envelope of this issue is adorned with a large **red dot** or circle: this decoration means that you will not be mailed any more issues until a technical contribution has been received by me.

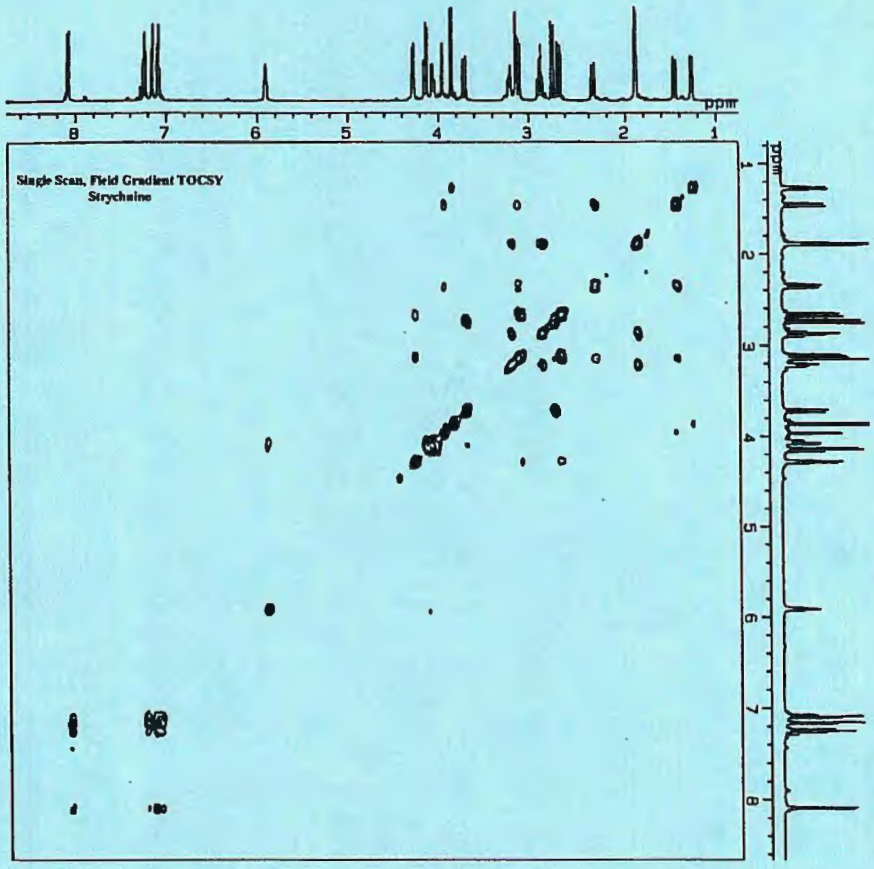
Page Length Instruction

Attention overseas subscribers: If you must use paper which is longer that 11", *please* take care that all material (including signatures, addresses - everything!) ends no more than 10" from the top of each of your pages. It is costly to make reductions, and henceforth I reserve the right to chop the excess length off any page, no matter what the result. Beware of the dreaded guillotine! Your cooperation in this matter will be greatly appreciated.

NMR

FROM JEOL USA

Gradient Probes



The above data is a 512 by 512 *TOCSY* spectrum and was obtained on an **Alpha**- 500 in less than 5 minutes with **JEOL**'s gradient probe. This same probe is also designed to do reverse and water suppression experiments. Pulse shaping, three channel operation, dynamic Rf control, matrix shims, as well as unsurpassed field reliability are all standard with the **Alpha**.

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