THE NMR NEWSLETTER

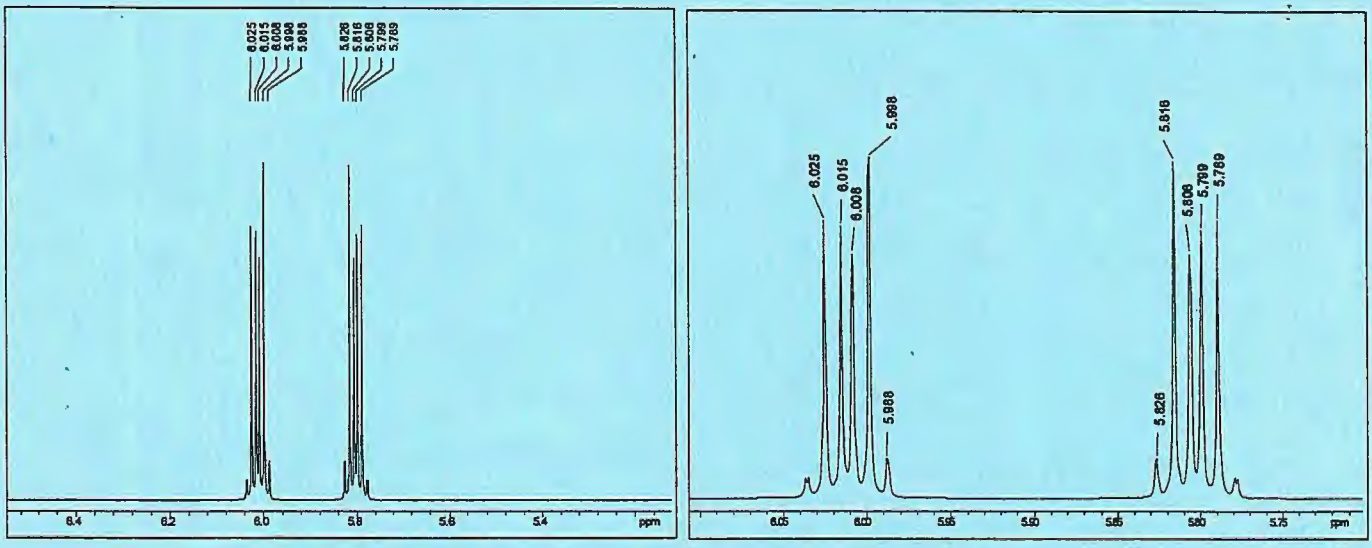
No. 472

January 1998

NUTS

NMR Data Processing Software

NEW! More options for displaying peak labels



Labels can be placed at the top of the plot or above each peak and can be frequency (Hz or ppm) or text input by the user.
Position of labels is automatically adjusted to prevent overlap, or can be adjusted manually using the mouse.

NEW! Listen to your FID, played through your computer's speakers!

See the website for
- Database of NMR Spectra
- Helpful Hints on Shimming
- Demo copies of NUTS

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Fremont, CA 94538

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Varian NMR Instruments

FORTHCOMING NMR MEETINGS

High Field NMR: A New Millenium Resource, Washington, D.C., **January 15 - 16, 1998**;Contact: Jo Ann Palmer, Workshop Coordinator, NHMFL, 1800 East Paul Dirac Drive, Tallahassee, FL 32310.

Tsukuba NMR 98, Tsukuba Science City, Japan, **March 10-12, 1998**. Contact: Professor Yoji Arata, Water Research Institute; +81-298-58-6183; Fax: +81-298-58-6166; e-mail: arata@wri.co.jp; http://www.wri.co.jp

39th ENC (Experimental NMR Conference), Asilomar Conference Center, Pacific Grove, CA, **March 22 - 27, 1998**; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87505; (505) 989-4573; Fax: (505) 989-1073; Email: enc@enc-conference.org. See Newsletter 460, 41.

Sixth Scientific Meeting and Exhibition, International Society for Magnetic Resonance in Medicine, Sydney, Australia, **April 18 - 24, 1998**. Contact: International Society for Magnetic Resonance in Medicine, 2118 Milvia St., Suite 201, Berkeley, CA 94704; 510-841-1899.

NATO ARW "Applications of NMR to the Study of Structure and Dynamics of Supramolecular Complexes", Sitges (Barcelona), Spain, **May 5 - 9, 1998**. Contact: Prof. M. Pons, Dept. Quimica Organica, Univ. de Barcelona, Mart I Franques 1, 08028 Barcelona, Spain; http://www.ub.es/nato/nato.htm; e-mail: miguel@guille.qo.ub.es.

^{13}C in Metabolic Research, Symposium at the University of Texas Southwestern Medical Center, Dallas, Texas, **May 7, 1988**; For more information, contact Jean Cody at 214-648-5886 or www.swmed.edu/home_pages/rogersmr.

Workshop on Magnetic Resonance of Connective Tissues and Biomaterials, Philadelphia, PA, **June 18-20, 1998**; For more information. Contact International Society for Magnetic Resonance in Medicine, 2118 Milvia Street, Suite 201, Berkeley, CA 94704; (510) 841-1899; fax (510) 841-2340; info@ismrm.org; http://www.ismrm.org.

Fifth International Conference on Heteroatom Chemistry, London, Ont., Canada, **July 5 - 10, 1998**. For details, see Newsletter 468, 40.

XIVth International Conference on Phosphorus Chemistry, Cincinnati. OH, **July 12 - 17, 1998**. For details, see Newsletter 468, 40.

Continued on p. 44

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Skokie, Illinois 60077
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Dr. Bernard L. Shapiro
966 Elsinor Court
Palo Alto, CA 94303

December 11, 1997
(received 12/15/97)

LOW COST 'DIGITAL' GAUSSMETER

Dear Barry,

SEARLE

Many years ago, one of the astute engineers from Bruker introduced me to a low cost 'digital' gaussmeter. The low cost parts consist of two 10 pfenning pieces (German coins). When one is in close proximity (a foot or so) to a superconducting magnet, grasp one of the coins using two *digits* of the right hand (it's easy if the thumb and index finger are selected), and hold the coin vertically (as if standing on its edge). Using the left hand, position the other coin to touch the first coin (once again, in a vertical plane). The magnetic field will hold the coins together, so you may now move your left hand out of the way.

Now, if you move away from the magnet slowly, with a steady hand, the coins will cling together until you reach the 10 gauss line. At the 10 gauss line the coins will separate. I have compared the results of measurements using this technique with measurements taken with an electronic magnetic field measuring instrument and found them to be quite reliable.

One can also use two 5 Peso pieces (Mexican coins) to accomplish this type of experimental measurement. These coins, however, will cling together out to the 5 gauss line. They do, however, require a much steadier hand.

One can also determine the approximate vertical center of the magnetic field using this type of digital gaussmeter. When the two coins are 'attached' and within a few inches from the superconducting magnet, change the position of the hand holding the coin along the vertical axis of the magnet. The 'magnetically held' coin will change its position from below the hand-held coin to above it. At the center of the magnetic field the 'magnetically held' coin will rotate to the quadrature position.

(There are probably a few people that can use left hand '*digits*' for these measurements.)

With best regards,

Robert Dykstra

PLAYING AROUND WITH RADIATION DAMPING

RADIATION DAMPING COMPENSATION UNIT

We have recently introduced the new **R**adiation **D**amping **C**ompensation **U**nit (**RDCU**) developped in collaboration with Professor Jean-Yves Lallemand's group (Ecole Polytechnique Palaiseau) on the AVANCE spectrometers.

Using the **RDCU** it is possible to compensate the natural radiation damping or to enhance it. Thus the **R**adiation **D**amping **C**ompensation **U**nit can operate during evolution,mixing and acquisition periods in 1D, 2D and 3D experiments.

The NOESY is one of the most widely used experiment in the biological area which is affected by radiation damping. If we have a look at the behaviour of the exchangeable NH proton peak intensities of a protein or a peptide as a function of the nOe mixing times (figure 1) on an **AVANCE 400** in presence of natural radiation damping. When radiation damping is compensated or enhanced, we see that we can take advantage from this situation to record fast NOESY experiments by compensating/enhancing the natural radiation damping every two scans with a specific phase cycle. Thus for small mixing times (<= 50 ms) **only the exchangeable protons are observed**

At higher fields (800 Mhz), the radiation damping effect is much more pronounced as is shown in figure 2. The intensities of the peaks of all these protons is restored when radiation damping is compensated.

The **R**adiation **D**amping **C**ompensation **U**nit (**RDCU**) is suitable for 3 channel AVANCE spectrometers.

- The **RDCU** is inserted in the AQX rack.
- The **RDCU** is easy to use and to calibrate.
- The **RDCU** can be used for radiation damping compensation for other nuclei (e.g. ^{19}F) and non biological samples for which the phenomenon is observed.

Call your local Bruker office, and ask for more details.

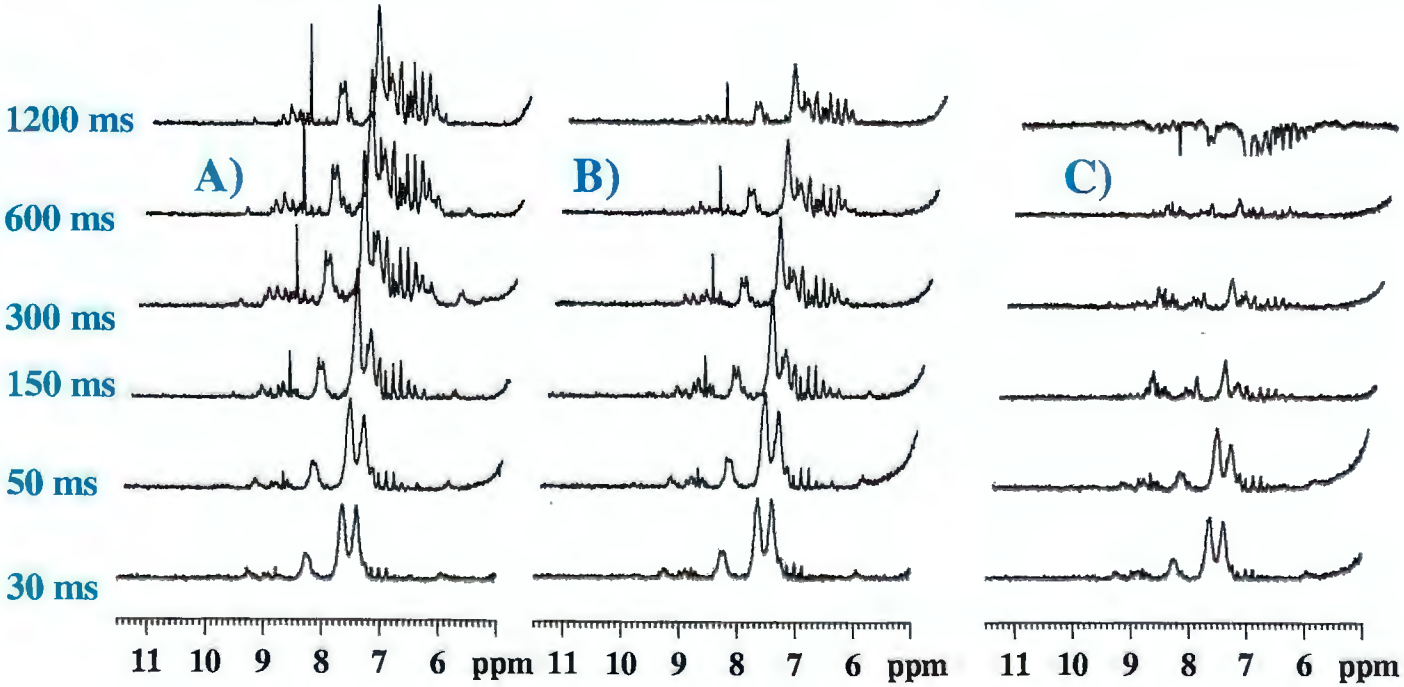
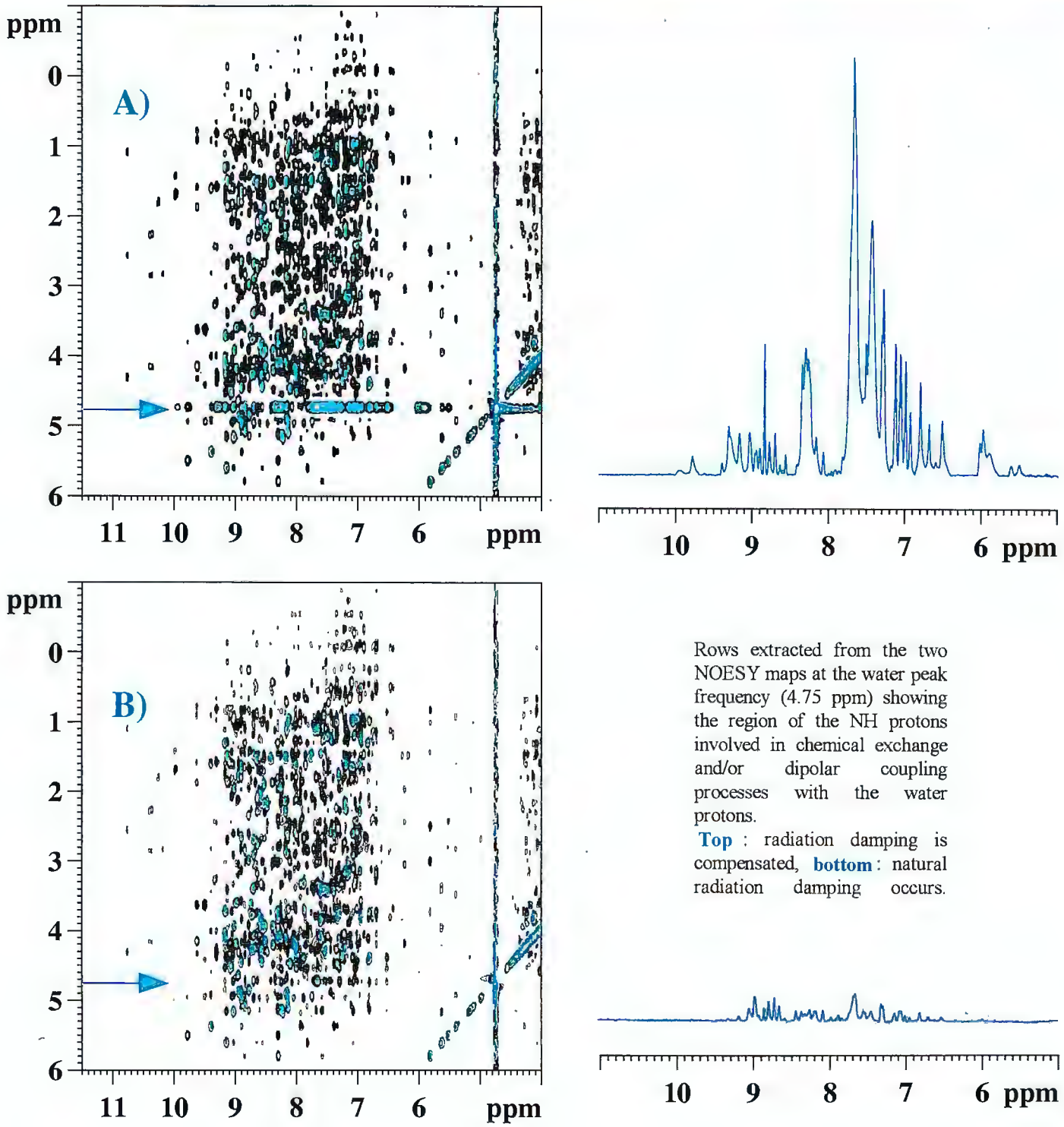


Figure 1: Rows extracted from 2D NOESY maps recorded on an **AVANCE 400** at 300 K at the frequency of the water peak. **A)** the radiation damping is compensated, **B)** natural radiation damping occurs and **C)** the radiation damping is enhanced. The sample is the 2 mM Lysosyme sample in 90/10 H2O/D2O.



Rows extracted from the two NOESY maps at the water peak frequency (4.75 ppm) showing the region of the NH protons involved in chemical exchange and/or dipolar coupling processes with the water protons.
Top : radiation damping is compensated, **bottom** : natural radiation damping occurs.

Figure 2: 1H-1H NOESY maps recorded on the 2 mM Lysosyme sample in 90/10 H2O/D2O (Mixing time = 150 ms) at 300 K on an **AVANCE 800**. Eight transients per 512 complex increments are taken in t1. The water elimination is done with a WATERGATE sequence. Both maps have been processed in the same manner (f1, f2 Fourier transformation with a $\pi/3$ shifted sinebell filter for both dimensions).
A) the NH, CH_α and CH_β region is shown when radiation damping is compensated during the evolution and mixing times, **B)** the same region when natural radiation damping occurs. Note the quasi perfect water peak elimination in both experiments.

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Dec. 9, 1997

A "Standard" NMR Data Format (Is it JCAMP-DX ???)

Dear Barry,

There have been numerous requests by NMR operators around the world for all NMR vendors to adopt a standard (*i.e.*, the same) format for NMR data files. There have been *ad hoc* meetings at the ENC about this issue with agonizingly slow progress (none??). In today's world, where file formats have become directory structures, there is little hope that the NMR vendors are moving toward a common data format. About the only progress towards a common data format is that Varian bought Chemagnetics' NMR business, thereby reducing the number of data formats by one.

Some of the reasons we have heard for the lack of progress in this area border on conspiracy theories that would put the "black helicopters" and "men in black" stories to shame. Many of these theories openly state that the lack of progress towards a common NMR data format is to the benefit of the NMR vendors by making direct comparisons between data from different vendors more difficult. Then there are the NIH theories (Not Invented Here), which state that the vendors all agree that a common NMR data format would be nice as long as it is theirs. Regardless of these and other stories, it does not appear that a common NMR data format is close at hand!

Our NMR data processing program, NUTS, chose the only non-vendor (public??) NMR data format available when it was created. We started with the "New Felix Format" used by Dennis Hare and his gang and extended it to add more information. Even today, NUTS can read a New Felix Format file without an import filter. The result just lacks some very useful parameter information. This format is well-documented in many places including the NUTS help files, but it has not become an industry standard format. Since NUTS imports a wide variety of NMR data formats, the NUTS format has been used by some groups as a data format standard. It is, however, unlikely that an expanded version of the NUTS data format will be adopted by NMR vendors as a "standard". If all NMR vendors were to export to and import from NUTS data format then the NMR community would have a *defacto* standard. This approach is also unlikely to succeed.

A group of people has developed a standard data format for IR and Mass Spectral data called JCAMP. Some efforts have been made to include NMR data in this standard.
See **http://lolita.colorado.edu/faq/data.html** for leading references.

This format is very poorly adapted to NMR data and its only use to date, of which we are aware, has been limited to the display of spectra across a network connection. There are Netscape plugins and Java applets for the display of NMR data in JCAMP-DX format available on the Internet. The documented JCAMP-DX format has some parameters and the real half of the data points of transformed spectra in an ASCII format (example 1 below). We can find no examples of this format for time domain data (fids) or for real/imaginary pair data pairs. The Bruker data format claims to be JCAMP-DX in its header, but the format used by Bruker is not documented anywhere in the JCAMP references or on the Internet

URLs we have found relating to JCAMP-DX. Bruker's data is not in ASCII format, but is in a binary file called fid (1D) or ser (2D) and currently is in a directory structure as opposed to a single file.

The only example of a JCAMP-DX file with real/imaginary data pairs that we were able to obtain was from Glenn Sullivan at Chemagnetics (now Varian) (example 2 below). It uses something called NTUPLES. This file format fails to be recognized by the Internet JCAMP viewers. We cannot find the documentation for NTUPLES, so if anyone has some information please email the information to **support@acornnmr.com**. We have not yet found examples of NMR time domain data in JCAMP-DX format. Repeated requests for clarification of the NMR dialect for JCAMP-DX data format to those involved in this "standard" definition have not yet yield any additional information.

So we have a "standard" data format. This format seems to be actively supported and used for IR and Mass Spectrometry. There has been little attention paid to NMR data by the JCAMP development people. The result is a poorly defined "standard" not yet usable by NMR spectroscopists. Perhaps it is time for the NMR community to take an active role in further defining this standard.

Some of the open issues are:

- What is the format for real/imaginary pair data?
- What is the format for time domain data?
- Listing the data points in ASCII integer format loses precision. Should the data points be in floating point (single or double precision)?
- The entire data file is ASCII. This is readable by every computer platform, but wastes space. At this time, it seems that cross platform support is more important than space. Space, however, is already a problem for 3D data, so some prevision needs to be in the data format definition to support binary data points. This should probably be floating point with some information about byte order.

There are probably other open issues which need to be discovered, documented and addressed. Therefore, we actively request people to provide any information they have about JCAMP-DX NMR data format. They are also requested to provide their ideas about the proper solutions to the open issues.

So the answer to the question "Is JCAMP-DX the coming standard for NMR data?" is "not yet". It is mostly used to display NMR spectra across networks after all processing is complete. Unless considerably more attention is paid in the JCAMP definition to NMR data, JCAMP-DX will probably not become the common standard NMR data format for which many NMR spectroscopists are wishing. We will see if this editorial stirs up any activity in either the JCAMP-DX group or by NMR spectroscopists.

Send comments and suggestions to: **support@acornnmr.com** or to the NMR Newsletter.

Sincerely,

Woodrow W. Conover

Virginia W. Miner

JCAMP-DX Example 1 (frequency domain, real data points only)

```
##TITLE= pyridine (in CDCl3)
##JCAMP-DX= 5 $$Exported Data File
##DATA TYPE= NMR SPECTRUM
##DATA CLASS= XYDATA
##ORIGIN= XYZ University
##OWNER= public domain
##SPECTROMETER/DATA SYSTEM= QE-300
##INSTRUMENTAL PARAMETERS= 1H
##.OBSERVE FREQUENCY= 300.1495
##.OBSERVE NUCLEUS= ^1H
##NPOINTS= 3088
##XUNITS= PPM
##YUNITS= ARBITRARY UNITS
##FIRSTX= 10.001
##LASTX= -.042
##XFACTOR= 1.0
##YFACTOR= 11.92E-8
##FIRSTY= 1.081
##MAXY= 144.45
##MINY= .09
##XYDATA= (X++(Y..Y))
10.001 9068084 8665431 8849980 8766095 9042918 8858369
9.982 8438939 8539602 8665431 8472493 8489270 8975810
9.9624 8413772 8824815 8413772 8766095 8455715 8908700
9.9429 8371829 9319743 8657043 8824815 8682208 8866758
... Lots of data points
.0064409 8145337 7834959 7893680 7172259 5872025 754974
-.01308 36624660 17255366 13514046 12968786 12213811 11215568
-.032602 11265899 10661920 10770971 10309599
##END=
```

JCAMP-DX Example 2 (with real/imaginary data pairs)

```
##TITLE= sbc_1pulse
##JCAMP-DX= 5.00 $$Spinsight convert
##DATA TYPE= NMR SPECTRUM
##DATA CLASS= NTUPLES
##ORIGIN= CMX INFINITY
##OWNER= Otsuka Electronics / Chemagnetics
##.OBSERVE FREQUENCY=300.068954
##.OBSERVE NUCLEUS=
##.DELAY= (0.000, 0.000)
##.ACQUISITION MODE= SIMULTANEOUS
##SPECTROMETER/DATA SYSTEM= CMX INFINITY
##NTUPLES= NMR SPECTRUM
##VAR_NAME= TIME, SPECTRUM/REAL, SPECTRUM/IMAG, PAGE NUMBER
##SYMBOL= X, R, I, N
##VAR_TYPE= INDEPENDENT, DEPENDENT, DEPENDENT, PAGE
##VAR_FORM= AFFN, ASDF, ASDF, AFFN
##VAR_DIM= 32, 32, 32, 2
##UNITS= PPM ARBITRARY UNITS, ARBITRARY UNITS,
##FIRST= 0.000, -1.3120, 0.4640, 1
##LAST= 0.000, -0.8962, 0.2782, 2
##MIN= 0.000, -33.6640, -25.1115, 1
##MAX= 0.000, 3.6967, 156.8160, 2
##FACTOR= 1.000000e+00, 1.567602e-08, 7.302314e-08, 1
##PAGE= N=1
##DATA TABLE= (X++(R..R)), XYDATA $$ Real pnts
0.0000-83694704-29555432-44119632-51479888-23924518-35100136
0.0000-11789181-33827132-9186003 2752932 104837400 66971412
0.0000 122453656-14232522 235815616 80699632-2147483648 89761352
0.0000-549367744 93478304-198580896-122850768-134741344-116147688
0.0000-103087376-97841808-74758216-96842000-55089620-70555072
0.0000-64789076-57167340
##PAGE= N=2
##DATA TABLE= (X++(I..I)), XYDATA $$ Imag pnts
0.0000 6354150 9275533 14858612 13730503 18151878 14308278
0.0000 18808544 16455404 20157994 27840268 21846360 24249316
0.0000 11798834 94674656 49889416 405111840 2147483647-343884512
0.0000-50997528-51053832-31736612-9120279-7301009-2871321
0.0000 3067521-1675790 269603 4245550 5291639-1762439
0.0000 8717814 3809665
##END NTUPLES= NMR FID
##END
```

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Central Research

NMR Spectroscopy

December 18, 1997 (received 12/19/97)

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

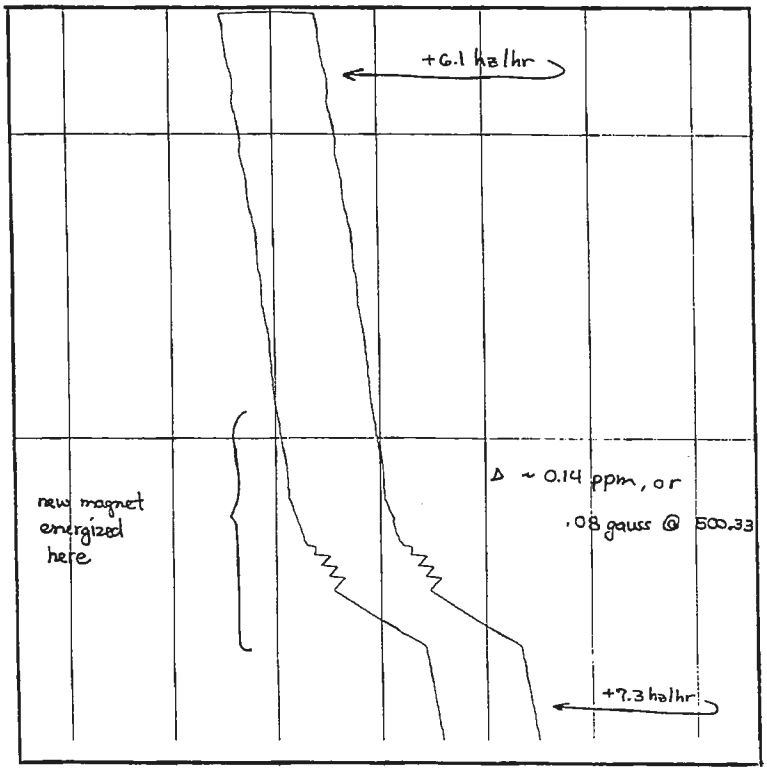
Measuring the Superconducting Magnetic Field Shielding Effect

Dear Dr. Shapiro,

Our colleagues in Analytical R&D bought a new Bruker DRX-500 with an actively-shielded Magnex magnet earlier in the year, and it was time for the magnet to be energized. What to do? We had designed the site so that the magnet immediately adjacent (which was an identical actively-shielded Magnex 500 MHz magnet) was 11' distant so that the 5 gauss circles would just touch, but we also knew that there would be a significant field perturbation one our magnet when the new magnet was energized. Colleagues at Magnex advised that the stray field 11' distant (about 3.4 meters) from magnet center would be 0.76 gauss (thanks to Geoff Seward for providing this information), which should yield a frequency change of

$$0.76\text{gauss} / (500.35\ \text{MHz} \times (11.744\ \text{tesla}/500.00\ \text{MHz}) \times 10{,}000\ \text{gauss/Tesla}) \times 10^6 = 6.47\ \text{ppm}$$

if we were observing protons on our 500.35 MHz system. So, that's exactly what we did. During the time that the new magnet was energized, we put our standard 2mM sucrose in 90% H_2O/10% D_2O sample in our spectrometer, turned the lock subsystem off, and watched the frequency of the water signal change at one minute intervals (single scan per minute):



The observed frequency change for the water signal over the two hours that the magnet was ramped from no current to ~145 amps (full field) was 0.14 ppm, which represents about 2% of the full effect of 6.47 ppm if there were no superconducting shielding present.

Walt

Sincerely,

Walter Massefski, Jr.
860-441-5962
860-441-0207 (FAX)
email: wwm@pfizer.com

Pharmacia&Upjohn

Gary E. Martin, Ph.D.
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December 4, 1997 (received 12/8/97)

Bernard L. Shapiro, Ph.D.
Editor, The NMR Newsletter
966 Elsinore Court
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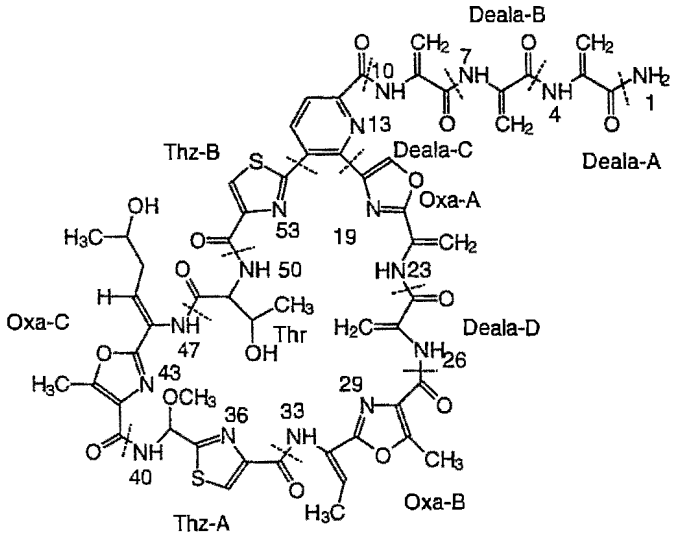
Long-Range ^{1}H-^{15}N NMR at Natural Abundance-- Weak Correlations to the Oxazole & Thiazole Residues of Sulfomycin-I

Dear Barry,

We currently in the process of doing some reconfiguring of the NMR instruments used by the Rapid Structure Characterization Group here in Kalamazoo. We've decomissioned one of our Bruker AMX-400's and are installing a three channel Varian Inova-400 console on our other 400 MHz instrument. We've also nearly completed the installation of a Varian Inova-600 in a newly renovated laboratory.

Aside from the changes in equipment, we've been continuing with some further investigations of long-range ^{1}H-^{15}N heteronuclear correlation at natural abundance. One of the molecules we have recently been studying is the thiopeptide antibiotic sulfomycin-I, whose structure is shown below.[1] As a fermentation product, sample availability isn't a problem -- convenient when acquiring even inverse-detected ^{15}N spectra of a molecule of this size at natural abundance. Sulfomycin-I is related to the more intensively investigated thiopeptide nosiheptide.[2] In a recent communication by Gasmi, Massiot, and Nuzillard,[3] which reported a series of new 2D NMR experiments, it was suggested, based on the much earlier work of Chen, von Philipsborn, and Nagarajan[4], that long-range correlation responses to the non-protonated heteroaromatic nitrogen atoms of nosiheptide's thiazole rings would be difficult to observe since the couling constant was expected to be of approximately the same magnitude as the proton linewidth, ~2 Hz. Indeed, no long-range coupling data to the thiazole nitrogens of nosiheptide were reported.[3]



Using the assigned ^{15}N resonances of nosiheptide as a chemical shift model, the thiazole nitrogen chemical shifts of sulfomycin-I were expected to be in the range of ~305-325 ppm.[2] Unfortunately, none of the thiopeptides with ^{15}N assignment data available contain oxazole-derived residues. Hence there wasn't an "accurate" chemical shift model available for the Oxa-A residue. The chemical shift of oxazole, however, has been reported at 255 ppm in DMSO.[4]

Based on the premise that the long-range couplings to the Oxa-A, Thz-A, and Thz-B residues would be relatively small, a 3 Hz optimized GHNMQC[5] spectrum of sulfomycin-I was acquired using a sample of ~100 mg (~8 mmoles) of sulfomycin-I dissolved in 550 µl of DMSO.

Pharmacia & Upjohn
Kalamazoo, MI 49001-0199
USA

Telephone (616) 833-4000

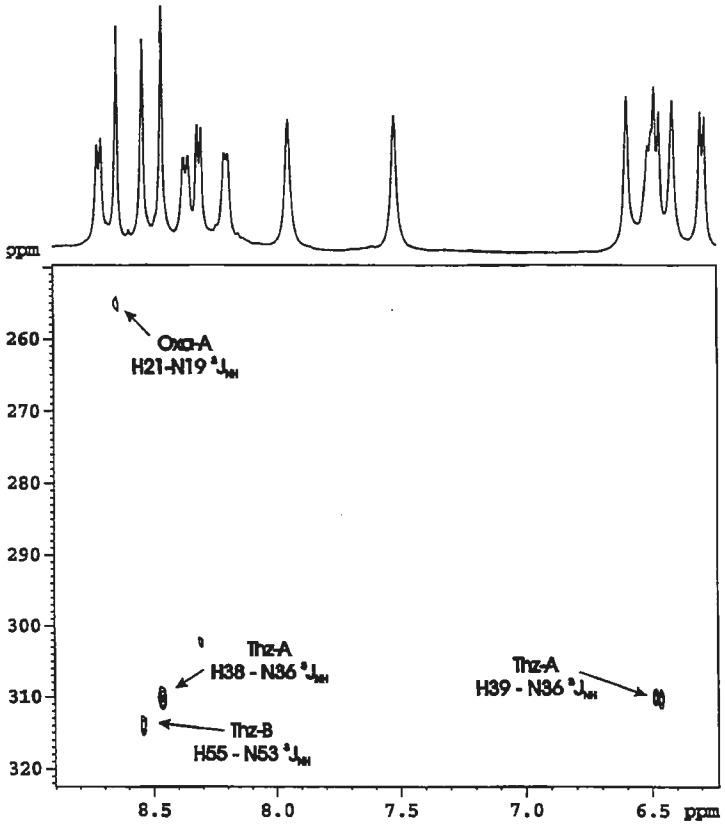


Figure 1. Segement of the GHNMQC[5] spectrum of sulfomycin I acquired at 500 MHz and optimized for 167 msec (3 Hz). Long-range correlation responses are observed for both of the thiazole residues from their respective H3 protons, and for the oxazole-A residue whose annular nitrogen resonates somewhat upfield of those for the thiazoles. Data were acquired in three days over a weekend. (The authors would like to thank K.A Farley for acquiring these data.)

The downfield region of the 3 Hz GHNMQC spectrum of sulfomycin-I from 250-320 ppm acquired using a Bruker AMX-500 and equipped with a 5 mm Bruker gradient triple resonance inverse-detection probe is shown in Figure 1. The Thz-A annular nitrogen has two long-range coupling pathways available. Responses for both were observed. H38 at the 3-position of the thiazole ring and resonating at 8.45 ppm was long-range coupled to a nitrogen, N36, resonating at 310.6 ppm. The proton doublet resonating at 6.45 ppm, which has been assigned as the H39 proton of the Thz-A residue was also long-range coupled to N36. The H55 proton at the 3-position of the Thz-B residue, resonating at 8.55 ppm, was long-range coupled to a nitrogen resonating at 314.3 ppm that may be assigned as N53. Finally, H21, at the 3-position of the oxazole ring was long-range coupled to the N19 oxazole annular nitrogen. The resonance was observed upfield of the thiazole resonances at 255.8 ppm.

Long-range ^1H-^{15}N heteronuclear shift correlation at natural abundance is a viable technique that allows investigators convenient access to nitrogens contained in the skeletons of biologically interesting molecules as a structural probe. Long-range correlation experiments are readily conducted on smaller samples (~25 mg, ~2 mmole in 150 µl of DMSO using a 3 mm probe) even at 400 MHz when less difficult to observe coupling pathways are being studied. A typical example would be the correlations from the exomethylene protons of the dehydroanalnine residues to their respective nitrogens. In the present case, however, the weak long-range couplings to the annular nitrogens of the oxazole and thiazole residues necessitated the much higher sample concentrations.

1.) GD Fate, CP Bonner, SH Grode, and TJ Gilbertson, *J. Am. Chem. Soc.*, **118**, 11363 (1996).
2.) U Mocek, AR Knaggs, T Tsuchiya, T Nguyen, JM Beale and HG Floss, *J. Am. Chem. Soc.*, **115**, 7557 (1993).
3.) G Gasmi, G Massiot, and JM Nuzillard, *Magn. Reson. Chem.*, **34**, 185 (1996).
4.) BC Chen, W von Philipsorn, and K Nagarajan, *Helv. Chim. Acta*, **66**, 1537 (1983).
5.) KA Farley, GS Walker and GE Martin, *Magn. Reson. Chem.* **35**, 671 (1997).

Regards,

Gary E. Martin

Kenneth D. Visscher

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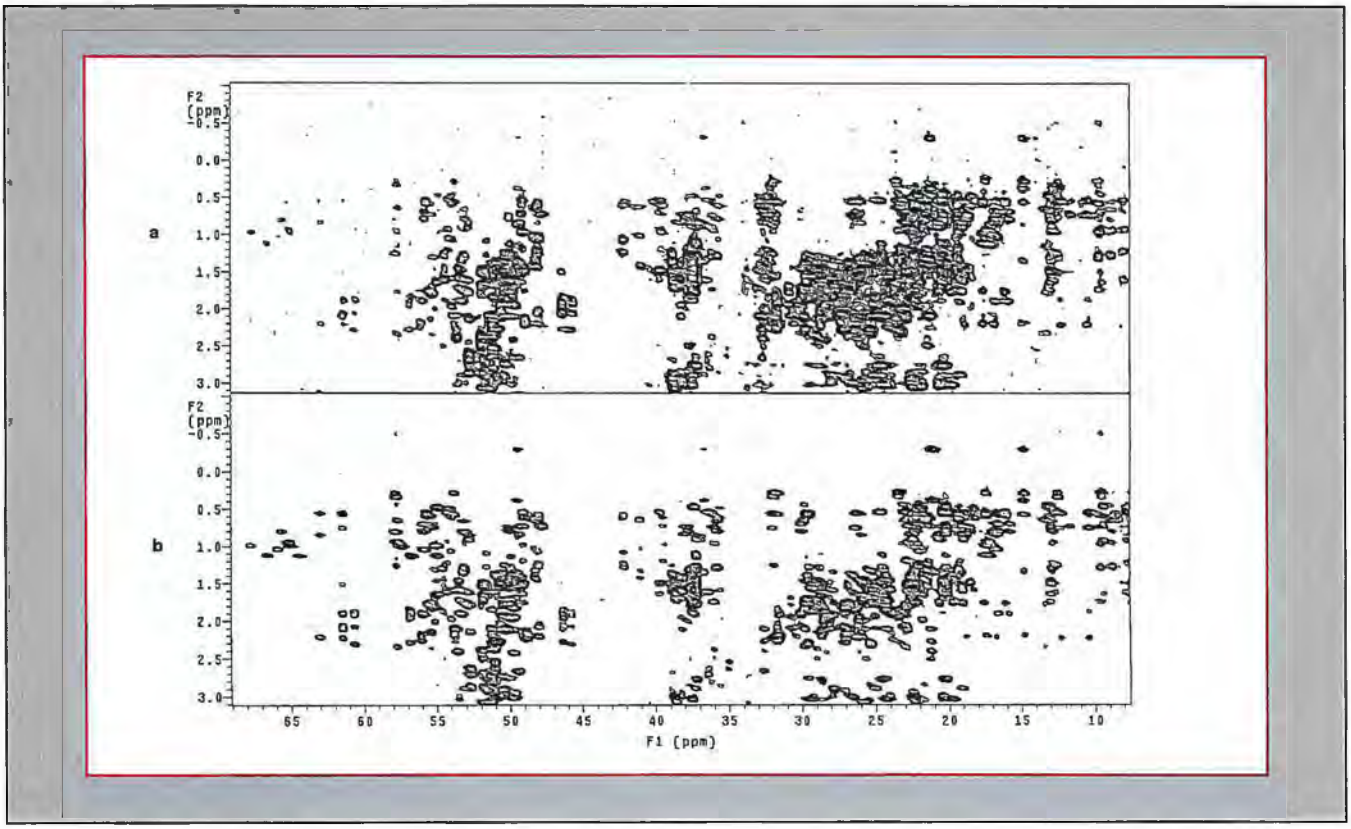

HCCH-TOCSY[1] spectra of 1 μM [^{13}C,^{15}N]-labeled ubiquitin in 90% H_2O/10% D_2O. Data acquired using a 5 mm $^1H\{^{13}C/^{15}N\}$ X,Y,Z PFG Triple Resonance probe, a 600 MHz UNITY*INOVA* spectrometer, and a.) INEPT ^{13}C-^1H polarization transfer and b.) Hartmann-Hahn ^{13}C-^1H polarization transfer (γB_2: 7 kHz ^{13}C and ^1H). Both sets of spectra employed a ^{13}C-^{13}C DIPSI spin lock of 15 ms at 40 watts. Z-gradient strengths of up to 70 gauss/cm were utilized. No presaturation or post-acquisition solvent suppression was used to remove the residual water.

High Power ^{13}C-^{13}C and ^{13}C-^1H Spin Lock and Gradient-Based Water Suppression

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[1]Kay, L.E., Xu, G-Y., Singer, A.U., Muhandiram, D.R., and Forman-Kay, J. D., *J. Magn. Reson., Series B*, 101, 333-337 (1993).

World Headquarters, Varian NMR Instruments, Building 4, 3120 Hansen Way, Palo Alto, California, USA 94304-1030
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B. L. Shapiro, Publisher
The NMR Newsletter

Lilly Corporate Center
Indianapolis, Indiana 46285
(317) 276-2000

December 1, 1997
(received 12/16/97)

Dear Barry,

Subject: **SAR by NOE?**

The field of drug design has undergone significant change over the last few years with the implementation of combinatorial chemistry and high throughput screening technology. Recently Steve Fesik and his group at Abbott Labs have introduced their own breakthrough in drug design methodology: "SAR by NMR". This method looks for ligands that bind simultaneously to a target protein. Ligands are then optimized using standard SAR techniques; then are followed by NMR studies to understand the structure of the co-complexes. Using the structural information, linked compounds are proposed. These methods have produced nanomolar antagonists for FK-506 binding protein [Shuker et al., Science 274, 1531 (1996)] and stromelysin [Hajduk et al., JACS 119, 5818 (1997)]. We have attempted to gain some experience with the method by studying the biotin-streptavidin complex. As you may know, this complex represents one of the highest known affinities between a protein and a small molecule, registering in the upper femtomolar (10^{-14}) range. While studying the interactions of biotin fragments with streptavidin, transfer NOEs were detected between the ligands catalyzed by the protein. This will potentially provide another route into the complex field of drug design.

As an initial test case, an analog of biotin, D-desthiobiotin, was used. Despite its lack of a sulfur atom, the analog still has an appreciable affinity with streptavidin (20 pM). Moreover, a simple methylene disconnect of the target provides two readily available fragments: methylimidazolidone (MI) and valeric acid (VA). By watching the high and low field signals of streptavidin, cooperative binding constants were determined to be ~1 mM. Using saturating concentrations of MI and VA on a streptavidin solution, a NOESY spectrum revealed numerous inter-ligand NOEs (Figure 1), the largest of which is between the methyl of VA and the methylene proton syn to the methyl group on MI. The presence of these transfer NOEs requires that both ligands bind simultaneously to proximal sites on the protein and suggests that the fragments are binding as the parent molecule does. Also, the NOE correctly identifies the ligand proton that should be replaced with the methylene linker in order to construct the target, desthiobiotin. The control spectrum, run without streptavidin, shows no such crosspeaks and proves that the NOEs observed are describing the streptavidin co-ligand complex. This transfer NOE phenomenon is reasonably robust, showing up as well between numerous analogs of the fragments MI and VA.

Another ligand, important for its use in measuring streptavidin's activity is 2-(p-hydroxyphenylazo)benzoic acid. Fragmenting this reagent can give phenol and benzoic acid. Protein chemical shifts indicated that both aromatic species bind to streptavidin, though likely in a competitive manner. NOESY spectra gave no inter-ligand NOEs consistent with the competitive binding. On a whim, VA was added to the sample. Protein shifts gave no indication that at 50 mM the acid was even binding. The NOESY spectrum (not shown), however, contained NOEs between the VA methyl and the meta positions of both aromatic ligands. Here the NOE is detecting VA binding before saturation has likely occurred. The NOEs from VA to both aromatic ligands imply that phenol and benzoic acid are competing for the same site, and both would benefit from the addition of a VA appendage to its meta carbon. The value of detecting NOEs from mixtures of ligands cannot be overstated, particularly with the need to screen large libraries of compounds. At equilibrium the protein is sampling the available ligands, and those with the highest affinities will have the longest residence times and hence the strongest NOEs. The higher NOE intensity to the phenol suggests that it is preferred over benzoic acid. The only linked species that was commercially available was 5-(3-hydroxyphenyl)valeric acid (direct link between VA and meta phenol position) which has a measured affinity of 160 μM with streptavidin. While this linked species is still a weak ligand, it is orders of magnitude stronger than the affinities of the fragments that make it up. Remember that no SAR has been performed on either the fragments or the linkage, thus the full potential of this ligand series has yet to be achieved.

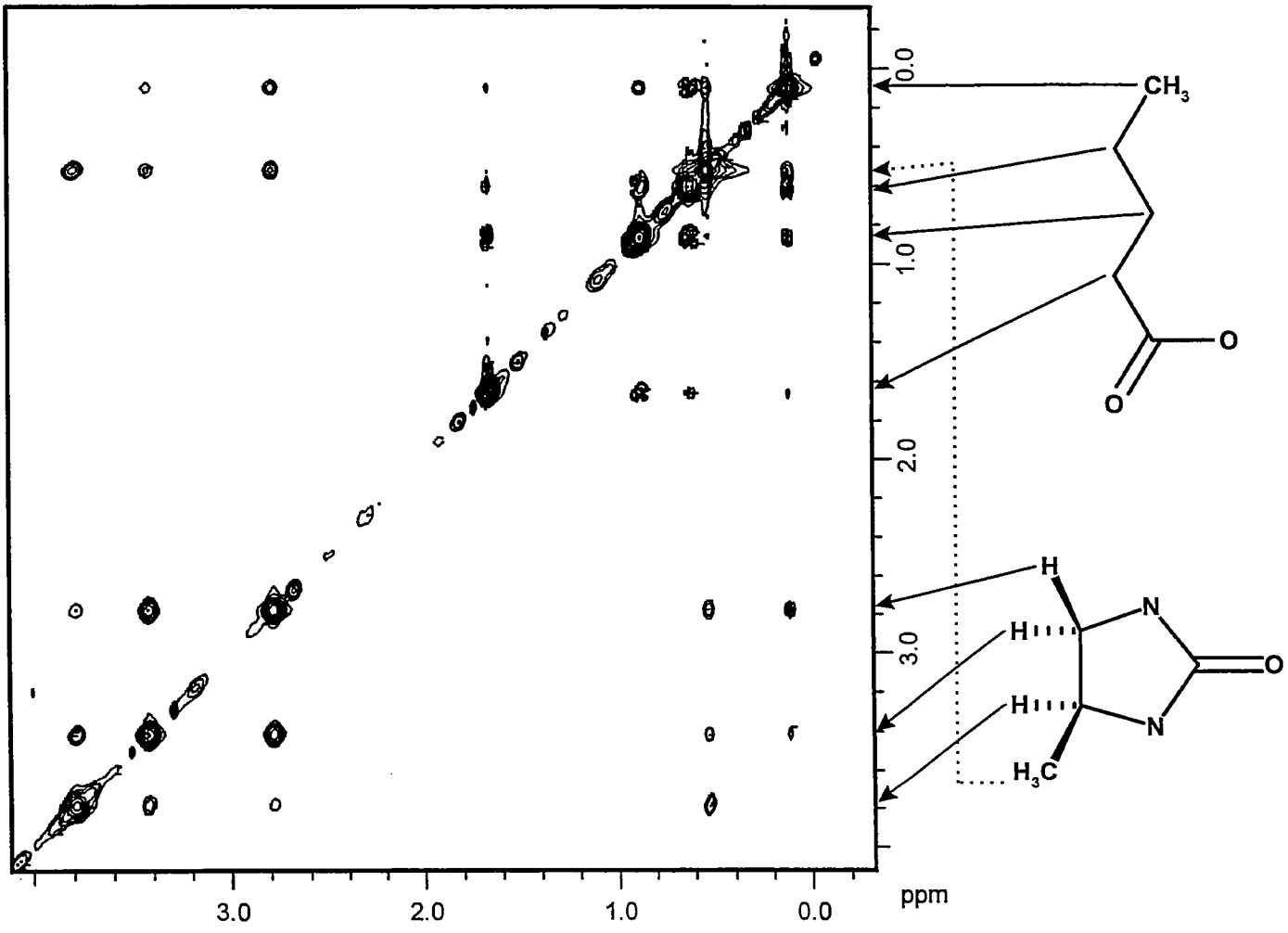
The observation of this inter-ligand transfer NOE has several implications for the "SAR by NMR" methodology. Since it depends on observing interactions between ligands and does not directly involve the protein, the requirements of protein isotopic enrichment, of the collecting and assigning numerous multidimensional NMR spectra, and of calculating 3D structures, can be eliminated, representing a great time and labor savings. More

importantly the molecular weight barrier that plagues solution state NMR is less problematic for this transfer NOE experiment. For this case of streptavidin, the tetrameric protein has an overall molecular weight above 60 kd. That weight would create severe limitations on collecting assignable NMR spectra and would preclude a standard implementation of "SAR by NMR". However, the transfer NOE is able to detect these vital interactions between ligands that ultimately guide the linking chemistry. This easing of the molecular weight barrier suggests that perhaps NOEs could be detectable between ligands involved with much larger proteins, such as membrane bound receptors. This would open up an important new classes of drug targets to solution-state NMR studies.

Because of the ramifications of this enhancement to "SAR by NMR", I am seeking a Ph. D. scientist to develop the potential of the technology to drug design. Interested applicants for this postdoctoral position should have background in solution state NMR, either protein or small molecule, and experience in synthetic organic chemistry. Training in protein cloning and purification is also an advantage. Interested parties should submit their CVs and a list of references to: **Manager, Chemistry-Research Technologies & Proteins, Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285-1533**. Be sure to indicate the position you are applying for.

Allen Kline Gianpaolo Bravi Jim Wikel

Figure 1: NOESY spectrum of MI & VA in the presence of 0.24 mM streptavidin. Arrows indicate assignments.



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A small protein bound to a big one

Wednesday, December 10, 1997
(received 12/15/97)

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

Dear Barry:

The *Bacillus subtilis* bacteriophage PBS1 and 2 exhibit a unique genetic system that naturally contains uracil in place of thymine in a double-stranded DNA genome. Stable incorporation of uracil residues into phage DNA is achieved by substituting dUTP for dTTP as a precursor for DNA synthesis and by inactivating the host uracil-mediated base excision DNA repair pathway. In order to block uracil-DNA repair and protect the uracil-containing DNA from degradation, an early phage gene is expressed that inhibits B. subtilis uracil-DNA glycosylase.

The PBS2 ugi gene encodes a small (9,474 dalton), monomeric, heat stable protein of 84 amino acids that inactivates uracil-DNA glycosylases (Ung) from diverse biological sources. Stopped-flow kinetic studies of the Ugi interaction with *E. coli* Ung indicate that complex formation is accomplished through a two step binding reaction. In the initial step, the association between free Ugi and Ung is characterized by a rapid pre-equilibrium reaction with a dissociation constant Kd=1.3 M. The final step leading to irreversible complex formation is characterized by the rate constant of 195 s-1. Thus, Ung-Ugi complex formation occurs initially through a "docking" interaction that facilitates optimal alignment between the two proteins. If correct alignment between Ung and Ugi does not occur, a reversible association will transpire. Conversely, if proper alignment is achieved then a "locked" complex is established.

Our previous studies have shown that wild type Ugi undergoes a considerable conformational change upon binding to Ung. The structure of the wild type complex was determined by the use of conventional methods as well as with the incorporation of extensive heteronuclear chemical shift information. To determine which part is involved in the locking step we have begun examination of mutant forms of Ugi bound to wild type Ung with a focus on the proteins which bind, but do not form a locked complex. The spectra that follow show that high quality spectra can be obtained on the mutant complexes that are formed with only the Ugi isotopically labeled. The molecular weight of the complex is about 34,000. The spectra are of quite high quality given the molecular weight of the system. The results indicate that the structural changes that the wild type undergoes upon binding are similar to, but not identical to those the mutant E28L form undergoes upon binding.

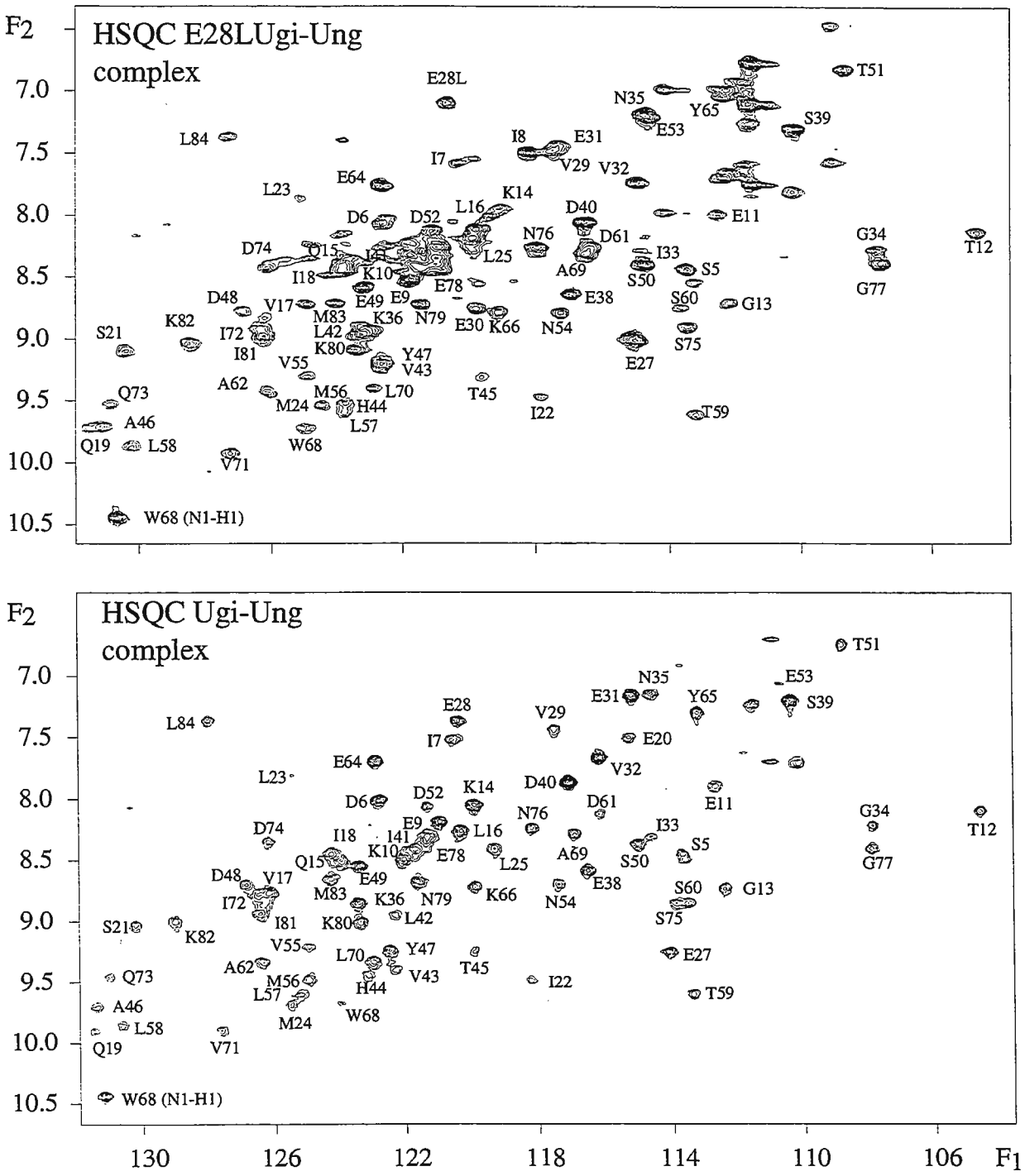
Sincerely,

Richard Beger

Richard Beger

Philip Bolton

S. Balasubramanian, R. W. Beger, S. M. Bennett, D. W. Mosbaugh, P. H. Bolton (1995) "Secondary structure of uracil glycosylase inhibitor protein", *J. Biol. Chem.* **270**, 296.
S. Balasubramanian, R. W. Beger, S. M. Bennett, D. W. Mosbaugh, P. H. Bolton (1995) "Tertiary structure of uracil glycosylase inhibitor protein", *J. Biol. Chem.* **270**, 16840.
R. D. Beger, P. H. Bolton (1997) "Protein ϕ and ψ dihedral restraints determined from multidimensional hypersurface correlations of backbone chemical shifts and their use in the determination of protein tertiary structures", *J. Biomolecular NMR* **10**, 129.
A. J. Lundquist. R. D. Beger, S. E. Bennett, P. H. Bolton, D. W. Mosbaugh (1997) "Site-directed mutagenesis and characterization of uracil-DNA glycosylase inhibitor protein: Role of specific carboxylic amino acids in complex formation with Escherichia coli uracil-DNA glycosylase", *J. Biol. Chem.***272**,21408.



UNIVERSITY OF ILLINOIS
AT CHICAGO

College of Medicine at Urbana-Champaign

Department of Medical Information Sciences
BIOMEDICAL MAGNETIC RESONANCE LABORATORY (MC-008)
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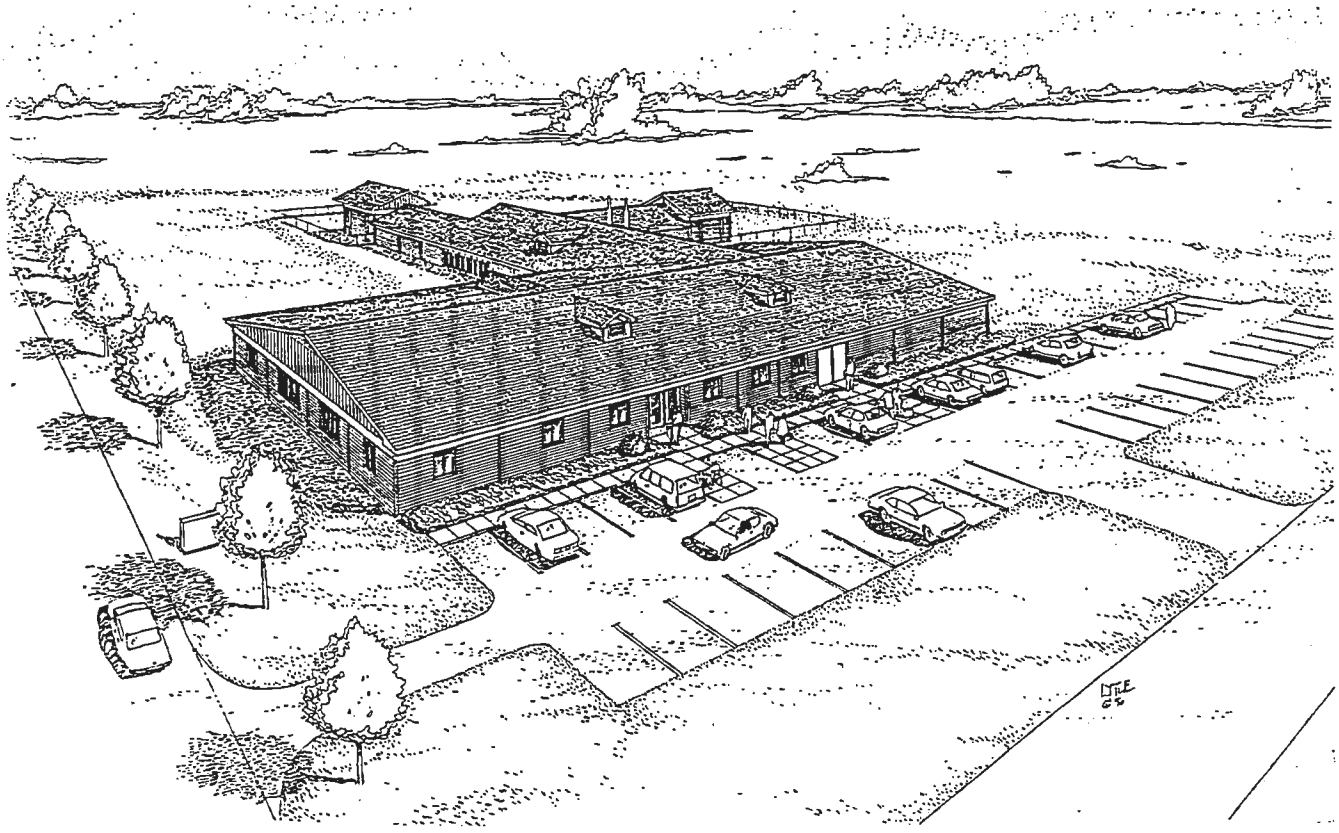
December 18, 1997
(received 12/19/97)

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

Dear Barry:

The Chaos and the Ivy

Your importunities come at a dreadful time. We have just moved our offices, labs and magnets from leased and other temporary facilities and are consolidating many of our activities in a new building on the Southern Marches of the campus. Some of our machines and people are down, and controlled chaos is our current mode of operation through at least this holiday season.



New Biomedical Magnetic Resonance Laboratory facility. It's not located on virgin prairie - the renderer has left out the cows, corn and outbuildings nearby. Note the magnet pods extending south and east, surrounded by fences enclosing the 5 gauss fringe fields.

Urbana • Phone (217) 244-0600 • Fax (217) 244-1330
E-mail: bmrl@bmrl.med.uiuc.edu • URL http://bmrl.med.uiuc.edu:8080/

Let me take this opportunity to summarize our programs and facilities, with a view to stimulating collaborations and applications for postdoctoral and research assistant positions.

The new 15,500 ft^2 building provides space for our 2.0T/1.05m magnet-based system. It is based on an SMIS console, and is being assembled from a variety of previously-used components. The B_o field looks pretty good, the gradients work, and images of phantoms have been produced. The system will be used to allow us to apply to humans techniques that we have hitherto been able to use only on vermin. These include reduced-encoding methods and real-time functional brain imaging and interactive imaging over the World Wide Web. We are also arranging some clinical collaborations, locally and elsewhere.

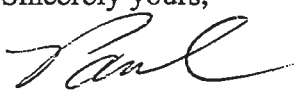
Our new facility also houses a Varian-SISCO-Oxford 4.7T/33cm horizontal bore imaging spectrometer and an SMIS-Magnex 4.0T/31cm horizontal bore system, both with probes for microscopy, and interfaced for interactive "real-time" operation over the World Wide Web. They are used for technique development and animal studies, as well as for microscopy, and in collaborations, with the Agricultural Engineering Department for studies of grain and of food processing and with the Mechanical Engineering Department for studies of flow.

Elsewhere on campus, in the laboratories of Prof. M. Joan Dawson, are a Tecmag/GN300 multinuclear wide-bore spectrometer and a Varian 500 MHz narrow-bore instrument with microimaging probes. Both are used for molecular and isolated tissue studies, and the 500 MHz machine in particular for microscopic diffusion imaging of polymers and nerves.

We also have an IBM Research Field-Cycling Relaxometer, used primarily in contrast agent development and now sited in the Beckman Institute for Advanced Science and Technology. ESR studies are carried out in collaboration with the Illinois ESR Research Center on this campus.

Graduate students, undergraduate students and postdocs at the BMRL come from many science and engineering departments and programs, and from the very large and diverse M.D./Ph.D. program maintained by our branch of the College of Medicine. Varied and exciting opportunities are available here, in new and expanded facilities. Drop in to see us sometime, should a flight be diverted to our airport, before our new building is covered by ivy.

Sincerely yours,



Paul C. Lauterbur
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Head, Department of Medical Information Sciences
Research Professor, Department of Radiology and
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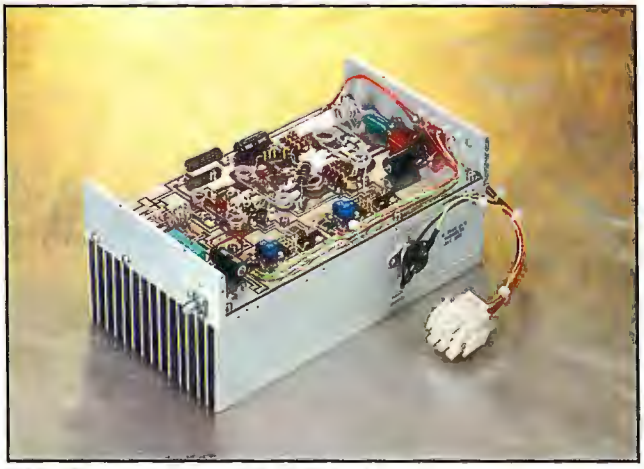

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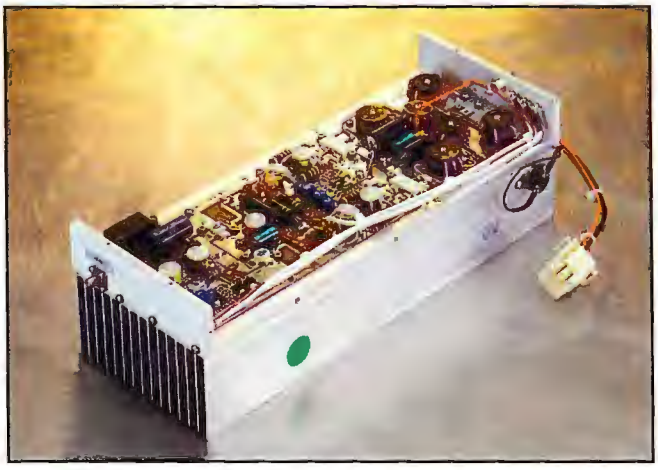

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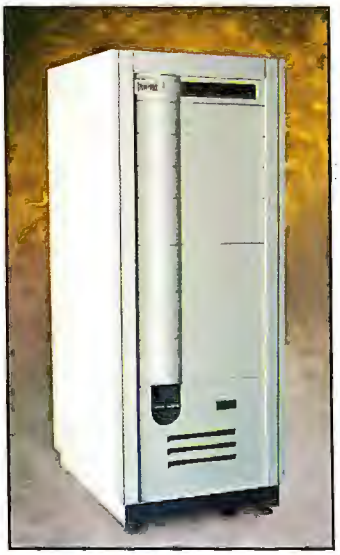

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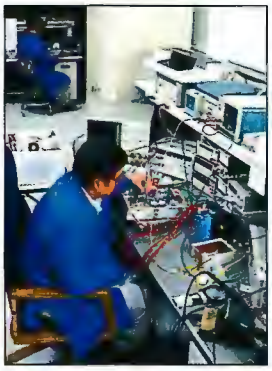
AMT's scientific products are used extensively in Nuclear Magnetic Resonance (NMR) systems. These amplifiers cover the frequency ranges of 6 MHz to 950 MHz, with power levels as high as 2.0 kW peak power at 10% duty cycle.

AMT's medical products are employed in Magnetic Resonance Imaging (MRI) systems. These amplifiers cover the frequency ranges of 10 MHz to 200 MHz with power levels as high as 8.0 kW peak power at 10% duty cycle.

All amplifiers have dual mode capability and can be operated in either a pulsed or CW mode. Scientific and Medical customers include both OEM system manufacturers and end users.

SCIENTIFIC & MEDICAL PRODUCTS



COMPANY

AMT designs, develops and manufactures custom radio frequency (RF) and microwave power amplifiers for the wireless, scientific/medical and application specific industries. The company has been in business since 1984 and currently has over 60 employees, including 20 experienced engineers.

AMT has a worldwide reputation as a leading supplier of high power, solid state power amplifier products that operate at frequencies between 1 MHz and 3 GHz and provide RF power from several watts to several kilowatts. Its products are noted for their exceptional performance, highest quality and superior reliability.

The company's products are sold to numerous major corporations, universities and research centers throughout the world.

FACILITIES

AMT is located in Anaheim, California and occupies a 25,000 square foot facility allocated to engineering, manufacturing, quality assurance, marketing/sales, administration and finance.

Engineering areas include an R & D laboratory, a tool and die shop, mechanical design and drafting areas, an environmental testing laboratory and document control. The R & D laboratory is equipped with all of the latest design and testing equipment including intermodulation distortion simulators, network analyzers, spectrum analyzers, signal generators, noise figure meters and infrared (IR) scanners. The environmental testing laboratory includes equipment to simulate shock, vibration and thermal environments.

Manufacturing areas include a controlled access stock room, a 10,000 square foot assembly area and a production test area employing automatic testing. Also included is an environmental laboratory used for environmental stress screening of production products.

PRODUCTS

AMT's products vary in complexity from single modules, to rack-mounted amplifiers, to complete transmitter systems. The rack-mounted amplifiers and complete transmitter systems typically include detection/protection circuitry, built-in power supplies, front panel metering and digital and/or analog interface controls. Both forced air and/or water cooling are used, depending on the customer's requirements.

AMT's products feature highly reliable technical solutions designed for producibility and reliability. Producibility is enhanced through the use of surface mount components and circuit designs that eliminate the need for excessive alignment during the production cycle. High reliability is accomplished through the implementation of conservative thermal and RF circuit design and sophisticated self-protection schemes. Reliability is further enhanced during the design phase by employing detailed environmental testing.

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

December 19, 1997

AutoTest : An Automated Instrument Tracking Protocol

Dear Barry,

As requirements for GLP (Good Laboratory Practices) become increasingly relevant to NMR, many users are put into a position of defining or obtaining testing protocols that verify instrument compliance. This is increasingly important in those labs serving the health-care industry. The trend can only be one toward more verification and documentation. The ubiquitous "ISO9000" label carries the message of rigorous testing and record keeping. The NMR spectroscopist can fall back on the "classical" purchase specs for the spectrometer, but these are really insufficient to verify instrument performance in today's wide variety of experiments.

Apart from satisfying bureaucrats, the NMR spectroscopist has other reasons for testing the spectrometer. Periodic testing can reveal slow degradation, or performance characteristics that don't get measured by the traditional specifications. In a multi-user environment, periodic testing can serve as testimony that the "instrument is OK but your sample is bad", when the "customer" challenges the hardware because the spectrum does not give the desired result. True hardware problems can be recognized early and dealt with before critical experiments suffer.

While many labs have their own set of "standards", it is difficult to compare results or communicate with the NMR vendor because there is no shared common base of experience such as is with the samples and tests used for specifications. D2O doped with a paramagnetic relaxation agent and a small amount of H2O has been used widely because of its ease of preparation and short T1 and T1 relaxation times (permitting rapid pulsing and large enough linewidth to make shimming uncritical). Many tests can be performed on this sample and we have used it in our factory for over a decade for stability tests.

Just as important as a proper sample and test is the ease with which it is done. If it takes a skilled spectroscopist a full day of "at the console" operation in setting up experiments, doing them, plotting the data and doing statistical analyses, it won't get done! Therefore, such a test has to be fully automated. In addition, it should be short enough to not affect the productivity of the instrument for "real" samples. In my opinion, an hour or so would be short enough to permit even weekly testing without using too much time, particularly if the operator can be doing something else.

A few years ago I put together an automated test (AutoTest) for people evaluating spectrometers in demonstrations. This has been augmented and refined since then and a wider class of "potential beneficiaries" has expressed interest in using it. Service people have used it for checking out instruments in the field. Recently, the factory has adopted the test for installation testing and has consequently substantially increased the number of standard specifications used for new system installations. A number of customers have used it to track their instrument's performance. This last usage will be the predominant use in the future.

AutoTest is designed as an instrument performance tracking tool for any UNITY^{plus} or INOVA spectrometer at any field strength. Tests have been designed to test only one aspect of performance and one piece of hardware at a time, as much as possible. Historical trends in performance are easily displayed. Plots include spectra, statistical results, regression analysis plots and explanatory text. If parameter sets are requested, separate sheets are printed including full parameter sets, pulse sequence diagram and text files indicating the purpose and value of the test. .

AutoTest uses a sample of 1% H2O in D2O, 0.1% 13CH3OH and enough GdCl3 to lower the water T1 to 50-75 msec (typically 0.3 mg/ml). The following operations are performed: An initial experiment at large SW permits determination of the proper frequency for H2O. Then, calibrations are made of parameters such as 1H pw90 using channels 1 and 2, and the 13C pw90 (via indirect detection using the methanol 13C). Printouts of pw90 and B1 for all attenuator values are made for both 1H and 13C. High and low band amplifiers are tested for compression at the specified "hard pulse" power level by redetermining the pw90's at a power levels lower by 12 dB. Probe RF homogeneity is measured by running a series of single-pulse experiments in which the pulse width is regularly incremented. This permits easy examination of the phase stability (no arcing) and allows measurement of the RF homogeneity values (1H 450/90, 810/90 and 13C 360/0, 720/0). Once the 1H pw90 is determined, the optimal gain is determined, and T1 measured. The T1 value is used to set the recycle delay in subsequent experiments to at least 10T1. The observed linewidth is used to set an adequate value of the acquisition time for subsequent tests.

The test is designed to quantitate instrument performance on a firm, unbiased, and operator-independent statistical basis. Hence, stability/reproducibility measurements are made (typically 20 independent trials), and the average values and standard deviations measured, reported, and stored. These tests include high-power 90 degree pulse stability (rectangular and gaussian), 30 degree pulse stability, and phase stability via the "13-degree" test (rectangular, gaussian and phase-ramped gaussian). A sensitivity measurement is done using the 90 degree pulse stability data.

Other tests produce a regular intensity output (exponential or linear), such as when an attenuator (e.g., tpwr) or modulator (e.g. tpwrf) value is varied. These results are subjected to a regression analysis and correlation coefficients and standard deviations are reported.

Functionality of receiver gain and s/n as a function of gain for both normal- and over-sampling is tested. Small-angle phase shifters, pulse turnon times, attenuator power control, linear modulator power control, and pulse shaping are tested. Tests requiring waveform generators are done if the proper waveform generator is present.

Linearity of the total RF system is tested by examining the excitation profile of a gaussian pulse. RF predictability is tested by comparing the amplitudes of signal following rectangular, gaussian and EBURP 90 degree pulses with identical peak amplitude, where the pulse widths of the gaussian and EBURP pulses are calculated theoretically from the value for the rectangular pulse.

All of the above tests are done both on channels 1 and 2 since independent hardware is used (synthesizer, transmitter, rf control board, waveform generator, and coarse attenuator) in each channel. Common hardware starts at the power amplifier for 1H tests. The pulses are done on either channel but signals are detected using channel 1.

Various aspects of hardware are checked, including lock performance (measured by phase-cycle cancellation efficiency), image cancellation, built-in phase modulator 13C decoupling efficiency (mlev16, waltz16, xy32, and garp-1), waveform decoupling efficiency (wurst and stud), sample heating under 13C decoupling and 1H spinlocks, and variable temperature response in a 5 degree temperature jump. The lock channel power and gain control is checked and quantified.

Gradient performance is checked on all active axes. Gradient DAC values to generate 10G/cm strengths are determined for all active axes. The tests include signal amplitude stability following a pulse done 100 usec after a gradient pulse, or a pulse followed by a bipolar gradient pair generating a gradient echo. Field recovery is measured by doing a gradient (rectangular and half-sine, positive and negative) followed by a variable delay and an RF pulse. Gradient stability is measured by performing a CPMGT2 experiment with and without gradients within the spin echos, including one in which the gradient levels are mis-matched by 1%. The phase-cycle cancellation test is repeated, except that a gradient is done 100usec before the pulse. Spectral purity is measured by obtaining data over a large SW with no pulse (only noise should be visible).

As tests are performed, spectra are plotted (if specified), fids are stored, and calculated results are stored in appropriate text files. At the end of the test a single-page report is printed summarizing the test results. If desired, the test can be made to repeat until aborted to acquire multiple runs (during an overnight period, for example). A single run will last from ~40 to 120 minutes, depending on the options chosen. If test cycling is selected, the last full AutoTest data set is stored separately for later examination. On the order of 3300 separate fids are generated in a full AutoTest run.

While the test is running the operator may abort an acquisition at any time and restart with "au". All macros and new pulse sequences begin with the letters "AT" so that they are easily identified. No compiled programs outside of standard VNMR are used. Therefore, any of the AutoTest macros may be examined to verify exact operation.

As results are determined the values are written into the text file that is subsequently printed, and into specific text files organized by test. Thus, separate text files for image cancellation, 90 degree stability, etc., are stored with new lines added each time AutoTest is run. Therefore, the operator can examine a historical record (including dates) for each result.

After several runs have been done, histograms showing all previous values of a parameter may be viewed, under menu control, for example, all previous z-axis gradient calibrations, along with the average of all these results and their standard deviations. Of particular interest is a parameter exhibiting a sudden change in value, or a steady increase or decrease. These results can give an early signal of a hardware problem. These histograms may be plotted automatically at the end of an AutoTest run for a permanent record. In addition, a summary of all results is printed for all results, including average values and standard deviations for the entire period covered in the HISTORY file.

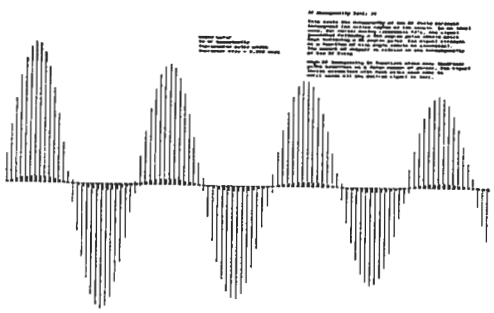
AutoTest is now available in the on-line userlib (nmr.varian.com) as well as in /vnmr/userlib of VNMR version 6.1, or via email request at userlib.request@nmr.varian.com. The package of macros, parameter sets, pulse sequences and menus features automatic installation. The README file contains full descriptions of the tests, including a protocol defining each test and a description of the reason for the test.

A few examples of AutoTest output are illustrated in the Figures.

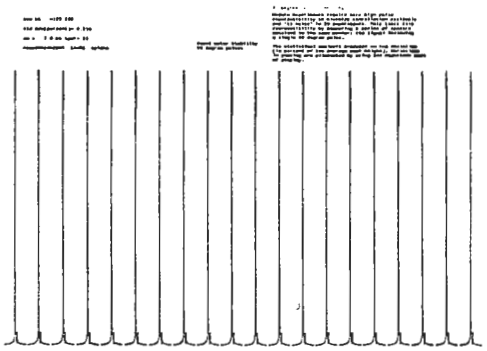
Sincerely,

George A. Gray, D-298
NMR Applications Laboratory
Varian Associates
Palo Alto, CA 94304-1030

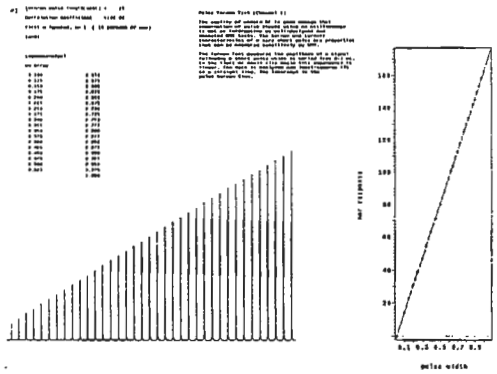
1H RF Homogeneity



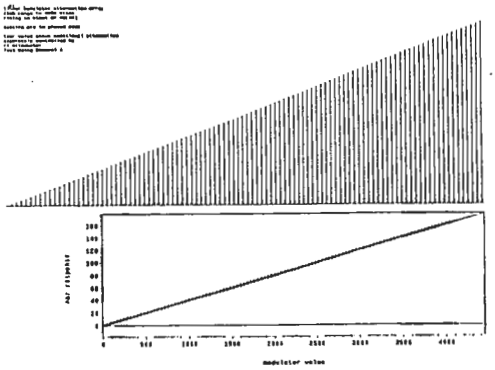
90 Degree Pulse Stability



Pulse Turnon Time (0-1 usec)



Linear Modulator Test



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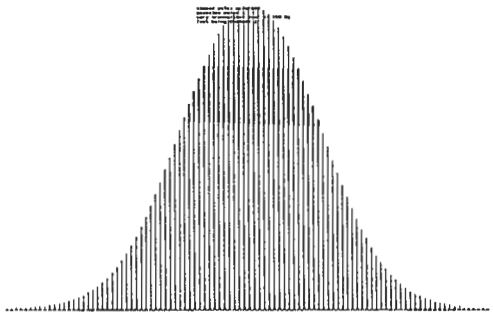
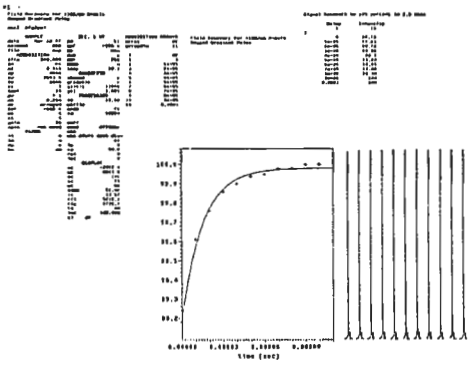
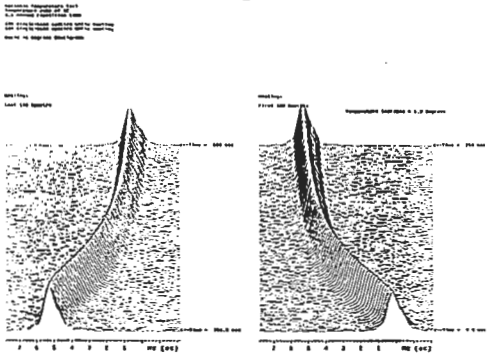


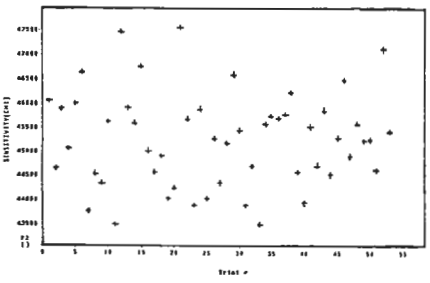
Gradient(Field) Recovery



Temperature Jump and Cool-Down



Sensitivity Histogram (autoscaled to fill Y-axis)



UNIVERSITY OF VIRGINIA
DEPARTMENT OF CHEMISTRY
McCORMICK ROAD
CHARLOTTESVILLE, VIRGINIA 22901

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

December 5, 1997
(received 12/10/97)

Converting Varian Unity Data to Tecmag MacNMR Format

Dear Dr. Shapiro:

Our NMR lab is a departmental resource and currently consists of three 300 MHz, one 360 MHz and two 500 MHz spectrometers. Two of the 300's (GN-300 and QE-300) and the 360 (NT-360) were upgraded with Tecmag acquisition systems and Macintosh computers, and therefore are controlled by using the Tecmag MacNMR program. One of the major advantages of the Tecmag/Mac upgrades was that the data on the spectrometer Macs could be readily transferred to remote Macs for storage and processing. Last summer we acquired Varian UnityInova 300 and 500 spectrometers and temporarily inherited a Varian UnityPlus 500. Part of the planning for efficient integration of the Varian spectrometers into our lab included deciding what to do about processing the Varian data on computers other than those which run the spectrometers. It seemed to us that the best solution was to use our existing set of Macs running MacNMR. Two problems had to be solved in order to implement this solution. The first was providing an efficient way to move the data from the spectrometers to the Macs and the second was data conversion from Varian Unity to MacNMR format. The first problem was solved by obtaining TCPConnect4 from InterCon Systems Corp. This software contains a NFS client for Macs. Thus the Macs could access files on the spectrometers as if they were local files. The second problem was solved by Tom Egan of Tecmag who supplied us with a Mac program called VariantoMacNMR that converts Varian Unity data to the MacNMR format.

Data transfer and conversion become one process when using TCPConnect4 and VariantoMacNMR. When the user starts VariantoMacNMR he/she is prompted for the locations of the Varian fid and procpar files. The files are selected by using the standard Mac file opening format and the fact that the Varian files are not local is not noticed by the user due to TCPConnect4. The NMR users do not even have to save their data on the spectrometer due to the fact that this is done automatically in the acqfil subdirectory of the experiment directory that was

used during data collection. This method of transferring data from the Varian acqfil directory immediately after data acquisition is facilitated by the fact that we have placed a NMR data station Mac running TCPConnect4 and VariantoMacNMR adjacent to the Sun workstation which is controlling one of the Varian spectrometers. We have been using TCPConnect4 and VariantoMacNMR running under MacOS 7.6.1 for several months and the combination has performed well.

Several programs including commercial and freeware packages are available for processing Varian NMR data (see http://www.chem.umu.se/divisions/fk/EduNMRSoft.html for a compilation). MacFID1D is a program created by Tecmag that has the same 1D processing capabilities as MacNMR, is free (it can be obtained at http://www.tecmag.com) and has the ability to process data created by the many commercial NMR spectrometers. We preferred MacNMR to MacFID1D because the former can process both 1D and higher D data and because MacNMR has been installed on many Macs in our department and has been routinely used to process data acquired on the older spectrometers mentioned above. Using TCPConnect4, VariantoMacNMR and MacNMR for remote transfer, conversion and processing of Varian Unity data has major advantages for us over alternative possibilities. The main advantages are the low hardware and software cost and the ability to utilize software (MacNMR) that was familiar to our NMR users. We only had to buy a couple of Macs to use as data stations (we obtained Power Computing Power Base 200's) and TCPConnect4 ($240/copy from MacWarehouse).

Unfortunately, two of the products mentioned above may be difficult to obtain. Power Computing is no longer selling Mac clones however some models are still available (see http://www.warehouse.com) as are many alternatives (see http://www.macdirectory.com/pages /Hardware.html). Also, TCPConnect4 may no longer be available. Intercon Systems Corp., the maker of TCPConnect4, has been obtained by Ascend Communications Inc. Ascend has a product that contains a NFS client for Mac but it also includes many other features and may not be a good option for those looking only for a Mac NFS client (see http://www.ascend.com/2405.html). Another possible solution may be Pathway NFS client for Mac from Attachmate Corp. (see http://www.atm.com/OSG/nfsclientmac/default.asp).

Best Regards,

Jeff Ellena

Jeff Ellena
Senior Scientist

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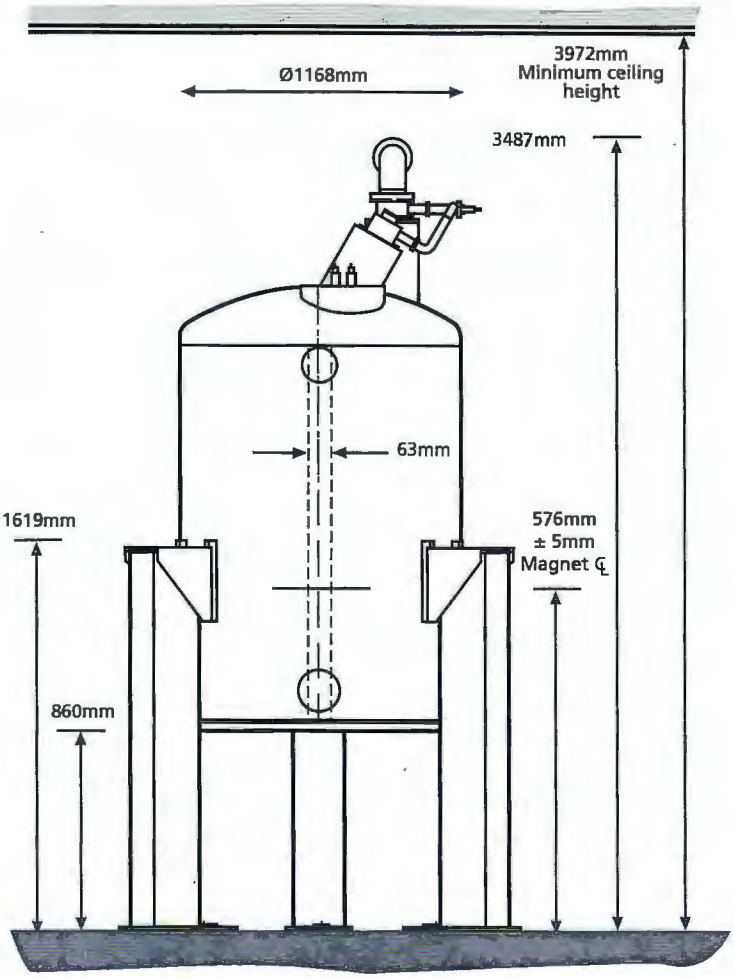

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PROF. RAY DUPREE

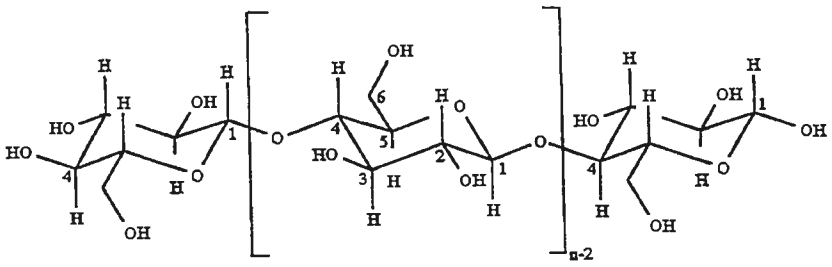
(received 12/10/97)

Prof. B. L. Shapiro,
966 Elsinore Court
Palo Alto. CA 94303, USA
2nd December 1997

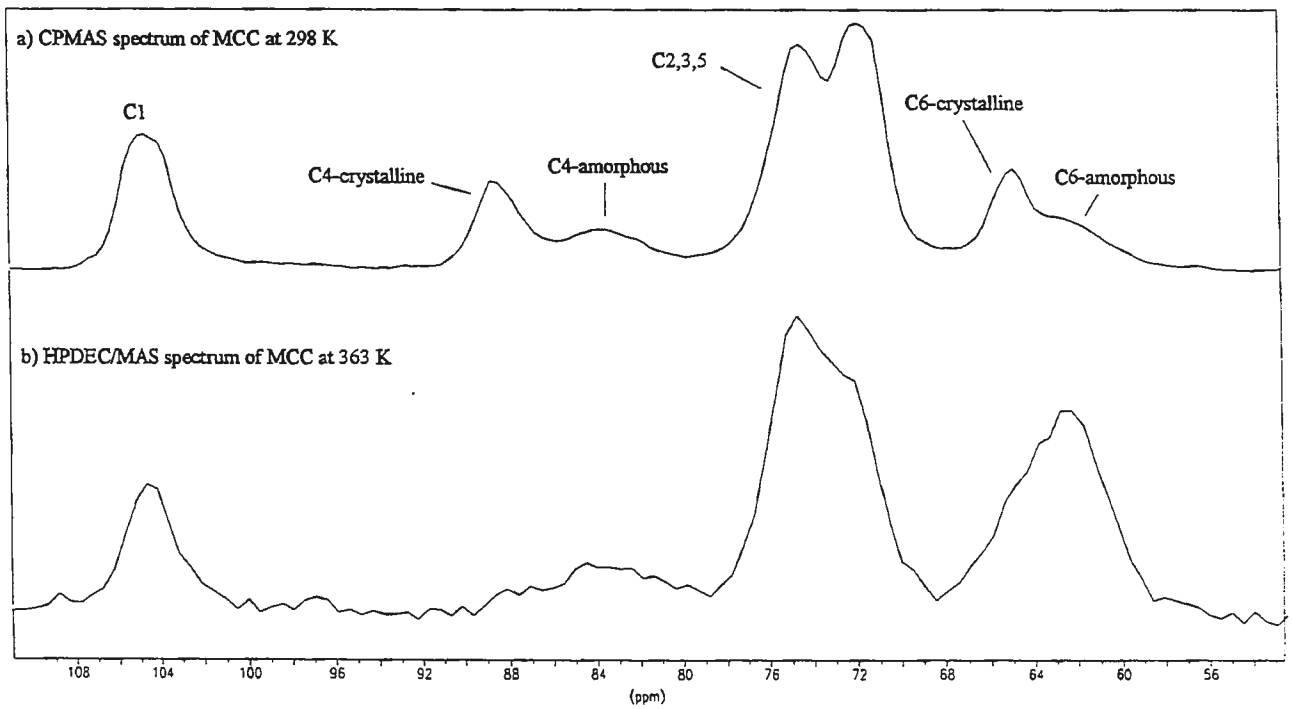
Watching Cotton Shrink - by Microscopy and by ^{13}C NMR

Dear Barry,

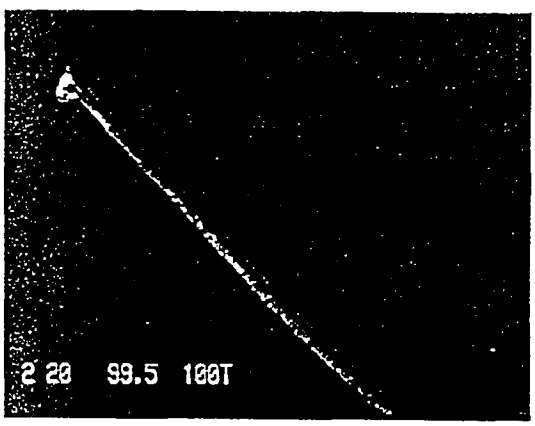
Roger Ibbett (of Courtaulds Research), Michael Michael and I have been trying to understand how cellulose dissolves in the proprietary amine oxide solvents that Courtaulds now use in the manufacture of *Tencel*. The crystalline parts of cellulose fibres are constructed from 1,4-polyglycosyl chains that are remarkably linear. They achieve this linearity with an eclipsed conformation at each glycosidic bond, as shown below.



As it happens, this conformation minimises all possible γ-*gauche* interactions between the rings, and thus gives quite unusually high carbon shifts at C-1, C-4 and C-6. Thus, as an example, C-4 appears at 89 ppm in the CPMAS spectrum of the crystalline regions of microcrystalline cellulose, obtained from a cotton source. In contrast, the same sample gives a C-4 peak at 84 ppm, from its solid but amorphous regions. In dissolved cellulose, the same resonance shifts further, down to 80 ppm. Evidently the cellulose chains bend once they are freed, even partially, by the solvent. This assessment is supported by several independent studies of the glycosyl bond. We also have evidence that several near-solvents boost the 'amorphous' peaks at the expense of the 'crystalline' ones.



If the cellulose chains start to bend as they absorb solvent, then the microcrystals must swell laterally to the chain axis, but also shrink in the direction parallel to it. We may be able to observe this directly, by optical microscopy. Some of the better-formed fibrils (individual fibre components) have well-formed, uniform sections, often with a central linear hole, called a lumen. The two photomicrographs below show first the original fibril, and then the same fibril after 5 minutes at 100 °C in the solvent. In the second picture, solvent has penetrated the outer layers, and also widened them, so as to make them scarcely visible, even in the original photo. However, the lumen is now clearly visible as a shattered zigzag. This is just what one predicts if the solvent has not yet penetrated to the crystalline regions that surround the lumen and render it rigid. This rigid part becomes compressed like a conjurer's wand by the shrinkage of the outer layers.



×90.

Of course, more complex explanations are also possible, involving hierarchies of structure within the fibril. But even so, something like the above explanation must be necessary to explain the data.

Best wishes,

Oliver

****

- continued from p. 31

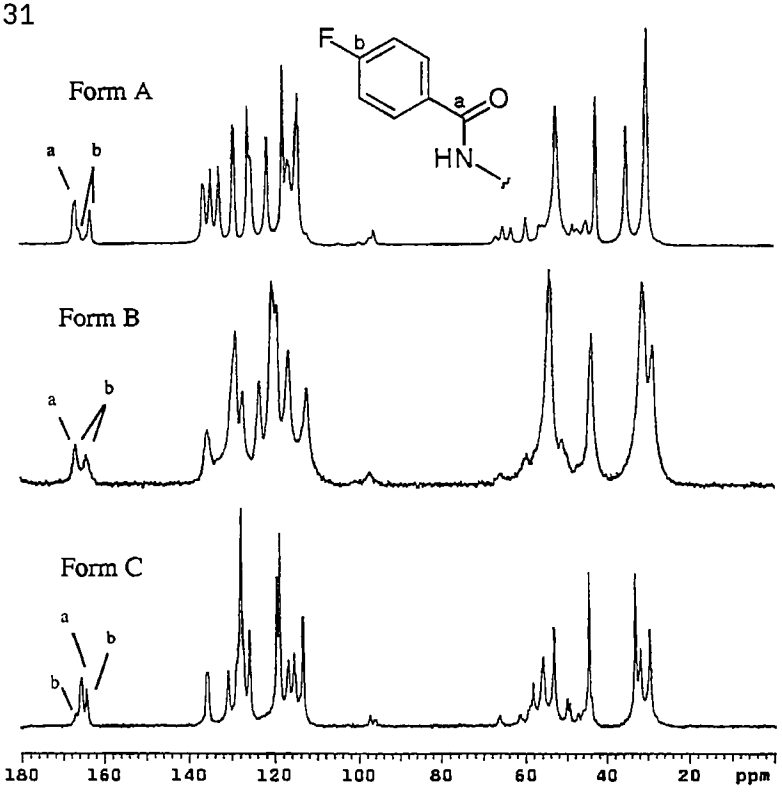


Figure 1. 100.6 MHz ^{13}C CP/MAS NMR spectra of three pharmaceutically-relevant forms of Compound I.

Lilly

Lilly Research Laboratories
A Division of Eli Lilly and Company

Lilly Corporate Center
Indianapolis, Indiana 46285
(317) 276-2000

December 2, 1997
(received 12/5/97)

Interpreting the ^{13}C CP/MAS NMR Spectra of Three Drug Crystal Forms

Dear Dr. Shapiro,

We routinely use solid-state ^{13}C NMR (SSNMR) spectroscopy to characterize crystallinity, polymorphism, and solvation in our pharmaceutical solids. From the crystal structural information obtained in SSNMR spectra, we are oftentimes able to understand and rationalize the physical properties of drug crystal forms.[1] Recently, we obtained ^{13}C CP/MAS NMR spectra of three pharmaceutically-relevant crystal forms of Compound I, Figure 1. The spectra of these materials feature relatively sharp resonances to suggest that they are crystalline. Since the solid-state ^{13}C chemical shifts reflect not only the molecular structure of I, but also its electronic environment(s) in the crystal forms, polymorphism (or hydrate or solvate formation) is inferred by different chemical shifts of equivalent ^{13}C nuclei.

Once the crystallinity of a drug material is established, it is usually trivial to determine the number of unique electronic environments, i.e. molecules in the asymmetric unit of each crystal structure, from SSNMR spectra. In each crystal form, Compound I is considerably immobilized, likely forcing the achiral drug molecule to adopt (at least) one asymmetric conformation. Consequently, the resonances of symmetry-equivalent carbons, such as the *para*-fluorophenyl CH carbons, are split in the ^{13}C CP/MAS NMR spectra. Additional splitting is observed in the 160-170 ppm region. The amide carbonyl carbon, $^{13}C_a$, resonance is slightly split (J ~ 50 Hz) in Form C due to residual dipolar coupling between the amide carbonyl ^{13}C and quadrupolar ^{14}N (Note: this splitting is not obvious in the other crystal forms). The $^{13}C_b$ carbon resonance, on the other hand, is split into two peaks (J ~ 280 Hz) in each SSNMR spectrum due to scalar $^{13}C,^{19}F$ spin-spin coupling. The unusual, uneven peak intensities of the ^{19}F-coupled ^{13}C resonances are attributed to the overlap of the isotropic chemical shifts and the anisotropy pattern of the $^{13}C_b$ carbon.[2] Because the magnitudes of the splittings caused by the different coupling interactions are readily discernable, there is no ambiguity in the peak assignments.

Peak splitting is observed in the three crystal forms of Compound I due to crystallographic inequivalence, and scalar and residual dipolar coupling, yet fewer resonances are observed in the solid-state spectra than in the corresponding solution-state spectra. These data suggest that a single molecular conformation (and environment) is present in each crystal form.

Please credit this contribution to the account of Dr. Doug Dorman.

Sincerely,

Susan M. Reutzel

Susan M. Reutzel

-continued on p. 30

[1] Bugay, D. E., *Pharm. Res.*, **1993**, *10*, 317.

[2] Carss, S. A.; Harris, R. K.; Fletton, R. A., *Magn. Reson. Chem.*, **1995**, *33*, 501.

UNIVERSITY OF DELAWARE

Department of Chemistry and Biochemistry
Newark, Delaware 19716-2522

Professor Cecil Dybowski
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FAX: (302) 831-6335
dybowski@udel.edu

December 11, 1997 (received 12/15/97)

NMR Calculations with MATHCAD

Dear Barry,

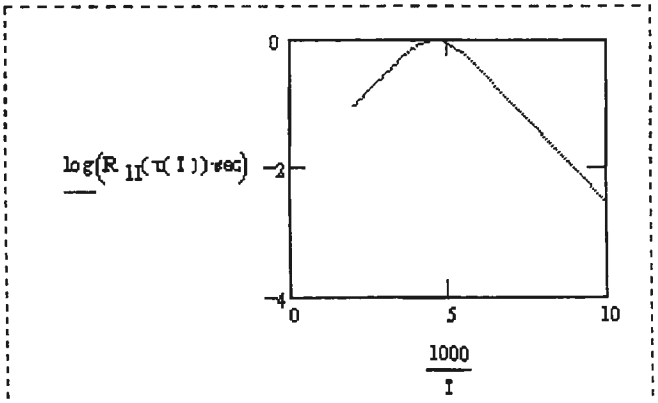
I have recently been interested in the spin-lattice relaxation of ^{207}Pb in solids. Relaxation theory is relatively well studied, so it is tough to find new tricks to report every eight to ten months. However,I point out that a program like MATHCAD is a convenient tool for visualization of data and simple theoretical calculations. I particularly find graphs make trends easy to visualize. Below is an example in which I look at the temperature dependence of T_1 due to the chemical-shift-anisotropy mechanism described by Farrar and Becker. What is convenient is the ease with which one may change parameters to determine the effect on the observed temperature dependence of relaxation times.

Relaxation of a Spin 1/2 by Chemical Anisotropy

$\omega_I := 62.778 \cdot 2 \cdot \pi \cdot 10^6 \cdot sec^{-1}$ $\quad \Delta\sigma := 50 \cdot 10^{-6}$ $\quad \Delta E := \frac{10000}{8.31451}$ $\quad B_0 := 7.04 \cdot tesla$

$\tau_0 := 1 \cdot 10^{-11} \cdot sec$ $\quad \tau(T) := \tau_0 \cdot \exp\left(\frac{\Delta E}{T}\right)$ $\quad R_{1I}(\tau) := \omega_I^2 \cdot \Delta\sigma^2 \cdot \left(\frac{2 \cdot \tau}{1 + \omega_I^2 \cdot \tau^2}\right)$

$T := 100, 110 .. 500$



Yours truly,

Cecil

NEW MEXICO RESONANCE
A nonprofit research corporation

Diffusion Imaging with Hyperpolarized ^{3}He Gas

(received 12/16/97)

Dear Barry,

We have used MRI of hyperpolarized ^{3}He to demonstrate some novel aspects of gas diffusion. Two different techniques were used. First, a slice was burned into a one-dimensional image by inverting the spins in the slice and diffusion was studied by measuring the magnetization as it filled the depleted slice. A diffusion coefficient was determined by the fit of these data. Second, one-dimensional diffusion images were made using a Stejskal-Tanner PGSE method. This was done with and without a temperature gradient present, showing that the effect of temperature can be dynamically monitored by such diffusion images.

The experiments were done in a imager/spectrometer (Nalorac Cryogenics Corp.) with a 1.9 T, horizontal bore, superconducting magnet (Oxford) having a bore diameter of 31 cm. The gas was hyperpolarized in the fringe field of the 1.9 T magnet at a distance of 2m by laser optical pumping in the presence of rubidium molecules (1,2). The He gas was at 7 atm pressure in a cylindrical cell with inner dimensions 7.0 cm long and 2.2 cm inner diameter. The polarization time constant of the cell (T_1) was about 15 h and with 4 h of optical pumping a polarization of about 5% was achieved. This is over 3 orders of magnitude greater than that from conventional thermal polarization and significantly improves the ability to image the gas.

A series of one-dimensional images over time was obtained in which the diffusion of two populations of nuclei could be seen in a manner similar to that of (3). First the magnetization of nuclei in a thin central section of the cylinder was inverted. Then, images were taken every 0.2s for a total of 5s, using a constant flip angle of 4.5° . These images are normalized to the same total intensity because a fraction (sin(4.5°)) of the magnetization is lost with each image acquisition. The He diffusion coefficient was measured with these data using a simple model. A delta function spike in density will, through diffusion, form a density profile which is Gaussian whose variance is proportional to the diffusion coefficient and the time over which diffusion has taken place. We therefore modeled each one-dimensional image by convolving the first image with a Gaussian whose variance V was proportional to a candidate diffusion coefficient D times t; the time interval separating the two images is $V = 2Dt$. We searched for the value of D which minimized the error between the predicted and measured values. A comparison of the data to the modeled data with the best-fitting value of D is shown in Fig. 1 for a few selected time intervals. A value of $D = (21.3 \pm 0.4)$ mm^2/s was obtained.

Next, one-dimensional diffusion images were made using the technique of Stejskal and Tanner (4). A diffusion coefficient was calculated at each point in the image by taking the ratio of the image intensities with an without a previous bipolar magnetic field gradient in place. D can be calculated from the signal ratio e^{-ibD} where b is known from the bipolar gradient parameters. Diffusion can be affected by physical boundaries as well as by temperature or pressure. Diffusion images were made both at thermal equilibrium (Fig. 2a) and with a thermal gradient (Fig. 2b) produced by holding the right end (as viewed in the figure) of the cell in a liquid nitrogen exhaust plume for a few minutes. Error bars in Fig. 2a are larger than those in Fig. 2b because the data of Fig. 2a were obtained after a shorter polarization time and therefore had lower signal. The diffusion measured from the ends of the cylinder is consistent with that measured from observing the diffusion of a section of inverted magnetization, described above. The plot on the right shows a diffusion image when the cylinder had a thermal gradient. We see that the diffusion coefficient decreases with temperature.

We have presented two different experimental techniques which yielded the ^{3}He self-diffusion coefficient of (21.3 ± 0.4) mm^2/s. A previous NMR measurement (5) at 300 K and at 1 Torr yields a self-diffusion coefficient of (27.1 ± 1.5) mm^2/s at 7 atm when scaled by a factor of 5320, assuming a linear pressure dependence. Many measurements of He-He diffusion have been made at atmospheric pressures using techniques other than NMR, as summarized in (6). All of these agree with each other to within 3% and after

2425 Ridgecrest Drive, SE; Albuquerque, NM 87108
Phone: 505-262-7575, ext. 5025. Fax: 505-262-7043

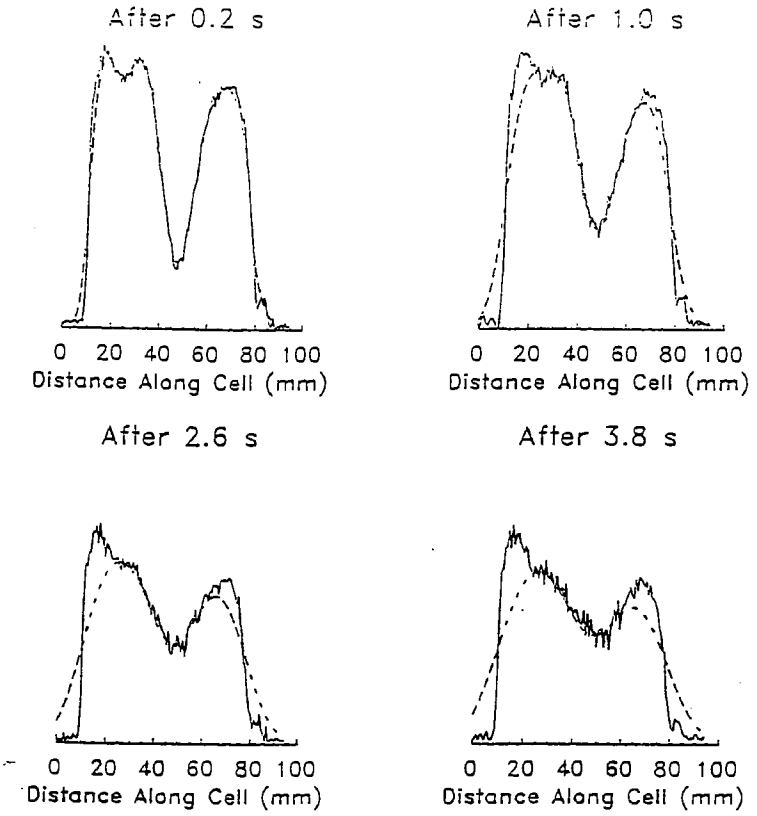


Fig 1

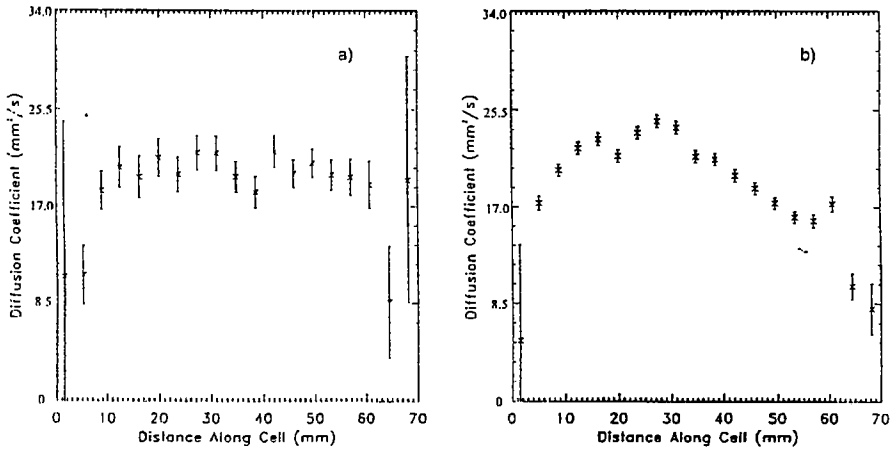


Fig. 2

correction for isotopic mass dependence and for pressure dependence yields a value of 28.0 mm^2/s at 7 atm. Considering the type and scale of corrections needed to compare our results to previous results, and the uncertainty of the pressure in our cell, the difference may not be significant.

References

1. N.D. Bhasker, W. Happer, and T. McClelland, Phys. Rev. Lett. 49, 25 (1982). **2.** W. Happer, E. Miron, S. Schaefer, D. Schrelber, W.A. van Wijngaarden, and X. Zeng, Phys. Rev. A 29, 3092 (1984). **3.** M. Pfeffer and O. Lutz, J. Magn. Reson. A 113, 108 (1995). **4.** E.O. Stejskal and J.E. Tanner, J. Chem. Phys. 42, 288 (1965). **5.** R. Barbé, M. Leduc, and F. Laloë, J. Phys. 35, 935 (1974) **6.** J.C. Liner and S. Weissman, J. Chem. Phys. 56, 2288 (1972).

Best regards,

A. Caprihan E. Fukushima

D.M. Schmidt[1], J.S. George[1], S.I. Penttila[1], A. Caprihan, and E. Fukushima
[1]Los Alamos National Laboratory, Los Alamos, NM 87545

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FAX: (302) 995-4117

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

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

Dear Dr. Shapiro:

NMR Analysis of Compositionally Heterogeneous Polymers

Almost all copolymers exhibit some degree of compositional heterogeneity. Thus, for a given copolymer, if one sorts and plots the compositions of individual polymer chains, one may get a chemical composition distribution (CCD) curve that look like Figure 1. Such heterogeneity may arise from different sources[1]. Some common types are summarized in Table 1. Depending on the nature of heterogeneity, the CCD may be either symmetric or non-symmetric.

Compositional heterogeneity can also influence polymer microstructure, and NMR data may reflect this heterogeneity. In the past this effect was usually ignored in polymer NMR analysis. However, a number of workers did recognize this problem and have made attempts to address it[2-9]. For example, a few years ago I reported the use of the perturbed first-order Markovian model[6] to treat the NMR data of polymers that exhibit symmetric CCD curves. Recently I have generalized the treatment[9] to include cases where the CCD is non-symmetric. Computer programs have also been written to facilitate such analysis.

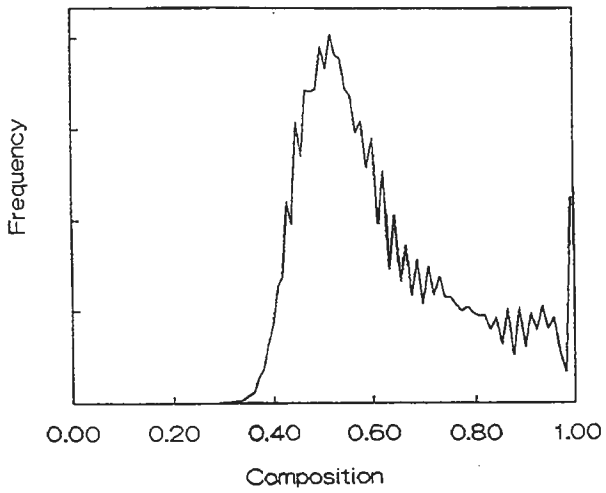

Figure 1. Chemical composition distribution (CCD) curve for a polymer exhibiting conversion heterogenity

Table 1. Different Kinds of Compositional Heterogeneity

Kind	Possible Origin of Heterogeneity	CCD Curve
Statistical	Instantaneous copolymer composition fluctuation	Symmetric
Conversion	Different comonomer reactivities	Skewed or "tent"-shaped
Multi-state	1. Polymerization occurring at different sites or phases 2. programmed monomer feeds	Skewed to multi-modal
Process	Fluctuations in polymerization process conditions	Symmetric or slightly skewed

In order to illustrate this methodology, I shall summarize the treatment for an exponentially modified Gaussian (EMG) distribution. The EMG function can be expressed mathematically as follows:

$$f(z) = \frac{N}{\tau\sigma\sqrt{(2\pi)}} \int_o^\infty \exp\left[- \frac{(z-P'_{BA}-t')^2}{2\sigma^2} - \frac{t'}{\tau} \right] dt'$$

where $z = P_{BA}$ = 1st order Markovian probability; P'_{BA} is the average value of the Gaussian distribution (of z) without the exponential modification. Moreover, N is the area, τ the time constant for the exponential modifier, σ the standard deviation of the Gaussian, and t' is the dummy variable of integration. The function is graphically depicted in Figure 2.

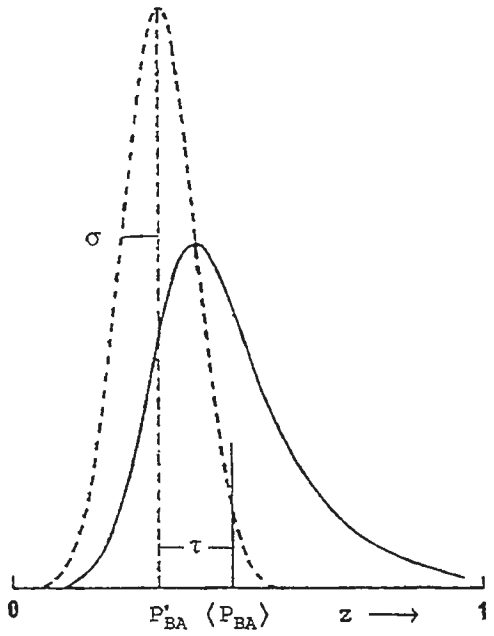

Figure 2. Exponentially modified Gaussian function

Through some mathematical manipulations, one can derive the theoretical expressions corresponding to the copolymer composition, diad, triad, and tetrad sequences. Some of these expressions are given in Table 2. Four parameters characterize this model: P'_{BA}, P'_{AB}, σ, and τ. Equivalently, these expressions may be re-cast as a function of average probabilities $\langle P_{BA} \rangle$ and $\langle P_{AB} \rangle$.

The expressions in Table 2 can now be used to treat experimental NMR data. A computer program, called PERTEMG, has been written for automated analysis[9]. As an example, the NMR triad data for a commercial vinyl chloride(A) / vinylidene chloride(B) copolymer sample as published in the

Table 2. Probability Expressions for the Perturbed First-Order Markovian Model Using Exponentially Modified Gaussian[a]

sequence	theoretical expression
⟨A⟩	$k\,(P'_{BA} + \tau)$
⟨B⟩	$k\,(P'_{AB} - \tau)$
⟨AAA⟩	$kP'_{BA}\,(1-P'_{AB})^2 + (k-2+s)\tau + (2k-2)K_2 + kK_3$
⟨AAB⟩	$2kP'_{BA}\,P'_{AB}\,(1-P'_{AB}) + 2(1-s)\tau + (4-2k)K_2 - 2kK_3$
⟨BAB⟩	$kP'^2_{AB}\,P'_{BA} + s\tau - 2K_2 + kK_3$
⟨ABA⟩	$kP'_{AB}\,P'^2_{BA} + K_2 - kK_3$
⟨BBA⟩	$2kP'_{AB}\,P'_{BA}\,(1-P'_{BA}) + 2\tau - (2k+2)K_2 + 2kK_3$
⟨BBB⟩	$kP'_{AB}\,(1-P'_{BA})^2 - (k+2)\tau + (2k+1)K_2 - kK_3$

[a] Four parameters characterize this model: P'_{BA}, P'_{AB}, σ, and τ. The other variables are defined as follows:

$K_2 = \sigma^2 + 2\,P'_{BA}\,\tau + 2\,\tau^2$

$K_3 = 6\,\tau^3 + 6\,P'_{BA}\,\tau^2 + 3\,(P'^2_{BA} + \sigma^2)\,\tau + 3\sigma^2\,P'_{BA}$

$k^{-1} = s = P'_{AB} + P'_{BA}$

Table 3. Fitting of the Triad Data for Vinyl Chloride (A)/Vinylidene Chloride (B) Copolymer by the EMG Perturbed First-order Markovian Model

		2nd ord.Markov	Perturbed 1st order Markov		
	I_{obsd}	I_{calc}	$I_{calc}(1)$	$I_{calc}(2)$	$I_{calc}(3)$
AAA	16.2	16.2	4.7	7.5	16.2
AAB	9.9	10.0	16.7	16.5	10.3
BAB	14.9	15.4	14.9	14.9	15.2
ABA	8.6	8.2	8.5	10.7	7.9
BBA	24.8	24.5	29.5	24.8	24.8
BBB	25.7	25.7	25.7	25.7	25.7
P'_{AB}			0.640	0.678	0.714
P'_{BA}			0.365	0.430	0.423
σ			0	0.188	0.324
τ			0	0	0.050
mean dev.		0.22	3.86	2.91	0.22

literature[10] are shown in column 2 (I_{obsd}) of Table 3. It has been reported[10] that the data did not fit well to Bernoullian and first-order Markovian models; the best fit was obtained only with a second-order Markovian model. Since the same copolymer system has been shown to obey first-order Markovian model for lab prepared copolymers[10], the need to invoke second-order Markovian model is surprising. An alternative computation has been done[9] by assuming that the commercial sample contains compositional heterogeneity. With program PERTEMG, the optimal values of the parameters were calculated for three cases (Table 3):

Case (1). A simple first-order Markovian model is used ($\sigma = \tau = 0$). The fit is poor as indicated by the large mean deviation.

Case (2). Symmetric Gaussian broadening is introduced ($\sigma > 0$; $\tau = 0$), but the fit is still poor.

Case (3). An EMG function is used, and an excellent fit is obtained.

Thus, one interpretation of the NMR data is that this commercial sample of vinyl chloride/vinylidene chloride copolymer has a broad (and skewed) CCD curve.

This methodology has been extended to generalized CCD distributions. More details and examples can be found in a recent paper[9]. Anyone interested in the computer programs may write to me.

Yours very truly,

H. N. Cheng

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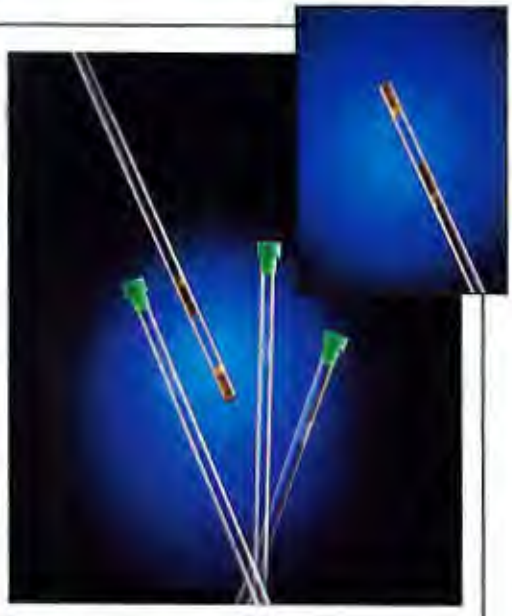

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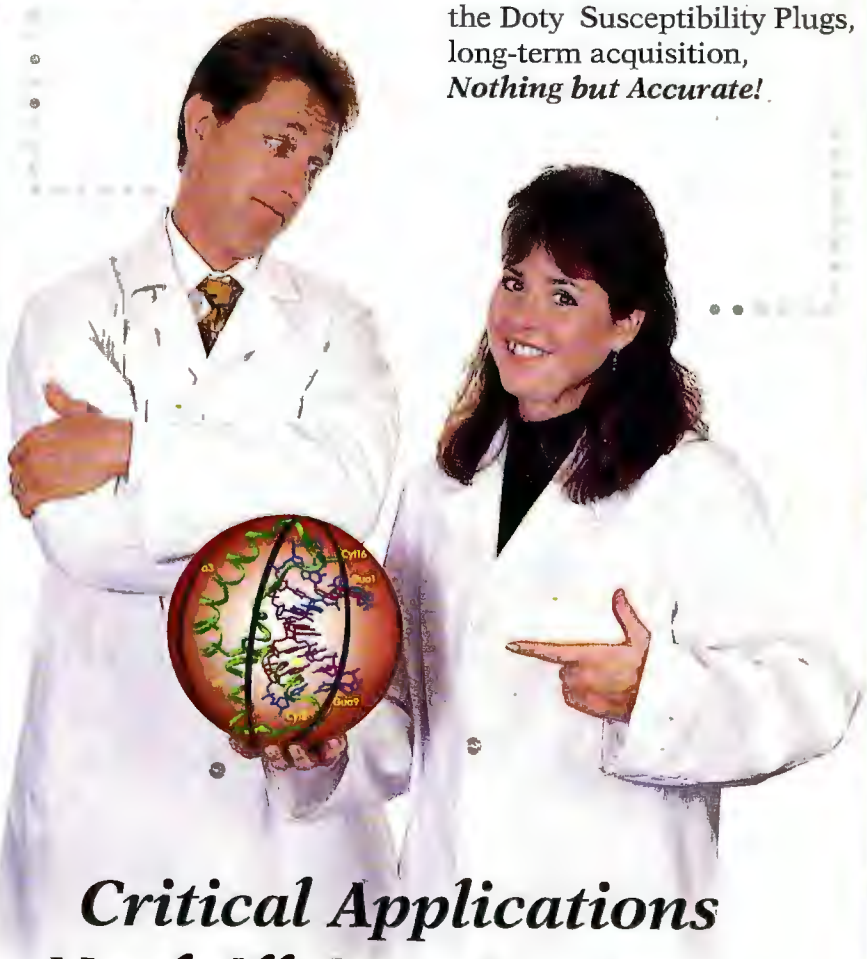

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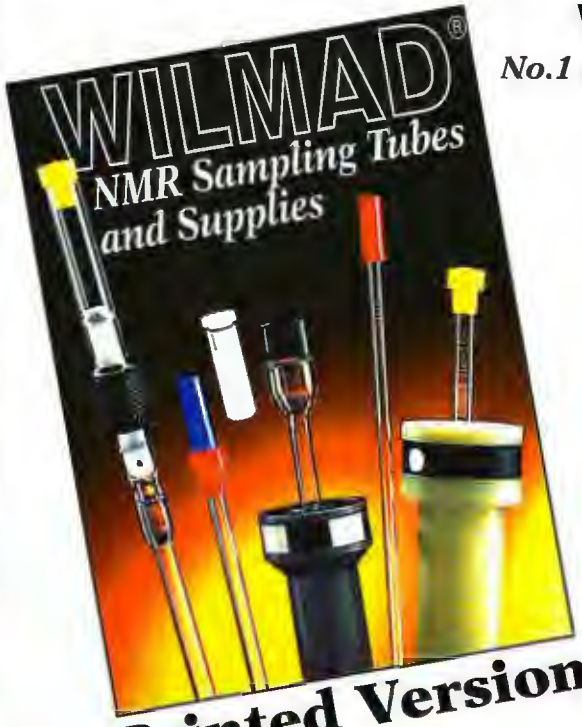

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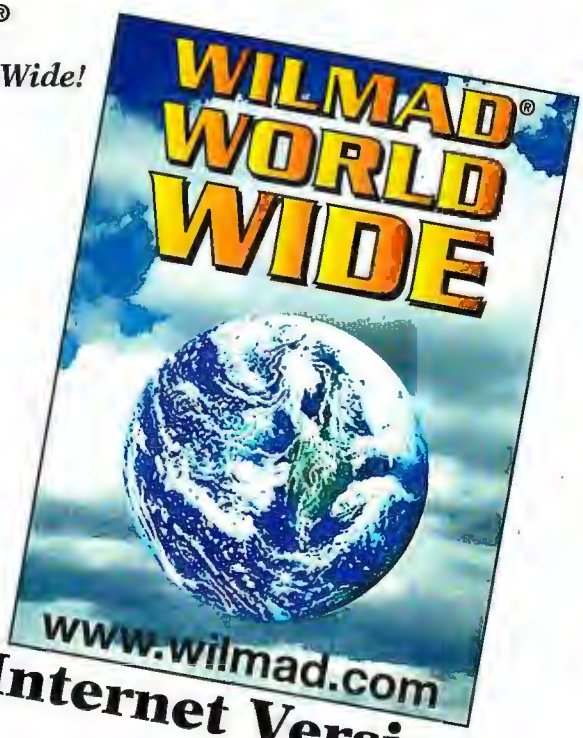

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(received 12/16/97)

Nijmegen, December 11, 1997

Filling magnets without worries.

Dear Dr. Shapiro,

As anybody will agree, filling supercons with nitrogen is a time consuming and boring activity. One has to stay with the nitrogen vessel, because walking away from it leads to chilled ceilings and cracked lino on the floor. One can buy automatic filling systems based upon the Pressure-Temperature characteristic of Xenon. Because our magnets are (fortunately) not very close to one another, this would involve a lot of hardware and money, especially if one also wants to use the same system for filling smaller dewars for low-temperature experiments. Therefore, we chose a much cheaper solution that we built ourselves. The setup shuts off the nitrogen supply as soon as liquid nitrogen is ejected from the outlet of the magnet. It consists of a normal composite resistor (NTC) as temperature sensing element, a cryogenic valve and a box containing the hardware. The cryogenic valve and the hardware travel with the vessel, every magnet or Dewar has its own sensor.

The biggest problem one encounters is to measure the liquid nitrogen in a stream of nitrogen gas at the magnet exhaust. Temperature-sensing seems impossible as both are almost equally cold. In principle, it is possible to put a known amount of heat in the sensing resistor by running a known electrical current through it. In this way one senses in fact the specific heat of the gas phase compared to that of the liquid phase. In the present case the calibration would be very tedious, however, as the flow rate of the gas differs each time. Moreover, the exhaust of the magnet will not immediately change from gaseous to liquid nitrogen.

We found a very easy way around this problem, however, by building a kind of "phase separator". In fact this is not much more than a piece of bent copper pipe with a hole in it and closed on the end with a Teflon chamber containing the sensing resistor. Fig. 1 roughly shows the design. The nitrogen gas coming from the magnet blows into the pipe at point A and escapes from it at point B. The velocity of the gas causes a slight overpressure in the pipe past point C so there will not be any flow of gas past this point. This prevents substantial cooling of the resistor by the nitrogen gas. The situation changes drastically, however, at the moment a few nitrogen drops leave the magnet. They will obviously not escape from the pipe but

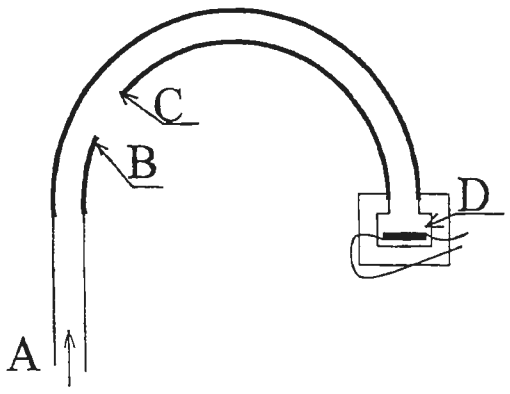

Figure 1

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are flung onto the sensing resistor in point D due to the centrifugal forces. Thus the sensor is cooled to near liquid nitrogen temperature very fast.

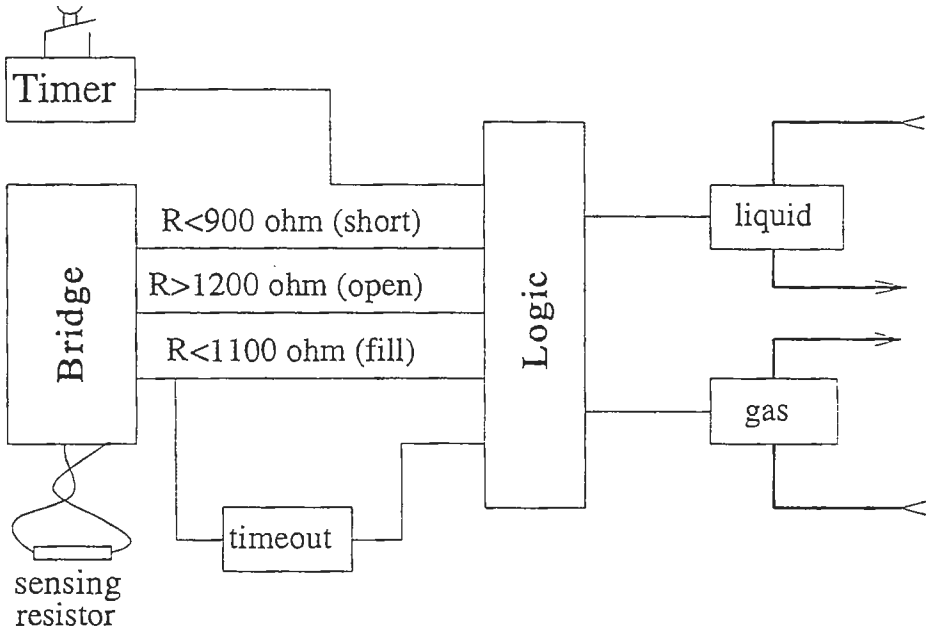

Figure 2

The hardware for the device to shut down the nitrogen supply is quite straightforward as can be seen from Fig.2. As a sensing resistor we use a resistor of nominal 1000 ohm at room temperature, going to over 1350 ohm at liquid nitrogen temperature. We implemented a test for open wires (>1200 ohm) and short circuited wires (< 900 ohm) before the device will start at all. The "cold condition" is set to 1100 ohm. When the wiretest is O.K., hitting the push-button will open the valve on the nitrogen vessel, and filling of the magnet will commence. As soon as the sensor cools down and its resistance exceeds 1100 ohm the valve will close.

The device is also equipped with a timer which allows us to keep dewars filled for low-temperature experiments. The timer will reopen the valve when it reaches its preset value, restarting the filling sequence all over again. We set it typically to a few hours to keep our Dewar for low-temperature MAS operation filled.

We added a safety procedure in case the valve freezes and does not close anymore. If the sensing resistor stays cold for more than one minute, it means the valve is frozen. This condition causes the (safety)gas valve of the nitrogen vessel to open, taking the pressure off the container. We have been using this device for quite some time now and it performed reliably. In fact, the safety valve never had to be activated. We hope that this idea will make life a little easier for all those responsible for keeping magnets and Dewars filled.

Sincerely yours,

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The NMR Newsletter - Book Reviews

Book Review Editor: István Pelczer, Dept. of Chemistry, Princeton University, Princeton, NJ 08544

"100 and More Basic NMR Experiments" A Practical Course

by

S. Braun, H.-O. Kalinowski, and S. Berger

VCH Publishers, Weinheim, Germany and New York, NY, USA; 1996
ISBN 3-527-29091-5 (pbk),418 pages; $49.96
Available through John Wiley and Sons, Inc.: http://www.wiley.com

Stefan Berger and his two partners came up with something practical and quite useful which had appeared – in pieces– only in manufacturers' manuals and in basic handbooks on NMR from Sanders and Hunter, Derome, or Fukushima and Roeder, all referenced frequently in this excellent book.

A systematic practical introduction to basics of high field NMR experiments has been long overdue and, as most NMR spectroscopists acquire their skills outside of official courses, will fill a significant hole in the literature in the field. The book offers a helping hand for novices in NMR to guide them through the forest of basic techniques, which make structural studies by NMR possible and so efficient.

The book consists of twelve Chapters in 418 pages, including two Appendices and an extensive Glossary and Index. Chapter 1 introduces us to the very basics of the NMR spectrometer: magnet, console, tuning and, the old-time ghost, shimming. Next, the entire Chapter 2 is spent on the determination of pulse length, while Chapter 3 presents a list of standard tests, well-known from installation procedures. The title of Chapter 4 is "Decoupling Techniques", but it also introduces us to 1D NOE difference spectroscopy, both homo- and heteronuclear. The next Chapter, No. 5, tells about temperature calibration and dynamic NMR spectroscopy.

Multipulse sequences start to show up in Chapter 6, comprising a diverse collection of relaxation measurements, various heteronuclear polarization transfer methods and water suppression. It is followed in Chapter 7 by – as I believe, Stefan's favorite – experiments which take advantage of selective pulses. Surely the message of this chapter should be well heard: if you can simplify your NMR approach to the lowest level of dimensionality and simplicity using selective pulses, do it! That is what will provide the highest sensitivity and efficiency.

Chapter 8 deals with lanthanide shift reagents, relaxation reagents, quantitative proton and carbon-13 NMR, and the CIDNP effect. In my opinion, this chapter about less frequently used 1D approaches could be moved to the end of the list, not because of less importance, but because the following chapters continue the lineup of the previous ones more naturally. Chapter 9 gives a succinct treatment of eight experiments for "heteronuclear" NMR, involving ^{15}N, ^{19}F, ^{29}Si, ^{119}Sn, ^{2}H, ^{11}B, and ^{17}O.Some of the heteronuclear experiments presented here connect very well to the polarization experiments from Chapter 6, for example.

continued

The fascination of NMR spectroscopists since the mid-seventies, multidimensional NMR spectroscopy, is introduced in the last three chapters (Nos. 10-12). These chapters present most of the basic homo- and heteronuclear two-dimensional correlation methods: for ROESY, the more advanced TR-ROESY is presented; variants using pulsed-field gradients; and some three-dimensional applications. The latter applications may seem to be the most attractive ones for many (as the title-page figure suggests, too), but the appearance of 1D and nD methods in this book is quite proportional to that in real life, especially if all routine studies in industry (now sparkling with combinatorial chemistry) and academic labs are concerned. Also, without the basics none of the more sophisticated methods can be made working well, even with the most user-friendly push-of-a-button software tools now in hand.

Every entry in the book is handily presented with detailed parameter settings, appropriate phase cycles, processing recipes, real-life examples, suggested literature, and space for your own observations.

There are only few things to criticize in this book. Basic rules in phase cycling, would have been useful to squeeze in, in order to provide better understanding what suggested phase cycles are good for. Also, I was missing applications of homonuclear multiple-quantum spectroscopy (other than MQ-filtering and INADEQUATE) from the list.

The book is perfectly ready for users of most, both older and more up-to-date Bruker instruments. Operators of other spectrometer makes and those using alternative data processing tools will still benefit a lot reading the Chapters – and Appendix 1, a unique cross-correlation table between keywords in various environments. This excellent handbook can be recommended to all, including more experienced users, but especially those who want to start with practical NMR spectroscopy and need a reliable guide in this journey.

István Pelczer
Department of Chemistry
Princeton University
Princeton, NJ 08544

Forthcoming NMR Meetings, continued from page 1:

NMR Symposium at the 40th Rocky Mountain Conference on Analytical Chemistry, Denver, CO, **July 27 - 30, 1998**. Contact: Dr. Robert A. Wind, Battelle/Pacific Northwest National Laboratory, P.O. Box 999, MS K8-98, Richland, WA 99352; (509) 376-1115; Fax: (509) 376-2303; Email: ra_wind@pnl.gov. See Newsletter 470, 8.

XVIIIth International Conference on Magnetic Resonance in Biological Systems, Tokyo Metropolitan University, **August 23-28, 1998**. Contact: Professor Masatsune Kainosho, Department of Chemistry, Tokyo Metropolitan University; +81-426-77-2544; Fax: +81-426-77-2525; e-mail: kainosho@raphael.chem.metro-u.ac.jp; http://icmrbs98.chem.metro-u.ac.jp

Additional listings of meetings, etc., are invited.

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Policies and Practical Considerations

(Slightly revised December 1997)

The NMR Newsletter (formerly the **TAMU NMR Newsletter**, the **IIT NMR Newsletter**, and originally, the **Mellon Institute NMR Newsletter**), now in its fortieth year of consecutive monthly publication, continues under the same general policies as in the past.

1. **Policy**:

The NMR Newsletter is a means for the rapid exchange of information among active workers in the field of NMR spectroscopy, as defined broadly, including imaging. As such, the Newsletter serves its purpose best if the participants impart whatever they feel will interest their colleagues, and inquire about whatever matters concern them. Technical contributions should always contain a significant amount of information that has not already been published or that will appear in the formal literature within a few weeks of the appearance in the Newsletter.

Since the subscriber/participant clearly is the best judge of what he or she considers interesting, our first statement of policy is "We print anything." (This is followed by the reservation, "that won't land us in jail or bankruptcy court.") Virtually no editorial functions are performed, although on rare occasions there is the need to classify a contribution as 'not for credit'. The Newsletter is not, and will not become, a journal. We merely reproduce and disseminate exactly what is submitted.

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3. **Participation is the prime requisite for receiving the Newsletter: In order to receive the Newsletter, you must make at least occasional technical contributions to its contents.**

We feel that we have to be quite rigorous in this regard, and the following schedule is in effect: Seven months after your last technical contribution, you will receive a "Reminder" notice. If no technical contribution is then forthcoming, nine months after your previous contribution you will receive an "Ultimatum" notice, and then the next issue will be your last, absent a technical contribution. Subscription fees are not refunded in such cases. If you are dropped from the mailing list, you can be reinstated by submitting a contribution, and you will receive back issues (as available) and forthcoming issues at the rate of nine per contribution.

Frequent contributions are encouraged, but no advance credit can be obtained for them. In cases of joint authorship, only one contributor may be credited. Meeting announcements, as well as "Position Available," "Equipment Wanted" (or "For Sale"), etc., notices are very welcome, but only on a not-for-credit basis, *i.e.*, such items do not substitute for a *bona fide* technical contribution.

4. **Finances**: The Newsletter is wholly self-supporting, and its funding depends on Advertising, Sponsorships, and individual Subscriptions. The **Subscription fee** for the October 1997 - September 1998 year is US$190, with a 50% academic or personal subscription discount. Subscriptions are available for a minimum of the twelve monthly issues which end with a September issue. However, a subscription can be initiated at any time, with the price for more than twelve issues being prorated.

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Another major, indeed most essential, source of funds for the Newsletter is **Advertising**. We earnestly encourage present and potential participants of the Newsletter to seek advertising from their companies. Our rates are very modest. Please inquire for details.

5. **Practical Considerations**:

a) All technical contributions to the Newsletter will be included in the next issue if received on or before the published deadline dates.

b) Please provide short titles of all topics of your contributions, to ensure accuracy in the Table of Contents.

c) Contributions should be on 8.5 x 11" (21 x 27.5 cm) pages, *printed on one side only*. Contributions may not exceed three pages without prior approval. Each page must have margins of at least 0.5" (1.3cm) on all four edges. Black ink for typing, drawings, etc., is essential. All drawings, figures, etc., should be mounted *in place* on the 8.5 x 11" pages. We are not equipped to handle pieces of paper larger than 8.5 x 11" (21 x 27.5 cm).

Please do not fold, clip, or staple your pages. Protect the condition of your letters from the ravages of the mails by enclosing what you send in a cardboard or plastic folder, etc.

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When formatting your contributions, please consider the following:

i) Try using a smaller type font: The body of this page is printed in 10 point type, which I believe is adequate for most purposes. Even 11 or 12 point type is acceptable if the particular font is not too large. Type smaller than 8 point should not be used.

ii) **PLEASE** avoid excessive margins. *Instruct your secretaries to avoid normal correspondence esthetics or practices, however time-honored or 'standard'!* This page has margins on both sides of 0.6" (*ca.* 1.55 cm), which is very adequate. Margins of the same size at the top and bottom are sufficient also, but don't worry if there is more space at the end of your document, for I can often use such spaces for notices, etc.

Also, please avoid large amounts of unused space at the top of letters. Give thought to the sizes of figures, drawings, etc., and please mount these so as to use the minimum space on the page.

iii) 'Position Available', 'Equipment Wanted', and Similar Notices. These are always welcome, but not for subscription credit. Such notices will appear, however, *only* if received with these necessarily rigid constraints: a) Single spaced; b) both side margins 0.6 - 0.7" (1.5 - 1.7 cm.)- NOT WIDER; c) the minimum total height, please, but definitely no more than 4.5" (11.5 cm.).

iv) AVOID DOUBLE SPACING LIKE THE BLACK PLAGUE ! ! ! This is extremely wasteful of space.

6. **Suggestions**: They are always welcome.

B. L. Shapiro
December 1997

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*Fax: 650-493-1348 (Do not use for technical contributions which are to appear in the Newsletter, for Fax quality is not adequate.)
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No. 476 (May) 24 Apr. 1998

No. 477 (Jun.) 22 May 1998

* Fax: 650-493-1348, at any hour. Do not use fax for technical contributions to the Newsletter, for the received fax quality is very inadequate.

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Mailing Label Adornment: Is Your Dot Red ?

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

How To Run JEOL's Eclipse+ Spectrometer

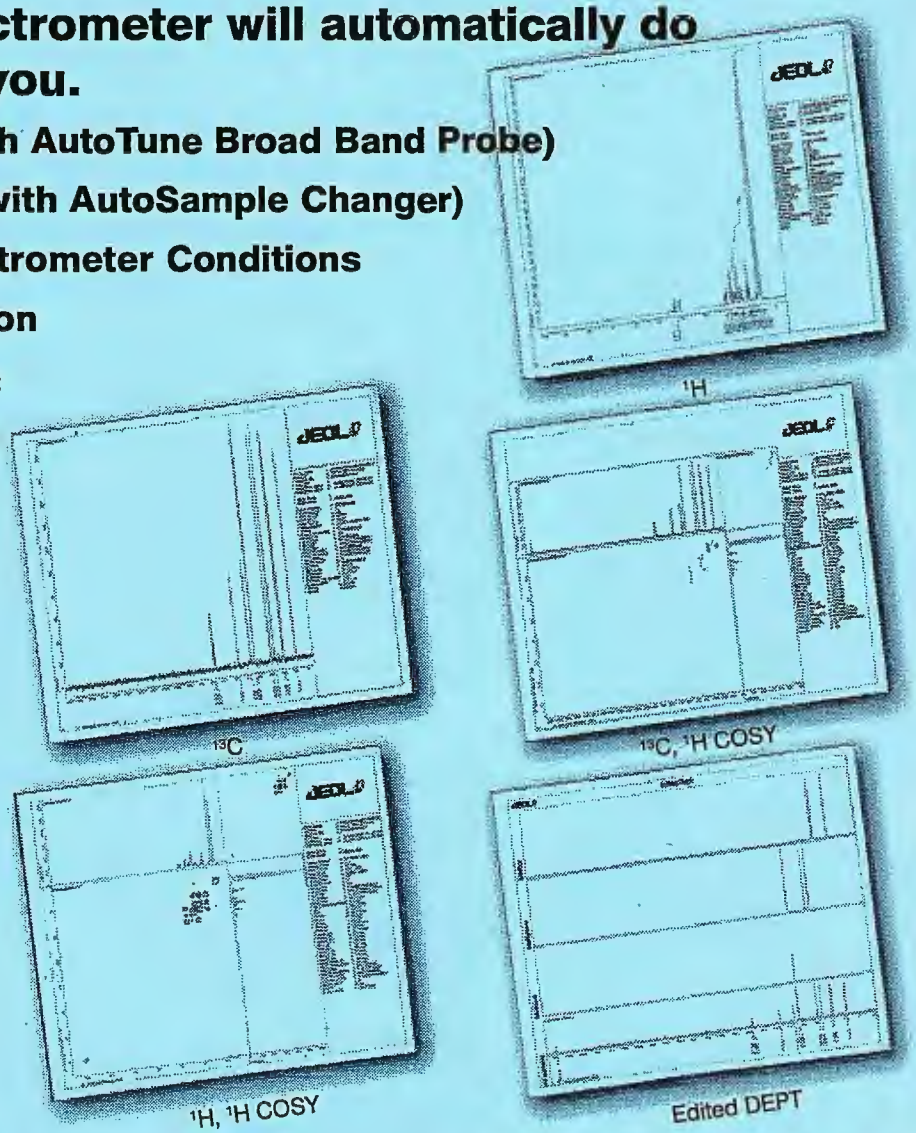
Step 1: Enter your sample name and the solvent.

Step 2: Click the mouse button on the data you want.

Step 3: Walk away with your data.

JEOL's Eclipse Spectrometer will automatically do everything else for you.

- ✔ Auto Probe Tuning (with AutoTune Broad Band Probe)
- ✔ Auto-sample Control (with AutoSample Changer)
- ✔ Auto Selection of Spectrometer Conditions
- ✔ Auto Baseline Correction
- ✔ Auto Data Presentation
- ✔ Auto Phase Correction
- ✔ Auto Digital Filtering
- ✔ Auto S/N Monitoring
- ✔ Auto Queue Control
- ✔ Auto Receiver Gain
- ✔ Auto Data Storage
- ✔ Auto Referencing
- ✔ Auto Processing
- ✔ Auto Peak Picks
- ✔ Auto Integration
- ✔ Auto Plotting
- ✔ Auto Shim
- ✔ Auto Lock



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