9th
EUROPEAN EXPERIMENTAL
NMR CONFERENCE

Hotel Post, Bad Aussee, AUSTRIA
May 16 - 20, 1988

Program and Abstracts
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Acknowledgements

The 9th EENC has been organized with financial support from many facturers of spectrometers and auxiliary equipments. Moreover, the presence of the manufacturers will strongly increase the interest in the meeting. Therefore we would like to thank:

Bruker Spektrospin S.A.
Campro Benelux
Doty Scientific Instruments
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We acknowledge a financial support from the goverment of Styria and a reception sponsored by the governor of Styria Dr. J. Krainer. In addition we have to thank the Marktgemeinde Bad Aussee - specially the mayor E.Meier, and the Fremdenverkehrverein - Mr.B.Seiberl - for financial support and the efficient and extremly helpful cooperation.

An important part of the organization of such a meeting is related to the cooperation with the hotel. In our case this cooperation was very kind and effective, so we have to thank the staff and the director of Hotel Post.

The local organizing committee.
PROGRAM

Monday, May 16th,

18.30 : Dinner

20.30 : Welcome mixer of the mayor of Bad Aussee.
Tuesday, May 17th:

Morning Session: Chairman: P. Servoz-Gavin
8.30: Opening of the meeting
8.40: J.M. Lhoste - Metabolic spectroscopy and high-field imaging in cancer research
9.30: C. Segebarth - Recent results in $^1$H image-guided localized MR-spectroscopy in humans.
10.20: Coffee break
10.45: J. Seelig - Deuterium imaging and $^{31}$P and $^{13}$C as tools for the study of in vivo metabolism.
11.10: D. Gadian - $^{31}$P and $^1$H NMR studies of brain metabolism in vivo.
11.35: A. Haase - Combined NMR imaging and NMR spectroscopy techniques.
12.30: Lunch

Afternoon Session: Chairman: R. Freeman
14.00: J. Jeener - Pulse spectroscopy with a quantized field.
14.50: M. Mehring - High resolution NMR on organic conductors.
15.15: B. Blmich - Nonlinear incoherent spectroscopy.
15.35: Coffee break
16.00: Poster session and exhibition
18.30: Lunch

20.00: Presentation by instrument manufacturers.
22.00: Swim-Jazz-Session
Wednesday, May 17th

Morning Session : Chairman : M.J.A. de Bie

8.30 : R.Kaptein : Protein structure determination by NMR.
9.45 : C.M.Dobson : The dynamics of protein folding

10.10 : Coffee break

10.30 : G.M.Clore : Determination of three dimensional structures of proteins in solution by NMR.
12.20 : J.Y.Lallemand : 1D and 2D spectra of peptides and oligo-nucleotides in H₂O , recent progress.
12.45 : G.La Mar : Nuclear Overhauser effects in paramagnetic molecules

12.30 : Lunch
14.30 : Sightseeing trip

18.30 : Dinner

20.30 : Round table discussion on software, networks, computer conferencing : Chairman : D.Ziessow
Thursday May, 19th

Morning Session : Chairman : P.Diehl
8.30 : A.Pines : Quantum phases in NMR and optics.
9.20 : W.S.Veeman : A new technique to obtain proton NMR images of rigid solids.

10.10 : Coffee break

10.45 : M.Levitt : Measurement of dipolar couplings in magic angle spinning solid-state NMR.
11.10 : J.Virlet : High resolution NMR of quadrupolar nuclei in solids.
11.35 : H.J.Jakobsen : SEMUT Spectral editing, calibration of field strengths and TOSS at high spinning speeds in 13C CP/MAS NMR of solids.

12.30 : Lunch

Afternoon Session : Chairman : J.Jeener

14.00 : M.Sauzade : Various applications of NMR imaging methods outside the medical field.
14.50 : E.Lippmaa :

15.35 : Coffee break
16.00 : Poster session and exhibiton

18.30 : Dinner

20.30 : Reception of the governor of Styria.
Friday, May 20th

Morning Session : Chairman : J.Reisse

8.30 : R.R.Ernst : Procedures for disentangling complex 2D NMR spectra
9.45 : A.Bax : New twists to some old experiments.

10.10 : Coffee break

10.30 : G.Bodenhausen : Non-trivial effects of relaxation in two dimensional spectroscopy.
11.20 : R.Freeman : Multidimensional correlation spectroscopy.
11.45 : D.Canet : Self diffusion measurements, spatial localization and signal suppression by the use of rf field inhomogeneity.

12.10 : Closing of the 9th EENC
12.30 : Lunch
LIST OF ABSTRACTS AND POSTERS
LIST OF ABSTRACTS AND POSTERS

The first two/three symbols belong to the number of the abstract or poster, the second symbol refers to the page within the proceedings.


A3, 4, In Vivo and In Situ Proton Spectroscopy using Volume Selection, M. Decorps, M. von Knielin, C. Remy and A. L. Benabid

A4, 5, Multiple Volume Selective Spectroscopy, Deuterium Imaging and 13C-NMR as Tools for the Study of In Vivo Metabolism. J. Seelig, S. Müller, J. Link and S. Cerdan

A5, 6, 31P and 1H NMR Studies of Brain Metabolism In Vivo, D. G. Gadian

A6, 7, Combined NMR-Imaging and NMR-Spectroscopy Techniques, A. Haase

A7, 8, NMR Studies of Protein Structure and Protein DNA Interactions, R. Kaptein

A8, 9, Some Rotating Frame Experiments in Solution, H. Kessler

A9, 10, The Dynamics of Protein Folding, C. M. Dobson

A10, 11, Determination of three-dimensional Structures of Proteins in Solution, G. M. Clore and A. M. Gronenborn

A11, 12, NMR and Macromolecular Structure, O. Jardetzky

A12, 13, Strategies for NMR Studies of Dilute Solutions, J. Y. Lallemand

A13, 14, The Utility and Manipulation of NOEs in Paramagnetic Molecules, G. N. La Mar, V. Thanabal and L. M. Dugad

A14, 15, Phase in Quantum Measurement; New Angles in Sample Spinning, A. Pines

A15, 16, Solid State NMR Imaging with Magic Angle Spinning, W. S. Veeman, D. G. Cory, J. W. M. van Os and A. Reichwein

A16, 17, Two Dimensional NMR Techniques for Studying Molecular Structure and Dynamics of Amorphous Solids, H. W. Spiess
A17, 18, Rotational Resonance in Spin-Pair Systems in Solids, M.H. Levitt, D.P. Raleigh, A.C. Kolbert, T.G. Oas and R.G. Griffin

A18, 19, Towards High Resolution NMR of any Nucleus in Solids: SMAQ Super Magic Angles for Quadrupolar (and other) Interactions, A. Llor and J. Virlet


A21, 22, Principles and Application of 3D NMR Spectroscopy, C. Griesinger, O.W. Sorensen and R.R. Ernst

A22, 23, New Twists to some old Experiments, A. Bax, D. Marion, L. Lerner and R. Tschudin


A24, 25, 3D NMR - Prospects and Limits, H. Armitage and D. Ziessow

A25, 26, Multidimensional Correlation Spectroscopy, R. Freeman, J. Friedrich and S. Davies

A26, 27, Self Diffusion Measurements, Spatial Localization and Signal Suppression by use of rf Field Inhomogeneity, D. Canet, D. Boudot and A. Belmajdoub

A27, 29, Pulse Spectroscopy with Quantized Fields, J. Jeener

A28, 30, Nonlinear Incoherent Spectroscopy, B. Blmich and J. Paff

A29, 31, High Resolution NMR on Organic Conductors, M. Mehring, H. Helmle, F. Hentsch, D. Kngheter and U. Rempel

A30, 32, Various Applications of NMR Imaging Methods outside the Medical Field, M. Sauzade

A31, E. Lippmaa no abstract
LIST OF POSTERS:

P1, 33, Speculations on Rotating Frame Heteronuclear NOE Experiments, G. Batta and K.E. Käver

P2, 34, DEPT Based Heteronuclear Correlations with Improvements, K.E.Kver and G.Batta

P3, 36, Pulse Shaping and Selective Excitation : The Effect of Scalar Coupling, R. Bazzo, J. Boyd and N. Soffe

P4, 37, Soft Pulses Applied to 2D NMR Experiments, R. Brscheiler, C. Griesinger, O.W. Sorensen and R.R. Ernst

P5, 38, New Approaches to the Computer Analysis of Two Dimensional NMR Spectra, B.U. Meier, S. Boentges, Z.L. Madi and R.R. Ernst

P6, 39, Optimization of Polarization Transfer Experiments taking into Account Transversal Relaxation, A. Bauer and N. Müller

P7, 40, Digital Quadrature Detection in NMR Spectroscopy, M. Rydzy, D. Ziessow and T. Keller

P8, 41, Transient Nutations for Pulsed RF Fields, A. Schfer and D. Ziessow

P9, 42, Phase Coherence and Solvent Suppression in Rotating-Frame Experiments, G. Esposito, W.A. Gibbons and R. Bazzo

P10, 43, HOMOTANGO - an Alternative to INADEQUATE ?, V.M. Zaloznik and L.A. Fedorov

P11, 45, ADPT (Adiabatic Polarization Transfer) - New Polarization Transfer Mechanism, which does not depend on J-Constant Value, V.M. Zaloznik and L.A. Fedorov

P12, 47, NOESY-TOCSY and ROESY-TOCSY: The Application of 2D-NMR Techniques Containing Spin-Lock Sequences for the Sequential Assignment of Peptides, H. Kessler, G. Gemmecker, B. Haas and S. Steuernagel


P14, 50, Improved One-Dimensional Correlation Experiments, L.D. Hall and T.J. Norwood

P15, 52, A Simple rf Phase Cycling Scheme, J. Higinbotham, D.D.M. Allan and I.C. Malcolm


P17, 55, Interproton Distances in Proteins from NMR Crossrelaxation in Laboratory and Rotating Frame, J. Fejzo, Z. Zolnai, S. Macura and J.L. Markley
P18, 56, Compression of 2D NMR Spectra, Z.Zolnai, S.Macura and J.L.Markley

P19, 57, 2D NMR Relaxation Spectroscopy: Theory and Applications, K.Miller, A.Schleicher and G.Kothe

P20, 59, Generalization of construction of correlation space (CCS), S.Szalma, I.Pelczer and G.Dombi

P21, 60, Indirect, Negative NOE on the Homonuclear Two-Dimensional Spectrum, I.Pelczer and Z.Rozsa

P22, 61, The E.COSY Experiment without "Small-Angle" Phase Shifts, P.Praestholm, H.Bildsoe and J.C.Madsen


P24, 63, Symbolic Algebra: A Useful Tool for NMR?, A.Khuen and P.Forster


P26, 66, $^1$H NMR Studies of Protein Folding, M.J.Bogusky and C.M.Dobson


P28, 68, Application of M.E.M. to the Study of a ten Base-Pair DNA Fragment, M.A.Delsuc, V.Stoven, E.Guittet and J.Y.Lallemand

P29, 69, Linear Prediction Applied to the Analysis of NMR-data from p21 Kinetics Measurements, H.Ruff, I.Schlichting and P.Rösch

P30, 70, Three-Dimensional Structure of Acyl Carrier Protein in Solution Determined by Nuclear Magnetic Resonance and a Combined Use of Molecular Mechanics, Molecular Dynamics and Distance Geometry, T.A.Holak, M.Nilgens, J.H.Prestegard, A.M.Gronenborn and G.M.Clore

P31, 71, Conformational Analysis of a cyclic all-L-Hexapeptide, M.Hofmann, M.Gehrke, H.Kessler and W.Bremel


P33, 73, The ROESY Experiment. Application to the Solution Conformation of the Synthetic Tubulin Fragment Ac-Tubulin Alpha (430-441)-amide, A.Otter and G.Kotovych
P34, 75, HI Two-Dimensional NMR Techniques as Applied to Globular Proteins-

P35, 76, Structure Confirmation of the Active Part of Haemophilus
Influenza Type B Vaccine, J.R. Melema, G.N.Wagenaars, C.W.Funke, P.L.Jacobs and
C.A.A. van Boeckel

P36, 77, Eromomycin - A Novel Glycopeptide Antibiotic; The Use of
Dipolar Interactions, G.Batta, K.E.Kver, T.F.Berdnikova and F.Sztaricskai

P37, 78, Vancomycin and Vancomycin-D-Ala-D-Ala Structures: an integrated
approach employing 2D NMR and energy minimisation
with distance constraints, L.Y.Lian, G.Hawkes, H.Molinari and K.Sales

P38, 80, 3D-NMR of Proteins in Solution, H.Oschkinat, P.J.Kraulis, C.Cieslar, A.M.Gronenborn and G.M.Clore

P39, 81, Sequence Specific $^1$H NMR Assignment and Secondary Structure
Determination of a 124 Residues "Large" Protein, S.Ludvigsen and F.M.Poulsen

P40, 82, $^1$H-NMR of the Rop Protein, W.Eberle, A.Kingswell, G.Cesareni and P.Roesch

P41, 83, Proton NMR of the P21.GDP.Mg$^{2+}$ Complex of the HA-RAS
Oncogene Product, I.Schlichting, J.John, A.Wittinghofer and P.Rsch

P42, 84, Structural Studies of the O-antigenic Polysaccharide of
Escherichia coli 0,86, with blood group B activity, N.Andersson, N.Carlin, K.Leontein, U.Lindquist and K.Slettengren

P43, 85, Structural Investigations of Head Activator by One- and
Two-Dimensional $^1$H-NMR Spectroscopy, R.Saffrich, H.R.Kalbitzer, H.C.Schaller and J.Postma

P44, 87, Refinement of the Three-Dimensional Solution Structure of Barley
Serine Proteinase Inhibitor 2 using Stereospecific Assignment, S.Hengyi, M.Kjr, O.W.Sorensen and F.M.Poulsen

P45, 88, Carbon-13 and Deuterium NMR Studies on the Liquid and
Solid Phases of Globular (CH$_3$)$_3$ CX Compounds, D.W.Aksnes and K.Ramstad

P46, 89, Magnetic Shielding Tensor Components in Highly Stained Phosphines, A.L.Barra and J.B.Robert

P47, 91, $^2$H-NMR-Spectroscopy of Oriented Phospholipid Bilayers, W.Hbner and A.Blume

P48, 93, Design of an Efficient Probe for Cross-Polarization
Variable-Angle Spinning NMR of Solids, P.Daugaard, V.Langer and H.J.Jakobsen
P49, 94, High Speed $^{27}$Al and $^{29}$Si MAS NMR Studies of Zeolite Structures and Clays. Determination of $^{27}$Al Quadrupole Coupling Constants and Accurate $^{27}$Al Chemical Shifts, H. Jacobsen, H. Bildsoe and H.J. Jakobsen

P50, 95, Spin Dynamics and Ferro to Paraelectric Transition in Polyvinylidene Fluoride-Trifluoro Ethylene Copolymer, B. Meurer, J. Hirschinger and G. Weill


P52, 98, Backbone Fluctuations of Proteins and Polypeptides as seen by Solid-State NMR Techniques: Proton Field Cycling NMR and Deuteron-NMR, W. Nusser, R. Kimmich and F. Winter

P53, 100, Two-Dimensional Quadrupolar Echo Spectroscopy of $^{13}$I Nuclei. Spin Relaxation in Anisotropic Systems, B. Halle, T.C. Wong and I. Furo

P54, 102, Domain Structure of Distinct Silicagels Revealed by $^{29}$Si-CP-MAS Spectroscopy, B. Pfleiderer, K. Albert, E. Bayer, J.W. de Haan and L.J.M. de Ven

P55, 104, Field-Cycling NMR Study of Collective Molecular Motions in Smectic Liquid Crystals, D. Pusiol and F. Noack

P56, 106, Phosphorous Homonuclear Correlation Spectroscopy in the Solid State, Han Xiwen and H. Ruegger

P57, 107, Deuteron 2D Exchange NMR of Solids, C. Schmidt, B. Blmich and H. W. Spiess


P59, 110, Linear Prediction, Application to High Resolution Solid State NMR Spectroscopy, D. S. Stephenson and A. Sebald

P60, 111, Linear Prediction, Spectral Transformation Using a Recursive Technique (STUART), D. S. Stephenson


P62, 113, $^{13}$C CP-MAS NMR Spectra of some Aromatic Sulfur Amides, A.M. Hkkinen and P. Ruostesuo

P63, 114, Distribution of Rotational Angles from Deuteron 2D-Exchange Spectra, S. Wefing, S. Kaufmann and H.W. Spiess

P64, 115, $^{2}$H and $^{13}$NMR Measurement of the Molecular Dynamics, Morphology, and Order in Stiff Macromolecules with Liquid Crystalline Mesophases, A.K. Whittaker, U. Falk, A. Adam and H.W. Spiess
P65, 116, Dynamic Nuclear Polarization Studies of a Molecularly Doped Polymer, R.A. Wind, L. Li and G.E. Maciel

P66, 117, Two-Dimensional $^{13}$C-MAS-NMR, Y. Yang, M. Schuster, A. Hagemeyer, B. Blmich and H.W. Spiess

P67, 118, $^{23}$Na NMR in Solids, K.M. Larsson, C.M. Dobson and S.R. Cooper

P68, 119, Tissue Characterization by Parameter Extraction from Nuclear Relaxation Dispersion Curves, H.W. Fischer, P.A. Rinck, A. Lowenthal, D. Karcher, L. Vander Elst, Y. van Haverbeke and R.N. Muller

P69, 121, High Resolution Heteronuclear Spectroscopy in Inhomogeneous Magnetic Fields, S.L. Duce, L.D. Hall and T.J. Norwood

P70, 123, Solid State NMR Imaging Using "Solid Echoes", J.J. Attard, P.J. McDonald


P72, 126, $^1$H COSY Spectra of Superfused Brain Slices of Rat: ex vivo Direct Assignment of Resonances, B. Gillet, S. Mergui, J.C. Beloeil, J. Champagnat and G. Fortin


P75, 130, Imaging of a Rotating Object, S. Matsui, K. Sekihara, H. Shiono and H. Kohno

P76, 132, Image Processing Techniques Applied to 2D NMR Spectroscopy, P. Sole, F. Delaglio and G. Levy


P78, 135, Device for Measuring the Contractile Force of Muscular Samples Designed for NMR Study, A. Schank and F. Baguet

P80, 138, Determination of Partial Structures of Dissolved Humic Acids by One and Two Dimensional $^1$H and $^13$C NMR Spectroscopy and by $^1$H NMR Spectroscopy, J.Buddrus, P.Burba, H.Herzog and J.Lambert

P81, 139, First High-Pressure, High-Resolution NMR Probe Working at 400 MHz, U.Frey, L.Helm and A.E.Merbach

P82, 140, Molecular Correlation in Liquid Solutions Studied by NMR of Magnetically Aligned Molecules, E.W.Bastiaan, E.T.J.Nibbering and C.MacLean

P83, 142, The Quadrupole Coupling Tensor of $^{95}$Mo in Mesitylene Molybdenum tricarbonyl, L.Huis, P.J.W.Pouwels and C.MacLean

P84, 144, La-NMR-Spectroscopy : Solution Studies on Triscyclopentadienyl La(III)-Derivatives, S.H.Eggers, M.Adam, E.T.K.Haupt and R.D.Fischer

P85, 145, Optical Purity Studies on some Dihydropyridinic Compounds, C.Marchioro, N.Fonte, M.Perchinum and R.Mica

P86, 147, A Portable NMR Spectrometer for Automatic Measurements in Plants, P.A.de Jager, J.E.A.Reinders, G.Polder and H.Van As

P87, 149, Determination of the Configuration and Conformation of Tetracyclic Partly Saturated Benzoxazines by NMR, P.Haas, J.Blach and W.Robien


P89, 152, Carbon-13 Relaxation in Sucrose Outside of the Extreme Narrowing Region, H.Kovacs and J.Kowalewski

P90, 153, Optical Purity Studies on some Dihydropyridinic Compounds, C.Marchioro, N.Fonte, M.Perchinum and R.Mica


P92, 155, Determination of the Configuration and Conformation of Tetracyclic Partly Saturated Benzoxazines by NMR, J.Pelzer, G.Dombi, L.Lazar, B.Flöp and G.Bernath

P93, 156, $^{13}$C NMR Applied to the Study of Lignin Structure, D.Robert, M.Bardet and K.Lundquist

P96, 160, Steady-State Inversion Recovery Technique for Slowly Relaxing Nuclei in Liquids,
W.Kreibich and A.Schwenk

P97, 161, Possible Conformational Dependence of Long Range Deuterium Isotope Effects on C-13 Chemical Shifts,
D.Vikic-Topic and Z.Meic

P98, 162, Assignment of Endgroup Resonances of Polyamide-4,6,
N.K.de Vries, H.A.J.Linssen and G.van der Velden

P99, 164, The ESR Investigation of the Core-Surface Relationship in the Low Density Lipoproteins,
J.Brnjas-Kraljevic, G.Pifat, J.N.Herak and G.Knipping

P100, 165, Molecular Dynamics of Crown Ethers,
M.Geringer and H.Sterk

P101, 166, On the Molecular Dynamics of Nucleosides and Nucleotides,
R.Konrat and H.Sterk

P102, 167, $^{29}$Si Doublequantum Coherence Spectroscopy (INADEQUATE)
A Powerful Method for the Structure elucidation of Silicon Framework,
F.Schrank, F.K.Mitter and E.Hengge

P103, 168, Nuclear Magnetic Relaxation Dispersion Measurement of the Dimerization of HEPW Lysozyme,
H.H.Raeymaekers, A.Verbeken, H.Eisendrath, Y.Vanhaverbeke and R.Muller

P104, 169, Determination of the $^1$H and $^{13}$C Shielding and $^{13}$C-$^{14}$N Spin-Spin Coupling Anisotropies for Methylisocyanide in Liquid Crystals,
J.Jokisaari, Y.Hiltunen, J.Lounila and A.Pulkkinen

P105, 170, Secondary Structure Analysis of the Initiation Factor of the Protein Biosynthesis of E.Coli IF1 by 2D NMR,
M.Paci, R.Boelens, C.L.Pon, C.Gualerzi and R.Kaptein
METABOLIC SPECTROSCOPY AND HIGH-FIELD IMAGING IN CANCER RESEARCH
Jean-Marc LHÔSTE, INSTITUT CURIE, Section de Biologie (U.219 INSERM),
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Methodological and instrumentation problems for metabolic investigations both in vivo, from mouse to rabbit, and ex vivo, in cultured cells and perfused organs, will be discussed from a practical two-year experience of imaging at high-field (4.7 Tesla) and multinuclear spectroscopy up to 9.4 Tesla.

The designing of probes specific for each animal and/or each organ to be explored is a prerequisite for a good sensitivity and a first step towards localized spectroscopic observations. Inductively coupled resonators have been developed for high resolution imaging as well as a large variety of surface coils or solenoids for spectroscopic investigations up to real-time kinetics.

Proton imaging at 4.7 Tesla requires routinely the spectroscopic separation of water and lipid signals since the chemical shift artifact may overcome the resolution by one order of magnitude. A technique named C4S (for chemical shift specific slice selection) has been developed for this selection. It is based on use of field gradient reversal for the specific detection of chemical species whose resonances are selectively excited in non overlapping slices.

The method has now been extended to sensitive volume localisation for spectroscopic observations at the resolution required in small animals. For this purpose, we combined the C4S method which offers the possibility to observe different lines at the same time in the same volume with well-known techniques of positive selective excitation (derived from ISIS and SPARS) resulting in the FRIVOL sequence (frequency interval selective volume localisation). For protons, this method eliminates easily the water signal and permits a good resolution, e.g. 1 ml for lactate observation in rabbit brain. Spin-echo techniques result however in loss of sensitivity for other nuclei. Localized observation by implanted surface coils or solenoids is still preferable; this will be illustrated by metabolic follow up of treated experimental tumors, by real time kinetics of phosphorylated metabolites in acute ischemia, by direct observation of metabolic transformation of fluoropyrimidines in rats by 19-fluorine NMR and of carbon-13 labeled metabolic precursors of glucose and aminoacids synthesis.

The in vivo metabolic investigations still suffer from quantitative uncertainties and physiological variability. They are favorably complemented by ex vivo spectroscopy in perfused organs and cells. A quantitative modelling of metabolic fluxes in then possible using carbon-13 precursors. This will be illustrated in the normal and leukemic liver of mice. Furthermore, a parallel in vitro analysis is required for a complete functional analysis including both resonance intensities and spin-spin couplings. Perspectives of development of comparable quantitative functional tests in living animals, or man, will be discussed.

(collaborators: J-L.DIMICOLI, F.MEGNIN, J-F.NEDELEC, J.MISPELTER, J.PATRY, E.QUIGNOU, B.TIFFON and A.VOLK)
Localized MR spectroscopy in humans

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Initially, MR spectroscopy studies on humans were performed using surface coils for mere RF excitation, and for detection of the ensuing signal. The advantage of surface coils is that they provide optimal sensitivity for superficial tissues. The use of surface coils has been particularly successful for the study, by means of 31P MR spectroscopy, of energy metabolism in muscle.

The MR spectroscopy examination of other human tissues requires the application of localization procedures in order to eliminate signal from overlying or surrounding tissues. A number of techniques have been proposed to achieve this (1). Each of these has certain advantages and disadvantages.

One class of experiments ("BI techniques") uses the inhomogeneous RF field of surface coils for depth selection. In practice, none of these experiments works satisfactorily when applied with a single surface coil. The shape of the sensitive volume follows the BI profiles of the surface coil used. And as a result the sensitive volume curves outward toward the plane of the coil. In addition, high flux regions near the coil wire may also contribute to the signal. The usual way to overcome the problems inherent to BI techniques is to apply a double coil configuration, whereby the outer coil is used for transmission, and the inner coil for reception only.

A second class of experiments ("BO techniques") uses selective RF pulses and switched magnetic field gradients. These techniques have the advantage that they are related to NMR imaging. With them, it is possible to define the sensitive volume for the spectroscopy examination on the basis of a 1H image acquired during the same session. A disadvantage of this type of techniques is that the use of switched gradients may adversely affect spectral resolution.

In this talk, we will present recent results of localized MR spectroscopy performed on a whole-body imager in a clinical setting. Phosphorus-31 localized MR spectroscopy of the human liver has been achieved following 2 different single surface coil approaches. In a first, the liver 31P MR spectra are obtained combining BI and BO selection (2). The BO selection serves to eliminate the undesired regions which are part of the sensitive volume defined by the BI
selection process. In a second approach, only BO selection is applied
("1H image-guided localized MR spectroscopy"). The temporal resolution
of the latter type of measurement is such that functional studies of the
liver can easily be undertaken (3). 1H image-guided techniques have
also been applied to 31P and 1H MR spectroscopy studies of human brain
tumors (4). Preliminary results will be shown.

References:

    75:345.
IN VIVO AND IN SITU PROTON SPECTROSCOPY USING VOLUME SELECTION

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The obtention of spatially resolved NMR spectra can now be achieved by using a number of methods. We describe the use of a technique derived from the Volume Selective Excitation experiment. The complete pulse sequence for three dimensional spatial localization consists of three symmetrical pulses sandwiches and is followed by a water suppressing pulse sequence for observation. Each sandwich is composed of a phase cycled selective refocusing pulse between two hard 90° pulses:

\[
\begin{bmatrix}
90_x, 180, 90_x \\
90_y, 180, 90_y \\
90_z, 180, 90_z \\
\end{bmatrix}
\]

The three sandwiches are applied in presence of x, y, and z \( B_z \)-field gradients respectively, giving rise to a three dimensional volume selection. Each refocusing pulse is phase cycled according to the Exorcycle scheme. Finally after a delay which is necessary for allowing unwanted transverse magnetization to dephase and eddy currents to decrease, the water suppressing hard pulse sequence is applied.

The sequence named SPALL has been used for in vivo experiments on experimentally induced intracerebral tumors in rat. A single-turn circular coil was chronically implanted on the skull. After measuring the coordinates of the tumor by imaging the rat brain, \(^1\)H NMR spectra of the tumor were acquired using a spin echo hard pulse sequence with delayed acquisition in order to avoid any first order phase correction:

\[
[1-\tau-1] - T_e - [2-\tau-2]_{tx} - (T_e+\tau/2) - Acq.
\]

The comparison of normal brain spectra with tumor spectra reveals drastic differences. These spectral findings have been confirmed by \( B_z \)-gradient localization methods using very small surface coils implanted in the vicinity of superficial brain tumors.
The main problem of in vivo spectroscopy is the precise selection of the region of interest with sufficient sensitivity. Image-guided, simultaneous spectroscopy of multiple volumes is possible via the application of frequency selective pulses composed of several excitation frequencies. The feasibility of multivolume $^{31}$P-NMR spectroscopy will be demonstrated for phantoms and for human heart. The availability of a rampable magnet allows routine MR-Imaging at 1.5 T and MR-Spectroscopy at 2 T with only 20 min intervals. Deuterium MR-Imaging will be introduced as a new method for contrast enhancement and flow. The application of $^{13}$C-NMR to the in vivo study of liver metabolism of rat will be discussed.
31P AND 1H NMR STUDIES OF BRAIN METABOLISM IN VIVO

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NMR spectroscopy provides a non-invasive method of studying energy metabolism, and has been widely used to monitor the metabolic changes associated with ischaemia (i.e. reduced blood supply) such as occur in stroke. A central issue is the relationship between regional blood flow, tissue metabolism and tissue function. In collaborative studies with colleagues at the Institute of Neurology and the MRC Cyclotron Unit, we have developed methodology whereby NMR spectra and regional blood flow can be measured concurrently and repeatedly in experimental animals. The flow measurements are made not by NMR, but by the hydrogen clearance method, while the combined use of 1H and 31P NMR permits measurements of ATP, phosphocreatine, inorganic phosphate, lactate, and intracellular pH. Among our results, we have found an abrupt change in the metabolic state of the gerbil brain when the regional cerebral blood flow falls to 20 ml/100g/min or below (1). This level of flow is similar to the threshold at which, in a variety of species, electrical failure ceases. We therefore suggest that the flow threshold for electrical function is a direct consequence of energy failure arising from an insufficient supply of oxygen.

Our colleagues at the Hammersmith Hospital NMR Unit have recently implemented the technique of four dimensional chemical shift imaging for 31P studies of human brain and liver metabolism (2). This allows simultaneous acquisition of spectra from multiple volume elements, and therefore permits direct comparison of spectra from healthy and diseased regions. In this lecture, we shall discuss the methods used for the animal and human studies, together with some recent results.

Spatially resolved NMR methods are used for non-invasive investigations of living matter. Two fields of research have been developed: (1) NMR-imaging using the $^1$H-NMR-signal to detect morphological information with a spatial resolution far less than 1 mm, and (2) NMR-spectroscopy using signals of $^{31}$P or $^{13}$C nuclei to investigate the biochemical state of tissues. The combination of both techniques became possible with the development of high-field large-bore magnets. We have described three methods to combine NMR-imaging and -spectroscopy: (1) Chemical shift selective (CHESS) imaging provides the spatial distribution of a well-defined substance within the tissue. The substance has to be identified by its NMR-resonance frequency. (2) Spectroscopic imaging which gives an NMR-spectrum for each image element. (3) Localised spectroscopy (LOCUS-spectroscopy) of a well-defined region of interest without affecting the spin-system by the localisation procedure. These techniques need long measuring times which have to be avoided for practical studies in living matter. Therefore, we have developed a rapid imaging sequence (FLASH-imaging). The combination of NMR-imaging and NMR-spectroscopy became possible by the application of this fast imaging method. Experiments for the combination of these techniques will be presented. A few of these techniques are now routinely used in medicine. First applications of spectroscopic FLASH imaging in animal studies will be shown.
NMR Studies of Protein Structure and Protein-DNA Interactions

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Recent developments in two-dimensional NMR spectroscopy allow the structure determination of small biomolecules in solution. These structures are based primarily on nuclear Overhauser effects (NOE's) augmented with information from J-coupling. The information from NOE's and J-couplings is converted via suitable calibration procedures into constraints on proton-proton distances. In order to improve the accuracy of the distances derived from NOE's an iterative procedure for structure determination has been devised based on the full relaxation matrix approach. Since the effect of spin-diffusion is fully taken into account, especially the longer distances can be determined much more accurately.

Algorithms for the structure determination on the basis of these distance contraints include Distance Geometry and Restrained Molecular Dynamics. A combination of these appears to be especially useful and this will be illustrated with the structure determination of the N-terminal DNA binding domain or headpiece of lac repressor for which no crystal structure is known.

2D NOE studies of complexes of lac headpiece with lac operator fragments will be presented. A large number of protein-DNA NOE's has been identified. These are all consistent with a single headpiece-operator complex. An interesting feature of the structure of this complex is that the orientation of the headpiece with respect to the pseudo two-fold symmetry axis of the operator is opposite to what has been found for other DNA binding proteins. Implications of the structure for the more general problem of protein-DNA recognition will be discussed.

References:
The introduction of spin-lock experiments (TOCSY and ROESY) offers a number of interesting applications. For medium sized molecules ROE allows to observe cross-relaxation for qualitative and quantitative through-space connectivity.

Also two-step magnetization transfers from the Relayed-NOESY type can be performed using TOCSY (homonuclear Hartmann-Hahn transfer via MLEV 17) instead of the COSY transfer and/or ROESY instead of the NOE mixing. For medium sized molecules a ROESY-TOCSY combination or the reversed sequence (TOCSY-ROESY) are recommended. These pulse sequences yield asymmetric spectra from which the symmetric part can be removed by special data handling procedures. For larger molecules also NOESY-TOCSY or TOCSY-NOESY, respectively, can be used. Their application for peptide sequencing is demonstrated.

Quantification of ROESY spectra for the determination of intramolecular proton-proton distances is possible, if contributions from J-coupling are minimized by a low lock-field and setting the carrier frequency adequately. Applications of thus obtained ROE values for structure determination of cyclic peptides in connection with molecular dynamics calculations are presented. For the MD calculation a charge release of the solvent exposed NH protons is recommended to take into account the effects introduced by calculating in vacuo. Side chain conformations are derived from homo- and heteronuclear vicinal coupling constants. The results are compared with results from X-ray analysis of crystalline samples.
NMR spectroscopy of proteins provides an experimental basis for developing an understanding of the manner in which a polypeptide chain folds to form the compact structure of a globular protein. A major objective is to characterise structural changes which occur during the folding process. One approach to this is to trap intermediate species during folding, and then to use NMR to explore their structural features. This approach has recently been used to study folding pathways of BPTI. An alternative approach which will be described here is to establish conditions where folded and unfolded or partially folded states exist in dynamic equilibrium. This enables the kinetics of their interconversion to be measured, by exploiting chemical exchange phenomena, and the structural relationships between the various states to be explored. Results for this presentation will be selected from recent studies of a variety of proteins including lysozyme, α-lactalbumin and staphylococcal nuclease.


DETERMINATION OF THREE-DIMENSIONAL STRUCTURES OF PROTEINS IN SOLUTION

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The determination of 3D-structures of proteins in solution using NMR spectroscopy comprises three stages: (i) the assignment of proton resonances by 2D-techniques to demonstrate through-bond and through-space connectivities; (ii) the determination of a large number of short (< 5Å) interproton distances using nuclear Overhauser effect (NOE) measurements; and (iii) the determination of the 3D-structure on the basis of these distances using distance geometry, dynamical simulated annealing and/or restrained molecular dynamics. This will be illustrated by the set of 3D-structures in solution that we have determined to date: purothionin, phoratoxin, hirudin, the globular domain of histone H5, growth hormone releasing factor, secretin, potato carboxypeptidase inhibitor, barley serine proteinase inhibitor 2, acyl carrier protein, the anaphylatoxin C5a and cecropin.
NMR provides a wealth of structural information about proteins in solution, but does not by itself permit an unambiguous determination of a unique structure. A method will be described for determining the family of protein structures compatible with a given set of NMR solution data. The method, encoded in an expert system called PROTEAN, systematically samples conformational space and excludes conformations which are not compatible with the data until only structures compatible with the data remain. The apparent computational intractability of this approach is reduced by assembling the protein in pieces, by considering the protein at several levels of abstraction, by utilizing constraint satisfaction methods to consider only a few atoms at a time, and by utilizing artificial intelligence methods of heuristic control to decide which actions will exclude most conformations. Examples of results will be presented.
STRATEGIES FOR NMR STUDIES
OF DILUTE AQUEOUS SOLUTIONS

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The conformational study of biological molecules in solution is nowadays one of the most exciting challenges facing N.M.R. spectroscopy. As most of the crucial information is brought by the exchangeable protons in these molecules (amide or amine protons of DNA/RNA bases, peptide bonds, amino acids...), it is necessary to record most of the spectra in non-deuterated water.

Two major problems have thus to be faced: i) the study of correlations involving protons giving signals in the proximity of the water line (such as $H_\alpha$ of the peptide bond), and ii) the limited dynamic range of the analog-digital converter: the reduction of the water signal has therefore to be realized before each acquisition (and not only by phase cycling).

As an alternative to classical solvent suppression methods (presaturation, WEFT, selective "soft" or gaussian pulses) one may devise sequences which are exclusively written with "hard" pulses. These modified sequences follow a binomial suppression scheme; the durations and phases of their pulses are set parallel to those of usual $1\,1\,2$ or $1\,3\,3$ pulse trains. One takes here advantage of their analogy to current $90^\circ-90^\circ$ or $90^\circ-180^\circ-90^\circ$ trains (for example). The suppression of water is thus achieved by the global sequence, and not by some included selective pulse; consequently, the only "suppressed" part of the 2D surface is, theoretically, a single point at $F_1 = F_2 = F_{\text{solvent}}$. Moreover, the experimental settings of the experiment proved to be easy: there are no delays or pulse power to adjust.

Thus, COSY, HOHAHA or INADEQUATE sequences may be fitted directly. The method may also be combined with older "classical" ones (such as WEFT), in order to cover the entire range of usual sequences (including, for example, the RELAYed experiment).

Even with such water reduction, the conditions remain of the "high dynamic range" type, and quantization errors are an important source of apparent "noise" in the spectrum. They may be reduced using the "oversampling" procedure, which consists in the recording of more points (better digitization) within the same total acquisition time. The windows of the analog "Butterworth" filters are kept constant, but the spectral width after FT is increased. The supplementary points recorded may exceed, during a 2D experiment, the memory available on current systems. It is nevertheless possible to keep only the significant part of the data, storing, for each experiment, the fictitious FID obtained by inverse FT of a truncated spectrum.

To summarize, these conditions are of the most demanding kind for the experimentalist. Many optimized approaches have therefore to be combined, including the very promising use of non-linear methods, such as the iterative spectrum reconstruction based on the maximisation of an "entropy" function.
The Utility and Manipulation of NOEs in Paramagnetic Molecules

by

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The presence of paramagnetic centers introduces an efficient alternate relaxation mechanism to \(^1\)H-\(^1\)H dipolar relaxation, and hence invariably leads to reduction in the magnitude of any nuclear Overhauser effects, often rendering them undetectable. For the special class of paramagnetic molecules for which the electron spin-lattice relaxation time provides the overwhelmingly dominant correlation time for the nuclear-electron relaxation, the paramagnetic relaxation is essentially independent of the frequency of overall molecular motion. Under such circumstances, it is possible to separately manipulate the \(^1\)H-\(^1\)H cross-relaxation rate, \(\sigma\), by changes in molecular reorientation time, \(\tau_c\), without significantly altering the \(^1\)H spin-lattice relaxation time. By decreasing the molecular reorientation time appropriately, the diamagnetic relaxation mechanism become more effective, with the result that the steady-state NOE is increased essentially linearly with \(\tau_c\). The results of the manipulation of the NOEs between similarly disposed pairs of protons using increasingly larger isostructural proteins, as well as a variety of agents to markedly increase solvent viscosity, will be described. In essence, valuable NOEs can be detected in any paramagnetic molecule under appropriate conditions. For macromolecules, the paramagnetism has the added advantage of suppressing spin-diffusion at very large molecular weights.
1. PHASE IN QUANTUM MEASUREMENT
2. NEW ANGLES IN SAMPLE SPINNING

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Topic number 1 is concerned with work in our laboratory by G. Chingas, K. Mueller, and D. Suter(1), and in collaboration with R.Y. Chiao of the Physics Department at Berkeley and M.V. Berry of the Physics Department at Bristol University. When a quantum measurement is performed, the state of the system "collapses" to an eigenstate of the observable associated with the measuring apparatus. An interesting question is whether there is any phase relationship between the collapsed and original quantum states. I shall show that such a phase relationship, first described by Pancharatnam, indeed exists, and that it has a geometrical interpretation related to the Berry and/or Anandan phases for cyclic evolutions(3). The relevance of these phases in NMR experiments will be illustrated.

Topic number 2 is concerned with work in our laboratory by C.J. Lee(2), A. Samoson (visiting from Tallinn, USSR), B.Q. Sun, D. Suter and T. Terao (visiting from Kyoto, Japan). Under magic-angle spinning (MAS), the first order (truncated) quadrupolar (or dipolar) interactions are averaged to zero, but the second order terms give rise to powder patterns which currently constitute the limitation to high resolution MAS of quadrupolar nuclei. By implementing more general mechanical circuits, such as nutation or hopping of the spinning axis, it is possible to average the second (and higher) order terms to their isotropic values, thereby producing a sharp spectrum. We term this approach SOS for Second Order Spinning or Second Order Spectroscopy. Related experiments are under way in the group of J. Virlet in Paris(4).


(1) Current address: Laboratorium für Quantenoptik, ETH, Zurich.
(4) J. Virlet, private communication.
Conventional NMR imaging of solids suffers from the large NMR linewidth in solids. To obtain high resolution very large field gradients have to be used and this implies low sensitivity (and for that reason low resolution) and makes uniform excitation difficult.

A solution to this problem is first to narrow the proton NMR linewidth by coherent averaging techniques and then apply NMR imaging. Since we did not want to loose the possibility to selectively excite and image certain solid components in a heterogeneous solid material, we choose to eliminate the anisotropy of the chemical shift by magic angle spinning and the proton dipolar interaction by MREV-8.

The conceptually easiest approach to NMR imaging in combination with these line narrowing techniques, is to employ a field gradient that rotates synchronously with the spinner [1,2]. Our experimental approaches to high-resolution NMR imaging of solids will be discussed and demonstrated with some results.

TWO DIMENSIONAL NMR TECHNIQUES FOR STUDYING MOLECULAR STRUCTURE AND DYNAMICS OF AMORPHOUS SOLIDS

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Solid state NMR offers unique possibilities for a detailed characterization of molecular order and dynamics in amorphous solids and therefore should provide the basis for a better understanding of those materials on a molecular level. In recent years, deuteron NMR has proven especially powerful in this area. Highly selective information without the need of isotopic labelling may also be obtained from $^{13}$C-MAS-NMR.

New developments in this area involve two-dimensional techniques. It will be shown that the jump angles resulting from molecular motions can directly be projected into 2D-exchange NMR spectra of $^2$H-spin alignment or $^{13}$C-stimulated echo spectra. This technique allows, therefore, the characterization of motional mechanisms without the need for interfacing a model. Rotor synchronized 2D-MAS-$^{13}$C-NMR will also be described. It offers a means to detect molecular motion, order and conformation of the individual residues of a macromolecule without the need of isotopic labelling.

The techniques will be illustrated on various polymers concentrating on the chain dynamics in the vicinity of the glass transition and the molecular order in PET-fibres and liquid crystalline polymers in their glassy state.
ROTATIONAL RESONANCE IN SPIN-PAIR SYSTEMS IN SOLIDS


Magic angle sample spinning has traditionally been performed on samples where the spin systems fall into one of two classes: magnetically dilute spins, and infinite networks of tightly coupled spins. In the former case, common for natural abundance $^{13}$C spectroscopy in the presence of $^1$H decoupling, the sample rotation only affects the spin eigenvalues, leading to the well-known phenomena of rotational echoes and spinning sidebands. In the second case, typical for $^1$H spectroscopy, the eigenstates are also strongly affected, and the sample must be rotated very rapidly to observe a narrowing effect. We have recently examined samples containing isolated pairs of dipolar-coupled spins. In general, their behavior is similar to the first group under normal circumstances, but under particular rotational resonance conditions, behavior associated with the second group is observed. Line splittings and broad spectral patterns are found which contain much information as to interaction tensors and their mutual orientation. The observations can be matched with numerical simulations. In heteronuclear systems, rotational resonance can be established by an applied rf field of intensity such that $\omega_{\perp} = n\omega_{r}$, where $\omega_{\perp}$ is the nutation frequency and $\omega_{r}$ the spinning speed. In homonuclear systems, the condition $\omega^{\text{iso}} = n\omega_{r}$ must be matched, where $\omega^{\text{iso}}$ is the separation between isotropic shifts. The onset of rotational resonance is indicated by dramatic spectral perturbations and strong enhancement of Zeeman-order exchange between the spins. Spin-pair systems can be engineered by selective isotopic labeling, so these experiments might become important for deriving certain types of elusive structural information.
"Towards High Resolution NMR of Any Nucleus in Solids:
SMAQ: Super Magic Angles for Quadrupole (and other) interaction(s)"

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In solids, High Resolution NMR spectra can be obtained by Magic Angle Spinning techniques (MAS, CRAMPS, etc...). These methods, which work nicely for spins 1/2, are unable to fully remove the broadening due to strong quadrupolar interaction of half-integer spins.

It is shown here that new averaging experiments can be devised which are able to remove not only, as MAS, the first order broadening, but also the second order or higher order broadenings.

Detailed theory and experimental realisation of such an experiment ("SMAQ") are described, which averages out the second order broadening of the central (-1/2 → 1/2) transition of half-integer quadrupolar spins.

Other ways of implementation of such methods are discussed.

A review is also given of some other sources of broadening where MAS averaging is ineffective and where this new class of experiments could be applied: amongst others, for spins 1/2, the dipolar coupling when the static magnetic field is low, or when the coupling is with a quadrupolar spin or a paramagnetic center.

Similar work is, independently, in progress at Berkeley.
SEhUT Spectral Editing, Calibration of Rf Field Strengths, and TOSS at High Spinning Speeds in $^{13}$C CP/MAS NMR of Solids

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Pulse techniques for spectral editing have become a popular tool for assignment of liquid state $^{13}$C NMR spectra. These experiments rely on multiplet labelling during a heteronuclear spin-echo editing fragment with evolution of scalar $^{13}$C-1H couplings. For solid powders, however, the performance of these simple schemes are hampered (or even ruined) because interactions from homo- and heteronuclear dipolar couplings and chemical shift anisotropies are not averaged. The key to the suppression of these disturbing effects is incorporation of techniques commonly applied for achieving high-resolution NMR spectra of solids.

This work describes extension of the concept of spectral editing to include 1D and 2D SEhUT editing of $^{13}$C CP/MAS NMR spectra for solids. Furthermore, as solid state NMR multipulse experiments in general are extremely sensitive to missetting of pulse timings we also report a 2D CP/MAS pulse sequence for fast and accurate calibration of rf field strengths in solids. Finally, we present new and improved four and six $\pi$-pulse TOSS sequences for efficient suppression of spinning sidebands under various experimental conditions. Compared to earlier sequences, the new TOSS schemes are advantageous for high-speed MAS experiments, for samples with short transverse relaxation times, or for efficient dipolar dephasing of protonated carbons in $^{13}$C CP/MAS NMR at high speeds. Experimental results obtained using our new sequences will be presented.

PROCEDURES FOR DISENTANGLING COMPLEX 2D NMR SPECTRA


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A critical assessment is given of techniques for disentangling 2D NMR spectra that are difficult to be analyzed directly. Techniques of multiple quantum filtering, spin topology filtration, and computer pattern recognition are analyzed and compared. Potential and limitations of experiments in the laboratory and in the rotating frame are discussed.
The introduction of a third frequency dimension in NMR spectra to encode further spectral information will be described. If an increase of resolution is of prime interest, chemical shift is the most useful parameter for the third dimension. The interrelations of three different spins can be mapped with 3D correlation experiments which can easily be designed by combination of two 2D experiments. Volume selection in the three-dimensional frequency domain by selective pulses or other means, that selectively excite a restricted chemical shift region, is exploited to reduce the otherwise excessive duration of the experiment and the huge amount of data. Methods to obtain pure phase 2D spectra can equally well be applied to 3D spectroscopy. 3D NMR is especially useful for large biomolecules when the overlap of cross peaks in 2D spectra becomes serious. Examples of 3D spectra of peptides and proteins will be shown.
NEW TWISTS TO SOME OLD EXPERIMENTS
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A number of modifications to existing 2D experiments are proposed. Improvements in sensitivity and resolution of the $^1$H-detected $^1$H-$^{13}$C-long range correlation (HMBC) experiment can be obtained by recording the spectrum in the absorption mode in the $F_1$ dimension and absolute value mode in $F_2$. A recipe for non-interactive phasing of this and all other types of 2D spectra will be presented.

A slightly different approach for suppressing zero quantum artefacts from NOESY spectra will be described and several mixing schemes for the HOHAHA/TOCSY experiment will be discussed and compared theoretically and experimentally. It is found that the optimal scheme depends on the electronics used for phase shifting, i.e., on the type of spectrometer used.
NON-TRIVIAL EFFECTS OF RELAXATION
IN
TWO-DIMENSIONAL NMR SPECTROSCOPY

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In its traditional form, two-dimensional exchange spectroscopy (NOESY) allows one to visualize the migration of Zeeman polarization from one site to another under the combined effects of chemical exchange and cross-relaxation (NOE). Various modifications involving multiple-quantum filtration can be designed to broaden the scope of the NOESY experiment. These techniques allow one to focus attention on the conversion of Zeeman order into longitudinal three-spin order $\langle I_{1z}I_{2z}I_{3z} \rangle$. This conversion occurs spontaneously as a result of the cross-correlation of pairs of dipolar interactions. The build-up rates should allow one to obtain a measure not only of internuclear distances, but also of angles subtended by internuclear vectors. Other forms of cross-correlation between various interactions (including the anisotropy of the chemical shifts) may give rise to anomalous coherences transfer processes that can be detected in two-dimensional correlation and multiple-quantum experiments.
3D NMR - Prospects and Limits

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A cross peak in a 2D spectrum determines two frequencies $F_A$ and $F_X$. For liquid samples and depending on the type of pulse sequence, they indicate that nuclei $A$ and $X$ directly couple ($J_{AX}$; 2-pulse COSY), or couple to an intermediate nucleus $M$ ($J_{AM}$, $J_{MX}$ and $J_{AX}=0$; 3-pulse relay COSY), or are part of an entire set of coupled nuclei in a molecule (isotropic mixing, HOHAHA). Connectivities of protons and/or carbons nuclei and thereby the molecular topology may thus be inferred from a sufficient set of frequency pairs $F_k,F_l$.

In large molecules, evaluation of pairwise connectivities is tedious and often hampered due to 2D spectral overlap. The addition of the third frequency dimension is thus of interest since connectivities of three nuclei may be directly obtained from 3D cross peaks and overlap problems are reduced. The 3-pulse sequence $90-t_1-90-t_2-90-t_3$(FID) achieves this goal but puts high demands on measurement time and data processing facilities. For sufficient resolution, of the order of $1K*1K*1K = 1$ Gigawords need to be be sampled. Even if the immense data array could be handled with future powerful NMR work stations, the problem of $N_1*N_2$ relaxation delays would remain.

Practical approaches therefore are

1. 3-pulse sequences with $N_1*N_2*N_3$ approximately equal to $N_1*N_2$ in a conventional 2D experiment, combined with non-linear spectral analysis (MEM and like) in order to obtain adequate spectral resolution in three dimensions.

2. 3-pulse sequences with soft pulses, for instance $90(soft)-90-t_2-90-t_3$(FID) with $t_1$ equal to zero which yields a 2D cross section through the 3D spectrum at the frequency selected by the soft 90 pulse.

3. Multi-Dimensional Stochastic Magnetic Resonance

These methods will be discussed and examples given.

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(3) G.W. Vuister u. R. Boelens, J. Magn. Reson. 73, 328 (1987)
MULTIDIMENSIONAL CORRELATION SPECTROSCOPY

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We propose an alternative approach to multidimensional spectroscopy which does not involve evolution periods \( (t_1,t_2...\text{etc.}) \) or Fourier transformation \( S(t_1,t_2... \rightarrow S(F_1,F_2...). \) Each new frequency dimension is explored by scanning the frequency of a highly selective radiofrequency pulse. Fourier transformation is only used in the final stage to convert a free induction signal into the observed spectrum. Two-dimensional correlation spectra (analogous to the well-known COSY experiment) can be obtained by this technique. They have all the familiar features of COSY spectra and an additional "zoom" capability which allows us to examine interesting features of the spectrum under very high digital resolution (1). We call this pseudo-correlation spectroscopy \( (\psi\text{-COSY}). \) Extension to three or more frequency dimensions (2) can be achieved without generating large data matrices or involving long experiments. In its extreme form the technique becomes an \( N \)-dimensional correlation experiment, designed to pin down the connectivity of a chain of \( N \) coupled nuclei, the result being presented as a one-dimensional spectrum in the difference mode.


Self diffusion measurements, spatial localization and signal suppression by use of rf field inhomogeneity

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Self diffusion measurements can be carried out by means of the axial gradient of the rf field produced by a single turn coil, positioned so that the sample experiences a linear gradient. The sequence also includes pulses of a homogeneous rf field delivered by a conventional saddle shaped coil orthogonal to the single turn coil. Denoting periods of application of the rf field gradient by braces, this experiment is schematized as:

\[ \{\mathcal{S}\}_{-\Delta/2-\{180^\circ\}_y-\Delta/2-\{\mathcal{S}\}_x-(90^\circ)_x}\text{-Acquisition} \]

The two rf channels corresponding to the two coils must be made phase coherent at the spin level. The resulting magnetization, as obtained after the last \((90^\circ)_x\) observing pulse, is given by:

\[ M(\Delta, \delta) = -M_0 \exp[-D\gamma g_1 \mathcal{S} (\Delta + 2\delta/3)] \exp(-\Delta/T_1) \left[ 2 - \exp(-\Delta/2T_1) \right] \]

\(D\) is the self diffusion coefficient and \(g_1\) the rf gradient; other symbols have their usual meaning. Measurements are best performed by varying \(\delta\) while keeping \(\Delta\) constant.

Another interesting application of this experimental arrangement concerns the spatial localization of a slice perpendicular to the single turn coil axis. Again, it makes use of both rf fields (homogeneous and inhomogeneous) and of a DANTE-like sequence which is the replica of the conventional DANTE sequence in the rotating frame:

\[ (\pi/2)_x(\mathcal{O})_{-\tau}(\mathcal{O})_y(\pi/2)_x(\text{Acquisition})_x \]

The angle \(\Theta\) is such that \(\Theta = \pi/2\) and \(\tau\) is chosen as a function of the abcissa \(a\) of the slice to be selected: \(\mathcal{D}_1(a) = 2m\). The repeated cycles \((\Theta)_{-\tau}(\mathcal{O})_y\) bring the magnetization of interest towards the \(z\) axis while magnetization corresponding to other regions is essentially kept along
the y axis. The capabilities of the conventional DANTE sequence may be transposed to the present experiment, especially the adjustment of selectivity (here spatial selectivity) as a function of n. This enables to obtain the spectrum pertaining to a slice whose both abcissa and thickness can be defined with great accuracy.

Finally, it can be pointed out that the inherent inhomogeneity of $B_1$ produced by a saddle shaped coil may be employed to suppress the longitudinal and one of the transverse components of nuclear magnetization. It suffices to apply for a time sufficiently long (in practice, of the order of several milliseconds) an rf field along, say the x axis of the rotating frame, for achieving a complete scatter of the magnetization in the yz plane, whereas the x component remains locked along the $B_1$ field. This feature can profit a lot of situations, especially when there is a need of suppressing a strong solvent peak; magnetization of the latter has simply to be taken along y while leaving other magnetizations (or part of them) along the x axis.
PULSE SPECTROSCOPY WITH A QUANTIZED FIELD

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The tradition of NMR is to use very elaborate (often "exact") time-dependent quantum mechanical calculations to deal with spin variables, and to approximate the electromagnetic field as a classical field. Also, the reaction of the spins on the field is usually ignored. These approximations become particularly questionable when the ideas of pulsed NMR are applied to pulsed optics.

In this presentation, I intend to indicate answers for questions of the following type:

- how can one construct a model for pulsed spectroscopy with full quantization of the field (including phase-sensitive detection of the FID's)?

- when an atom is excited by a short pulse, from which part of the pulse spectrum does the atom take the required energy?

- which are the merits and defects of the approximation in which "counter-rotating" terms are ignored?
NONLINEAR INCOHERENT SPECTROSCOPY

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With the advent of strong laser light sources nonlinear coherent spectroscopy became a field of active research for the experimenter. The coherence of the excitation, however, is no necessity for nonlinear spectroscopy but rather a result of the laser excitation. Incoherent or white light sources can also be used in principle. Commonly, however, they are not available with enough intensity unless one resorts to cyclotron radiation. This is the situation in optical spectroscopy. In magnetic resonance spectroscopy on the other hand, both coherent and incoherent excitation are readily available of sufficient power for nonlinear excitation. The nonlinear incoherent spectroscopy is known in NMR by the name of 'stochastic NMR spectroscopy'.\textsuperscript{1-3} Therefore stochastic NMR is the model for nonlinear incoherent spectroscopy.

In incoherent spectroscopy multidimensional spectra are derived from nonlinear cross-correlation functions of excitation and response by use of Fourier transformation. This procedure is known not only from stochastic NMR, but in the linear case also from Fourier transform infra-red spectroscopy. The resultant spectra are the resonant nonlinear susceptibilities. In stochastic NMR spectroscopy the Fourier transform of the cross-correlation algorithm has been applied in the past for the computation of 2D spectra in terms of 2D cross-sections through the 3D spectra of the third order nuclear magnetic susceptibility. This algorithm is being investigated and refined in D. Ziessow's group for its applicability to NMR problems of practical interest.\textsuperscript{4}

We are presently testing explicit time domain third order cross-correlation for derivation of 2D cross-sections through the 3D time correlation function, which after 2D FT lead to z-COSY or exchange and MQ type 2D spectra. This approach is of interest, since the evaluation can be executed in an analog fashion in parallel for each data point of the 2D time domain matrix. In this way the the multiplex advantage may be introduced to the additional dimension in 2D spectroscopy with the ultimate goal to measure a complete 2D spectrum within a few T. The procedure is presently being implemented in ESR spectroscopy aiming at dead time free 2D exchange ESR spectra for the investigation of molecular mobility of powdered and amorphous samples. There advantage is taken of the low power of the continuous stochastic excitation.

The relation between multi-dimensional stochastic NMR and nonlinear optical spectroscopy is reviewed. The state of the art of explicit time domain cross-correlation is described, and examples from NMR spectroscopy are given.

Several high resolution NMR techniques such as $^{13}$C-$^1$H cross polarization [1-3], multiple-pulse experiments of the WAHUHA type and derivatives [4] both with and without magic angle spinning have been applied to organic conductors recently. We will report on locally resolved Knight shifts in organic conductors. Besides the physical implications of the experimental results, details of the experimental techniques will be reported. This includes single crystal measurements and magic angle spinning experiments at low temperatures.


Magnetic resonance imaging has been mainly developed to obtain macroscopic images of the human body at a millimetric resolution. This method is now widely used as a major diagnosis tool but its rapid and successful development has partly precluded researches on other potential applications.

Among these, applied researches on diffused liquids in porous media and NMR microscopy, down to the micron-range spatial resolution, appear very promising. We will give some examples of such applications in low and high magnetic fields and will analyze the various parameters which control their feasibility.

In NMR microscopy, a high magnetic field intensity is needed to avoid too long experimental time, so a large magnetic susceptibility effect is induced. Different parameters intervene to limit spatial resolution. Among them, diffusion and magnetic susceptibility phenomena are the most important and we will discuss their relative influence with respect to the magnetic field intensity and the required spatial resolution.

The technical description of a microscopic imager operating at 8.6 Tesla will be given and a comparison of experimental results with theoretical predictions will be presented.
SPECULATIONS ON ROTATING FRAME HETERONUCLEAR NOE EXPERIMENTS

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Useful heteronuclear Overhauser enhancements are predicted for the case of heteronuclear CAMELSPIN experiment. Depending on the ratio of gyromagnetic factors of dipolarly coupled nuclei and the weight of dipolar relaxation mechanism a significant NOE polarization transfer is hoped even in the slow motion regime. Analogous 2D rotating frame experiments (HEROESY) seem feasible.
The 2D version of polarization transfer experiment DEPT(1,2) is an excellent tool for the detection of long-range $^{13}$C-$^1$H spin-spin couplings. However, due to spin-spin relaxation during the long intervals in the sequence a careful optimization of delay times is required. In an effort to facilitate the detection of long-range heteronuclear correlations we suggest some modifications on the basic 2D DEPT experiment.

A spin-echo sequence is proposed to eliminate magnetization transfer from the directly attached proton. The efficiency of this procedure has recently been verified in the case of 2D heteronuclear NOE and relay NOE experiments.

To suppress the modulation effects of response intensity due to the one-bond coupling (5,6) a BIRD sequence has been inserted in the middle of the final refocusing delay. A further important effect of BIRD sequence is that it ensures the suppression of the direct $^{1}J_{\text{CH}}$ responses, too i.e. it acts as a second (stage) filter resulting in a more efficient suppression of direct peaks even in the case of the spread of one-bond proton-carbon couplings.
Recently, a new technique, heteronuclear Zero-quantum carbon-shift correlation experiment (7) has been proposed for the determination of one-bond proton-carbon connectivities. The application of the method for detection of long-range couplings is straightforward. In comparison to the previous 2D DEPT sequence this experiment has an inherent advantage for it contains only two "long-range delay" periods. The inconveniences due to the unusual P1 dimension (zero-quantum frequencies) can be overcome with a modified experiment. To suppress the direct responses we applied the spin-echo sequence as before. Practical applications of these pulse sequences will be shown.

PULSE SHAPING AND SELECTIVE EXCITATION: THE EFFECT OF SCALAR COUPLING


Recently, a good deal of interest has centred around the use of shaped pulses for a variety of 1D and 2D experiments. Long low power rectangular pulses or waveforms shaped like Hyperbolic Secants, Sinc functions, Gaussian or half-Gaussian functions have been used in these experiments.

For very selective pulses the average r.f. power is adjusted to be the same order of magnitude as the J coupling. A theoretical description suitable for selective shaped pulses is given, which includes the effect of the scalar coupling. The excitation profile for the different coherence components of the irradiated spin will be shown for the commonly used shaped waveforms.
SOFT PULSES APPLIED TO 2D NMR EXPERIMENTS

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The use of frequency-selective pulses (so-called soft pulses) (1) in homonuclear 2D NMR experiments (2,3) introduces an additional degree of freedom in the experimental design with the following possibilities:

1) Economy of experiment and computer time by focusing on selected cross-peak regions.
A COSY-type experiment is presented, called soft-COSY, which has a higher signal-to-noise ratio compared to conventional COSY or E.COSY-type experiments. Furthermore it produces E.COSY-type cross-peak multiplets which allow an accurate and convenient measurement of coupling constants in the selected cross peaks.

2) Adaption of heteronuclear methods to homonuclear spin systems, for example $\omega_1$-decoupling.
The utility of selective $\omega_1$-decoupling in COSY- and NOESY-type experiments is demonstrated for the simplification of cross-peak structure and to prevent overlap of cross peaks.

These features are illustrated by 2D spectra of the decapeptide LHRH and the cyclic decapeptide antamanide.

NEW APPROACHES TO THE COMPUTER ANALYSIS
OF TWO DIMENSIONAL NMR SPECTRA

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Procedures are described for the analysis of two-dimensional correlation spectra by computer. COSY- and E.COSY-type spectra are discussed. The multiplet symmetry is exploited for the determination of coupling constants. For the construction of the coupling networks, characteristic multiplet patterns are used in a pattern match procedure. Strongly coupled spin systems are analyzed by iterative fitting algorithms.
Optimization of Polarization Transfer Experiments taking into Account Transversal Relaxation

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One and two dimensional polarization transfer pulse sequences, which are used routinely in heteronuclear NMR spectroscopy, involve precession delays that have to be adjusted accurately to obtain a maximum sensitivity enhancement. Especially in cases where linewidths and coupling constants are comparable (for example in biomolecules, paramagnetic species or if polarization transfer via long-range coupling constants is used to assign non-protonated nuclei) it is indispensable to take into account transversal relaxation during these delays. This has so far usually been done only based on rough approximations. We show exact expressions for the settings of delays in INEPT, DEPT and heteronuclear correlation (H-X-COSY) spectra for XH, XH2 and XH3 groups. The theoretically deduced formulae are experimentally confirmed by INEPT spectra, where transversal relaxation has deliberately been enhanced by addition of a paramagnetic reagent.

The application of optimized experiments is demonstrated by the assignment of non-protonated olefinic and aromatic carbons in a bile pigment derivative through H-X-COSY-experiments.

*This work has been supported by the "Fonds zur Förderung der wissenschaftlichen Forschung" project number P6213C.
Digital Quadrature Detection in NMR Spectroscopy

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Phase-sensitive detection involves the multiplication of an FID signal with a reference wave REF and subsequent elimination of output components at the sum frequencies f(FID)+f(REF). Up to now, these detectors work in an analogue way, i.e. using mixers, analogue filters and amplifiers. These operations may, however, also be performed in a digital manner provided that the intermediate frequency signal of the typical NMR detector can be sampled fast enough. This is in fact possible with flash A/D converters which have recently become available with sufficient vertical resolution at moderate cost (10-11 bit at 30 MHz). For instance, when an i.f. NMR signal at 6 MHz is sampled at a rate of 4x6 = 24 MHz, digital quadrature detection (DQD) amounts to multiplying subsequent samples with +1,+1,-1,-1 and +1,-1,-1,+1 for a sine (channel #1, real) and cosine (#2, imaginary) reference wave, respectively, followed by the addition of a suitable number of adjacent samples (digital low-pass filter).

The performance of DQD has been tested with hardware built around an Eurocard 8-bit A/D subsystem TDC 1007E1C from TRW Inc. which digitizes signals up to 1 V ptp from a 75 ohms source at rates up to 20 MHz. Samples are stored in a fast four-word memory and then added and subtracted with hardware made from standard 74Sxxx flip-flops and full adders. Real and imaginary data points are accumulated in two 16-bit registers the contents of which are read and cleared every other time period mt by an Aspect 3000 instrument computer. The number of acquired data varied from 1K to 256K. Further data processing (filtering, FT, etc) was done as usual.

Comprehensive tests were carried out for a sampling rate of 16 MHz and mt = 4 microseconds. The frequency of the input signal was selected in the range 3.980 to 4.020 MHz. The signal-to-noise ration amounts to 67 db and image peaks are below -60 db of the main signal in the range 3.9925 to 4.0075 MHz (+/- 7.5 kHz offset). For larger offsets, image peaks increase which is due to jitter of the convert pulse and imperfections of the moving-average filter.

Digital data processing thus allows quadrature phase detection with two almost perfectly orthogonal channels. In addition, the NMR spectral range may be selected after data acquisition with suitable digital filters which possess ideal linear phase characteristics and good sharpness of amplitude response. Since A/D conversion is performed at high bandwidth, the increased noise reduces the problem of dynamic range when strong solvent peaks are present.
Transient Nutations for Pulsed R.F. Fields

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In CW NMR spectroscopy, transient nutations (TN) have been used for the calibration of r.f. field strength, the investigation of coupled spin systems (homonuclear double resonance) and the determination of C-13 chemical shifts and C-C couplings constants at natural abundance (heteronuclear double resonance). For pulsed r.f. fields, such as a sequence of many closely spaced 90° pulses, NMR signals may be observed which are reminiscent of TN as well as FID signals. The features of these signals are discussed and it is shown that they may be used for the study of coupled spin systems. Simulations indicate that connectivity information can be extracted based on measurement times of the order of the relaxation time.
PHASE COHERENCE AND SOLVENT SUPPRESSION IN ROTATING-FRAME EXPERIMENTS.

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The routine application of rotating-frame experiments (1D and 2D cross-polarization and cross-relaxation) is faced by a number of theoretical and instrumental problems. An essential prerequisite for these experiments is the phase coherence among the different r.f. sources and the receiver, a feature being currently implemented in last generation spectrometers. In most operating commercial spectrometers, however, the receiver reference phase is given by the main transmitter r.f., the decoupler r.f. being generated by a separate, independent device. Therefore it is not possible to run rotating-frame experiments using a decoupler generated mixing r.f. field and transmitter generated hard pulses, without undermining the experimental performances.

In order to avoid sample overheating and damages to the probe, ensuing when the main transmitter is used as a single r.f. source, the decoupler adjustable high power output (2-5 Watts) can be employed, in the 'reverse detection' mode (i.e. receiver reference phase provided by the decoupler).

If solvent presaturation is required, a DANTE pulse train can be employed during the preparation period. This avoids any switching of the decoupler power level, a valuable feature for the spectrometer hardware, especially when such a switching is to occur several thousand of times, as during the collection of 2D spectra. The general applicability of DANTE presaturation schemes was successfully tested in many rotating-frame experiments, with excellent results.

The phase coherence of the r.f. field throughout any rotating-frame experiment is mandatory, and when the specific sequence explicitly demands the use of different power levels or even r.f. sources, additional hardware may be required.

For example, 2D HOHAHA sequences entailing complete loss of phase memory both at the beginning and at the end of the mixing period, fail when carried out using two different r.f. sources not phase locked.

HOMOTANGO – an alternative to INADEQUATE?

Zaloznikh V.M., Fedorov L.A.

Now INADEQUATE is well established for suppressing the strong isolated nuclei signal in coupled homonucleous systems. This powerful technique suffers from difficulty which is often ignored. Presence of strong signal in FID requires perfect linearity of the receiver system. Now it is considered that this problem is not too serious even with regard to noise characteristics and in the case of protons spectroscopy. Recently, Wimperis and Freeman [1,2] have proposed the TANGO sequence which acts as a 90° pulse for distant protons leaving protons directly attached to $^{13}\text{C}$ inverted. This is a heteronucleous technique like the BIRD pulse which was introduced in [3,4]. We have investigated a homonucleous form of such sequence:

$$45^\circ(\alpha) - \tau - 180^\circ(\beta) - \tau - 45^\circ(\gamma); \quad \tau = \frac{4}{\gamma_\text{C}} \cdot \frac{1}{2}\sqrt{\gamma_\text{C}}$$

It acts as 90° for coupled nucleous pairs, leaving isolated spins inverted. Thus already suppressed isolated nuclei signal is presented in FID.

Even in this simplest form it provides 30 - fold suppression of isolated nuclei signal in single-pulse experiment after fine adjustment. It is possible to achieve 70 - fold suppression with the help of simplest 16 step phase-cycling program, obtained by the addition of CYCLOPS to one which is given below:

$$\begin{array}{cccc}
\alpha & \beta & \gamma & \text{aqu} \\
\text{x} & \text{x} & -\text{x} & \text{y} \\
\text{x} & -\text{x} & -\text{x} & \text{y} \\
\text{x} & \text{y} & \text{x} & -\text{y} \\
\text{x} & -\text{y} & \text{x} & -\text{y} \\
\end{array}$$
More sophisticated sequences providing suppression comparable with INADEQUATE are presented. Sensitivity enhancement is demonstrated even in case of $^{13}\text{C}-^{13}\text{C}$ coupling investigation for common receiver.

REFERENCES

**ADPT (ADIABATIC POLARIZATION TRANSFER) - NEW POLARIZATION TRANSFER MECHANISM, WHICH DOES NOT DEPEND ON J-CONSTANT VALUE**

Zaloznikh V.M., Fedorov L.A.

Shaped selective $\pi/2$ and $\pi$ pulses produced in experiments with continuous r.f. field amplitude and phase modulation make it possible to transform any common technique with hard pulses into selective analog 1-3. Commercial devices for such experiments have been developed already. It may revive the application of earlier types of the selective NMR experiment also (SPT, SESET, SELECTIVE INEPT a.o.). However the continuous r.f. field amplitude and phase modulation possibility provides not only the successful excitation scheme but also has great potential for generating valuable experiments utilizing new concepts. There are only few polarization transfer mechanisms though they are of central importance in modern liquid-state NMR for nuclear spin systems exploration and for sensitivity enhancement (two-dimensional spectroscopy, multiple quantum spectroscopy and rare spin NMR): 1) J - Crosspolarization in the rotating frame by Hartman-Hahn matching of the applied r.f. field, by an adiabatic mixing process or isotropic mixing process; 2) Creating of multiple quantum coherence by rotations in the soft constant r.f. field (SPT, SESET a.o.); 3) Creating of multiple quantum coherence during the free time evolution (INEPT family, DEPT, POMMY, RELAY a.o.).

We propose a new mechanism, which does not depend on the scalar spin-spin coupling constant value. The multiple quantum coherence may be created in the single frequency r.f. field varing in time under special kind of adiabatic conditions:

\[
\begin{align*}
\text{H}_{13} & : \\
\text{C}_{13} & : \\
\text{H}_{13} & :
\end{align*}
\]
Such experiments may be easily described with the help of our model for vector representation of spin density operator in the case of weak coupling [4]. For example, for the density operator part describing spin X in heteronucleous spin system AX Liouville equation partitions into two vector equations (in double rotating frame):

\[
\begin{align*}
\frac{d}{dt} \hat{\rho}_X^+ &= \hat{P}_X^+ \times \hat{h}^+; \\
\frac{d}{dt} \hat{\rho}_X^- &= \hat{P}_X^- \times \hat{h}^-;
\end{align*}
\]

\[
\hat{P}_X^\pm(t) = (\rho_{X}^\pm(t)\hat{e}_X + \rho_{Y}^\pm(t)\hat{e}_Y + \rho_{Z}^\pm(t)\hat{e}_Z) \hat{e}_A^\pm;
\]

\[
\hat{h}^\pm = (\beta \hat{e}_Y \times \hat{e}_X + \gamma \hat{e}_X \times \hat{e}_Z) \hat{e}_A^\pm = \omega_{\text{eff}} \hat{e}_A^\pm; \quad \omega_{\text{eff}} = \sqrt{\beta^2 + \gamma^2};
\]

\[
\hat{h} = -2^{-1}(\beta \hat{e}_Y \times \gamma \hat{e}_X + \gamma \hat{e}_X \times \beta \hat{e}_Z \hat{e}_A^z \hat{e}_Z) = 2^{-1} \left( \hat{h}^z + \hat{h}^- \right); \quad \hat{e}_A^\pm = \hat{\rho}_A^+ = \hat{\rho}_A^- = 0.
\]

The experiment is designed so that the vector \( \hat{\rho}_X^\pm \) remains parallel to vector \( \hat{\rho}_X^\pm \) when \( \beta \) continuously decreases up to zero under adiabatic condition \( \Omega \ll \omega_{\text{eff}} \). If the initial state (immediately after first \( \pi/2 \) pulse with \( X \) frequency) is \( \hat{\rho}(0) = \hat{e}_X \) (e.g., \( \hat{\rho}_X^+ || \hat{\rho}_X^- \)) the vector \( \hat{\rho}_X^\pm \) will be antiparallel to \( \hat{e}_X^\pm \) and parallel to \( Z \)-axis when \( \beta = 0 \). Thus \( \hat{\rho}_{\beta=0} = \hat{e}_X \hat{e}_A \). When spin \( X \) is in weak coupling with \( \text{M} \) spins the Liouville equation partitions into \( 2^M \) Bloch equations with the help of \( \hat{\rho}(\rho) = 2^{-N} \sum_{n=0}^{N-1} (\hat{P}_n \hat{e}_A) \). \( \rho_A = \pm 1 \):

\[
\omega_{\text{eff}}(\rho) = \sqrt{\beta^2 + \gamma^2 (\hat{e}_A^z \sum_n \rho_n)^2}.
\]

Assuming the adiabatic condition is satisfied for the smallest J-constant, then for bigger ones it will be correct automatically. Homonucleous and heteronucleous forms of the methodic are discussed. The experimental results of these methodics simulation by special kinds of DANTE-type sequences are presented.

REFERENCES

NOESY-TOCSY AND ROESY-TOCSY: THE APPLICATION OF 2D-NMR TECHNIQUES CONTAINING SPIN-LOCK SEQUENCES FOR THE SEQUENTIAL ASSIGNMENT OF PEPTIDES

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New 2D-NMR techniques are proposed, which are based on the Relayed-NOESY experiment\(^{(1,2)}\). The presented NOESY-TOCSY and ROESY-TOCSY sequences make use of magnetization transfer in spin-lock periods showing several advantages over the original Relayed-NOESY technique.

The NOESY-TOCSY experiment\(^{(3)}\) is very favourable for large molecules with strong, negative NOEs. The antiphase structure observed in Relayed-NOESY cross-peaks usually leads to partial cancellation, especially in the case of relatively broad lines. In the NOESY-TOCSY experiment only in-phase signals are observed, therefore the signal-to-noise ratio of the desired cross-peaks is significantly improved compared with the Relayed-NOESY experiment. The application of the NOESY-TOCSY technique to a polycyclic nonadecapeptide clearly showed the advantages of this new pulse sequence.
The ROESY-TOCSY experiment is very useful for all compounds that do not exhibit significant NOEs, but show strong ROE (rotating frame NOE) effects. This is true for many small peptides containing 4-8 amino acid residues. The ROESY-TOCSY technique was successfully applied to several hexapeptides that could not be sequentially assigned with ROESY or COLOC due to severe overlap of the Hα-signals. Nevertheless, in all cases the sequence of the amino acid spin systems could be determined with the help of the NH-NH cross-peaks arising from the ROESY-TOCSY transfer.

In all Relayed-NOESY type experiments unwanted cross-peaks occur, which are due to other possible transfer pathways and cannot be filtered out by phase cycling. One attractive way of simplification is to create a symmetrized 2D matrix (using standard NMR software), which is then subtracted from the original 2D data matrix. Ideally a synthetic spectrum arises, which exhibits only the asymmetric contributions.

Determination of Heteronuclear Long-Range Coupling Constants
by Soft-\textsuperscript{1}H,\textsuperscript{13}C-COSY

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Homonuclear vicinal coupling constants $J_{\text{HH}}$ are used routinely for the determination of dihedral angles via Karplus relations (1). However, the determination of dihedral angles from $J_{\text{HH}}$ contains some ambiguity: up to four angles are consistent with each value for $J_{\text{HH}}$. Thus, a reliable determination of dihedral angles requires additional information, e.g. heteronuclear coupling constants $J_{\text{HC}}$ (1). Unfortunately, the extraction of these heteronuclear coupling constants from NMR spectra is rather arduous with non-$\text{^{13}C}$-enriched samples of biologically interesting molecules.

A possible method for the determination of $J_{\text{HC}}$ values is the hetero-E.COSY experiment (2), where all interesting $J_{\text{HC}}$ constants of a peptide can be extracted from a single cross-peak, i.e. the H\textsubscript{a}-C\textsuperscript{13} signal. The only disadvantage of this technique is its low sensitivity, which makes it nearly impossible to acquire a useful spectrum in a reasonable time.

Here we describe the soft-\textsuperscript{1}H,\textsuperscript{13}C-COSY experiment (a) and its inverse counterpart (b), which make use of selective excitation in the $\text{^{1}H}$ domain, thus resulting in the same multiplet pattern as the hetero-E.COSY technique, but being more sensitive (see below) (J):

\begin{center}
\begin{tikzpicture}
\end{tikzpicture}
\end{center}

In both experiments soft pulses on $\text{^{13}C}$ are not necessary for the selection of connected transitions, since $\text{^{13}C}$ is a dilute species. Nevertheless, selective excitation of $\text{^{13}C}$ is required for sequence 1b, because the exclusive recording of a small region in $F_1$ (necessary for a sufficient digital resolution) is impossible by analog filtering.

The sensitivity gain is $K_{\text{c}}=1$ for a soft-\textsuperscript{1}H,\textsuperscript{13}C-COSY. $K_{\text{c}}=2$ is the number of spins with resolved couplings to both active spins (2, J). For aromatic amino acids like Phenylalanin ($K_{\text{c}}=5$) the soft experiment has a 4 times higher sensitivity, resulting in a reduction of the measuring time by a factor of 16 (for equal signal-to-noise ratio).

Improved One-Dimensional Correlation Experiments

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Two-dimensional correlation experiments have largely displaced the use of 1D techniques in the analysis of all but the simplest molecules. Recently, however, there has been a renewal of interest in 1D techniques; many 2D experiments have been reduced to their 1D analogues (1-3), typically by replacing the first non-selective hard pulse of the 2D experiment by a semi-selective soft pulse and replacing the incremental delay $t_1$ by a period of constant duration. In such an experiment the connectivities of the spin selected by the soft pulse are investigated. It is usually a prerequisite that the multiplet of the spin under investigation be resolved from the transitions of its neighbours. Such experiments are often critically dependent upon experimental parameters, and consequently connectivities are frequently absent from the spectrum.

We have adopted an alternative approach to designing 1D correlation experiments which significantly reduces their dependencies on experimental parameters and results in a significant increase in overall signal intensity. In addition we demonstrate a quick spin selection procedure which only requires that a spin have a unique chemical shift to be able to select it,
and not a resolved multiplet as with previous techniques.

1. C.J. Bauer, R. Freeman, T. Frenkel and A.J. Shaka, 


A Simple rf Phase Cycling Scheme

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Phase shifting of the radio frequency (rf) irradiation applied to a nuclear spin system is required for many NMR experiments. For example, using quadrature detection to measure the spin-lattice relaxation time $T_1$, using the inversion recovery pulse sequence, some distortions can be eliminated using a 0 - 90° phase cycling scheme whilst most other distortions can be eliminated using a 0 - 180° phase cycling scheme implemented after the $\pi$ pulse.\textsuperscript{1,2} Hence, in order to eliminate both sets of distortions a 0 - 90 - 180 - 270° phase cycling scheme is required.

The 90° phase shift has been achieved by using the two outputs from a quadrature hybrid which are then fed, via rf gates, into a power divider. The quadrature hybrid outputs differ in phase by 90 ± 3°. The phase difference can be adjusted to 90.0 ± 0.1° by altering the length of the cable connected to one of the output ports of the quadrature hybrid. The rf gates are controlled by TTL pulses in such a way that either the first or the second rf gate transmits an rf pulse.

The 180° phase shift has been achieved by applying either a positive or negative bias to the output port of a balanced mixer. The characteristics of the balanced mixer were carefully measured and it was found that in order to produce a phase shift of 180° the positive and negative bias voltages had to be unequal in magnitude. A high degree of stability and reproducibility was achieved by designing a stable drive source. This stable source was then switched, by a TTL pulse, to provide the appropriate positive or negative bias to the balanced mixer. By making use of the measured characteristics of the balanced mixer in a standard test circuit, the phase shift could be set to 180 ± 1°. Final adjustment to 180.0 ± 0.1° was then performed by phase measurements with the balanced mixer in situ.

An essential feature of this scheme is the accurate measurement of the phase of both of the quadrature outputs for each of the four phases generated by the quadraphase shifter. First the background level is obtained by calculating the average level of the signal before the rf pulse for a large number of scans. The background level is then subtracted from the signal and the points, where the signal changes sign, are determined from the digitised signal. The zeros are then located by linear interpolation using the algorithm

$$t_i = t_j + T_s y_j / (y_i - y_{i+1})$$

where $t_i$ is the time of the $i$th zero, $y_i$ is the height of the signal at the last point before the signal changes sign, $t_j$ is the value of the time, measured from the centre of the pulse, associated with the signal value $y_j$ and $T_s$ is the sampling time interval. The period of the oscillations of the signal is then calculated using the Raleigh mean. Finally, the phase of the signal is calculated by calculating the number of periods between the centre of the pulse and the first measured zero, $t_1$. 

By making sets of about a dozen measurements, each of 1000 scans, the phase differences between the four phases were determined to within ± 0.1°. Because the phase differences can be measured and adjusted to within ± 0.1°, the calculated amplitude signal is independent of the differences in gain between the two quadrature detection channels. Hence, by using readily available components, a stable quadrature shifter, controlled by two TTL inputs, can be constructed, enabling most signal distortions to be eliminated.

References


Acknowledgements

This work was supported by a grant from the Association for International Cancer Research.
Graphical means are presented that have been developed in order to visualize phenomena in multidimensional Liouville space. The parameter dependence of effective Hamiltonians and the time dependence of the density operator in multiple-pulse experiments are graphically represented in a perceptive manner. Illustrative examples are taken from rotating frame spectroscopy.
INTERPROTON DISTANCES IN PROTEINS FROM NMR CROSS-RELAXATION IN LABORATORY AND ROTATING FRAME

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Interproton distances in small proteins in solution could be obtained by 2D exchange spectroscopy from cross-relaxation in the laboratory (NOESY) and the rotating (ROESY) frame. In the initial build-up rate regime (short mixing times) integral intensities (volumes) of cross-peaks are proportional to the inverse sixth power of the proton-proton distances. Unfortunately, several difficulties precluded quantitative measurements of the distances. In the protein spectra the most serious are spin diffusion, overlap of cross (and diagonal) peaks in the overcrowded spectral regions, and baseplane distortion.

In order to enable quantitative measurements of interproton distances from protein spectra in solution we have developed the methods for the elimination of some of these difficulties.

Influence of spin diffusion was monitored and avoided by the combined use of ROESY and NOESY experiments and base plane distortion was corrected by two-dimensional spline method.

It was found that deterioration of measured peaks volumes by their partial overlap could be reduced by the measurements of their heights only. In the cases where linewidths of cross-peaks were considerably different, they were measured in both domains and heights were corrected accordingly.

In order to verify experimentally our approach we have performed several NOESY (tau 30, 45, 60, 90, 120, 150 and 180 ms) and ROESY (tau 15, 30, 60 and 90 ms) experiments for turkey ovomucoid third domain, a small globular protein of 6062 in 15 mM solution (90/10 D_2O/H_2O) at room temperature and pH 4.0. Inter and intra residual proton-proton distances were measured for 91 assigned proton pairs. An average experimental error for corresponding distances obtained from ROESY or NOESY was of the order of 10%. Most of these distances fit well (within 10%) to the distances obtained from x-ray crystallography.

We anticipate that increased accuracy in the obtained distances would improve performances of distance geometry programs that are used for reconstruction of the secondary structure of proteins in solution.
It is well recognized that two-dimensional (2D) NMR spectroscopy is an essential tool for the study of biological macromolecules at atomic detail in solution. Structural and dynamic information about small proteins and nucleic acids can be obtained through the analysis of a variety of homo- and heteronuclear 2D NMR experiments. Since processing and manipulating 2D NMR data sets are rather time consuming, a standard practice is to store processed data sets along with the original time domain data. As a result, more demands are placed on storage, adding to the costs of long-term storage and to the time required for data retrieval and presentation.

Since the important information in a high-resolution 2D NMR spectrum is localized in a small fraction of the overall data block, the data files should be subject to compression by suitable manipulation. Data compression proposed here is based on elimination of background noise: data points that fall within the peak-to-peak noise range will be zeroed. Peak-to-peak noise is determined separately for each spectral column. This procedure enables the simultaneous observation of all cross-peaks and diagonal peaks from a single plot, even if very strong noise bands were present in the original spectrum. Spectra processed in this way are amenable to automatic computer analysis and can be compressed into smaller storage size.

By this method one can obtain a compression factor of 10-100 without significant loss of spectral information content. The method is particularly suitable for the storage of massive data sets. One or more two-dimensional spectra can be reduced and stored on one floppy disk. These methods and their versatility are demonstrated via compression of 2D NMR spectra of a small protein (turkey ovomucoid third domain).
Dynamic solid state $^2\text{H}$ NMR methods have successfully been employed to the studies of complex biological and chemical systems. In particular, the combination of various pulse sequences (inversion recovery, quadrupole echo, Jeener-Broeckaert) allows the detection of molecular motions over a large dynamic range. /1/

In this contribution we report on a promising extension of these NMR methods applying 2D procedures. Here, a second Fourier transformation is performed with respect to a relaxation period ($t_1$), which yields a 2D representation of the particular relaxation experiment. From this, angular dependent relaxation rates can be determined, which simplifies the characterization of complex molecular motions.

The advantages of these 2D NMR methods are demonstrated by representative model calculations. Furthermore, applications to the investigations of liquid crystal polymers are presented. /2/

Figure 1. Experimental 2D NMR relaxation spectrum of L-alanine-d$_3$ at $T = 203$ K, obtained with the quadrupole echo sequence (stack and normalized contour plot).
Generalization of construction of correlation space (CCS)\(^1\)

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Construction of correlation space (CCS) from various, routinely accessible 1D and 2D NMR spectra may lead to a specially ordered and extremely compressed data space which is easy-to-handle in the same time. Possibilities to make slices and projections for substracting certain connectivity informations, hidden in the single original spectra available.

In comparision with real nD measurements the CCS method does not suffer from close practical limitations of them both in the experimental procedure and data handling.

Present work deals with construction of an algorithm and a program based on NMR spectroscopy which makes automated (partial) chemical structure elucidation and/or similarity search. The method provides a two-dimensional chemical graph or under favourable conditions a flexible three-dimensional graph with distance constraints by means of no or limited previous knowledge about constitution and configuration.

The CCS method involves four essential steps, as follows.

1. Transformation of the measured spectra to a vector space which can be transformed to Boolean algebra for visualization,
2. construction of the correlation solid,
3. derivation of sections,
4. spin graph construction and representation.

The CCS provides possibility of automation and it is easy to connect it to already existing NMR softwares. Examples of the combination possibilities using 1D and homonuclear and heteronuclear 2D correlation spectra will also be presented.

INDIRECT, NEGATIVE NOE ON THE HOMONUCLEAR TWO-DIMENSIONAL SPECTRUM

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Appearance of indirect effects in the NOE spectrum are known and demonstrated for 1D homo- and heteronuclear, as well as, more recently for two-dimensional heteronuclear cases but no, according to our best knowledge, experimental example of homonuclear 2D one has been presented yet.

During our work on new sesquiterpene esters, isolated from Euonymus species indirect, negative effects were detected on the homonuclear phase sensitive 2D NOE spectra. We present here some examples of them and discuss the extra information about the conformation and relative configuration involved. There are some notes on possible misinterpretation of these type of cross peaks, too.
THE E.COSY EXPERIMENT WITHOUT "SMALL-ANGLE" PHASE SHIFTS

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One of the most interesting versions of the COSY experiment is E.COSY (1,2). In this experiment cross-peaks are found only between connected transitions. In comparison to the basic COSY experiment E.COSY thus displays much simpler cross-peaks, which allow for more accurate determination of coupling constants, and even very small couplings (below 0.2 Hz) can be resolved. In addition relative signs of the coupling constants can be determined.

The E.COSY pulse sequence is $90^\circ_{\beta}-t-x-90^\circ_{\beta}-t_2$, with $\beta$ being changed in steps of e.g. 45° or 60°. Unfortunately, many NMR spectrometers are not equipped with hardware for these "small-angle" phase shifts. In the poster two solutions to this problem are described and illustrated with experimental spectra. One is to employ a "phase pulse" (3), i.e. a small delay between the $90^\circ_{\beta}$ and $90^\circ_{-x}$ pulses in which the transmitter frequency is changed. For the second solution we note that a $90^\circ_{\beta}-90^\circ_{-x}$ pulse pair is the composite equivalent of a $\beta_y$ pulse. The E.COSY sequence given above is thus equivalent to $90^\circ_{x}-t_1-\beta_y-t_2$; i.e. the "small-angle" phase shift has been replaced with a change in pulse width. This simpler version was mentioned in (1).

Varian XL and VXR spectrometers use simple calculations to generate phase settings and time intervals (delays, pulse widths), when they are changed between scans. It is obvious that the very irregular pattern for the $\beta$ values in the E.COSY sequence is not suited for this procedure, and a table must be used instead. The pulse sequence code for E.COSY, displayed in the poster, shows how the standard software with a few tricks can accommodate tables.

SUPPRESSION OF THE LINESHAPES AND BASELINE DISTORTIONS ARISING IN THE NMR SPECTRA FROM THE FIRST-ORDER PHASE CORRECTION

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In order to obtain properly phased spectra, it is inevitable in most NMR experiments to apply a first-order phase correcting routine. This may pose a problem especially in cases when the point of maximum coherence of frequency components lies outside the measured signal. The use of a standard linear phase correction algorithm leads then to distortion of lineshapes and to the baseline roll. Methods trying to overcome this problem, such as MEM or LPSVD, are much more complicated than the linear phase correction and yet the results are often more troublesome to interpret, either due to amplitude distortion or to the peak detection reliability.

The proposed iterative technique is less general than the two methods mentioned above, yet it is advantageous in its simplicity and clarity of the preliminary knowledge it uses. Moreover, usually a single iteration step is sufficient to remove the distortions arising from the absence of the initial part of FID signals.

The gist of the method consists in combining the linear phase correction procedure with a method of estimating the missing parts of the time signal. After a distorted spectrum is obtained, the highest peaks are found and used to estimate the missing initial part of the FID signal. The measured signal being completed by this estimate serves as the starting point for the next iterating step. In such a way it is possible to remove most of the distortions. It has been shown that no amplitude distortions nor convergence difficulties arise as long as certain reasonable conditions considering line widths and signal-to-noise ratio are met.
Symbolic Algebra: A useful tool for NMR?

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Handling formulas in NMR can be quite cumbersome. The question is: How can existing symbolic algebra systems be employed in NMR? What systems are available and how can they be adapted to specific problems?

Known systems are MACSYMA, REDUCE, SCRATCHPAD, MuSIMP/MuMATH etc. Most systems are based on LISP, a language appropriate for symbolic manipulations. They contain a parser to transform mathematical statements into equivalent LISP expressions which are then evaluated by LISP systems.

MuMATH [1] is a system available on microcomputers running under the CP/M or MS/DOS operating system. We implemented this system in XLISP [2] and Cambridge LISP [3] to use it in a more powerful environment (ATARI-ST computer and UNIX systems).

Usually these systems must be extended to be useful in NMR. We enhanced the matrix-package of MuMATH as well as of REDUCE [4] with the trace operation and with the direct product of matrices [5] to allow symbolic handling of first order spin systems in Hilbert space or in superoperator (Liouville) space.

Nowadays systems like MuMATH and REDUCE are available on microcomputers and can be accessed from the spectroscopist's desk. If the personal workstation is connected to a computer network larger symbolic problems can be solved on the main frame computers. Afterwards the solutions can be further simplified at the desk.

Examples of handling problems of pulse NMR are given.
Literature:


Toward a Computer Assisted Analysis of NOESY Spectra: A Multivariate Pattern Recognition Analysis of DNA and RNA NOESY Spectra

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Two dimensional NMR spectra of biomolecules present us with a wealth of data. However, if we wish to access this information on a routine basis, automated methods for spectral assignment are essential, since the spectra are so complex. A 2D NOESY spectrum of a relatively small DNA or RNA fragment can contain several hundreds of cross-peaks. The situation is especially critical for RNA spectra, which typically include several regions of severe overlap or minimal resolution. Therefore, even the most basic task of selecting the peaks to be included in an initial analysis is difficult and time-consuming. For the same reasons, the actual procedures of manual assignment are also difficult.

In this study we show that several of the above issues can be addressed by appealing to multivariate representations of the NOESY spectra. The analysis generates projections of the multivariate space by calculating principal components, with rather remarkable consequences. These projections directly identify relevant spectral bands. Subsequent multivariate analysis can provide peak assignment information according to type of base or conformation, and can even supply reliability estimates for proposed assignments, based on previously assigned spectra.

The techniques are illustrated for separation of different structural segments of an RNA duplex NOESY spectra of (CACAUGUG)2.

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Many proteins can be reversibly folded and unfolded in solution provided that the correct experimental conditions are found. Under these circumstances, there are several ways in which NMR spectroscopy can be exploited to characterize the kinetics and thermodynamics of the folding transition. Of particular interest are magnetization transfer techniques which also permit the correlation of resonances between the different states of the protein.\textsuperscript{1,2}

Methods designed to exploit magnetization transfer phenomenon in one- and two-dimensional NMR studies of proteins will be described. Several applications will be presented involving detailed characterization of the folding and unfolding transitions of proteins ranging in molecular weight from 7K to 50K daltons. Examples of both cooperative and noncooperative folding transitions will be presented.


"A.T.P."
A PROGRAM FOR
REAL TIME PROCESSING AND ANALYSIS OF THE N.M.R. SIGNAL

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To follow a biological process by N.M.R., it is necessary to collect signal intensities (or areas) (e.g. phosphocreatine in $^{31}$P, lactate in $^1$H ...), chemical shift variations (e.g. inorganic phosphate in $^{31}$P) or line widths. These data permit the obtention of the evolution of fundamental biological parameters in the studied organism (metabolite concentration, pH).

Generally, it is highly desirable to adapt the experimental conditions to the evolution of the biological parameters. Therefore it is necessary to process N.M.R. spectra as soon as acquired.

We have developed a program named "A.T.P." (Automatic Time-shared Processing) that can follow, at the same time, signal intensities, chemical shifts and line widths. It is able to analyze alternated multinuclear experiments (e.g. $^1$H/$^{31}$P). Results are presented as graphical evolution curves on a drum plotter. It can also be used for postponed processing of a series of spectra.
APPLICATION OF M.E.M. TO THE STUDY OF A TEN BASE-PAIR D.N.A. FRAGMENT

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The growing complexity of the spin systems studied by N.M.R. spectroscopists (D.N.A. fragments, proteins, etc...) often leads to very crowded spectra containing many overlapping signals after regular data processing such as zero filling, apodization by suitable functions and Fourier Transform.

It thus has become mandatory to implement methods which increase simultaneously sensitivity and resolution. In this purpose, maximum entropy processing (M.E.M.) permits to deconvolute spectral lines, and has recently been introduced in N.M.R.

For that reason, we have developed an iterative M.E.M. technique, derived from the Gull and Daniell algorithm. However, compared to Gull and Daniell's algorithm, it exhibits both stability against noise or high dynamic range, and good convergence speed. We show on this poster its efficiency in the processing of 1D as well as 2D spectra.

An example is given on a dilute sample of a ten base-pair (non auto-complementary) platinated D.N.A. fragment in H_2O. Our M.E.M. algorithm has proven to be very efficient in the present case to study exchangeable protons. This approach has been used for the study of imino and amino N.O.E. correlations of our duplex. Former attempts with classical approaches had proven unsuccessful.
Linear Prediction applied to the analysis of NMR-data from p21
kinetics measurements

Linear Prediction is a method developed in the last few years (1 and 2) for analysing time-domain signals without using a conventional Fourier transformation. One advantage of the technique is that it can handle even highly truncated signals, as in contrast to Fourier transformation, it does not require the whole time-domain signal as an input, but just a number of successive data points (in practice, between 512 and 1024 complex points have been used).

Also, preprocessing of the data is not necessary. Another advantage of LP is that it yields directly the signal parameters (frequencies, damping factors, amplitudes and phases).

The major drawback of the method is the long computing time required, as the CPU-time increases with the third power of the number of data-points used.

The algorithm we have employed is based on an improved method using the "state space" formalism (3).

p21 is a guanine nucleotide binding enzyme with some sequence homology to other guanine nucleotide binding enzymes, like the bacterial elongation factor Tu. It regulates the hydrolysis of p21-GTP to p21-GDP and phosphate. For the measurement a fivefold excess of GTP was added to p21-GDP.

The solution conformation of acyl carrier protein from *Escherichia coli* (77 residues) has been determined on the basis of 423 interproton distance restraints derived from NMR measurements. The interproton distances were obtained from volume integrals of NOE cross-peaks. A simple method for the calculation of volume integrals of cross-peak in two-dimensional NOE spectra was devised. The method relies on an appropriate combination of one-dimensional row and column integrals. A total of 12 structures were computed using a hybrid method combining metric matrix distance geometry with either dynamical simulated annealing or molecular mechanics programs. The polypeptide fold is well defined with an average backbone atomic rms difference of 0.20±0.03 nm between the final structures and the mean structure obtained by averaging their coordinates.
Conformational Analysis of a cyclic all-L-Hexapeptide

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The conformation of the all-L-Hexapeptide cyclo(-Pro-Thr-Lys-Trp-Phe-Phe-) is analyzed using latest 2D techniques and MD-calculations.

Starting with a proton detected C,H- and relayed C,H-correlation most of the spin systems were identified, while an inverse correlation optimized for heteronuclear long range couplings yielded the sequence assignment. To discriminate the strongly overlapping peaks of the aromatic amino acids a soft 2D-C0SY was used. Due to the high resolution of this spectrum it was possible - in connection with a 600 MHz spectrum - to simulate the three spin systems. The coupling constants thus obtained were then used to determine the side chain conformations. Based on NOE data from a 2D NOESY spectrum the conformation was finally calculated using Molecular Dynamics calculation.
THE INTERACTION OF LAC REPRESSOR HEADPIECE WITH ITS OPERATOR.
A TWO-DIMENSIONAL NMR STUDY.

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The molecular basis for the recognition of specific DNA sequences by proteins still constitutes a major problem in molecular biology. Progress in this area has been made by the determination of the crystal structures of several DNA binding proteins (CAP, trp repressor, phage lambda proteins cro and cl) and of the complex of the 434 repressor with its operator. This has led to several proposals for the interaction of these proteins with their respective DNA binding sites. A common feature of all these models is a helix-turn-helix superstructure of the protein, the second helix interacting with the major groove of DNA. However, genetically and biochemically the E.coli lac operon remains the best characterized system. A detailed model for the lac repressor-operator complex has been proposed on the basis of the supposed analogy with a model for the cro-OR3 complex [1]. Recently, the spatial structure of the "headpiece 51", the 51 N-terminal amino-acid residues of the lac repressor [HP51], has been derived from 2D NMR data [2].

By two-dimensional 500 MHz proton NMR the complexes of lac repressor HP 56 and 59 with a 11, 14 and 22 basepair lac operator fragments were studied. Headpiece binds specifically to the lac operator fragments [3]. The complexes are in fast exchange with its constituents on the NMR time-scale. Furthermore, a large number of resonances in the NMR spectra of both headpiece and the lac operator fragment in the complexes have been assigned. Analysis of NOE's observed between protein and DNA shows that the second helix of the headpiece ("the recognition helix") binds in the major groove of DNA, but that the orientation of this helix is approximately 180° different from the proposed model [1]. By a combination of molecular docking and restrained molecular dynamics using the NOE's as constraints, models of the lac headpiece-operator complex were built. These models are in good agreement with recent genetic data, which indicate a similar orientation in the complete lac system as well [4].

References
The conformation of small peptides and proteins is conveniently studied using the nuclear Overhauser effect (NOE) experiment. However, the observation of the NOE often fails for molecules of the size of the tubulin fragment, irrespective of the internuclear distances involved, because the tumbling rate for this molecule (dodecamer) is close to that at which the maximum possible NOE passes through zero. Consequently, sequential assignments along the peptide backbone and the observation of interproton distances that reveal secondary structures are impossible.

With the application of the rotating frame NOE experiment (1-5), this problem could be completely overcome. Our results, based on phase-sensitive COSY and ROESY experiments indicate that the dodecamer under study,
N-acetyl-Lys\textsuperscript{1}-Asp\textsuperscript{2}-Tyr\textsuperscript{3}-Glu\textsuperscript{4}-Glu\textsuperscript{5}-Val\textsuperscript{6}-Gly\textsuperscript{7}-Val\textsuperscript{8}-Asp\textsuperscript{9}-Ser\textsuperscript{10}-Val\textsuperscript{11}-Glu\textsuperscript{12}-NH\textsubscript{2}, in 80\% CD\textsubscript{3}OH/20\% D\textsubscript{2}O solutions, has an \( \alpha \)-turn at the N-terminal half of the molecule, from Lys\textsuperscript{1} to Glu\textsuperscript{5}. The remainder of the molecule is extended with a fold-back due to a kink between residues Asp\textsuperscript{9} and Ser\textsuperscript{10}. This creates a close proximity of the C-terminal end of the molecule and amino acids 3 to 5 of the \( \alpha \)-helical turn. The results are in good agreement with previously-published CD data (6).


\textbf{Acknowledgements}

We thank Dr. John M. Stewart and Ms. Eunice J. York for the synthesis of the peptide.
Specific assignment of the spectrum of a protein is the first essential step in detailed studies of the conformation and dynamics of proteins and their complexes with inhibitors and drugs. Standard methodology for obtaining these assignments has been developed. However, the behavior of individual proteins differs from each other in solution as a result of, for example, localized conformation alterations or changes in the degree of flexibility or aggregation. This means that experiments must be tailored to a specific protein or types of protein.

We discuss in this poster the two-dimensional $HI$ NMR studies of three globular proteins—Ribonuclease A (m.w. = 13700), Phospholipase A$_2$ (m.w. = 14000) and Dihydrofolate Reductase (m.w. = 18000).

Ribonuclease A
Ribonuclease A and its complexes have long been used as a simple model for protein-nucleotide interactions. However, specific interactions of different nucleotides are not the same and the information on the kind of structural changes that nucleotide binding induces is limited. The proton NMR lines are narrow and two-dimensional techniques have been applied exhaustively. The protein does not aggregate at the typical concentrations required (3mM). The amide proton exchange rates at pH 3.8, 310K, are slow enough for many of the resonances to be observed, hence affording the possibility of full sequential assignment of the polypeptide backbone protons.

Phospholipase A$_2$
Phospholipase A$_2$ catalyses the first step in the Arachadonic Acid cascade resulting in the production of prostaglandins and leukotrienes implicated in many inflammatory conditions.

The abundance and ease of purification of these enzymes coupled with their relatively small size, pH and temperature stability and the fact that the X-ray crystal structures have been determined would appear to make this class of enzymes ideal for NMR investigation. However, problems do arise because of the tendency of the protein to aggregate at concentrations above 2mM. Even at low concentrations of 1mM, spectral lines are still quite broad; this is attributed to a short $T_2$. The large amount of secondary structure in these proteins suggested that sequential assignment techniques could be applied; however, the $T_2$ problem has precluded the acquisition of required information by the relayed coherence transfer method. The $T_2$ problem is however, somewhat eliminated in the spin-lock experiments.

Bacterial Dihydrofolate Reductase
Despite its molecular weight, the NMR lines of this protein are narrow. Assignments to date have been confined to the high-field methyl and aromatic regions, by reference to the crystal data. The 32 residues that have been assigned represent a useful set of reporter groups. The enzyme and its conformationally locked complexes do not tolerate high temperatures (>40°) and are insoluble above 4 mM. These factors, together with the size of the protein, severely limit the success of the sequential assignment methods. We are exploring modified assignment strategies.
STRUCTURE CONFIRMATION OF THE ACTIVE PART OF
HAEMOPHILUS INFLUENZA TYPE B VACCINE.

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Abstract:

The molecule under investigation is expected to be active as a Haemophilus Influenza type B vaccine when coupled to a tetanus toxoid protein.

\[
\begin{align*}
\text{ribose (3)} & \quad \text{ribose (2)} & \quad \text{ribose (1)} & \quad \text{spacer} \\
\text{riboitol (3)} & \quad \text{riboitol (2)} & \quad \text{riboitol (1)} & \quad \text{phosphate (3)} \\
& \quad \text{phosphate (2)} & \quad \text{phosphate (1)}
\end{align*}
\]

Structural integrity of the molecule is determined before linkage to the protein by means of combination of various NMR techniques and Fast Atom Bombardment mass spectrometry. Proton NMR assignments were achieved by \(^1\)H-\(^1\)H (phase sensitive) COSY and \(^{31}\)P-\(^1\)H correlated spectroscopy. The \(^{31}\)P spectrum was easily assigned by recording a \(^1\)H-coupled spectrum. The \(^{13}\)C resonances were assigned by means of a DEPT analysis and a \(^{13}\)C-\(^1\)H correlated spectrum using \(f_1\) decoupling. Information on the molecular weight and some structural elements was obtained by Fast Atom Bombardment mass spectrometry.
EREMOMYCIN - A NOVEL GLYCOPEPTIDE ANTIBIOTIC
THE USE OF DIPOLAR INTERACTIONS

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Primary structure including the carbohydrate [\(\alpha\)-L-
  -eremosaminyl(4'-epi-vancosaminyl) and 2-0-\(\alpha\)-L-
  -eremoseaminyl-\(\beta\)-D-glucosyl] moieties of this antibiotic
  has been clarified by \(^1\)H and \(^13\)C NMR methods. The molecular
  motions are outside the extreme narrowing regime even at
  70°C in D\(_2\)O solutions. Heteronuclear NOE effects and \(^13\)C
  spin lattice relaxation are analyzed.
Vancomycin and Vancomycin-D-Ala-D-Ala Structures: 
an integrated approach employing 2D NMR and energy minimisation 
with distance constraints.

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Glycopeptide antibiotics belonging to the Vancomycin class have 
received an increased amount of interest due to their efficacy against 
methicillin resistant Gram-positive bacteria. These antibiotics exert 
their biological action by inhibiting bacterial cell-wall synthesis by 
binding to cell-wall precursors terminating in D-Ala-D-Ala. We report 
here a study, using NMR and force field calculations, on the 
conformational properties of the antibiotic Vancomycin and its 
interactions with D-Ala-D-Ala - a model for the site of action of the 
antibiotic.

Williams and his co-workers have reported extensive 1D NMR studies 
of Vancomycin, but a detailed quantitative approach employing 2D NMR 
data and restrained molecular dynamics calculations (for the 
determination of the three-dimensional structure of the free Vancomycin 
and its complex) has not yet been published.

A combination of 2D experiments, such as COSY, homonuclear 
Hartman-Hahn (HOHAHA), NOESY and Rotating frame Overhauser enhancements
(ROESY) have been performed on both the antibiotic and its complex in order to obtain structural information. The multispin analysis of the cross peak volumes, measured from a set of NOESY experiments performed at different mixing times, led to the determination of proton-proton distances which were used as constraints in the molecular dynamics calculations. Constraints on dihedral angles may also be obtained from J coupling data. Further NMR information derived from heteronuclear \(^{13}\text{C},^{15}\text{N}\) experiments has been analysed in the light of possible structures.

\[ \text{(1)} \]

**REFERENCES**


Two pulse sequences for three dimensional NMR-spectroscopy are presented: HOHAHA-NOESY and a combination of inverse $^{15}\text{N}-^{1}\text{H}$ COSY and the NOESY experiment. Both sequences have been applied to a solution of 5mM γ1-Purothionin in $\text{H}_2\text{O}/\text{D}_2\text{O}$. The merits and limitations of both sequences will be discussed.

The first sequence was applied to the sequential assignment of the NH-resonances. An advantage of this sequence is that the water resonance can be suppressed easily by using a 1-1 pulse at the end.

The second sequence represents a novelty in 3D-NMR spectroscopy, because no frequency selection is necessary to record 3D-spectra involving a $^{15}\text{N}-^{1}\text{H}$ coherence transfer. Consequently, the pulse sequence will not contain any soft pulses. The relationship of this sequence to corresponding 2D-relayed techniques will be discussed.
SEQUENCE SPECIFIC $^1$H NMR ASSIGNMENT AND SECONDARY STRUCTURE DETERMINATION OF A 124 RESIDUE "LARGE" PROTEIN

by

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With the aim to determine the three-dimensional structure of a 124 residue "large" protein by 2D NMR spectroscopy sequence and stereo-specific assignment of the $^1$H NMR spectrum has been obtained. The assignment procedure employed relies on data obtained by 2D NMR techniques such as COSY, Double Quantum Filtered COSY, NOESY, Double Quantum Spectroscopy, Relayed COSY, TOCSY and E. COSY.

The first stage of the assignment procedure identifying spin systems of the amino acids is completed. The sequential assignment process is presently in progress and a substantial part of the secondary structure has been determined to consist of a four-stranded anti-parallel beta-structure and two alpha-helices.

The poster will give an up to date description of the sequential assignment and the secondary structure of the protein which is among the largest proteins so far being studied with these techniques.
'H-NMR OF THE ROP PROTEIN

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We are investigating the rop (repressor of primer) protein found in the ColE1 plasmid. The wildtype protein structure differs appreciably from the mutant rmk6 as evidenced by their proton NMR spectra. rmk6 lacks the C-terminal 6 amino acids. Both forms of the protein are very acid and temperature stable, so that we can perform two-dimensional measurements at 40°C and pH 2.3, a good basis for structural investigations. As the x-ray structure of the wildtype protein is known at 1.7 Å resolution, we have a good starting point to investigate the solution structure of this protein. The spectrum of the wildtype shows an unusually large number of very slowly exchanging protons, probably due to the extremely high α-helix content of the protein. The rmk6 mutant has not been crystallized yet. We are currently exploiting spin system assignments of both proteins and hopefully will get secondary structure information quite soon.

Literature:
PROTON NMR OF THE P21.GDP.Mg\(^2+\) COMPLEX OF THE HA-RAS ONCOGENE PRODUCT

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The gene products of the ras family are highly related proteins of molecular weight 21 kDa termed p21 with a chain length of 189 amino acids. They bind guanine nucleotides with high affinity and specificity and exhibit low GTPase activity [for a review see e.g. (1)]. Since no X-ray structure of p21 is available by now, two dimensional NMR studies were performed on the c-Ha-ras encoded proto-oncogene product p21\(_c\). COSY and NOESY spectra of the p21\(_c\).GDP.Mg\(^2+\) complex show that the ribose H1' proton of the bound GDP is in close proximity to the aromatic side chain of a phenylalanyl residue (2). Site directed mutagenesis is used to elucidate which phenylalanyl residue in the sequence is the one in question.

Literature:

Structural studies of the O-antigenic polysaccharide of *Escherichia coli* O86, with blood group B activity.

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ABSTRACT

The structure of O-antigen polysaccharide of *Escherichia coli* O86:K2:H2 has been investigated using methylation analysis, specific degradations and n.m.r. spectroscopy. For the assignment of signals standard homo- and heteronuclear COSY, relayed COSY, double relayed COSY and NOESY pulse sequences were used. It is concluded that the O-antigen is composed of pentasaccharide repeating-units having the following structure. The structure represents the biological repeating unit.

\[ \alpha-L-Fucp-(1 \rightarrow 2)-\beta-D-Galp-(1 \rightarrow 3)-\alpha-D-GalpNAc-(1 \rightarrow 3)-\beta-D-GalpNAc-(1 \rightarrow 4)-\alpha-D-Galp \]
1D and 2D $^1$H-NMR experiments have been performed at 500 MHz to elucidate the solution structure of the neuropeptide head activator from hydra. The head activator, an endekapeptide with the sequence p-Glu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe was first isolated from Hydra attenuata where it initiates head specific growth and differentiation, but it can also be found in higher organism. The biological activity of head activator is strongly dependent on its concentration; at concentrations higher than $10^{-11}$ M the neuropeptide is desactivated by dimerization. The $^1$H-NMR spectrum of the head activator in aqueous solution has been completely assigned by two-dimensional NMR spectroscopy. The apparent pseudo first-order exchange rates $k_{ex}$ of the backbone amide protons and the correspondent activation enthalpies $\Delta H$ could be determined. The exchange rates decrease and the activation enthalpies increase monotonically from the N-terminal to the C-terminal part of the peptide. The exchange rates vary from $21 \text{ s}^{-1}$ to $0.3 \text{ s}^{-1}$ at 274° K, the activation enthalpies from $60 \text{ kJ mol}^{-1}$ to $75 \text{ kJ mol}^{-1}$ The $pK_a$ values of the terminal carboxy group and of the lysyl amino group have been estimated as 3.3 and 10.3, respectively. These NMR results are in line with a dimeric structure in antisymmetric arrangement of the subunits, forming an antiparallel $\beta$-pleated sheet between C-terminal segments from amino acid 7 to 11 of the two subunits. The structure appears to be very stable; in the temperature and pH range studied, i.e. from 274° K to 338° K and from pH 0.9 to pH 11.6, there were no spectroscopic indications for a global structural isomerisation.

Due to the unfavourable rotational correlation time $\tau_c$ of the molecule no NOE's could be obtained by NOESY spectroscopy, only the correspondent experiment in the rotating frame (ROESY) permitted the observation of a number of mutual NOE's. From the semiquantitative evaluation of these
NOE's follows that the prolyl peptide bonds in the N-terminal part of the sequence p-Glu-Pro-Pro are in the trans-conformation. NOE's between p-Glu and Phe suggest strongly that the C-terminus and the N-terminus of the peptide are in close proximity. Because of the symmetry of the dimeric structure, in principle, all observed NOE's can be interpreted as intramolecular or intermolecular effects, a fact which makes any structural determination very difficult.

Only extensive calculations which were based on restraint molecular dynamics and on energy minimization algorithms adapted to the special problem allowed the determination of the three-dimensional structure of the dimer in solution.
REFINEMENT OF THE THREE-DIMENSIONAL SOLUTION STRUCTURE OF BARLEY SERINE PROTEINASE INHIBITOR 2 USING STEREOSPECIFIC ASSIGNMENT

By:

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We have recently described the three-dimensional structure of BSPI-2 as determined by $^1$H NMR spectroscopy and restrained molecular dynamics (1-4). The present paper describes the stereospecific assignment (5) of the AMX beta-methylene hydrogen- and valine gamma-methyl hydrogen resonances of BSPI-2 as obtained using a combination of E. COSY experiments (6) to measure coupling constants and NOE time developments to measure intra-residue NOE's. The symmetry projection operator method (7) was applied to measure the intensities of the NOE's. Given the additional stereospecific assignments and the determination of the torsion angles phi and chi, a refined structure was calculated using distance geometry and restrained molecular dynamics.

REFERENCES:
Carbon-13 and Deuterium NMR Studies on the Liquid and Solid Phases of Globular \((\text{CH}_3)_3\text{CX}\) Compounds.

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Compounds of the \((\text{CH}_3)_3\text{CX}\) type with \(X = \text{Cl}, \text{Br}, \text{NO}_2, \text{CN}, \text{and SH}\), exist, owing to their almost globular shape of molecules, in an orientationally disordered crystalline high-temperature phase. In this so-called plastic phase the molecules undergo rapid reorientational motions and the phase is of high symmetry. The \(\text{t-butyl}\) compounds listed above demonstrate in their solid states at least two phase transitions which separate phases featuring different lattice symmetry and molecular dynamics.

The orientational disorder and dynamics of the solid phases of globular compounds have been studied by various methods such as NMR, X-ray, neutron scattering and dielectric measurements. Unfortunately, the interpretation of the accumulated data has produced some inconsistent results. A powerful feature of the NMR experiment is the possibility to monitor and study the resonances of different nuclear species. In organic solids, the nuclei which have been most widely studied are \(^1\text{H}\) and \(^{19}\text{F}\), whereas \(^2\text{H}\) and in particular, \(^{13}\text{C}\) have received more limited attention.

The results of \(^2\text{H}\) and \(^{13}\text{C}\) NMR linewidth and spin-lattice relaxation time measurements on the liquid and solid phases of some \(\text{t-butyl}\) compounds will be presented. The aim of this work is mainly to study the temperature behaviour of globular compounds and to characterize the molecular motions occurring in the various phases.
MAGNETIC SHIELDING TENSOR COMPONENTS IN HIGHLY STRAINED PHOSPHINES
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The $^{31}$P magnetic shielding tensor components of 1,2,3-Triphenylphosphirene have been measured using the MAS method, with several spinning speeds, at 80 MHz. In this highly strained three-membered ring molecule, the valence bond angle at the phosphorus has a very peculiar value ($k_2'$ intracyclic) as compared to unstrained phosphines (100° typically).

The MAS method has proved to be definitely more precise in this case for the measurement of the $\sigma$ tensor. This results of the large anisotropy of the tensor. A programme has been developed, on the basis of Herzfeld and Berger analysis, to determine the principal values of the $\sigma(31^P)$ tensor. These values, with positive values at high field and respect to $H_3P_O$, 85%, are quoted below:

$$\sigma_{11} = -58 \text{ ppm}, \sigma_{22} = 28 \text{ ppm}, \sigma_{33} = 636 \text{ ppm}$$

To the best of our knowledge, the anisotropy $\Delta\sigma = |\sigma_{11} - \sigma_{33}| = 694 \text{ ppm}$ is the highest value measured in a three-coordinate phosphorus compound. In unstrained phosphines $\Delta\sigma < 100 \text{ ppm}$.

The crystal structure of 1,2,3-Triphenylphosphirene shows that the local symmetry at the phosphorus is very close to $C_s$, with the local symmetry plane $\pi$ bisecting the three-membered ring. Thus one principal direction of the $\sigma$ tensor is in the plane of the ring and perpendicular to $\pi$. From a calculation using the IGLO (Individual Gauge for Localized Orbitals) method the high field component $\sigma_{33}$ is found to be perpendicular to the ring.

The $\sigma_{11}$ and $\sigma_{22}$ values correspond to normal resonance frequencies for a phosphine, conversely to the $\sigma_{33}$ value which is at very high field. Thus it is this unique principal value which is responsible of the high field resonance observed in the isotropic phase. It has to be pointed out that such a behaviour has also been reported for the aliphatic $^{13}C$ of cyclopropene. The high field resonance in the isotropic phase of this $^{13}C$ results of a unique high
field principal value, perpendicular to the ring.

As a consequence, we anticipate that the high field resonance of phosphiranes is the consequence of a similar behaviour. Experiences are underway in order to ascertain this statement.
Analysis of $^2$H-powder spectra of specifically labeled phospholipids have yielded valuable information on the motional and conformational behaviour of lipid bilayer membranes, particularly when combined with a complete line shape analysis and simulations of the experimentally observed spectra (1,2). While these $^2$H-nmr line shapes are very sensitive to motions in the intermediate exchange regime, certain situations can occur, where the spectra cannot unambiguously be simulated with only one particular type of motional mechanism.

Studies of oriented systems have then the advantage of giving additional information by having the possibility of recording spectra of the oriented system at different orientations of the director axis with respect to the magnetic field. With this technique a discrimination between different types of molecular reorientations giving nearly identical powder spectra should be possible.

We have succeeded in preparing ordered lamellar systems of specifically labeled dipalmitoyl-phosphatidylcholine (DPPC) and dimyristoyl-phosphatidylethanolamine (DMPE). $^2$H-nmr spectra were recorded with different orientations of the bilayers with respect to the magnetic field and at temperatures above and below the phase transition of the respective phospholipid. Spectra taken in the liquid-crystalline phase showed the expected dependence of the quadrupole splitting on the angle of orientation. Notable differences were observed in the gel phase spectra. In DMPE multilayers the molecular axes are oriented parallel to the bilayer normal. Gel phase spectra of DMPE could be quantitatively simulated using the six-fold jump model described previously (1,2), using a Gaussian distribution of director axes with a half width of ca. 9°. In DPPC bilayers in the L$_{g'}$-phase the molecules are tilted with respect to the bilayer normal. This is clearly evident
from the spectrum taken with the bilayer normal oriented parallel to the magnetic field. Because of this molecular tilt, the reorientation mechanism of the molecules seems to be more complicated than in the case of DMPE, preventing up to now a satisfactory simulation of spectra of oriented DPPC bilayers taken at other director orientations.

DESIGN OF AN EFFICIENT PROBE FOR CROSS-POLARIZATION VARIABLE-ANGLE SPINNING NMR OF SOLIDS

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High-speed, stable spinning is a desirable technique in multinuclear magic-angle spinning (MAS) NMR experiments of solids. In variable-angle spinning (VAS) experiments of quadrupolar and spin-1/2 nuclei high-speed spinning capabilities are especially important because of the broadening observed for the $m = +1/2 \leftrightarrow m = -1/2$ transition. High-speed VAS experiments are of particular interest since spin-1/2 chemical shift anisotropies, quadrupolar coupling constants, and the corresponding asymmetry parameters can be accurately obtained using this technique (1,2).

We describe the design of a CP/VAS probe capable of high-speed spinning at any angle between 0° and 90° with respect to the magnet field. The spinning performance of this new design is even better than for another spinner design recently published by us (3), i.e. for 5 and 7 mm o.d. rotors the rotational frequencies are larger than 15 and 10 kHz, respectively, using ordinary air for both drive and bearing. The spinner assembly which consists of three cylindrical parts held together by a press-fit is also very suitable for variable temperature studies.

The simplicity of the spinner design and the electronic efficiency of the probe will be demonstrated along with its applications to VAS NMR experiments of some inorganic materials.

HIGH-SPEED $^{27}$Al AND $^{29}$Si MAS NMR STUDIES OF ZEOLITE STRUCTURES AND CLAYS. DETERMINATION OF $^{27}$Al QUADRUPOLE COUPLING CONSTANTS AND ACCURATE $^{27}$Al CHEMICAL SHIFTS

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In recent years $^{27}$Al and $^{29}$Si high resolution MAS and CP/MAS NMR spectroscopies have proven useful tools in structural studies of zeolites, layered silicates, and clay minerals. Especially the $^{29}$Si chemical shift, which may be determined with a high accuracy, has found useful applications in that it correlates (i) silicon-oxygen tetrahedra and their linkage, (ii) the number of next nearest neighbour $^{27}$Al-atoms, i.e. Si(nAl), (iii) Si-Si/Si-Al bond lengths and Si-O-Si/Si-O-Al bond angles. Although $^{27}$Al chemical shifts are extremely useful in distinguishing between octahedral (0-12 ppm) and tetrahedral (ca. 50-80 ppm) Al-sites, determinations of accurate $^{27}$Al chemical shifts are more difficult. The reason is that observed $^{27}$Al resonances ($m = 1/2 \leftrightarrow m = -1/2$) may be very broad and its center of gravity is shifted from the exact chemical shift because of second order quadrupolar effects. Since most studies have not taken these effects into account, the reliability of a vast number of literature $^{27}$Al chemical shifts must be taken with great caution.

In this work we report that using very high-speed spinning (i.e. spinning speeds up to and above 10 kHz) the determination of accurate $^{27}$Al chemical shifts, quadrupole coupling constants (QCC) and asymmetry parameters ($\eta$) is greatly facilitated. For sufficiently large $^{27}$Al QCC's these parameters may be determined from computer lineshape simulations. Examples of such simulations of $^{27}$Al MAS lineshapes observed at high-speed spinning will be presented. For compounds with small QCC's the lineshape approaches a usual Lorentzian lineshape, and an approximate value (within ca. ±15%) for the QCC may be determined using the spinning sidebands for the satellite transitions. For this purpose very high-speed spinning is of great advantage in order to increase the intensities of these spinning sidebands. These two methods for the determination of the QCC parameters have been applied to: (i) a series of ion-exchanged sodalites for which a 1:1 linear relationship between $^{27}$Al and $^{29}$Si chemical shifts, and linear relationships between bond lengths/bond angles and $^{27}$Al (or $^{29}$Si) chemical shifts is observed; (ii) the determination and confirmation of structures for the zeolite Li-ABW-H$_2$O, anhydrous Li-ABW, and the three polymorphs α-, β-, and γ-Eucryptite; (iii) natural clays from oil wells in the North Sea, and for which QCC's for the two Al-sites have been determined from spinning sideband patterns of the satellite transitions.

The spin dynamics of hydrogen and fluorine in a 70/30 VF$_2$/TrFE copolymer have been investigated by the measurements of the nuclear spin-lattice relaxation times in the laboratory ($T_1$) and rotating frames ($T_1^\theta$), the transient Overhauser effect (TOE) and the free induction decay (FID).

A simple model treating cross-relaxation and spin diffusion on an equal footing was found to be appropriate to describe all the experimental data. Moreover, it provides useful information about the molecular motion in the amorphous phase. $T_1^\theta$ results then demonstrate the presence of local motions within the crystallites implying the existence of crystalline "defects".
We have been exploring the use of paramagnetic ions to probe the structures of 3-D continuous solids using magic angle spinning (MAS) NMR. Despite extensive studies of paramagnetic species in solution, there are few examples of such studies in the solid state.

Here we describe investigations using $^{119}$Sn NMR of the lanthanide stannates $\text{Ln}_2\text{Sn}_2\text{O}_7$, a series of isostructural compounds (they all adopt the pyrochlore structure). Massive chemical shifts were observed whose direction and magnitude could be rationalised as resulting primarily from a Fermi contact (through-bond) mechanism. The solid solutions $\text{Ln}(1)_{2-x}\text{Ln}(2)_x\text{Sn}_2\text{O}_7$, where $\text{Ln}(1)$ is a diamagnetic and $\text{Ln}(2)$ a paramagnetic ion, have been studied in particular detail. In these cases the chemical shifts of the $^{119}$Sn nuclei were found to be extremely sensitive to the adjacent paramagnetic ions, with each successive substitution of a paramagnetic ion for a diamagnetic ion into the local coordination sphere around the tin atom producing a large additive shift. By exploiting the different relaxation properties of nuclei close to paramagnetic species, the resonances resulting from $^{119}$Sn nuclei containing paramagnetic ions in their local coordination sphere could easily be observed, despite these nuclei often being in very low concentration in the solid. When limited solid solutions were formed, it also proved possible to calculate the extent of solid solution in these systems using intensity data, which had not previously
been possible from X-ray data. In addition, dipolar (pseudocontact) shifts resulting from the substitution of paramagnetic ions in more distant sites, separated by four bonds from the $^{119}$Sn nuclei were observed.

The general utility of paramagnetic shifts in HASNMR is also discussed, and illustrated with examples involving other nuclei.

REFERENCES

(1) S. Ganapathy, V. P. Chacko, R. G. Bryant and M. C. Etter,

(2) A. K. Cheetham, C. M. Dobson, C. P. Grey, and R. J. B. Jakeman,
Backbone Fluctuations of Proteins and Polypeptides as seen by solid-state NMR techniques:
Proton field-cycling NMR and deuteron-NMR

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Introduction. The dynamic behaviour of proteins is of considerable interest with regard to the functional kinetics of this class of biomolecules. Therefore, many studies have been devoted to this problem with different experimental methods (1,2). In this work we have studied protein dynamics in the time scale of solid-state NMR (3,4) by proton field-cycling relaxation spectroscopy and after deuterium exchange - by solid-state deuteron NMR.

Techniques. The field-cycling relaxation data were recorded with a home-built apparatus using a superconducting magnet with a detection field of 1.2 T corresponding to 52 MHz proton resonance frequency (9). The deuteron spectra were recorded with a Bruker MSL 300 solid-state NMR spectrometer operating at 7 T corresponding to 46 MHz deuteron resonance frequency. The RF-coil was directly wound on 5 mm sample tubes to achieve 90° pulse-lengths of 2.5 μs. The deuteron powder spectra were recorded using the conventional solid echo pulse sequence with a pulse delay of 20 μs. Quadrature detection and CYCLOPS phase cycling were applied in all cases. T1 was measured by the aid of a saturation pulse prior to the solid echo pulse sequence. The Jeener-Broekaert decays were recorded using the known 3-pulse sequence (10,11).

Proton experiments. The proton field-cycling data of polyglycine and polyalanine (backbone protons) - and for comparison of D2O-hydrated α-chymotrypsin - can be described in a frequency range of almost 5 decades by a simple power law (5,6)

\[ T_1 = C \omega^{0.75} \]  

with \( C = 4.7 \times 10^{-7} s^{1.75} \). This coincidence of the \( T_1 \)-dispersion slopes near room temperature of the different samples demonstrate that any influence of distributions of sidegroup correlation times can be excluded at low frequencies. Polyglycine in particular lacks of any sidegroups at all. The finding that near room temperature the power law eqn. (1) describes the \( T_1 \)-dispersion of globular proteins as well as fibrillar proteins lead to the conclusion that it is the chain character rather than the primary, secondary, tertiary or quaternary structure which determines the dynamic behaviour in our frequency window. In other words, the macromolecules behave as quasi one-dimensional systems.

Deuteron experiments (7,8,9) The deuteron powder spectra of exchange deuterated bovine serum albumin shows two components. In analogy to the proton relaxation data we attribute them to rotating sidegroups and to the backbone amide groups. The narrow component has a spin-lattice-relaxation time of about 70 ms. Decreasing the temperature from 294 K to 165 K this component disappears without any line-broadening. This behaviour is typical for thermally activated processes. The broad component relaxes much more slowly (\( T_1 = 1.9 \ s \) at room temperature) and can be described by the standard theory for the rigid lattice case with the conclusion that the backbone is orientationally rigid in the time scale < 10^{-5} s. An extension of this time scale is provided by the Jeener-Broekaert (JB) experiment (10,11). The time constants of the JB
and saturation-recovery curves of the backbone signal (both non-exponential) coincide within the limits of the evaluation limits. The conclusion is that the JB experiment does not indicate any backbone reorientation within a time scaled by the time constants of the spin-lattice relaxation. In general, the deuteron experiments permit us to draw two conclusions. First, the backbone dynamics is heterogeneous as revealed by the multiexponential relaxation curves. Second, there are no perceptible rotational motions in the time scale of the JB experiment and, hence, of the proton $T_1$-dispersion range.

Discussion. In the above sections we have shown that proton and deuteron data lead to the following discrepancy: The proton $T_1$-dispersion indicates strong backbone fluctuations in the correlation time scale $10^{-10} s < \tau_H < 10^{-5} s$, while the deuteron experiments do not reveal any perceptible backbone motions in a range of rotational correlation times $\tau_D < 1s$. The key for a solution of the problem must be sought in the different interactions which protons and deuterons underline. Among protons the internuclear dipolar interaction dominates as a relaxation mechanism, while in the case of deuterons the quadrupole coupling governs the coherence evolution and the relaxation. With deuteron NMR, one therefore can detect only rotational fluctuations, while the dipolar interactions among protons are influenced by translational displacements in addition. Translational fluctuations therefore can directly be investigated with proton NMR.

Assuming the existence of dilating defects in the protein backbone structure one now provides an explanation of such translational fluctuations in the absence of rotational motions as concluded from proton and deuteron NMR. The dilating defects are assumed to diffuse along the backbone, i.e. in one dimension, and cause translational displacements of the interacting protons by a widening of the local structure. The orientation of the local electric field gradient tensor governing the deuteron quadrupole coupling hereby must be assumed to remain virtually unaffected (7).

References
1) R. Porter, M. O'Conner, J. Whelan (Eds.)
   Mobility and Function in Proteins and Nucleic Acids
2) M. Karplus and J. A. McCammon
   CRC Critical Reviews in Biochemistry, 1981; 9, 293
4) K. P. Pauls, A. L. McKay, O. Södermann, M. Bloom, A. K. Tanjrea, R. S. Hodges
   Eur. Biophys. J., 1985; 12, 1
7) W. Nusser, R. Kimmich, F. Winter submitted for publication
9) G. Schauer, W. Nusser, M. Blanz, R. Kimmich
Two-Dimensional Quadrupolar Echo Spectroscopy of I > 1 Nuclei. Spin Relaxation in Anisotropic Systems.

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The quadrupolar echo (QE) pulse sequence \{((\pi/2)_x - \tau - (\pi/2)_y - \tau - acq)\} has been used extensively to extract the powder spectral pattern and to determine the spectral densities for I = 1 nuclei such as $^2\text{H}$ and $^{14}\text{N}$ in anisotropic phases. The application of the QE pulse sequence to I > 1 nuclei has so far received little attention, particularly for relaxation purposes. The determination of the motional spectral densities $J_k(\omega_0)$ (k = 0,1,2) characterizing the fluctuating quadrupolar interaction for nuclei such as $^{17}\text{O}$ (I = 5/2) and $^{23}\text{Na}$ (I = 3/2) is of great importance in the study of the aqueous regions in heterogeneous systems, lyotropic liquid crystals and lipid bilayers. However, there have been few useful and convenient NMR techniques for such studies, particularly of $J_0(0)$ which reports on slow diffusional processes in these systems. The QE pulse sequence is potentially a suitable technique for this purpose.

We have examined the response to the QE pulse sequence of a nuclear spin system of arbitrary half integral I (I > 1) in an anisotropic system. The state multipole formalism was used to describe the effects of the radio-frequency pulses and the evolution, quadrupolar precession and relaxation of the spin system. The main objective of the study is the extraction of the spectral densities. The response of the spin system in the frequency domain as a function of the evolution time can be best presented in the two-dimensional NMR format.

Experiments have been performed for $^{23}\text{Na}$ (counterions) and $^{17}\text{O}$ (water) in two types of anisotropic systems, i.e.

a. Partially oriented systems (i.e. systems with a preferential macroscopic orientation with respect to the magnetic field but also having a non-negligible orientational distribution).
b. Randomly oriented (powder) systems.

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The observed spectra for these two types of systems in the F1 dimension are in agreement with the theoretical description, and their common characteristics can be summarized as follows.

1. All information pertaining to the static quadrupolar interaction is contained in the F1 dimension. This is true even if only the central \((1/2 \leftrightarrow -1/2)\) transition in the F2 dimension is detected.

2. The number of satellites appearing in the F1 dimension is increased. The exact number depends on the spin quantum number \(I\) and the frequency in the F2 dimension. The satellites appear in the F1 dimension at frequencies \(\pm n/2 \omega_Q\). Furthermore, the total satellite intensity is enhanced relative to that of the central transition.

3. Second-order quadrupolar broadening does not appear to affect the width of the central line in the F1 dimension.

4. From the linewidths of the central transitions in the F1 dimension taken from cross-sections along \(\omega_2 = 0\) and \(\omega_2 = \pm n/2 \omega_Q\), the spectral densities \(J_0(0), J_1(\omega_0)\) and \(J_2(2\omega_0)\) can be determined in combination with the measurement of inversion-recovery and the linewidth of the central line in the F2 dimension. The \(J_0(0)\) thus determined is free of the effect of small orientational distribution and/or distribution of the quadrupolar coupling constant.

5. The linewidth of the satellite transitions in the F1 dimension are influenced by the orientational distribution. However, its contribution to the width can be calculated more accurately than in the case of conventional 1-D spectra of such systems.
The reasons for the selectivity of commercially available silica gel phases in the chromatography are not fully understood.

Insight into the surface structure - which determines the separation processes - and the dynamic behaviour of silica can be gained by \(^{29}\text{Si-NMR-solid-state-spectroscopy}\) combined with magic angle spinning (MAS) and crosspolarization (CP). The geminal silanol-(Q2), the silanol-(Q3) and the siloxane groups (Q4) can be identified.\(^{(1)}\)

To achieve maximal magnetization we varied the contact time on different silica gels (Nucleosil, Zorbax), and found that, in addition to the broad maximum of the siloxanes (Q4) at about 25-30 ms, our CP-curves show a second maximum at about 8 ms. Thus the curve is a superposition of several maxima, resulting from two or three types of siloxane units with different relaxation time. Two different \(T_{\text{Si}^H}\) and \(T_{\text{Si}^H}\) values can be obtained. They are small \((T_{\text{Si}^H} = 1-2\text{ms}, T_{\text{Si}^H} = 22 - 25\text{ms})\) and large \((T_{\text{Si}^H} = 21-38\text{ms}, T_{\text{Si}^H} = 40 - 50\text{ms})\). In comparison with \(^{13}\text{C-CP-MAS relaxation measurements of polybutadiene}\), the values of the crystalline- \((T_{\text{Si}^H} = 1-3\text{ms}, T_{\text{Si}^H} = 18-20)\) and amorphous domains \((T_{\text{Si}^H} = 21-32\text{ms}, T_{\text{Si}^H} = 35 - 40\text{ms})\) are of comparable magnitude.

This behaviour may be explained by the existence of two types of siloxane units, one being partially cristalline (β-christobalite) and one partially amorphous. The flexibly bound silicon atoms of the amorphous region require more energy for polarization than the more rigidly bound silicons of the crystalline region and so the \(T_{\text{Si}^H}\) and \(T_{\text{Si}^H}\) times are longer.
These findings provide a further evidence for the model that silicas are not homogenous systems; but consist rather of domains with differing mobilities.

References:
As was first predicted by Pincus, Blinc et al., and later derived more precisely by Freed, the Larmor frequency dependence of the longitudinal spin-lattice relaxation time $T_1(\nu)$ may reflect, under favourable conditions, the presence of collective molecular motions such as order fluctuation of the director (OFD). For nematic (N) liquid crystals the expected square-root law $T_1(\nu) \sim \nu^{1/2}$ has been verified experimentally by various research groups using special NMR techniques like fast field-cycling. Obviously, at standard high Larmor frequencies the experimental verification of this relaxation dispersion suffers from the decreasing relaxation rate of the collective contribution, which hence becomes masked by other processes. By now proton NMR studies carried out on numerous nematogen liquid crystals have verified that at Larmor frequencies lower than approximately 1 MHz OFD is the dominating relaxation mechanism.

In the case of smectic (S) liquid crystals, theoretical models predict a different relaxation dispersion by OFD, namely a linear variation $T_1(\nu) \sim \nu$, but so far no systematic experimental studies of this behaviour are available. We have studied the proton's $T_1$ frequency dependence in some smectogen liquid crystals (azoxybenzenes, cyanobiphenyls, oxycyanobiphenyls) over a broad frequency range (200 Hz to 100 MHz). The figure illustrates the results for one of the compounds (HpAB) at two temperatures in the S-phase, and for comparison, also in the N-phase. In the range $10^4$ Hz $\leq \nu \leq 10^5$ Hz one sees the transition from the $\nu^{1/2}$-dispersion for the nematic state to the expected $\nu$-dispersion for the smectic state. This behaviour is superimposed (1) by five quadrupolar dips due to the coupling between proton and nitrogen spins, (2) by a low-frequency plateau due to a cut-off of the OFD modes, and (3) by a shallow high-frequency dispersion due to non-collective molecular reorientations. The dips have not been observed previously in nematic liquid crystals, but they are well-known at usual high Larmor frequencies in the solid state of such compounds. An analysis of the quadrupolar frequencies $\nu_0$ reveals that there exist at least two non-
equivalent nitrogen positions in the molecular arrangement. The temperature dependencies of the $\mathbf{v}_{ij}$s are much stronger than described by standard NQR theory.

Organometallic complexes containing phosphiniligands are used to catalyse various organic transformations. In many cases it would be of interest to support these homogeneous catalysts on a polymer to facilitate the separation from the reaction products. A feasible method to analyse these supported catalysts in the solid state consists in the application of Phosphorous Cross-Polarization and Magic Angle Spinning techniques. In practice, however, an unambiguous assignment was often hampered by the presence of a manifold of resonance lines due to different crystalllographic sites and homonuclear couplings of two or more phosphorous nuclei on the same metal rendered inequivalent in the solid state. To identify the chemical shifts and the homonuclear couplings we replaced the first 90° pulse of the COSY experiment with a spin lock pulse and employed CW decoupling throughout the evolution and acquisition period. With this experiment, we were able to assign from the cross-peaks the chemical shifts and the homo- and heteronuclear coupling constants.

Figure: \( ^{31}\text{P-CP-MAS-COSY} \) spectrum of the model compound \( [\text{EtCl}(\text{P}('\text{Bu})_3)(\text{P}('\text{Bu})_2\text{CMe}_2\text{CH}_2)]. \) The phosphorous which is part of the metallocycle exhibits a much higher chemical shift anisotropy.
Deuteron 2D Exchange NMR of Solids

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Deuteron 2D exchange spectra of polycrystalline and amorphous solids can provide direct information about molecular reorientations in the slow motion limit. For deuterons the quadrupole interaction is large compared to the dipolar coupling so that sharp powder spectra are obtained. The singularities of a 2D powder spectrum form characteristic geometrical patterns ("ridges").

In the case of simple jump motions the angles of reorientation can be obtained by a direct analysis of the ridge pattern. This is demonstrated for the six-fold rotation of hexamethylbenzene. The 2D exchange spectrum of hexamethylbenzene shows a splitting of the ridge pattern, which can be explained only by two different relative orientations between the principal axis systems of the electric field gradient tensors of the methyl groups. This confirms earlier experimental results which give evidence of a two-fold rather than three- or six-fold symmetry of the hexamethylbenzene molecules in their crystalline environment.

For more complicated motions with continuous distributions of reorientational angles, as they may be encountered for example in polymers above the glass transition, the 2D lineshape has to be analyzed. This requires undistorted 2D powder spectra with pure phase in both dimensions. It is shown how such spectra can be obtained for a spin I=1 system. Details of the experiment and the data processing are discussed.

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In liquid crystals with space dependent orientation of the molecular director a specific relaxation mechanism induced by molecular translational self-diffusion arises. It is called "translational diffusion induced rotation" (TR) and is based on the modulation of the orientation of the intramolecular internuclear vector with respect to the external magnetic field as the molecule diffuses from one preferential direction to the other. Spatially non-uniform orientation of the molecular director is known to take place in several cases: i) twisted nematic liquid crystals, where the molecular chirality induces the helical twist of the director. Similar is the situation in the chiral smectic C phase and in the blue phase. ii) Lyotropic liquid crystals with aggregates with curved surfaces. iii) Nematic liquid crystal confined into spherical microdroplets embedded in a solid polymer. Here the surface interactions impose either bipolar or radial configuration of molecular director if the magnetic coherence length is smaller than the radius of the droplet R.

In this paper we evaluate the dependence of the TR relaxation rate on the molecular diffusion constant, on the Larmor frequency $\omega_L$ and on the length characteristic for the non-uniformity of the system. The spectral densities of the autocorrelation functions of the dipolar interaction between two protons, $J_{kl}(\omega)$, are calculated using the probability function $P(\vec{r}, \vec{r}', t)$. It gives the probability that a molecule which is initially at $\vec{r}'$ arrives in time $t$ to $\vec{r}$ and changes its orientation accordingly. $P(\vec{r}, \vec{r}', t)$ is the solution of the continuous diffusion equation under the initial condition $P(\vec{r}, \vec{r}'', 0) = \delta(\vec{r} - \vec{r}'')$ and satisfying the boundary condition $\nabla P = 0$. The small anisotropy of the self-diffusion tensor is neglected. After performing the space and time integration the calculated spectral densities yield the following relaxation rates for a proton pair with $r_{ij}$ being the interproton distance:

i) In the twisted nematic phase:

$$ (T_{1}^{-1})_{TR} = \frac{9 \gamma^4 n^2}{4} \frac{\tau_{TR}}{r_{ij}^2} \left( \frac{1}{1 + 4 \omega_L^2 \tau_{TR}^{-2}} \right) . $$

The relaxation rate is here determined by only one correlation time $\tau_{TR}$.
equal to $p^2/16\pi^2 D$, $p$ is the pitch of the helix and $D$ the diffusion constant in the direction of the helix axis.

ii) In the micellar isotropic phase with roughly isotropic micelles \(^3\)

$$\left( T^{-1}_1 \right)_{TR} = \frac{3\gamma^4 n^2}{10r_{ij}} \left( \tau_S \gamma + \frac{4\tau_S}{1 + \omega_L^2 \tau_S} \right). \quad (2)$$

The correlation time $\tau_S$ is given by $d^2/16\pi$, where $d$ is the diameter of the micelle and $D$ the diffusion constant for lateral diffusion along the surface of the micelle. If the micelle is discoidal the relaxation rate is expressed in terms of three correlation times.

iii) In nematic microdroplets

$$\left( T^{-1}_1 \right)_{TR} = \frac{6\pi \gamma^4 n^2}{10} \sum_{s, ij} A_{s, ij} \left( \frac{\tau_{Zs}}{1 + \omega_L^2 \tau_{Zs}} + \frac{4\tau_{Zs}}{1 + 4\omega_L^2 \tau_{Zs}} \right). \quad (3)$$

Here a number of correlation times arises due to the spatial confinement of the liquid crystal. $\tau_{Zs}$ is given by $R^2/\xi_{Zs}^2$, where $\xi_{Zs}$ is the $s$-th zero of the first derivative of the $Z$-th spherical Bessel function. The coefficients $A_{s, ij}$ are determined numerically. For the radial structure only the coefficients $A_{s, ij}$ with $Z=2$ are different from zero. The values of $\xi_{Zs}$ and $A_{Zs}$ are:

<table>
<thead>
<tr>
<th>$s$</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
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<tr>
<td>$\xi_{Zs}$</td>
<td>11.12</td>
<td>53.14</td>
<td>112.65</td>
<td>191.71</td>
</tr>
<tr>
<td>$A_{Zs}$</td>
<td>0.154</td>
<td>0.009</td>
<td>0.0017</td>
<td>0.0006</td>
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It is shown that the dominant contribution comes from the term with $\tau_{Zs} = R^2/11.12D$. In the droplet with bipolar structure, on the other hand, the leading term is $\tau_{Zs} \approx R^2/40D$.

In order to determine the diffusion constant in the above systems the measurements of the relaxation rate should be performed at Larmor frequencies with $\omega_L^{-1}$ being of the same order of magnitude as the correlation time, i.e. in the kHz frequency range. From the dispersion of the relaxation rate the correlation times can be determined yielding directly the diffusion constant if the characteristic dimension of the system is known from other studies (optical, X-rays).

Up to now the TR relaxation mechanism has been experimentally determined in phospholipid membranes analogous to lyotropic liquid crystals and in nematic microdroplets with bipolar structure. \(^4,5\)

LINEAR PREDICTION
Application to High Resolution Solid State NMR Spectroscopy

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A problem in High Resolution Solid State NMR Spectroscopy, despite improvements in instrumentation (probe heads), remains a restriction on the length of the acquisition times, particularly under high power decoupling conditions. Consequently the observed FID is often severely truncated. Conventional Fourier Transformation then yields a spectrum in which each line is convoluted by a "sinc" function ("wiggles"). Under these circumstances vital information such as satellites from J-coupling (\(^{29}\)Si, \(^{13}\)C, \(^{119}\)Sn etc.) may be masked.

However, linear prediction methods, which make no assumptions about the form of the FID outside the measurement period, are ideally suited to the distortionless spectral analysis of such time domain data.

High Resolution Solid State \(^{119}\)Sn Spin Echo data serve as an example of this application.

References
R.K.HARRIS and A.SEBALD, to be published.
LINEAR PREDICTION
Spectral Transformation Using A Recursive Technique
(STUART)

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It is well known that Fourier transformation of a severely truncated FID produces a distorted spectrum. For this reason various methods based on linear prediction of the data have been advocated. Recursive methods which equate the forward and backward prediction coefficients are fast but either yield low resolution spectra or suffer from spurious line splitting and intensity distortions. On the other hand a full least-squares solution for the forward or backward coefficients using matrix diagonalisation or inversion is very costly.

A recursive solution of the least-squares equations provides a fast method of determining both forward and backward prediction coefficients independently and its application to truncated and to noisy data is demonstrated.

References
Some Interesting Dynamic Properties of Crystalline Penicillins by Variable Temperature CP/MAS NMR

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A combination of lineshape analysis and rotationally synchronised one-dimensional magnetisation transfer experiments in variable temperature, cross polarisation, magic angle spinning $^{13}$C NMR spectroscopy on some crystalline penicillin aromatic ester derivatives has shown that both the phenoxy and benzyl aromatic rings in each molecule are executing 180° flips about their local C$_2$ axes. Specifically, the motion leads to line broadening either when its rate is comparable to the frequency difference of the isotropic chemical shifts which describe the exchanging sites ($\approx 10^2$s$^{-1}$) or when it is comparable to the $^{13}$C-$^1$H dipolar decoupling frequency ($\approx 10^5$s$^{-1}$). The lineshapes have been described by a single correlation time two-site exchange model of the motion. Similarly, rotationally synchronised one-dimensional magnetisation transfer experiments over a range of mixing times (0.5s-8.0s) have provided kinetic data on the motions in the slow site exchange limit ($\leq 10^1$s$^{-1}$). Combining the lineshape analysis and magnetisation transfer kinetic data has enabled the determination of the activation parameters for both aromatic rings which are consistent over a motional frequency range of $10^{-2}$-$10^6$s$^{-1}$.

The observation of single correlation time ring flips raises questions on the mechanism of such large amplitude motions in densely packed and well ordered molecular solids. A recent simulation of ring motions in an idealised array of aromatic rings has suggested that ring flips may be cooperative events$^1$ and it is interesting that the activation parameters of the phenoxy and benzyl ring flips are different in both compounds and consequently the phenoxy and benzyl ring flip events apparently occur with no direct correlation between these two motions within each crystal.

Sulfur amides are of particular interest due to the variation of oxidation state of sulfur atom, and as model compounds for study of the functional groups existing in some pharmacological compounds. The solid state $^{13}$C CP-MAS NMR spectrum exhibits features that are averaged out in the corresponding solution spectra and it presents exactly the same conformation as the X-ray crystal structure does. In view of this we studied several sulfur amides by $^{13}$C CP-MAS NMR spectroscopy and compared the results with the solution state NMR spectra and crystal structure determination of the compounds.

In the solid state CP-MAS NMR spectra, the signals of $^{13}$C nuclei attached to the nitrogen atoms of sulfonamide groups are split into doublets by the $^{14}$N quadrupole interactions. Distinctive $^{13}$C splittings are also observed due to restricted rotation of methyl groups about the N-C bond. Differences between the doublet patterns of the different sulfur amides are related to the oxidation state of sulfur and to type and location of the substituents.

There are also some differences in the chemical shifts of $^{13}$C between solid state and solution spectra. Some of the differences are greater than those attributable to the solvent effect, which, however are small in the case of carbon nuclei. The chemical shifts of $^{17}$O, $^{15}$N or $^{14}$N nuclei exhibit more variation with the solvent and are sensitive as well to other electronic changes around the nuclei. The $^{13}$C CP-MAS NMR spectra reflect the electronic distribution in the molecule better than the corresponding $^{13}$C solution spectra.
DISTRIBUTION OF ROTATIONAL ANGLES
FROM DEUTERON 2D-EXCHANGE SPECTRA

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A systematic description of the 2D-NMR exchange experiment for studying slow molecular motions in static powder samples will be presented. In particular it will be discussed to what extent angular information can be extracted from 2D-exchange spectra without the need of assuming a motional model. It will be shown that each 2D-exchange spectrum is a two-time distribution function. Thus the whole three-dimensional data set represents a direct image of a motional process.

Theoretical as well as experimental examples will be presented. The model calculations include standard models like isotropic rotational diffusion or overall isotropic reorientation combined with discrete internal rotational jumps aimed at the description of molecular dynamics in polymers. Experimental deuteron 2D-exchange spectra will be presented for polystyrene deuterated at the polymer backbone or the phenyl side group, respectively, both above and below the glass transition.
When flexible alkyl side chains are attached to stiff macromolecules such as aromatic poly(ester)s and poly(amide)s, a variety of systems can be generated which exhibit liquid crystalline mesophases. The rigid chains can then be oriented either in an external magnetic field, or by mechanical forces, and offer a means of molecular support of polymers. $^2$H NMR studies of selectively labeled materials provide information on the molecular dynamics and order of the backbone chain, and of specific sites in the side chains. These measurements are complimented by $^{13}$C MAS NMR studies of conformation and motion in isotropic samples, and order in mechanically oriented films.

The molecular mobility of poly(ester)s and poly(amide)s with C$_{16}$H$_{33}$ and C$_{10}$H$_{21}$ side chains was probed by the temperature dependence of the $^2$H NMR lineshape, and by measurement of $T_2$. The chain dynamics depends on the position of labelling: at the C$_7$ position the motion is somewhat independent of the main chain structure, however, at the C$_1$ position the motion shows considerable coupling to the rigid main chain.

Mechanical drawing of the poly(ester) films results in a layered biaxial structure, as confirmed by rotor-synchronised $^{13}$C 2-D MAS NMR. $^{13}$C NMR also shows great sensitivity to the conformation and morphology of the alkyl side chains. Finally, the dynamics of the unsubstituted phenylene ring has been studied using a modified TOSS experiment.
Small organic, charge-transporting molecules doped into inert polymer matrices offer many advantages as model systems for the study of electronic processes in amorphous materials. We have studied samples of bisphenol-A-polycarbonate doped with various amounts of trianisylaminium perchlorate and trianisylamine using proton DNP and C-13 DNP/CPMAS and DNP/FIDMAS experiments. The H DNP experiments indicate that the electron-proton interactions have both a time-independent and a time-dependent component. The former lead to enhancements due to the solid-state and thermal mixing effects, the latter to an Overhauser enhancement. The Overhauser enhancement is positive, indicating that scalar electron-proton interactions dominate. The addition of free amine reduces the proportion of Overhauser enhancement.

The C-13 DNP/FIDMAS experiments indicate differential proton nuclear Overhauser enhancement as well as a mixture of solid-state, thermal mixing and Overhauser enhancement due to the unpaired electrons. The implications of these observations for charge mobility and small molecule clustering in the polymeric matrix will be discussed.
TWO-DIMENSIONAL $^{13}$C-MAS-NMR

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Rotor synchronized $^{13}$C-MAS-NMR experiments will be presented designed for studying molecular order and dynamics as well as spin diffusion in solids. If the mechanical rotation is combined with rotations in spin space the spinning sidebands can be modified or suppressed, e.g. by the TOSS sequence in a preparation period. During the subsequent mixing period molecular motion or spin diffusion can change this delicate spin state, leading to partially restored sidebands in the detection period.

For isotropic samples sidebands indicating slow molecular motions can be generated in a simple 1D version of such experiments. For partially ordered samples, e.g. drawn fibres, the sidebands cannot be suppressed completely by the TOSS sequence in the preparation period. If combined with rotor-synchronization, the sidebands can, however, be confined to a single line in a 2D sideband pattern. Modification of the spin state in the mixing period will then generate exchange sidebands in the 2D spectrum, which can only occur if the carbon position detected is involved both in the macroscopic order and the dynamic process. Thus possible correlations between these features can directly be studied.

The methods are illustrated on solid polymers, where both slow molecular dynamics and spin diffusion have been detected.
Solid-state NMR on quadrupolar nuclei with half-integer spin is becoming increasingly popular and we present some results from static and MAS $^{23}$Na NMR experiments for a series of sodium-containing compounds. The quadrupole coupling constants (QCC's) and asymmetry parameters ($\eta$'s) were determined from comparison with theoretically calculated lineshapes and correlated with structural and motional properties.

We also discuss and illustrate some important experimental aspects of $^{23}$Na NMR in solids, such as choice of $B_0$-field and how the lineshapes depend on QCC's and the strength of the r.f. field.
One of the major goals of medical NMR from its very beginnings has been the detection and characterization of diseased regions or states inside the human body. One central topic was the distinction between normal and tumorous tissue by their relaxation times $T_1$ and $T_2$. However, up to the moment no generally applicable rules have been found. Especially the discrimination between tumors and other pathologies seem to be difficult or impossible. This is not so astonishing in view of the great variety of pathologies and the small number of available diagnostic parameters ($T_1$, $T_2$ and rho, the proton density), their biological variability and the uncertainty in their measurement. The variation of $T_1$ with the magnetic field strength makes the situation even more complicated.

We use a new approach, which increases the number of parameters, reduces the experimental error and overcomes the problem of field dependence of $T_1$ by taking advantage of it. The method used is field cycling relaxometry, which means in our case the measurement of $1/T_1$ at field strengths between 0.0002 and 4.7 Tesla in vitro on fresh or carefully stored tissue samples, combined with the determination of $1/T_2$ at fixed field and of water content. The result
of the field-cycling experiment, the longitudinal relaxation dispersion, then undergoes a numerical curve-fitting procedure, from which parameters and their variance are extracted. As the dispersion curves of tissues in the observed field range always resemble single-step, four parameters are obtained using the equation

\[
\frac{1}{T_1} = A + \frac{B}{(\text{freq.} / C)^D}
\]

with

- \(A\) : baseline
- \(B\) : height of the dispersion step
- \(C\) : inflection frequency
- \(D\) : steepness of the dispersion step.

Until now, we do not assign any physical meaning to these parameters.

Thus, the total number of parameters is 6 instead of 3, and we hope to improve tissue specificity this way. A large number of normal human brain samples has been investigated. The evaluation is still under way, but first results show e.g. a clear distinction between grey and white matter for all parameters. Pathological samples are currently, under investigation, recent results will be compared and discussed.
High Resolution Heteronuclear Spectroscopy in Inhomogeneous Magnetic Fields

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The resolution of an NMR spectrum is severely degraded by any inhomogeneities in the static magnetic field or in the magnetic susceptibility of the sample. However high resolution heteronuclear spectra may be obtained under these adverse conditions by performing an experiment that incorporates a heteronuclear coherence transfer echo. An experiment of this type utilising double quantum coherence has previously been demonstrated (1).

We have designed a family of pulse sequences based upon the initial generation of transverse proton magnetisation to maximise the sensitivity. This is subsequently transferred to the $^{13}$C nuclear spins to which the protons are coupled. The heteronuclear coherence transfer echo is then observed using the $^{13}$C channel, thus avoiding the need for water suppression. The pulse sequences are kept as short and simple as possible to minimise both relaxation effects and also the dependents on $B_1$ inhomogeneity.
These experiments produced high resolution spectra even in an inhomogeneous magnetic field. By tailoring the basic pulse sequence the resonances observed can be made to display either proton-proton scalar couplings or proton-carbon scalar couplings or no couplings at all. We envisage that this technique will make it feasible to perform heteronuclear in vivo spectroscopy even in an inhomogeneous field providing the molecules of interest do not have prohibitively short transverse relaxation times.

SOLID STATE NMR IMAGING USING "SOLID ECHOES"

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The NMR imaging of liquid and biological samples has been well established since the early 1970's and high quality images are now routinely obtained. Only recently has attention been focused on the possibility of achieving comparably resolved images in the solid state. The increasing number of papers describing the many available techniques testifies to the interest being shown in this research field. The lack of any high resolution images, despite the many techniques, emphasises the need for further development in what is a rapidly expanding field.

Solid echoes in a wide class of materials can be produced using a 90°-τ-90° pulse sequence. The echo intensity is encoded with spatial frequencies using gradient fields, and the evolution of the echo peak (at t=τ) is then used to produce an image. We have used this method to image materials with a wide variety of spin configurations, ranging from isolated spin ½ pairs in CaSO₄·2H₂O and spin ½ triads in C₆(CH₄)₆, to more complex configurations in materials such as adamantane and polytetrafluoroethylene.

The experimental investigations, and corresponding theoretical back up studies, suggest that imaging of all these classes of materials has the form:

\[ \rho(z) = \frac{1}{IM_0} \int \left\{ \left[ \langle I_y(g) \rangle - J \langle I_y(0) \rangle \right] \cos(2\gamma g z \tau) + \langle I_x(g) \rangle \cos(2\gamma g z \tau) \right\} dg \]

where the scaling factors I and J are spin configuration dependent. We are led to the conclusion that for a sample containing mixtures of spin configurations, some of these configurations will be preferentially imaged.

Since the available encoding time for solid imaging is at least two orders of magnitude less than that available for liquid imaging, a problem arises from the need to switch the gradient fields on and off within this encoding time. An investigation into the combined effect of the fields due to the radio frequency pulses and the gradients on the spin motion has also been made.
A high frequency E.C. (equal current) 'whole rat' coil using transmission line elements

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Basic element of construction
An excellent strategy to construct RF probes for NMR imaging and spectroscopy at high NMR frequencies (> 100 MHz) is the application of transmission lines as described in reference 1.

The probe design is based on a standing wave in an open-ended transmission line. The RF excitation field (B1) can be created by exposing the inner conductor at 1/4 lambda from the open end of the line. This can be realized by enlarging the outer conductor, which subsequently acts as a faraday shield. At this position the standing wave generates a current anti-node coinciding with a voltage node, yielding a maximum magnetic field and minimum dielectric coupling (Fig 1). The result is an ideal basic element for the construction of several kinds of RF probes.

Simple body coil
Since in general the main magnetic field in a superconducting magnet is parallel to the bore of the magnet, the RF excitation field has to be perpendicular to the bore axis. For that reason a long solenoid, which produces the required homogeneous RF field, is impractical because of difficult access. Another way to create a homogeneous B1 field is by parallel axial conductors on a cylinder generating a cosine current distribution [2]. A good approximation of this current distribution is to place six conductors 60° apart, where four conductors are carrying currents of equal magnitude. Two conductors are carrying no current, and therefore can be omitted. Such a probe is essentially the saddle coil, a very common type of body coil. This approximation of the current distribution to create a body coil can, even for high frequencies, be realized very easy by placing four of the described transmission line segments axial on a cylinder 60° apart. The outer conductor of the four lines can be common to create one faraday shield (Fig 2) [3].

Another method is to use one long transmission line which passes the 'coil' four times at current anti-nodes in the line, where the currents are of equal and maximum magnitude (take care of the direction) and the dielectric coupling is minimal.
E.C. coil

To obtain a more homogeneous B1 field the cosine distribution has to be approximated closer. This can be realized by more conductors on the cylinder. Usually the rods are placed equidistant and the required current distribution is achieved by travelling or standing waves. This design is commonly known as a bird-cage coil [4]. However for frequencies above 100 MHz these methods give rise to many problems. The current distribution can also be approximated by conductors carrying currents of equal magnitude. The positions of the conductors should then be non-equidistant.

An illustrative way to show how the conductors should be placed to achieve the optimal homogeneity is to draw one period of a cosine and divide the 'surface' by the number of wanted conductors as depicted in Fig. 3.

We performed computer simulations (taking reflections of the Faraday shield into account) to study the resulting homogeneity for different designs. The homogeneity for a birdcage coil with 10 (-2) conductors and an E.C. coil with 8 conductors are comparable. These results will be presented.

For higher frequencies it requires special care when designing the probe to generate currents of equal magnitude in each conductor. It is, for instance, not correct to connect two or more conductors with the same dc resistance and drive them in parallel. The impedances for high frequencies will be completely different due to coupling and parasitic capacitances. This design will fail due to non-equal distribution of the current over the parallel conductors. The correct way to construct an equal current coil (E.C. coil) is to use for each conductor a transmission line segment. The impedances of the exposed parts of the lines in the coil will differ. However, the current of the standing wave in a segment is determined by the input impedance of the transmission line segment. When the transmission lines are driven in parallel there will be a near equal current distribution.

Results

We build several coils using the transmission line segments according to the different designs described above. Some results will be presented. Especially the E.C. coil with 8 rods turned out to be very sensitive and homogeneous. (inner diameter 7 cm, Q\text{empty}=450, Q\text{filled}=125)

We used the RF coils to measure in vivo images and spectra from anesthetized rats at 6.3 T (proton Larmor frequency of 270 MHz).

REFERENCES

We have developed a superfusion chamber in a 5 mm diameter tube in order to run $^1$H N.M.R. spectra of superfused brain slices. Superfusion was obtained by suction of the liquid by a peristaltic pump. A good suppression of the huge water resonance was obtained by presaturation and acquisition after a spin-echo which simultaneously suppresses the wide phospholipid peaks.

COSY spectra of superfused tissue can be obtained by selective presaturation of the water resonance and insertion of delays to increase water signal reduction and suppress phospholipid correlations. In the case of spectra of living tissue, the sample is not homogeneous, giving poor resolution. Therefore COSY off-diagonal signals which are of anti-phase character tend to mutually cancel. Diagonal peaks are in-phase, giving a too large diagonal/off-diagonal intensity ratio. The introduction of two J-tuned spin-echos symmetrical with respect to the second pulse of the COSY sequence ("SUPERCOSY") (1) reverses the phase character of diagonal and off-diagonal signals giving excellent 2D COSY spectra of metabolites in superfused brain slices.

Using the technique described above, we have obtained assignment of metabolite signals, directly on the living tissue, by superimposition of COSY spectra. In this way, signals of lactate, alanine, GABA, N-acetyl-aspartate, aspartate, glutamine/glutamate, creatine, phosphocreatine, taurine, inositol, choline/ethanolamine have been easily assigned. Threonine was detected in dead slices, but not in living ones. A fast COSY spectrum ($^1$H) can be used for assignment of the $^1$H N.M.R. spectrum of living tissues, organs and cell suspensions, before or during biological experiments.

Streptococcus faecalis: A model for studying the mode of action of carboxylic polyether ionophorous antibiotics by "in vivo" NMR.

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The aim of this work is to study more precisely the mode of action of carboxylic polyether ionophorous antibiotics on a Gram(+) bacteria.

The chosen bacteria model is a Streptococcus faecalis. To avoid the interference of the systems of transport of the bacteria, which are all ATP-dependant, with the ionophorous antibiotics, the bacteria must be de-energized. This "resting model" can be considered as a simple membrane system. An addition of glucose allows ATP synthesis, bacteria are energized again ("active model").

- The validation of this bacterial model for the two states "resting" and "active" is presented.

- In preliminary experiments, the ionophore monensine is tested on this model:
  
i) Ions fluxes across the bacterial membrane are studied on the "resting model". H+, Na+ and K+ are followed by $^{31}$P, $^{23}$Na NMR and atomic absorption ($^{29}$K NMR is not sensitive enough)

  ii) The modification of the metabolism in the presence of monensine is studied on the "active model" by $^{31}$P and $^{12}$C NMR.

In conclusion, this model will allow in the future to study more precisely these processes. In particular different types of ionophorous antibiotics (or analogs) will be tested. Different ionic concentrations and doses will be investigated as well.
THE DETERMINATION OF THE REGIONAL RESPONSE TO RENAL HYPOTENSION USING A SPECTROSCOPIC IMAGING TECHNIQUE

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Due to the heterogeneity of the kidney, non invasive studies for evaluating cell damage in acute renal failure have been difficult. The structures of the thick ascending limb are particularly sensitive to hypoxic cell damage in this syndrome (1). While the medulla is the most likely region to become hypoxic, this has not yet been directly demonstrated. Phosphorus NMR indicates an important role for the hydrogen ion, and lactic acid is probably the major source of protons for these tissues that are at risk (2).

If the potential of 31P NMR as a clinical modality for monitoring acute renal failure is to be realized (2, 3, 4) localization of the phosphorus signal to the required volume is essential. Several techniques have been developed to define the region of interest in vivo, the most useful of which have been those employing pulsed field gradients (5-10). Of these methods, SPARS (7) Stimulated Echo (8) and PRESS (9) are unsuitable for P31 NMR studies since the short T2 of the in vivo phosphorus metabolites preclude the use of spin echo techniques. In addition, the concentrations of the metabolites of interest are of the order of 1 - 5 mM, and the spectra are often complicated by overlapping resonances, therefore the use of high field instruments is necessitated. Thus, localization techniques requiring the use of frequency selective r.f. pulses in the presence of Bo gradients suffer from spatial error due to chemical shift artifacts and are therefore not suitable.

Because of these difficulties, and because of the need for a simple technique for volume localization of phosphorus metabolites at 4.7 T, we have applied a spectroscopic imaging technique (11-13) to examine hypotension and the effect of treatment in isolated perfused pig kidneys at 4.7 T. Localized 2 dimensional P31 NMR spectra were obtained using a 4 cm surface coil placed over the kidney so that signals could be obtained from the cortical and medullary regions. The pulse sequence consisted of a non selective r.f. pulse followed by a phase encoding gradient in a plane perpendicular to the surface coil. Cortex and medulla were distinguished in the 2 dimensional spectra by the presence of glycerophosphoryl choline (GPC) only in the medulla. (Pi) was significantly higher in the medulla than the cortex in the normal kidney. During hypotension (renal blood pressure was dropped to half the normal value) the medulla showed a more marked increase in (Pi) and drop in (ATP), and a greater intracellular acidosis than did the cortex. Addition of
the diuretic drug Furosemide (10^-4 M final concentration) prior to hypotension showed a protective effect on medullary and cortical (ATP) loss as well as a less severe increase in (Pi). Intracellular acidosis occurred to the same extent as in untreated kidneys.

In conclusion, using the spectroscopic imaging technique localized 31P NMR indicates that the changes during renal hypotension occur earlier in the medulla than the cortex supporting the view that it is the outer medulla which is immediately susceptible to the onset of acute renal failure. Addition of the diuretic drug Furosemide protects against loss of high energy phosphates and increase in intracellular Pi in both cortex and medulla during hypotension.

IMAGING OF A ROTATING OBJECT

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We describe an approach to obtaining a static image of a rotating object. Preliminary experimental results of proton imaging on a rotating phantom are presented.

When a whole object is moving, one must consider the field gradient in the moving object frame, $\mathbf{G}(t)$, (not in the laboratory frame, $\mathbf{G}(r)$). The NMR signal from the moving object observed under the applied gradient results in scanning in the object-frame $k_r$ space (spatial frequency domain). The scanning pattern is given by

$$k_r(t) = \int_0^t \mathbf{G}(t') \mathbf{G}(t') dt' = \int_0^t D_G(t') \mathbf{G}(t') dt'.$$

Here, $D_G$ is a transformation depending on the object motion. The principle of our approach is to apply field gradients in the laboratory frame such that the signal results in an appropriate scanning pattern to cover the $k_r$ space as uniformly as possible. A static image of the object can then be obtained from such a scanning pattern by suitable data processing [1,2].

In our two-dimensional $(x,z)$ proton imaging experiment on a phantom (two water-filled capillaries of $\sim 1.5$ and $2 \text{ mm i.d.}$) rotating about the $Y$ axis at an angular frequency $\omega_c$, $D_G$ is given by

$$D_G = \begin{pmatrix} \cos \omega_c t & 0 & \sin \omega_c t \\ 0 & 1 & 0 \\ -\sin \omega_c t & 0 & \cos \omega_c t \end{pmatrix}.$$

In order to obtain a spiral scanning pattern, $k_r(t) = (Y_G tsin \omega_c t, 0, Y_G tcos \omega_c t)$, a gradient sequence, $\mathbf{G}(t) = (G_0 \omega_c t, 0, G_0)$ was applied in the laboratory frame. The measurement sequence is shown in Fig. 1 and the resultant image is displayed on an $64 \times 64$ matrix interpolated from an $8 \times 8$ matrix. (Fig. 2). The image distortion observed may result from field inhomogeneity.

References

Fig. 1 Measurement sequence for obtaining a static image of an object rotating about the Y axis at $\omega_s$.

Fig. 2 Static image of the rotating phantom consisting of two water-filled capillaries.
IMAGE PROCESSING TECHNIQUES
APPLIED TO 2D NMR SPECTROSCOPY

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In this poster we illustrate the use of three algorithms derived from the field of image processing to increase visibility of important spectral features for an easier interpretation of 2D NMR spectra.

1. Spectral Modeling by Histogram Analysis: Examining the statistics of the noise is vital in order to predict the usefulness of deconvolution procedures and in the selection of contour levels. To automate the estimation of the noise mean and variance, we have found it useful to study the histogram of only those points which are detected as local maxima. Noise parameters are derived from this function, and they are used to estimate a lower bound to the first contour level. A completely different approach of separating peaks from background uses a segmentation procedure, also based on the histogram analysis, to select an adequate threshold for peak extraction. This automatic method selects a level which maximizes a measure of the separability of the peaks and the background. This procedure can be iterated to generate a series of contour levels to achieve adequate feature definition.

2. Resolution Enhancement of NMR Spectra: The deconvolution problem has been extensively studied for blur occurring with usual image formation techniques. Its application in spectroscopy is to improve the signal-to-noise ratio and spectral resolution. We have obtained some interesting results with a constrained restoration procedure. Several smoothness constraints have been tested, such as the amplitude of the second derivative of the spectrum, and an approximation of the maximum entropy criterion. The derivation of the filter is then a straightforward use of Lagrangian minimization implemented in the frequency domain. An interesting feature of this approach is that it can naturally treat spectra with positive and negative signals.

3. Processing symmetric spectra: Our approach generalizes symmetrization procedures previously proposed in the literature. The discrimination between peaks and noise uses the neighborhood of each point, and of its symmetric partner. For this purpose the matrix is folded over the diagonal. The result is an intercalation of points coming from above and below the folding axis, forming a hexagonal network. Because of this intercalation procedure, peaks and their symmetric points will form a surface twice as large as that of either peak. By contrast, noise which has no symmetric correlation will be spread and weakened in the folded data set. This process insures the success of an opening morphological filter which smooths the data by first selecting the local minima and then the local maxima on the hexagonal domain.
VOLUME SELECTIVE SPECTROSCOPY
In Combination With Water Line Suppression, Spectral Editing And Post-Detection Signal Processing (VOSING)

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Purpose
A method has been developed permitting the record of in vivo-MR-spectra by excitation of sharply bound volume elements. The method allows precise and flexible localisation and can be combined with various spectral line suppression and spectral editing techniques. Additional post-detection signal processing is desirable for enhancement of the detection sensitivity.

Abstract
A series of test experiments with the VOSY technique for volume-sensitive NMR spectroscopy using the stimulated echo has been carried out. It is demonstrated that several volume elements can be probed in a single-scan experiment so that concentrations of metabolites, for instance, can be related to other regions of interest or to a calibration sample. The VOSY method can be combined with spectral line suppression and spectral editing procedures such as the spin-echo double-resonance sequence for the selective investigation of J-coupled groups. A new volume selection/spectral editing pulse sequence (VOSING) has been developed. The special features specific to this technique are that the volume selection and the spectral editing interval coincide and additional that no decoupling is necessary. Phantom experiments with lactate solutions and human blood serum led to water suppression factors of 20000. A post detection signal processing method has been implemented. The final sensitivity for lactate determination could thus be improved by a factor of more than 4. At present, the lower detection limit of lactate is 1 m mol/l for a (1.2 cm)³ voxel and 32 scans in a 4.7T/40cm magnet.

References
1. Kimmich R., Höpfeld D.
2. Kimmich R., Schnur G., Höpfeld D., Ratzel D.
3. Höpfeld D., Schnur G., Kimmich R.,
4. Knüttel A., Kimmich R.
5. Macovsky A., Spielman D.,
6. Knüttel A., Rommel E., Clausen M., Kimmich R.
lactate solutions
32 scans
(1.2 cm)³ voxels
spectral editing

\[ \text{mMol/l} \]

VOSING spectra (32 scans of (1.2 cm)³ voxels) of Ringer lactate solutions with 1, 5 and 20 mmol/l lactate.
Left: Without signal processing.
Right: After signal processing.

Results of the dilution series of Ringer lactate solution. The amplitudes have been evaluated from 32 scans/(1.2 cm)³ VOSING spectra after signal processing. The dotted line indicates the uncertainty due to noise.
DEVICE FOR MEASURING THE CONTRACTILE FORCE OF MUSCULAR SAMPLES DESIGNED FOR NMR STUDY

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Chemical agents can induce contraction (Acetylcholine) or relaxation (5-hydroxy tryptamine) of molluscan smooth muscles.

Their metabolism is influenced by these chemical mediators. $^{31}$P NMR is a convenient method for following metabolic variations in vivo. On the other hand, the contractile force developed by the muscles will also be influenced by the effect of these compounds. For measuring on the same muscular samples the physiological and the metabolic variations we have designed a small device that can be introduced in a 15 mm diameter NMR tube. The muscles are maintained at constant length and, at the upper part of the support, a strain gauge can be connected for recording the isometric force.

NMR and force measurements are presented and discussed.
STRUCTURALLY NEW MACROCYCLES FROM THE RESORCINOL-ALDEHYDE CONDENSATION REACTION. CONFIGURATIONAL AND CONFORMATIONAL ANALYSES BY MEANS OF DYNAMIC NMR, NOE AND T1 EXPERIMENTS.

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ABSTRACT

Three stereoisomeric macrocycles 1, 2 and 3 are obtained by the acid-catalyzed condensation of resorcinol with aliphatic aldehydes under appropriate conditions. The product distribution is controlled by the relative solubility of the different isomers in the reaction medium. Detailed conformational and configurational analyses have been carried out on these compounds by means of \textsuperscript{1}H-NMR. In particular variable temperature spectra give informations on the dynamic and symmetry of macrocycles. 1 has a boat-like conformation with C\textsubscript{2v} symmetry, 2 a chair-like conformation with C\textsubscript{2h} symmetry and 3 a diamond-like conformation with C\textsubscript{s} symmetry. This last one has never been observed before. The configuration of R substituents has been assigned on the basis of NOE enhancements and T\textsubscript{1} relaxation times. The three isomers have all the R substituents in axial configuration, which is the less hindered one. The presence of the new macrocycle 3 indicates the complete axial stereocontrol for this condensation reaction. The thermodynamically more stable boat isomer 1 can be obtained selectively in high yields.
(50-80%) exploiting the different solubilities of the stereoisomers and the reversibility of the condensation reaction, by changing temperature and solvent.
One and two dimensional $^{13}$C nmr spectroscopy is applied to determine partial structures of humic acids dissolved in water. Conventional $^{13}$C nmr spectra are taken by the spin echo technique rather than by the one pulse technique, because phase corrections and thus quantitative measurements are easier to perform. DEPT and QUAT pulse techniques provide the ratio of the fragments $\text{CH}_3$, $\text{CH}_2$, $\text{CH}$ and $\text{C}_{\text{quart}}$. Two dimensional J-resolved $^{13}$C nmr spectroscopy confirms these results and provides some CH-coupling. Spin-spin relaxation rates are determined and reveal complexation of paramagnetic metal ions with carboxylate ions. - Somewhat unexpected $^{17}$O nmr spectra of good signal-to-noise ratio are produced showing two separate bands, the origin of which is discussed.
FIRST HIGH-PRESSURE, HIGH-RESOLUTION NMR PROBE WORKING AT 400 MHz

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In the course of our studies on chemical exchange at high-pressure we developed a high-pressure, high-resolution probe for $^1$H-NMR working at 400 MHz. The probe fits into a 9.4 T wide-bore (89 mm $\varnothing$) NMR magnet and is used with a Bruker AM-400 spectrometer.

The probe consists of a thermostated Berylco-25 pressure vessel, topped by a plate and a cap, arranged in a flat Bridgemann seal. The plate is equipped with four feedthroughs for RF and T-measurement (100$\Omega$ Pt resistor) filaments. The RF tuning and matching circuit sits just above the pressure vessel inside the magnet. The pressure is transmitted via a connector at the bottom of the bomb and measured outside the bomb with a Heise-gauge. We use a commercially available two-turn saddle coil (Bruker) wound around a glass tube and high quality variable capacitors (Polyflon, Johanson). The probe is double tuned to 400 MHz and 61.4 MHz, and allows therefore homogeneity adjustment and field locking on $^2$H during $^1$H observation.

The sample is contained in an ordinary 5 mm $\varnothing$ sample tube cut to 47 mm length and closed with a specially designed movable seal to permit volume changes due to pressure variations. The sample can not be rotated.

Until now we have tested this probe between -40 and +70°C. The temperature gradient as well as the stability over the whole sample is less than ± 0.2 °C. The resolution as measured with 0.18% C$_6$H$_6$ in CD$_3$NO$_2$ is better than 0.5 Hz over the entire pressure range from 0.1 to 200 MPa. Signal-accumulation over 30 min. does not broaden the signal. The signal to noise ratio obtained with one transient and the sample described above is 107:1. The 90° pulse length is about 40 $\mu$s.

More information about technical details as well as first results of N,N'-dimethylformamide(DMF) exchange on Mg(DMF)$_6^{2+}$ under pressure will be given.
Magnetic field alignment effects in NMR spectra provide information about molecular properties and interactions in liquid solutions [1]. Via temperature dependent studies the influence of correlations between the molecules is clearly revealed.

Introduction
The magnetic field of the spectrometer exerts orienting torques on molecules that have an anisotropic magnetic susceptibility ($\chi$). In liquid solutions, these torques are counteracted by the Brownian motion and a very incomplete dynamic orientation results. This may lead to detectable residual electric quadrupolar and/or magnetic dipolar couplings in high-field (> 5 T) high-resolution NMR spectra. The anisotropic couplings show up as splittings $\Delta v$ of the NMR lines. For molecules having effective axial symmetry

$$\Delta v = c \Gamma_z S_{zz} ; \quad S_{zz} = g_2 \frac{\Delta \chi B^2}{15 \mu_0 K T}$$

Here $c$ is a nuclear coupling constant and $\Gamma_z$ a molecular geometry factor. The order parameter $S_{zz}$ describes the degree of orientation of the molecules. For alignment by a magnetic field, $S_{zz}$ is related to the molecular susceptibility anisotropy ($\Delta \chi$), to the square of the magnetic induction (B), to the absolute temperature (T) and to the angular correlation factor $g_2$, which accounts for intermolecular interactions. Typical values for $S_{zz}$ are $10^{-6} - 10^{-5}$ at $B = 10$ T.

Examples
11.75 T ($^1$H: 500.1 MHz; $^2$H: 76.75 MHz) NMR spectra: Normal (left) and resolution enhanced (right). The doublets arise from incomplete averaged anisotropic couplings.

$^1$H-spectrum of 1 mol% 9H-fluorene-$d_8$ in acetone-$d_6$: Dipolar coupling between the CH$_2$ protons.

$^2$H-spectrum ($^1$H dec.) of 5 mol% benzene-$d_4$ in diethyl ether: Coupling of the deuteron nuclear quadrupole with the surrounding local electric field gradient.
**Interpretation of \( g_2 \).**

For molecules with effective axial symmetry in a non-aligning solvent

\[
g_2 = 1 + \rho \Omega^{-1} \int \int \frac{3}{2} \cos^2 \theta_{ij} - \frac{1}{2} g^{(2)}(r_{ij} ; \omega_{ij}) \, dr_{ij} \, d\omega_{ij}
\]

Here \( r_{ij} \) and \( \omega_{ij} \) specify the relative distance and orientation of the molecules \( i \) and \( j \); \( \theta_{ij} \) is the angle between their molecular z-axes; \( \Omega = \int d\omega \); \( \rho \) is the particle density. The angular correlation function \( g^{(2)} \) characterizes the average local structure around any molecule.

\[
g^{(2)}(r_{ij} ; \omega_{ij}) = \exp(-w^{(2)}(r_{ij} ; \omega_{ij})/kT)
\]

Here \( w^{(2)} \) is the potential of mean force in the liquid solution.

To find the explicit temperature dependence of \( S_{zz} \), \( w^{(2)} \) is separated in a strictly radial part \( V_R \) and an angular part \( V_A \). Usually \( V_A \ll kT \) and the angular factor in \( g^{(2)} \) can be expanded in a Taylor series. Representing \( V_R \) by a square-well potential:

\[
V_R = \infty (r < \sigma); \quad V_R = -\epsilon (\sigma \leq r \leq \lambda \sigma); \quad V_R = 0 (r > \lambda \sigma),
\]

an approximate expression for \( S_{zz}(T) \) is obtained

\[
S_{zz}(T) = \frac{\Delta \chi B^2}{15 \mu_0 k} \left[ \frac{1}{T} + a \frac{1}{T^n} \left\{ b \exp(\epsilon/kT) + c \right\} \right]
\]

The parameters \( a \) and \( n \) depend on the angular potential; \( b \) and \( c \) depend on the radial force parameters \( \sigma \) and \( \lambda \sigma \).

**Results**

The figure below illustrates some typical temperature curves for \( S_{zz} \). Although only a limited temperature range is accessible for liquid solutions, deviations from the ideal \( 1/T \)-behaviour clearly show. The interpretation, however, is extremely difficult.

For the benzene solutions quadrupolar interactions between the benzene molecules might be important. Representing the angular potential by the electrostatic quadrupole-quadrupole energy, it follows that: \( n = 3; a = 4\theta^4/(4\pi \varepsilon_0 k)^2; b = c(\lambda^2 - 1); c = 4\pi \rho \sigma \lambda^2 \) where \( \theta \) is the molecular quadrupole moment. From literature data for benzene: \( \theta \approx 2 \times 10^{-39} \text{ Cm}^2; \rho = 3 \times 10^{-17} \text{ m}^{-3}; \lambda = 1.4; \sigma = 5 \text{ Å}; \varepsilon \approx 620 \) K. Using these values, the behaviour of \( S_{zz}(T) \) for benzene is well reproduced (deviations \( \approx 5 \% \)).

![Graph showing \( S_{zz} \) vs. \( 1/T \) for some aromatic compounds.](image)

**Reference**

ENANTIOMERIZATION BARRIER ENERGY OF N-ISOPROPYSALICYLALDIMINATE BERYLLIUM AND ZINC COMPLEXES

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It is well established that β-diketonate complexes of beryllium (II) and Zinc(II) assume a tetrahedral idealized $D_{2h}$ structure in solution and in the solid state. In such a conformation bis-chelate complexes derived from unsymmetrically substituted ligands can exist as only one $dl$ pair. In order to obtain a direct measure of the inversion barrier at the tetrahedral Beryllium and Zinc centers we investigated the DNMR behaviour of bis (N-isopropylsalicylaldiminato)beryllium (I) and bis (N-isopropylsalicylaldiminato)Zinc(II) under conditions in which the molecular interactions are weak and, more important, in which the reversibility of the process can be readily ascertained. In these compounds, the isopropyl group functions as a diastereotopic probe, in the sense that under conditions in which the chirality of the structure is preserved the two methyl groups within each isopropyl moiety are magnetically nonequivalent, giving rise to two doublets in the methyl region of the $^1H$-NMR spectrum. Any process which destroys the chirality of the structure or results in an averaging of the two enantiomers will render the two methyl groups equivalent on the NMR time scale, and the two doublets will collapse to a single doublet. Through variable temperature $^1H$-DNMR experiments we measured the barriers for the enantiomerization process in (I) and (II); the values are 22.18 Kcal/mol and 15.16 Kcal/mol, respectively, in deuteronitrobenzene as solvent.
With the purpose of investigating the effect of the nature of the substituents on the aromatic ring of the ligand on the energy of the enantiomerization process, we studied the dynamic stereochemistry of Be and Zn bis-chelate ring substituted N-isopropylsalicylaldiminate complexes. The free energy of activation for the enantiomerization process as a function of the $\sigma$ of Hammett of the ring substituents gave a satisfactory linear relationship both for the Be and Zn complexes. The experimental values are:

$$\Delta G^* = 1.81 \sigma + 21.96$$  for the Be Complexes
$$\Delta G^* = -2.48 \sigma + 15.44$$  for the Zn Complexes

These relationships are correlated with the nature of the bond which is present in such complexes.

References

Aaaq.

Numerous members of the chemically well-characterized family of Triscyclopentadienyl-Lanthanum(III)-adducts of the type 

\[
[(\eta^6-C_{5}H_{5})_3La^{139}La]^{\pm}
\]

\(n = 0, 1 \text{ or } 2\) and \(q = 0 \text{ or } -1\)

have been examined by \(^{139}\text{La-NMR-Spectroscopy}\) in solution. The poster presents the extension of earlier studies \(^{1}\) with respect to the metal-bonded element of the ligand \(L (P, C, S \text{ etc.})\), the electric charge of \(L\) and a few exchange processes.

At present, the variation of \(L\) may lead to \(\delta(\text{La})\) values within the rather wide range of -380 to -620 ppm, while \(W_{1/2}\) may lie between \(30\text{Hz}\) and undetectably broad signals. The lowest \(W_{1/2}\)-value so far recorded is around 22 Hz (for \(L\)=DMSO) and offers the opportunity to introduce an occasionally more practical, new \(^{139}\text{La-NMR standard.}\)

Some examples demonstrate that the generally very short relaxation time of the \(^{139}\text{La nucleus (\(\approx 10^{-3}\text{ s}\)) causes some problems for the study of chemical equilibria and related exchange processes.}\)

\(^1\) S.H. Eggers, M. Adam, E.T.K. Haupt, R.D. Fischer
The quadrupole coupling tensor of $^{95}$Mo in Mesitylene Molybdenum tricarbonyl

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The quadrupole coupling tensor of $^{95}$Mo in Mesitylene Molybdenum tricarbonyl has been determined in benzene solution with NMR of the dielectrically oriented molecule.

Introduction

NMR of liquid systems that are partially aligned by a static electric field can yield valuable information about anisotropic spin interactions. In case of polar molecules the alignment is a result of the coupling between the dipole moment and the electric field, which is chosen parallel to the magnetic field. Spectra of quadrupolar nuclei attached to such an oriented molecule will consist of 21 lines with an equal separation, given by:

$$\Delta \phi = \frac{3}{2I(2I-1)} \frac{eQ}{h} V_{zz} S_{zz} \tag{1}$$

$I$ is the spin of the nucleus, $Q$ its quadrupole moment and $V_{zz}$ the component of the field gradient tensor along the dipole moment. The averaged orientation is represented by $S_{zz}$:

$$S_{zz} = < \frac{3}{2} \cos^2 \Theta - \frac{1}{2} > E = \frac{1}{15} \left( \frac{p_{ef} E_d}{kT} \right)^2 \tag{2}$$

$\Theta$ is the angle between the magnetic field and the dipole moment, $p_{ef}$ the effective dipole moment in solution and $E_d$ the internal electric field [1]. For dipolar molecules, typical values of $S_{zz}$ are $10^{-3} - 10^{-4}$.

Determination of quadrupole coupling tensors

An application of the method described is the determination of nuclear quadrupole coupling tensors. This will be demonstrated for $^{95}$Mo ($I = \frac{5}{2}$) in Mesitylene Molybdenum tricarbonyl (fig. 1). For this type of experiment a so-called "reference nucleus" is needed, with a known quadrupole coupling tensor and of course attached to the same molecule. A very suitable nucleus for this purpose is deuterium ($I = 1$). When the molecule is oriented in the electric field, the $^{95}$Mo resonance will split into a quintet and the $^2$H resonance into a doublet (fig. 2). The ratio of the observed splittings gives a direct relationship between the components of the quadrupole coupling tensors along the dipole moment for both nuclei:

$$\frac{\Delta \phi (^{95} \text{Mo})}{\Delta \phi (^2 \text{H})} = \frac{1}{10} \frac{(eQ/h)V_{zz}(^{95} \text{Mo})}{(eQ/h)V_{zz}(^2 \text{H})} \tag{3}$$

It is obvious from the molecular symmetry that the principal axis system of the $^{95}$Mo quadrupole coupling tensor coincides with the molecular axis system. Because the molecule has effective axial symmetry along the $z$-axis, the principal components $V_{x^*}x^*$ and $V_{y^*}y^*$ are equal.
Results

The experiments were performed with a solution of mono-deutero-Mesitylene Molybdenum tricarbonyl in benzene (0.07 M). Using a quadrupole coupling constant of \(186 \pm 6 \text{ kHz} \) \((eQ/h)\) and an asymmetry parameter \(\eta\) of \(0.05 \pm 0.02\) for deuterium [2]
\[(eQ/h) = \eta V_x V_z, \quad \eta = V_x V_y V_z / \left( V_x^2 + V_y^2 + V_z^2 \right), \]
the principal components of the 95Mo quadrupole coupling tensor were calculated to be: \((eQ/h) V_x V_z = (947 \pm 60) \text{ kHz}\), \((eQ/h) V_y V_y = (474 \pm 30) \text{ kHz}\).

References


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*The investigations were supported by the Netherlands Foundation for Chemical Research (SON), with financial aid from the Netherlands Organization for Scientific Research (NWO).*
A portable NMR spectrometer for automatic measurements in plants

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Introduction

The study of plant-water relations using NMR is part of the research program of our department. The spectrometer used in our laboratory has been described recently. Here we present a home-built portable NMR spectrometer for automatic measurements of relaxation times and sapstream in stems of intact plants. This spectrometer is computer controlled and equipped with an internal magnetic field-lock system for unattended operation over long periods of time in greenhouses in which temperature, humidity and light intensity show large variations.

Methods

For the determination of sapstream in stems of plants the repetitive pulse method is used: pulse sequence \((90^\circ - \tau)_n \tau = 300 \mu s n = 1000\), linear magnetic field gradient \(G\) in the direction of the flow \(G = 5 \text{ mT/m}\), detection along the direction of the \(90^\circ\) pulses. Both volumetric and linear flow rate can be determined using this method. \(T_2\)-measurements, which give information on the water content of the plant, are carried out using the CPMG method.

Hardware

The hardware is housed in three units.

1. A 30 kg U-shaped permanent magnet of 0.23 T (air gap: 2 cm) with field shift coils. In order to be easily accessible for plants, the probe can be removed from the magnet to be opened. Then the hinged Helmholtz r.f. coil (inside diam.: 1 cm) is opened and folded around the plant stem. After closing the probe the r.f. coil is well shielded against r.f. interference.

2. The spectrometer consists of a 10 MHz transmitter and receiver (modified Bruker Minispec electronics). A duty cycle limiter is included in the transmitter for protection.

3. A TBM PC containing a LabMaster (Scientific Solutions) analog and digital input output board controls the spectrometer. This unit provides the data acquisition (ADC), the field shift control (DAC) and the pulse generator with field gradient on/off control.

Software

The software is written in ASYST (Macmillan Software). ASYST is a scientific software package and furnishes comprehensive procedures for mathematics, statistics, graphics, signal analysis and data acquisition. Furthermore it includes a driver for the LabMaster board. We have developed a collection of words (routines) like FLOW, \(T_2\), FID, LOCK, CALCULATE etc. The user can create his own words e.g. for a new pulse sequence. These words combined with the standard ASYST words are a highly flexible programming tool. The user interface is implemented as a menu structure. It has two modes. In the direct mode it is possible to call these words interactively. In the program mode you can create your own application using these words and the standard ASYST words.
Results

This figure is a typical result obtained with this spectrometer. It shows the linear flow velocity in a Cucumber plant during 24 hours in a climate control chamber.

References
Complete proton resonance assignments of two representative lysergic acid peptides ergosine (ESN) and its epimer ergosinine (ESNN) in CDCl₃ has been made by combining conventional 1D techniques with phase sensitive DQ COSY and NOESY experiments. The conformation of D ring was determined from 1D 1H spectra and from NOESY experiments. Two intramolecular H-bonds N20H...N6, and 12'-OH...OC18 stabilize mutual orientation of the ergolene and peptide subunits.

**Fig. 1. Molecular structure of lysergic acid peptide.**

Assignment. The proton resonances of N2OH, N1H, 12'-OH, 2'-Me and c'-Me are readily assigned from 1D spectra. Some of the D ring proton resonances and that of the proton 5' were assigned by decoupling experiments considering the coupling network. However, there are three critical regions where proton signals overlap: (i) the region around 2 ppm where signals of the tripeptide moiety protons 9', 10', a' and b' are expected; (ii) the region around 3.5 with expected signals of 8', and 11', overlapped by some of the D ring
proton resonances; (iii) the signal of N6Me at 2.6 ppm may mask some signals of D ring protons.

PS DQ COSY experiment eliminates the N6Me and 2'-Me signals. The experiment separates 5 groups of spin systems that overlap in one dimension. Comparison of PS DQ COSY spectra with PS NOESY spectra permits the identification of NOE's between J-coupled hydrogen atoms. For example, NOE occurs between c'-Me and b', 9 and 8, 8 and 7α, 7β, 7α, and 7β in the case of ESN only, N1H and 2, 11' and 10', 9' and 8', 4α and 4β, 5α and 4α, 4β. Combination of the results of both experiments leads to the assignement of the proline ring protons, the leucine fragment protons, and the D ring protons 4α, 4β, 5α, 7α, 7β, 8, and 9 on the basis of connectivity relations for separated groups of cross peaks.

Structural inferences. Several structural features already emerge from the analysis of 1D spectra (1). The conformation of the D ring of both epimers is γ-chair. N6 is above the approximate plane of ring D of ESN with the C8 substituent in β-axial orientation while it is below the plane with ESNN and the C8 substituent is α-axially orientated. These conformations are stabilized by intramolecular H-bonding N20H...N6 indicated by N20H chemical shifts (9.36 ppm with ESN and 9.86 ppm with ESNN). Another intramolecular H-bond exists between the C18 carbonyl and 12'-OH (δ12'-OH = 6.9 ppm with both epimers). These hydrogen bonds are instrumental in stabilizing the mutual orientation of the ergolene and peptide subunits. Cross peaks in the NOESY spectrum of ESN between N20H and 9, 8, 7α, 7β and N6Me reveal the close proximity of N20H and the D ring protons confirming the results of 1D spectral analysis. ESNN shows NOE's between N20H and, 9, 7α, 8, N6Me, which also is in agreement with the analysis of 1D spectra.

Experimental. 1D 1H NMR spectra were recorded on Varian XL-200 and WXR 300 spectrometers using 60 mM solutions. PS COSY and NOESY with mixing times 0.5 s, 1 s, and 1.5 s were recorded on VXR 300 spectrometer by standard methods. Temperature was 22°C.

THE ASSIGNMENT OF THE POSITION OF $^2$H- AND $^3$H LABELS IN CYCLOOCTYLACETIC ACID BY MEANS OF $^1$H-, $^2$H-, $^3$H- AND $^{13}$C NMR.


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Abstract:
Isotopic labelling of cyclooctylacetic acid (COA) with deuterium and tritium yielded an unexpected distribution of the label over the molecule.

In order to assign the $^2$H- and $^3$H-NMR spectra the $^1$H spectrum was assigned via the $^{13}$C spectrum. The interpretation of the $^3$H spectrum was helped by a $^3$H-$^3$H COSY spectrum. It followed from this spectrum that the main $^3$H intensities are arising from mono- and ditritiated species. The label has been spread out over the whole molecule, except that the presence of label at position 6 could not be proven from the analyses.
Carbon-13 spin-lattice relaxation times and nuclear Overhauser enhancement factors are reported for sucrose in the 2:1 solvent mixture of D$_2$O and DMSO-d$_6$ in the temperature range of -25°C to +30°C at two magnetic fields, 9.39 and 6.34 Tesla. In this solvent system, it is possible to obtain data which correspond to both sides of the minimum in the $T_1$ versus temperature curves and to test various models describing the motional behaviour of molecules in solution. This study is an extension of the recent work of McCain and Markley (1) on the rotational correlation functions for aqueous sucrose solution.

Lanthanide shift reagent has been used by us to analyze and quantify the optical purity of two enantiomers of a 6-substituted dihydropyridine.

These measurements indicated that the up-field shift induced on the 2-methyl signal by Pr (tfc)$_3$ is a good probe for examining chiral dihydropyridines.

The data have been confirmed also through quantitative derivatization with (R)-mandelic chloride.
The kinetics of sodium binding to ATP

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The biochemistry of ATP is controlled by association with various species, especially divalent cations, and consequently these complexes have been studied frequently. In a previous paper (U Pilatus, A Mayer, W Offermann, D Leibfritz, BBA 926 106 [1987]) we showed, that the line width of the $^3$P-NMR signal of $\gamma$-ATP is broadened by complexation with sodium ions at 8.4 Tesla. The extend of broadening is sensitive to the pH of the solution, which we explained by a theoretical model that takes into account pH dependent kinetics for the sodium association to ATP.

\[
\begin{align*}
&H^+ + ATP^{4-} + Na^+ \\
&\quad \xrightarrow{k_5} k_1 \quad \xrightarrow{k_6} k_2 \\
&H^+ + NaATP^{3-} \quad \xrightarrow{k_7} k_3 \\
\end{align*}
\]

Here we report a detailed analysis of the pH dependent line broadening, which led to the exchange rates of sodium association and dissociation. We titrated a Na$_2$H$_4$ATP solution with sodium chloride at different pH values and monitored the $^3$P-NMR spectra. In order to analyze the broadening of the $\gamma$-ATP line we used a density matrix formalism that takes into account J-coupling (K V Vasavada, J I Kaplan, B D N Rao, JMR 41 467 [1980]).

From the resulting lifetimes we deduced following our model the kinetic parameters $k_5$ and $k_7$ for the association and dissociation of sodium to de- and monoprotonated ATP.

The formation and nature of higher complexes in the presence of a large excess of sodium is discussed.
Determination of the configuration and conformation of tetracyclic partly saturated benzoazines by NMR

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The partly saturated 1,3-benzoxazino[3,2-c]benzoazines \(2a-2\) were prepared in a one-pot synthesis from trans-2-aminomethylcyclohexanol (1).

On standing in CDCl\(_3\), a fast epimerization was observed in position 5a, and if R=H in position 11, too. Consequently in the equilibrium mixture the presence of two or four diastereomers could be observed. The diastereomers could be identified in the mixture without separation of them if the configuration and conformation of the theoretically possible individual structures were taken into account.

By means of modern NMR methods (MQF COSY, spin-lock methods, phase-sensitive 2D NOE, and heterocorrelation) a complete analysis of the mixture spectra was possible and the dominant conformation could be unequivocally determined.
C NMR APPLIED TO THE STUDY OF LIGNIN STRUCTURE.

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Lignin, an amorphous and reticulated polyphenol is one of the main components of wood. Each step in the technical development of $^{13}$C NMR has brought an improvement in the knowledge of the very complex chemical and macromolecular structure of this natural polymer. With qualitative $^{13}$C NMR, it is easy to classify the very many different kind of lignins according to their origin. Quantitative $^{13}$C NMR is the only technique which give, without degrading the samples, reliable data about the ratio of specific chemical fragments, and combined with the use of the DEPT sequence, it is possible to get information about the degree of condensation of the aromatic ring. The 2D-INADEQUATE sequence reveals itself a very efficient technique: for the first time, one can get direct assignments, that is determination of the connectivities between the carbons, along the complex carbon skeleton, without having to rely on the comparison with dimer model compounds. In addition, a detailed study of the 2D-spectra in the region of the aliphatic propane side chain has been made. It has been possible to assign on the contour plot of the correlation peaks, the position of the C- and C-carbons of the 16 possible diastereoisomers with the threo and erythro forms of the alkyl-aryl ether dimers which constitute the main fragment of the lignin structure.
Structure elucidation of organic compounds is mainly performed by application of spectroscopic methods, like mass-spectrometry, NMR- and IR-spectroscopy. During the last decade, $^{13}$C-NMR spectroscopy has developed into a routine technique for structure elucidation, even for small amounts. The interpretation of carbon-NMR data is mainly based on comparison with suitable reference data taken from the literature. The complete information contents of C-NMR cannot be utilized by manual interpretation. This tedious and time-consuming task can be done by appropriate computer-programs. Using a knowledge-base of some 25000 $^{13}$C-NMR spectra, we have focused our effort on the development of an efficient technique for interpretation of spectral data in terms of substructural fragments. The algorithm applied creates fragments of different size, depending on the reference data available. Complete chemical structures are assembled by a model builder program using restrictions known to the expert. Besides the automatic interpretation of $^{13}$C-NMR spectra a software package for handling this large amount of data will be presented on a graphics workstation. Also a new algorithm for assigning resonance lines to the corresponding carbons within a large set of similar structures, which circumvents the problem of bad representation of the query-structures by the database itself, has been developed.
APPLICATION OF Cu(2,4-DICHLORO-BENZOATE)₂ AS SELECTIVE PARAMAGNETIC RELAXATION AGENT IN THE ¹³C NMR SPECTRAL ANALYSIS OF N-HETEROCYCLICS.

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Cu(2,4-dichloro-benzoate)₂ turned out to be an selective "shiftless" relaxation reagent¹. The effect of Cu²⁺ ions on the ¹³C NMR spectral lines of a number of N-heterocyclics (simple mono- or bicyclic compounds and alkaloids) have been studied in the metal/ligand range of 10⁻³ - 10⁻⁵.

The interaction operates through electron-nuclear hyperfine coupling, however, chemical exchange processes slow on the NMR time scale also contribute significantly. The magnitude of hyperfine coupling contents. A depends also on the distance of the interacting centres. Two-bond couplings are generally larger than three-bond ones. By these empirical relationships binding sites of metal ions can be determined, e.g.in the Cu(II)-Penicillin-G complex, contrary to earlier reports, we found that the Cu²⁺ is bonded to the carboxylate cation exclusively.
Other application possibilities also have been looked for. On the basis of the observed selective broadenings it is possible to assign carbon atoms one or two bonds away from a basic nitrogen and the relative basicity of different nitrogen atoms can also be judged.

In favourable cases it can be used to settle stereochemical problems making use of the observed dependence of the three-bond hyperfine coupling constants on the spatial vicinity of the nitrogen lone pair and the interacting nuclei.

While its main advantage is simplicity the main drawback is that it is limited to molecules capable to form complexes with Cu$^{2+}$ ion.

Steady-State Inversion Recovery Technique
for Slowly Relaxing Nuclei in Liquids

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A new technique is pointed out to determine relaxation times of very slowly relaxing nuclei by observing the buildup of the steady-state. Applying periodic equal and coherent rf-pulses with the optimum flip-angle \( \Theta = \arccos \left( \frac{T_1/T_2 - 1}{T_1/T_2 + 1} \right) \) the steady-state is built up exponentially with the time constant \( T^* = (T_1 + T_2)/2 \). To increase the dynamic range of this buildup, the experiment is started with negative longitudinal magnetization rather than with the unmagnetized sample.

This initial state of the experiment, which must be repeated frequently to achieve a sufficient signal/noise ratio, is established by inverting the longitudinal magnetization of the nearly built up steady-state with the aid of a \( \pi \)-pulse. The transverse component of the steady-state magnetization is destroyed irreversibly by spin-diffusion in a homogeneity spoiled field \( B_0 \). By this, a steady-state of the longitudinal magnetization is maintained over the total time of all such buildup experiments, necessary to accumulate a sufficient signal/noise ratio; whereas the steady state of the transverse magnetization, i.e. the phase-coherence of the spins, is destroyed after each such buildup.

This technique allows an observation of the nearly full dynamic range of the buildup of the steady-state of the spin system and yields an optimum signal/noise ratio in a given measuring time: NMR signal can be accumulated during nearly the total spectrometer time, i.e. no time without signal acquisition is required neither to wait for the thermal equilibrium or zero magnetization as initial state nor to wait for the progress of the relaxation process. Experimental examples with the low-\( y \) spin \( \frac{3}{2} \) nuclei \( ^{57}\text{Fe} \), \( ^{103}\text{Rh} \), and \( ^{109}\text{Ag} \) are presented.

1. The ratio \( T_1/T_2 \) must be determined in a previous experiment:

POSSIBLE CONFORMATIONAL DEPENDENCE OF LONG RANGE DEUTERIUM ISOTOPE EFFECTS ON C-13 CHEMICAL SHIFTS

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A series of specifically deuterated binuclear aromatic conjugated compounds (Fig.) has been investigated by C-13 and H-1 NMR spectroscopy (at 100 MHz and 400 MHz, respectively).

The bridging groups (B) are: -CH=CH- and -CH=N-, i.e. the compounds are trans- and cis-stilbenes and trans-N-benzyldenedianilines.

Beside usual deuterium isotope effects on chemical shifts a few positive (deshielding) and through-space deuterium effects were also found. For para- and bridging group deuterated compounds a relationship between long range deuterium effects on C-13 chemical shifts and molecular dihedral angle was revealed. Some other aspects of deuterium effects upon chemical shifts and coupling constants will be discussed as well.
ASSIGNMENT OF ENDOGROUP RESONANCES OF POLYAMIDE-4,6

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DSM Research BV

Introduction

No unambiguous $^{13}$C NMR chemical shift assignments exist for the main chain and endgroup resonances of the polyamides-6, -4,6 and -6,6, mainly due to the failure of chemical shift increment data in polar solvents. We have used oligomers or polymers of PA-4,6 (STANYL©) to establish these assignments in the $^1$H and $^{13}$C spectra via 2-D NMR methods. Results are compared with data for high molecular weight PA-6 and -6,6.

Results

All resolved resonances are assigned via $^1$H-$^1$H and $^1$H-$^{13}$C correlation techniques, using formic acid as the solvent and using a 400 MHz NMR spectrometer (see Fig. 1 and 2). This establishes the assignments for 1 to 4 and for $\alpha_A$ to $\delta_A$, and $\alpha_C$.
The resonances for the remaining endgroups $\beta_C$ to $\delta_C$ overlap with the resonances for the main chain groups. Assignments were made via a HHC relayed coherence transfer experiment (see Fig. 3) and via a long-range $^{13}$C-'H correlation (COLOC) over three bonds (Figs. 4 and 5).

Results of the $^{13}$C measurements for the three polyamides are given in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<th>$\delta_A$</th>
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<td>40.78</td>
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<td>41.30</td>
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<tr>
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<td>40.86</td>
<td>28.64</td>
<td>26.40</td>
<td>24.66</td>
<td>34.23</td>
<td>-</td>
<td>40.30</td>
</tr>
</tbody>
</table>

Conclusion

Endgroup resonances in PA-6 and -6,6 have been assigned on the basis of an interpolation of the chemical shifts of the endgroups of PA-4,6. As can be seen in the table, there is a fair agreement between the chemical shifts of carbon atoms having a similar chemical environment.

Literature

3) See for example: P.A. Mirau and F.A. Bovey, Macromolecules 19, 210 (1986).
THE ESR INVESTIGATION OF THE CORE-SURFACE RELATIONSHIP IN THE LOW DENSITY LIPOPROTEINS

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The Mn(II) ion has proven itself suitable for the ESR investigation of the structure properties of the low density lipoprotein particle (LDL) because of its two characteristics.

First- in the LDL structure investigation Mn(II) may be the substitute for the biologically more important Ca(II) and Mg(II) ions, and that because of its similarity in binding properties to the LDL surface.

Second- Mn(II) is paramagnetic (S=5/2) and when it is free in the solution it gives rise to a sextet ESR spectrum. The amount of Mn(II) bound to the LDL particle surface could be determined from the difference in the amplitudes of free Mn(II) in the solutions in the presence and absence of the LDL.

LDL are particles consisting of the hydrophobic cholesteryl esters reach core and of polar outer shell composed of phospholipids and protein. The shell is responsible for the solubility of the lipoprotein and its interaction with the arterial wall.

It has been established that some structural features of the LDL core are sensitive to the dietary fat, and could influence the rate of formation the atherosclerotic lesion what would primarily depend on the state of the LDL surface.

Our intention was to investigate the influence of core structure changes into the surface state by monitoring the changes in the binding capacity for the Mn(II) ion. The causal relationship between the phase transition temperature of the core and the binding capacity change has been demonstrated for the two porcine LDL fractions with changable cholesterol content.

Those core-surface correlation could be an important step in understanding how the composition of a fat diet can influence the structural and metabolic alterations that ultimately would lead to atherogenesis.
Crown ethers are known to show remarkable internal molecular dynamics, at least as long as they are not hosts for cations. As these internal molecular dynamics are mixed with overall dynamics of the system, the determination of the internal mobility is a non trivial problem. To get some insight into the motional behavior, measurements using benzosubstituted crown ethers have been performed. Thereby the aromatic substituent of the crown ether allows an unambiguous definition of the overall motion. The intrinsic internal motion is treated as the difference between the measured and a calculated relaxation rate of the crown CH₂-groups, whereby the calculation was based on the CH₂-vectors and the overall motion exclusively. The so obtained relaxation rate was further on used as a measure to fit an AX₂-case as well as used in a simple oblate rotational ellipsoid formalism. The before mentioned calculations of the overall diffusion were performed by treating the system as an AMX-case whereby the Inversion-Recovery as well as the proton inversion and carbon recovery where analysed by a multidimensional fit procedure within the scope of normal modes.

Ref.:
The relaxation behaviour of various nucleosides and nucleotides has been investigated by means of the "magnetization modes" formalism proposed by Grant. The time evolution of the magnetization, which determines the relaxation behaviour, is measured via inversion recovery experiments. The diffusion constants as well as the random fields are fitted by means of a least-squares procedure. To gain verification for these values a cross-check with different independent measurements has been employed. It will be shown that this method is useful with respect to applications in biochemical problems as far as the molecular dynamics are concerned. To show this applicability a complex of Guanosine-5'-monophosphate and Uridine-5'-monophosphate has been investigated. Our investigation exhibits that the lifetime of the formed complex is greater than the correlation time of the rotational diffusion.

(3) Vold, R.L.; Vold, R.R. Prog. NMR Spectrosc. 12, 79, (1978)
29Si DOUBLEQUANTUM COHERENCE SPECTROSCOPY (INADEQUATE)
A powerful method for the structure elucidation of silicon frameworks

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The INADEQUATE pulse sequence which is a standard method for the structure elucidation of carbon systems has not been used for the investigation of silicon frameworks up to now. There is only one publication dealing with heteronuclear INADEQUATE for the assignment of 13C-29Si-couplings.

Recently we succeeded in applying the one- and two-dimensional 29Si-INADEQUATE-NMR experiment to assign Si-Si connectivities of cyclic silanes. The standard pulse sequence is modified by replacing the usual broadband noise decoupling by gated decoupling in order to reduce the signal attenuation caused by the negative NOE on silicon. The period of time between the beginning of the decoupler pulse and the acquisition time has to be minimized in order to guarantee total decoupling on one hand and reduction of the NOE on the other hand. In the most unfavourable case to NOE still causes extinction of signals, therefore one has to examine each single case carefully.

The efficiency of 29Si-INADEQUATE-NMR spectroscopy is demonstrated on four known cyclic silanes, namely: Trimethylsilylnonamethylocyclopentasilane, Si₅Me₉-SiMe₃; Dimethylsilylnonamethylocyclopentasilane, Si₅Me₉-SiMe₂H; Bis(undecamethylcyclohexasilanyl), (Si₆Me₁₁)₂ and Bis(undecamethylcyclohexasilanyl)dimethylsilane, (Si₆Me₁₁)₂SiMe₂.

Nuclear Magnetic Relaxation Dispersion measurement of the dimerization of HEW Lysozyme.

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²University of Mons, Faculty of Medicine, Mons, Belgium

The study of the magnetic field dependence of the NMR relaxation times (Nuclear Magnetic Relaxation Dispersion: NMRD) in protein solutions has provided an efficient means for determining the rotation correlation time of the proteins, through the following relation (1):

\[ \nu_c = \sqrt{3/2} \pi \tau \]

\[ \nu_c = \text{dispersion inflection frequency} \]

\[ \tau = \text{protein rotation correlation time} \]

We measured the longitudinal relaxation dispersion of protons in Hen Egg White Lysozyme solutions, at different pH (fig. 1), in order to visualize the known pH-dependent dimerization of the lysozyme (2).

The dispersion profiles obtained at pH=4 and pH=9 show inflection frequencies that correspond very well with the calculated protein rotation correlation times of the lysozyme monomer (pH=4) and dimer (pH=9) respectively.

Lysozyme (2g/100ml) 4C

![Graph showing NMR relaxation dispersion](image)

References:


DETERMINATION OF THE $^1$H AND $^{13}$C SHIELDING AND $^{13}$C→$^{14}$N SPIN-SPIN COUPLING ANISOTROPIES FOR METHYLISOCYANIDE IN LIQUID CRYSTALS

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NMR spectra of molecules partially oriented by liquid crystals (LC) yield information not only on the molecular geometry and orientation (via dipolar couplings) but also on the nuclear shielding and spin-spin coupling anisotropies. Often, however, the experimentally derived anisotropy values have appeared sensitive to the LC solvent [1].

In the present study, we determined the $^1$H and $^{13}$C shielding anisotropies, $\Delta \sigma = \sigma_{zz} - \frac{1}{3}(\sigma_{xx} + \sigma_{yy})$, for methylisocyanide

$$\begin{array}{c}
\text{H} \\
\text{H-C$_1$-N} \equiv \text{C$_2$} \rightarrow z \\
\text{H}
\end{array}$$

by applying the method which makes use of the mixture of two LC's with opposite anisotropy of the diamagnetic susceptibility. The results, $\Delta \sigma_H = 4.3 \pm 0.1$ ppm, $\Delta \sigma_{C_1} = 52.4 \pm 0.3$ ppm and $\Delta \sigma_{C_2} = 359 \pm 5$ ppm are found to be in good agreement with the theoretical ones.

The anisotropies of the $^{13}$C→$^{14}$N spin-spin coupling tensors were derived by allowing for the deformational contributions in the experimental dipolar couplings arising from the intermolecular interactions in LC's [2,3]. The results, which after applying the deformational corrections are not any more solvent-dependent, are the following: $\Delta J_{C_1N} = 8.7 \pm 1.7$ Hz and $\Delta J_{C_2N} = 42.8 \pm 2.8$ Hz.


Secondary structure analysis of the initiation factor of the protein biosynthesis of E.Coli IF1 by 2D NMR.

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\(^3\)Max Planck Inst.fur Molekulare Genetik Abt. Wittmann, Berlin, Germany;
\(^4\)Dep.of Cell Biology, Lab. of Genetics, Camerino, Italy.

Initiation factor IF1 is one among the three factors required for translational initiation of the protein biosynthesis of E.Coli mainly stimulating the activity of the two other factors IF2 and IF3 in the interaction with the 30S ribosomal subunit.

This effect can be explained by a kinetic role of IF1 in accelerating the rate of formation of the 30S initiation complex. Upon joining the 50S ribosomal subunit which generates the 70S initiation complex, IF1 is ejected from the 30S particle.

IF1 (MW=8119 Da) has a sequence of 71 aminoacids and the primary structure is known.

Sequential assignments were performed by different 2D NMR methods in combination, analysing NOESY, Double Quantum Filtered Cosy and HOHAHA spectra either in water with 10% D or in deuterium oxide (99.8% D) in order to individuate the sequential contacts as well the scalar connectivities and to identify the individual spin systems.

About 75% of the residues have been assigned and the analysis of the proximities obtained from NOE connectivities indicates that IF1 secondary structure consists of a large segment of \(\beta\) sheet and another short antiparallel \(\beta\) sheet.

Furthermore the end terminal domain probably contains a short helical domain. The detection of the resonances of slow exchanging amide protons confirms the presence of hydrogen bonds in these regions.
Cross-polarization for Quadrupolar Nuclei:
Proton to Sodium-23

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The nuclear magnetic resonance behaviour of quadrupolar nuclei, in the solid state, has recently generated considerable interest. Application of rapid sample rotation and heteronuclear decoupling techniques have allowed important insights to be gained in the investigation of the structure of inorganic powders.\(^1\) However, the inherent complications of the spectra which may arise as a consequence of the number of possible transitions and the linewidth contribution from any quadrupole moment, may obstruct interpretation of any data obtained.\(^6\) It has been shown that close attention must be paid to the coherent excitation of spin \(I = \frac{3}{2}, \frac{5}{2}, ...\) nuclei, if either the quantitative\(^7\) or qualitative\(^8\) measurement of an isotropic chemical shift is to be meaningful.\(^9\)

The spectral editing properties of the cross-polarization (CP) experiment\(^{10,11}\) have been demonstrated for spin \(I = \frac{1}{2}\) nuclei.\(^15\) It has been shown that the MAR experiment may impose a modulation on the CP behaviour, where the heteronuclear dipole-dipole interaction has been made non-static as a result of sample rotation.\(^{16} (\omega_{\text{rotor}} \geq |\Omega_{11}|)\) The nature of the double-resonance experiment, applied to quadrupolar nuclei has been discussed\(^{17}\) and experimentally verified\(^{18}\) for the 'low field' (\(|\omega_0| \gg |\omega_z|\)) case. Polarization transfer has also been demonstrated between spin \(I = \frac{1}{2}\) and \(I = \frac{3}{2}\) nuclei under 'high field' (\(|\omega_z| \gg |\omega_0|\)) conditions.\(^{19}\)

In this paper we present some preliminary experimental data which illustrates the application of the cross-polarization experiment between protons and the sodium-23 nucleus (\(I = \frac{3}{2}\)).

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The first two/three symbols refer to the abstract of a talk (A) or poster (P), the following symbols refer to the page number.

J. Arnold P34.75
D. W. Aksnes P45.88
K. Albert P54.102
J. J. Attard P70.123
A. Bax A22.23
A. L. Barra P46.89
E. W. Bastiaan P82.140
G. Batta P1, 33; P2, 34; P36.77
R. Bazzo P3, 36; P9.42
A. Bauer P6, 39
A. Belmadjoub A26.27
J. C. Beloéil P27.67; P72.126
A. H. Bergman P71.124
H. B. Bildsoe A19.20; P22.61; P49.94;
A. Blume P47.91
B. Bliznic A28.30; P57.107; P66.117
G. Boddenhausen A23.24
R. Boelens P32.72; P105.170
D. Boudot A26.27
M. J. Bogusky P26, 66
J. Brnjas-Kraljevic P99.164
R. Brschweiler P4, 37
J. Buddrus P80, 138
D. Canet A26, 27
G. M. Clore A10.11; P30, 70; P38.80
S. Davies A25.26
P. Daugaard P48.93
G. Dauphin P73.127
M. Decorps A3.4
F. Defaglio P25.65; P76.132
C. M. Dobson A9.10; P26.66; P51.96; P61.112; P67.118
G. Dombi P20.59; P92.155
S. L. Duce P69.121
W. Eberle P40.82
U. Edlund P25.65
U. Eggenberger A23.24
R. R. Ernst A20.21; A21, 22; P4, 37; P5.38; P16.54
G. Esposito P3.42
L. A. Fedorov P10.43; P11, 45
D. G. Gadian A5.6
M. Gehrke P31.71
G. Gemmecker P12.47; P13.49
H. Grahn P25.65
M. Geringer P100, 165
C. P. Grey P51.96
C. Grieseinger A20.21; A21, 22; P4, 37; P16.54
E. Guittet P28.68
A. Haase A6.7
Xiuwen Han P56.106
A. M. Hkkinnen P62, 113
E. Haupt P84.144
L. Helm P81.139
S. Hengyi P44.87
J. Higinbotham P15,52
M. Hofmann P31,71
L. Huis P85,145

H. Jacobsen P49,94
H. J. Jakobsen A19,20; P48,93; P49,94
P. A. de Jager P86,147
O. Jardetzky A11,12
J. Jeener A27,29
J. Jokisaari P104,169

R. Kaptein A7,8; P32,72; P105,170
S. Kaufmann P63,114
H. Kessler A8,9; P12,47; P13,49; P31,71
A. Khuen P24,63
J. Kidric P87,149
R. Konrat P101,166
H. Kovacs P89,152
K. E. Kver P1,33; P2,34; P36,77
G. Kotovych P33,73
J. Kowalewski P89,152

J. Y. Lallemand A12,13; P27,67; P28,68
G. La Mar A13,14
J. Lambert P80,138
R. Lamerichs P32,72
V. Langer P48,93
K. M. Larsson P67,118
M. H. Levitt A17,18
J. M. Lhoste A1,1
L. Y. Lian P34,75; P37,78
H. A. Linssen P98,162
S. Ludvigsen P39,81

S. Macura P17,55; P18,56; P87,149
Z. L. Madi A20,21; P5,38; P16,54
C. Marachino P90,153
S. Matsui P75,130
M. Mehring A29,31
B. U. Meier A20,21; P5,38;
Z. Meic' P97,161
J. R. Melema P35,76; P88,151
B. Meurer P50,95
H. Molinari P37,78
N. Morellet P27,67
K. Müller P19,57
N. Müller P6,39

N. C. Nielsen A19,20
T. J. Norwood P14,50; P69,121
W. Nusser P52,98

W. Offermann P91,154
H. Oschkinat P38,80

M. Paci P105,170
J. Paff A28,30
I. Pelczar P20,59; P21,60; P92,155
B. Pfeifer Würtenberg P54,102
A. Pines A14,15
P. Praestholm P22,61
F. M. Poulsen P39,81; P44,87
D. Pusiol P55,104
H. H. Raeymaekers P103,168
H.H.Raeymaekers P103,168
D.Robert P93,156
J.B.Robert P46,89
W.Robien P94,157
P.Rösch P29,69; P40,82; P41,83
H.Ruegger P56,106
H.Ruff P29,69
R.Saffrich P43,85
K.Sales P37,78
M.Sauzade A30,32
A.Schank P78,135
I.Schlichting P29,69; P41,83
C.Schmidt P57,107
G.Schnur P77,133
F.Schrank P102,167
A.Schwenk P96,160
A.Seibald P59,110
C.Seegebarth A2,2
J.Seelig A4,5
K.Slettengren P42,84
O.W.Sorensen A20,21; A21,22; A19,20; P4,37; P16,54; P44,87
P.Sole P76,132
S.Spera P79,136
H.W.Spiess A16,17; P57,107; P63,114; P64,115; P66,117
Z.Starcuk P23,62
D.S.Stephenson P59,110; P60,111
H.Sterk P87,149; P100,165; P101,166
S.Steurnagel P12,47
G.Szalonthai P95,158
J.M.Twyman P61,112
L.Vander Elst P68,119
W.S.Veeman A15,16
D.Vikic-Topic P97,161
M.Vilfan P58,108
J.Virlet A18,19
N.K.de Vries P98,162
S.Wefing P63,114
A.K.Whittacker P64,115
S.Wimperis A23,24
T.C.Wong P53,100
Y.Yang P66,117
D.Ziessow A24,25; P7,40; P8,41
Z.Zolnai P17,55; P18,56
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**Table 1: Sample Tube Available Grades**

<table>
<thead>
<tr>
<th>GRADE</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHz Rating</td>
<td>220-300</td>
<td>90-160</td>
<td>60</td>
<td>30-00</td>
</tr>
<tr>
<td>Concentricity (I.D. to O.D.)</td>
<td>±.001&quot;</td>
<td>±.001&quot;</td>
<td>±.0005&quot;</td>
<td>±.002&quot;</td>
</tr>
<tr>
<td>Carbomer (Tube O.D. axis)</td>
<td>±.0005&quot;</td>
<td>±.001&quot;</td>
<td>±.001&quot;</td>
<td>±.002&quot;</td>
</tr>
</tbody>
</table>
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AM 600 NMR Spectra

Current AM 600 specifications (subject to change):

<table>
<thead>
<tr>
<th>Probehead</th>
<th>Nucleus</th>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H selective (5mm)</td>
<td>1H</td>
<td>lineshape</td>
<td>10/20</td>
</tr>
<tr>
<td>Broadband (5mm)</td>
<td>1H</td>
<td>lineshape</td>
<td>6/15</td>
</tr>
<tr>
<td></td>
<td>13C</td>
<td>S/N (FORM)</td>
<td>25:1</td>
</tr>
<tr>
<td>Broadband (10mm)</td>
<td>13C</td>
<td>S/N (ASTM)</td>
<td>650:1</td>
</tr>
<tr>
<td></td>
<td>13C</td>
<td>S/N (10% EB)</td>
<td>375:1</td>
</tr>
<tr>
<td></td>
<td>15N</td>
<td>lineshape</td>
<td>6/15</td>
</tr>
<tr>
<td></td>
<td>15N</td>
<td>S/N (FORM)</td>
<td>90:1</td>
</tr>
</tbody>
</table>

Resolution (all probes) 0.25 Hz

Magnet drift: ca. <40 Hz/hr

- EB = ethylbenzene with 1H decoupling
- ASTM = 60% C6D6 in dioxane
- FORM = Formamide (1H decoupling without NOE)

LINESHAPE: 1H = CHCl3 linewidth at height of 13C satellite/10% this level
13C = C6H6 linewidth at 0.55%/0.11% of peak height
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Fig. A: Cross sectional image of a philodendron stem. Resolution $19\mu m \times 19\mu m \times 300\mu m$.

Fig. B: Cross sectional image of a mouse brain tumor. Resolution $100\mu m \times 100\mu m \times 500\mu m$.

Fig. C: A cross sectional image of a mouse eye, 3 mm in diameter. Resolution $20\mu m \times 20\mu m \times 250\mu m$.

Fig. D: Image of an ovum from laevis (frog egg). Resolution $10\mu m \times 10\mu m \times 250\mu m$.

Fig. E: Diffusion of water through a piece of nylon. Resolution $50\mu m \times 50\mu m \times 1000\mu m$.

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   b. RF pulse amplitude modulator
   c. Linear RF amplifier
5. Probehead with integral gradient assembly
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*Other nuclei available upon request

Micro-Imaging Accessory

Image graphics display processor

Display monitor

Gradient pre emphasis unit

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Sample

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