Nasjonalt NMR-møte

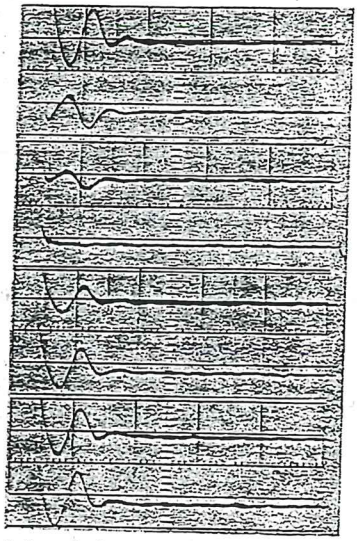

FIG. 1. A sample of raw data for measurement of proton T_1 in $CaSO_4 \cdot 2H_2O$. Each trace shows the free-induction decay following the second pulse of a 180°–90° sequence. The first trace shows the amplitude appropriate to full recovery of the magnetization. Succeeding traces correspond to times between the two pulses ranging from 0.015 to 0.30 sec.

Highland hotell, Geilo 5.-6. januar 2000

Nasjonalt NMR-møte

Highland hotell, Geilo

5. – 6. januar 2000

Oslo 27. desember 1999

Velkommen

På det forrige nasjonale NMR-møtet, arrangert på Fefor 7.-8 januar 1998 av Universitetet i Bergen, bestemte Samarbeidsutvalget for NMR at det neste møtet skulle arrangeres av Universitetet i Oslo. Vi har gjort som Bergen og lagt møtet i forkant av Organisk Vintermøte. Da 6. januar (trettende dag jul) er helligdag både i Sverige og Tyskland var det ingen derifra som kunne/ville komme til Norge så dennegang er det et rent nasjonalt møte. Da nå alle har en epostadresse, var møtet enkelt å arrangere. Det meldte seg dessuten akkurat tilstrekkelig mange foredragsholdere til å fylle den tilgjengelige tid. Det eneste vi har gjort er å ordne foredragene i grupper etter tema.

Utviklingen innen NMR fortsetter med stormskritt. Da den store instrumentanskaffelsen (kjøp av 12 spektrometere) fant sted i 1995 – for snart 5 år siden – begynner utstyret allerede å trekke på årene. Indymodellene som ble levert markedsføres ikke lenger, og Bruker har lansert en versjon av XWIN-NMR på PC, og hvordan utviklingen går videre er usikker. Heldigvis for oss kjøpere er det fortsatt to tunge instrumentleverandører på markedet. Det synes også som MRI for kjemikere kommer – i hvert fall vil det bli et økende behov for folk som kan både teori og praksis for opptak og tolkning av MR-bilder (MRI) og MR-spektra (MRS). Vi må ikke la andre fag stjele metodene fordi de hevder å ha rett til de gode problemstillingene! Det gjelder både biologi, medisin, materialvitenskap og geologi. Så vi må stå på for å holde standarden oppe. Det gjelder både for dem som bruker NMR og for dem som utprøver og utvikler nye metoder og som strever med å holde utstyret og programvaren oppdatert.

Bjørn Pedersern

Frode Rise

Program for NMR-møtet på Geilo 5-6. januar 2000

Onsdag 5. januar

13.00 - 14.00 Lunsj

14: 15 - 14: 20 **Åpning:** Bjørn Pedersen

14: 20 - 16: 30 **Fysikalsk NMR** ordstyrer: Einar Sletten

Foredragsholdere: Drabløs, Hansen, Holme, Gran, og Veliyulin

16: 00 - 16: 30 Kaffepause

16: 30 - 17: 30 **MRI & MRS** ordstyrer: Eddy Hansen

Foredragsholdere: Gribbestad, Hjelstuen, og Sitter

17: 30 - 19: 00 **Institusjonenes fremtidsplaner innen NMR**

Innledere: Jan Bakke, NTNU, Nils Åge Frøystein, UiB, Eddy Hansen, Sintef Oslo, Bjørn Pedersen, UiO og Tore Skjetne Sintef Unimed

20: 00 Middag

Torsdag 6. januar

9: 00 - 10: 00 **Dynamisk NMR** ordstyrer: Tore Skjetne

Foredragsholdere: Aksnes, Courivaud, Kristiansen og Seland

10: 00 - 10: 30 Kaffepause

10: 30 - 12: 00 **Bioorganisk NMR** ordstyrer: Jan Bakke

Foredragsholdere: Antonsen, Nerdal, McKinney og Torskangerpoll

12: 00 - 12: 40 **Biofysikalsk NMR** ordstyrer: Aurora Martinez

Foredragsholdere: Andersen og Molderheim

12: 40 - 12: 50 Avslutning

13: 00 - 14: 00 Lunsj

Postere henges opp så snart deltagerne kommer og tas ned før deltagerne reiser dvs posterne henger oppe fra lunsj onsdag til lunsj torsdag.

Sammendrag av Foredrag og postere er gitt nedenfor ordnet etter foredragsholderens eller posterpresantererens etternavn.

FOREDRAG

Foredrag

Navn	Institusjon	Tittel
Aksnes, Dagfinn, Gjerdåker, Lars, Kimtys, Liudvikas and Sørland, Geir H.	@kj.uib.no	Dynamic NMR investigations of organic molecules confined in porous materials
Andersen, Bjørn and Sletten, Einar	@kj.uib.no	NMR solution structure of a DNA 12/11-mer…
Skarstad, Anita, Anthonsen, Henrik W., Anthonsen, Marit W. and Johansen, Berit	@unimed.sintef.no	Production, purification and NMR Studies of CaLB
Courivaud, F., Hansen, E.W., Kolboe, S., Karlson, A. and Støcker, M.	@kjemi.uio.no	Influence of the surface properties and the pore filling on the diffusion of n-hexane in MCM-41 studied by Pulsed Field Gradient NMR
Drabløs, Finn	@unimed.sintef.no	Can ab initio and DFT methods predict reliable NMR shifts?
Gran, Hans Christian	@byggforsk.no	Undersøkelse av væsker i poresystemet til hydratisert sementpasta
Gribbestad, I.S., Sitter, B., Bakken, I.J., Axelson, D., Nilsen, G. and Kvistad, K.A.	@unimed.sintef.no	Combined in vitro and in vivo MR spectroscopy in biomedical research
Hansen, Eddy W.	@chem.sintef.no	Pore Size Distribution of Silica Gels Probed by ^{2}H-NMR Spin-Lattice Relaxation
Hjelstuen, Mari, Gribbestad, Ingrid S. og Haraldseth, Olav	@unimed.sintef.no	MR i prekliniske forsøk
Holme, Hilde K. og Kristiansen, Are	@hydro.no	Kvalitetssikring av NMR-instrument
Kristiansen, Per Eugen	@kjemi.uio.no	Phase Distribution and Molecular Motion Characteristics of Polyethylenes
McKinney, Jeffrey A.,	@pki.uib.no	The conformation of L-tryptophan

Teigen, Knut, Haavik, Jan, Knappskog, Per M., Frøystein, Nils Åge and Marti'nez, Aurora		and dihydrobiopterin bound to human tryptophan hydroxylase. Implications for the catalytic mechanism.
Moldrheim, Erlend, Sletten, Einar and Hannon, Mike	@kj.uib.no	Metallo-supramolecular biology: Interaction between supramolecular cylinders and DNA
Nerdal, Willy, Hindenes, Jan-Ove, Guo, Wen, Di, Li, Small, Donald M. and Holmsen, Holm	@kj.uib.no	PHYSICAL PROPERTIES OF THE TRANSMEMBRANE SIGNAL MOLECULE, *sn*-1-STEAROYL 2-ARACHIDONOYL GLYCEROL. Acyl chain segregation and its biochemical implications.[1].
Seland, John Georg	@chembio.ntnu.no	Diffusion measurements at long observation times in the presence of internal magnetic field gradients
Sitter, Beathe, Singstad, Trond E., Fjøsne, Hans, Kvistad, Kjell Arne og Gribbestad, Ingrid S.	@unimed.sintef.no	Sammensetning av brystkreftvev analysert med mikro MRI og høyoppløsning MAS spektroskopi
Torskangerpoll, Kjell	@kj.uib.no	NMR-studier av antocyaner i tulipan
Veliyulin, E., Aursand, M., Singstad, T.E., Erikson, U. and Gribbestad, I.S.	@unimed.sintef.no	NMR as a tool for optimization of high quality baccalao production

Dynamic NMR investigations of organic molecules confined in porous materials

Dagfinn W. Aksnes[1], Lars Gjerdåker[1], Liudvikas Kimtys[2] and Geir H. Sørland[3]

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In this work, the dynamics of organic probe molecules confined within porous materials are studied by high field (9.4 T) NMR, and the results are discussed with reference to the bulk substances. NMR line-widths, relaxation times (T_1, T_2) and diffusivities are reported as a function of temperature, as demonstrated for *tert*-butyl chloride (TBCL) in Fig. 1.

If the porous grains or crystallites are of reasonable size (~10 μm) intracrystalline NMR parameters can be obtained. However, unless the distance travelled by the molecules during the experiment is less than the dimension of the pore, the measured NMR parameters will be affected by boundary interactions and internal field gradients inside the pore. However, by using bipolar PFG spin-echo methods[1] and short diffusion times[2], we demonstrate that it is possible to extract the true intracrystalline diffusivity.

Both the phase behaviour and molecular dynamics of the adsorbed substances are drastically changed as a result of the confinement. Thus, the ^{1}H line-shape and T_2 measurements clearly reveal a two-component system consisting of a narrow line superimposed on a broad line at temperatures well below the transition point of the bulk material. The narrow component is attributed to the surface layer, while the broad component originates from the solid phase at the centre of the pores.

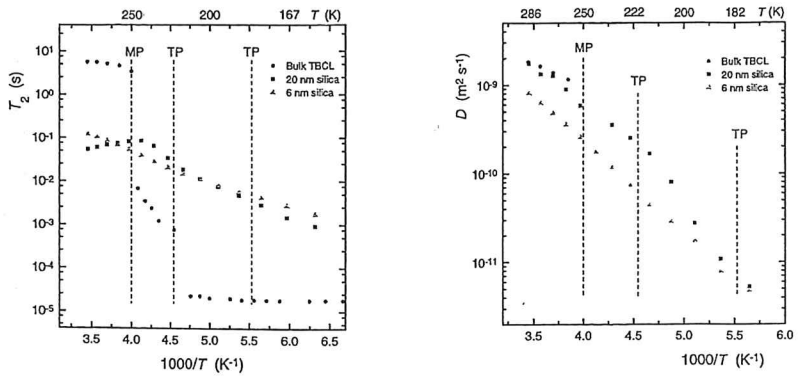

Fig. 1. Temperature dependence of T_2 and the diffusivity of *tert*-butyl chloride (TBCL) in porous silica.

References

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2. L. Gjerdåker, G. H. Sørland and D. W. Aksnes, *Microporous and Mesoporous Mater.*, **32** (1999) 305.

NMR solution structure of a DNA 12/11-mer: d(CTCCTGTGTCTC)·d(GAGATA-AGGAG) containing a transplatin interstrand G-N7/A-N1 cross-link; relevance to antisense strategy.

Bjørn Andersen and Einar Sletten, Department of Chemistry, University of Bergen,
Eloy Bernal-Méndez and Marc Leng, Centre de Biophysique Moléculaire, CNRS, Orleans

Abstract : In the so-called antisense strategy, the binding of oligonucleotides to mRNA leads to inhibition of translation or RNA metabolism [1]. However, a major constraint is that the oligonucleotide-RNA hybrids have to be stable enough to avoid dissociation by the cellular machinery. Cross-linking the oligonucleotides to their targets can prevent the dissociation. Recently, a convenient method has been proposed to cross-link specifically and irreversibly oligonucleotides to their targets in cell-free and cell media [2].

In this talk, the structure determination of a *trans*-diammineplatinum(II) interstrand cross-linked DNA duplex is presented. The system investigated in detail consists of an 12/11-mer hybrid d(CTCCTG*TGTCTC)·d(GAGATA*-AGGAG) (the asterisks indicate the cross-linked bases). The cross-linked duplex was prepared in two steps. First, the single-stranded d(CTCCTGTGTCTC) was reacted with *trans*-DDP and subsequently hybridized with the partially complementary oligonucleotide d(GAGATA-AGGAG) in which the d(AT) doublet facing the intrastrand GTG cross-link replaced the d(CAC) triplet in a regular duplex. The formation of the hybrid promoted the rearrangement of the (G1,G3)-intrastrand cross-link into an interstrand cross-link. NOESY spectra and chemical shifts indicated that the inter-strand cross-link is established between G6-N7 and A18-N1. Using all the NOESY data (215 constraints) a solution structure of the cross-linked duplex was obtained by NOE-restrained molecular dynamics/mechanics refinements. Only the central portion of the duplex deviates significantly from regular B-form geometry. The interstrand cross-link induces a bend of 21° in the duplex.

Acknowledgments Financial support by the European Commission BIOMEDII program (Contract BMH4-CT97-2485) and Agence Nationale de Recherches sur le Sida is gratefully acknowledged. Thanks are extended to the Norwegian Research Council for a fellowship to B.A.

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Production, purification and NMR Studies of CaLB

Anita Skarstad [1], Henrik W. Anthonsen [2], Marit W. Anthonsen [1] and Berit Johansen [1]

[1] Unigen, NTNU, Trondheim
[2] SINTEF Unimed MR, Trondheim

Abstract

Cytosolic phospholipase A2 ($cPLA_2$) is an enzyme that hydrolyzes phospholipids containing arachidonate in the *sn*-2 position. $cPLA_2$ is activated by a range of stimuli, e.g. proinflammatory cytokines, growth factors, thrombin and lipopolysaccharide. Activated $cPLA_2$ releases arachidonic acid, which is converted to eicosanoids, mediating inflammatory responses. The $cPLA_2$ consists of a catalytic domain, hydrolyzing phospholipids; and a Ca^{2+}-dependent, lipid-binding domain (CalB/C2), which mediates translocation to cellular membranes where the catalytic domain can act.

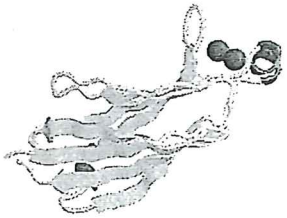

Figure 1: Schematic drawing of the crystal structure of the 14 kDa CaLB domain in $cPLA_2$ (Perisic, J Biol Chem, **273**, 1596 (1998)). The figure shows the overall backbone structure and two bound calcium atoms close to the only alpha-helical region in the protein.

In order to perform NMR-studies and to obtain insight into the structural basis for the Ca^{2+}-dependent lipid binding of CalB, we have cloned, expressed and purified CaLB. This procedure is being optimized for isotope labeling of CalB. Details of the protein production and initial results from NMR studies will be reported. Understanding of the interaction of CalB with lipid surfaces may allow development of novel, specific inhibitors of $cPLA_2$.

"Influence of surface properties and pore filling on the diffusion of n-hexane in MCM-41"

F. Courivaud[a], E. W. Hansen[b], S. Kolboe[a], A. Karlsson[b] and M. Stöcker[b]

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[b] SINTEF Applied Chemistry, P.O.Box 124 Blindern, N-0314 Oslo, Norway

The Pulsed Field Gradient NMR techniques offers a unique way of measuring molecular diffusion and surface dynamics in porous media.

The presence of pore walls in porous solids causes a deviation from the gaussian diffusion propagator at sufficient observation time, and is closely related to the physical properties of the porous host. This has been observed by PFG NMR in porous media such as sandstone or porous glass, and also in zeolites and mesoporous materials.

The mesoporous materials MCM-41 are well known as model systems for fundamental adsorption studies. Their regular channels can be shrunk in a controlled way by treating them with methyltrichlorosilane followed by water vapour exposure, leading to a methylated surface.

We have studied the influence of surface properties and the pore filling on the diffusion behaviour of n-hexane in MCM-41 particles. Starting with totally filled pores, the *n*-hexane diffusivity within MCM-41 particles is significantly enhanced as the pore filling is decreased until it reaches a maximum value. The diffusivity declines again at very low pore filling. This is rationalised by a three-component parallel diffusion mechanism, in which surface diffusion, "interface layer" diffusion (i.e.

The reliability of NMR shifts predicted by *ab initio* and DFT methods

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NMR shielding constants can be estimated from electronic structure calculations. The electronic structure methods use the laws of quantum mechanics as their basis, as described by the Schrödinger equation. There are three major classes of electronic structure methods; semi-empirical, *ab initio* and density functional theory (DFT) (although DFT methods often are classified as *ab initio*). In principle, *ab initio* methods are based solely on the laws of quantum mechanics – the first principles referred to in the name *ab initio* – and on the values of a small number of physical constants, whereas semi-empirical methods use parameters derived from experimental data to simplify the computation. However, for all but the simplest molecular systems the exact solution of the Schrödinger equation is not possible, and simplifying approximations have to be made also in *ab initio* calculations. Some of these approximations may be more or less fixed, like the Born-Oppenheimer approximation, which says that nuclear and electronic motions can be treated separately. However, other approximations are at least to some extent under user control. This study has looked at how two such approximations, basis set and electron correlation, may influence the quality of theoretically estimated NMR shielding constants.

The basis set approximation involves expressing the molecular orbitals as linear combinations of a finite number of pre-defined one-electron functions known as basis functions. These basis functions bear some resemblance to atomic orbitals. Often gaussian-type atomic functions are used, and linear combinations of *primitive* gaussians are used to form the actual basis functions, called *contracted* gaussians. Common basis sets are STO-3G and 6-31G.

The electron correlation approximation used in Hartree-Fock (HF) theory ignores correlation between the motions of electrons of opposite spin within a molecular system. Electron correlation methods try to treat this phenomenon by additional calculations. Relevant methods include Configuration Interaction (CI) and Møller-Plesset perturbation theory (MP). However, these methods are computationally demanding, and over the last few years an alternative approach known as Density Functional Theory (DFT) has become popular. Here electron correlation is modelled via general functions of the electron density. The non-classical energy of the charge distribution is usually divided into separate parts, exchange (same-spin interactions) and correlation (mixed-spin interactions). These terms are functionals of the electron density, and several alternative formulations of these functionals have been developed. Examples are LDA and BLYP. It is also possible to define hybrid functionals, where a mixture of HF and DFT exchange together with DFT correlation is used. An example of this is the B3LYP functional.

The total effect of this is that several combinations of basis set, theory (*ab initio* or DFT) and functional are possible, and it is important to understand how these combinations may influence the quality of predicted shielding constants. Some studies of this type have been published. However, they have mainly focused on a few specific methods, or at selected types of molecules. In this study several basis sets and functionals have been tested on a very general small-molecule data set, with some additional compounds

Undersøkelse av væsker i poresystemet til hydratisert sementpasta

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Gjennomgang av resultater fra undersøkelser av porevæsker i hydratisert sementpasta utført ved Byggforsk og SINTEF,Kjemi. Gjennomgangen omfatter presentasjon av Fluorescent Liquid Replacement (FLR)-impregneringsteknikk for tette porøse materialer [1]. Teknikken er basert på utskifting av vann i poresystemet med en organisk væske tilsatt fluorescerende fargestoff. NMR er anvendt for å bestemme diffusjonshastigheter og grad av impregnering [2,3,4] for hydratiserte sementpastaer med ulike opprinnelige blandeforhold. I tillegg gis en oppsummering fra bestemmelse av porestørrelsesfordeling ved tilpasning av T_1-data og frysing [5,6]. Emnet belyses med noen praktiske eksempler der NMR benyttes til å påvise forandringer i porestrukturen til sementpastaer som har vært utsatt for uttørking og frost [7].

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[2] E. W. Hansen and H. Chr. Gran, Carbon NMR used in probing the exchange of ethanol with water in water-saturated cement pastes, Magnetic Resonance Imaging, 14, 903 – 904 (1996).

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Combined in vitro and in vivo MR spectroscopy in biomedical research

I. S. Gribbestad[1], B. Sitter[1], I.J. Bakken, D.Axelson[1,3], G. Nilsen[2] and K.A. Kvistad[2]
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MR spectroscopy (MRS) has a unique role in clinical research as a technique for non-invasive monitoring of body metabolism. In vivo applications of MRS have reached the point where clinical trials are underway for a number of different applications (1). In vivo ^{1}H chemical shift imaging (CSI) on human brain tumors significantly improves the preoperative clinical diagnosis when combined with pattern recognition methods (2). Addition of CSI to MRI also improves the specificity of prostate cancer diagnostics (3). In a recent in vivo ^{1}H MRS study, we demonstrated high concentrations of choline compounds in breast carcinomas (4). The ability of in vivo ^{1}H MRS for differentiation of benign and malignant breast tumors is now being investigated (5) and also to detect the effect of chemotheraphy (6). Ex vivo MR spectroscopy of biopsies taken from suspicious thyroid, cervix, ovarian and colon tissue can be used for discriminating benign from malignant tumors (7,8) and also fast and slow growing prostate tumors (9). Recently, a study using ex vivo MR spectroscopy to investigate breast cancer biopsies reported a sensitivity of 95% and a specificity of 96% in distinguishing benign lesions from invasive breast cancer (10).

In an approach aimed at characterisation of breast tumors in vivo, we have reported ^{1}H NMR studies of perchloric acid (PCA) extracts of breast carcinomas and non-involved breast tissue (11). Better resolved spectra obtained from tissue extracts allow for more precise assignment of resonances and identification of components seen in the low-resolution in vivo ^{1}H spectra of breast tissue. This information is also of crucial importance in interpretation of ex vivo MR spectra of tissue biopsies. Our studies demonstrated large variations in phosphocholine (PC) and phosphoethanolamine (PE) content in malignant and non-involved breast tissue from the same origin (11). In conclusion, we regard the experience with in vitro MRS of crucial importance for the understanding and interpretation of in vivo MRS findings in breast cancer. Future work need to document the metabolic composition of tumors related to histopathology, in order to explore fully the potentials of MR spectroscopy as a tool in breast cancer diagnostics.

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Pore Size Distribution of Silica Gels Probed by ^{2}H-NMR Spin-Lattice Relaxation

E. W. Hansen

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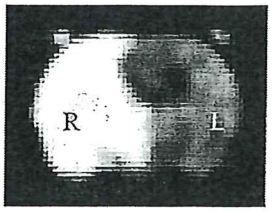
Abstract

Pore size distributions (PSDs) of commercial, porous silica gels saturated with water (D_2O) have been determined by deuteron spin-lattice relaxation rate ($1/T_1$) measurements at temperatures slightly below the normal freezing point of bulk water. The actual PSD was obtained by combining NMR and nitrogen adsorption/desorption isotherms. Some discrepancies between the PSDs obtained by the two techniques are noticed and discussed in the text. The benefit by applying ^{2}H-NMR relaxation technique in obtaining PSD relies on the short acquisition time (of the order of minutes) and the experimental simplicity.

MR i prekliniske forsøk

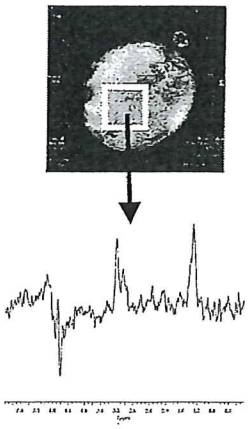
Mari Hjelstuen[1], Ingrid S.Gribbestad[1]og Olav Haraldseth[2]
[1]SINTEF Unimed, MR Senteret og [2]Institutt for anestesi og bildediagnostikk, NTNU, Trondheim

Prekliniske forsøk som omfatter bruk av dyr har vært utført ved MR-senteret i Trondheim de 10 siste årene. Den første tiden var slike forsøk konsentrert rundt utvikling av MRI og MRS metoder (diffusjon, perfusjon og ex vivo ^{13}C MRS) for deteksjon og metabolismestudier av hjerneslag og andre hjernesykdommer. En egen rotte hjerneslagmodell er etablert for disse forsøkene. Denne er også blitt benyttet til preklinisk effektevaluering av nye hjerneslagmedikamenter med MR for farmasøytisk industri.



Transversalt MR bilde av rottehjerne med hjerneslag i høyre hemisfære (R).

MR-senterets hovedfokus nå er kreft, og målet er å utvikle nye MRI og MRS metoder for bedre malignitetsgradering, prediksjon og evaluering av behandling. Dyreforsøk vil være et nyttig redskap i utvikling og uttesting av slike metoder før implementering i klinikken. Senteret har i dag en egen brystkreftmodell (MCF-7), og har planer om å etablere flere dyremodeller for kreft i tiden fremover. De fleste forsøk med rotter og mus har vært utført ved 2.35 T (Bruker Avance DBX 100, Biospec), men mulighetene for å analysere mus ved 4.7 T (Bruker Avance DMX 200) er nå etablert ved MR-senteret.


Volumselektiv ^{1}H-MRS (4.7T) av kreftsvulst grodd rundt venstre lårben. Bildet øverst viser et transversalt bilde gjennom låret med svulsten.

Fire prekliniske dyreforsøk pågår nå ved MR-senteret: Utvikling og testing av en ny MR sekvens til studier av biokjemien i kreftsvulster; evaluering av hvilken effekt MRI kontrastmidler har på MRS resultater; preklinisk uttesting av et potensielt tumor kontrastmiddel, og MRI benyttet til å evaluere hvilken effekt et intratumoralt injisert hyaluronsyrenedbrytende enzym har på blodvolum og diffusjon av fritt vann i kreftsvulster. Eksempler fra disse studiene vil bli vist.

Noen aktuelle referanser:

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Kvalitetssikring av NMR-instrument

Hilde K. Holme og Are Kristiansen

Pronova Biomedical AS (Hilde.K.Holme@hydro.com)

Abstract

I farmasøytisk industri er en underlagt ett meget strengt regelverk (GMP=Good Manufacturing Practice) for kvalitetssikring av produksjonsprosesser og av ferdige produkter. Kvalitets-kontrollen av ferdig produkt innebærer typisk bruk av en analytiske metode som krever ett eller flere analyseinstrumenter. Det kreves omfattende dokumentasjon for både metoden og for instrumentet. Foredraget tar for seg "kvalifisering" av analyseinstrument, samt "validering" av metode.

Kvalifisering av instrument omfatter:

i) Spesifisering av krav til instrumentet

ii) Kontroll og dokumentasjon av innstallasjon

iii) Kontroll og dokumentasjon av at kravspesifikasjoner er oppfylt

iv) Vedlikeholdsrutiner og dokumentasjon av at instrumentet er operativt i hht tiltenkt bruk.

Metodevalidering innebærer en bevisførsel for at en analytisk metode gir riktig resultat. Dette omfatter identifikasjon av kritiske parametre, vurdering av presisjon, nøyaktighet og usikkerhet, metodens reproduserbarhet og "robusthet". Metodevalidering forutsetter at analyseinstrumentene er kvalifisert.Basert på en nylig fullført innstallasjon av ett 400 MHz NMR-spektrometer hos Pronova Biomedical vil det bli gitt spesifikke eksempler på kvalifisering og metodevalidering, samt fallgruber. Kvalitetssikring av instrumenter og metoder bør ha interesse også utenfor farmasøytisk industri.

Phase Distribution and Molecular Motion Characteristics of Polyethylenes

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Abstract

Polyethylene (PE) is an important polymer, used in everything from plastic bags to boats. Crystallinity and phase distribution of PE is key properties that determine the possible use of a PE sample. The morphology of PE has been investigated by NMR spectroscopy. A new quantitative method for determining the crystallinity of PE from the free induction decay (FID) has been investigated. It has been shown that the crystallinity obtained from conventional spectral analysis is under-estimated by approximately 8 % due to signal loss during the pre-sampling delay.

The "FID-analysis" technique involves model fitting to three phases. The crystalline part of the FID is represented by an inverse Fourier transform of the Pake function. A fast decaying exponential function is assigned to the intermediate phase, and a Weibullian function is assigned to an amorphous phase. At room temperature the crystallinity derived by the "FID-analysis" is in excellent agreement with the crystallinity obtained by other methods.

The "FID-analysis" method has been used to gain *in-situ* information on phase distribution during melting and crystallization. These changes have been discussed in light of polymer theory. The average molecular mobility within the crystalline phase as a result of crystallization and melting has also been obtained. Moreover, differences in mobility between the phases of PE have been found.

The conformation of L-tryptophan and dihydrobiopterin bound to human tryptophan hydroxylase. Implications for the catalytic mechanism

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Tryptophan hydroxylase (TPH) catalyzes the hydroxylation of L-tryptophan (Trp) to 5-hydroxytryptophan, using tetrahydrobiopterin (BH_4) and dioxygen as additional substrates. This is the rate-limiting step in the biosynthesis of the neurotransmitter 5-hydroxytryptamine (serotonin). Together with tyrosine hydroxylase (TH) and phenylalanine hydroxylase (PAH), TPH makes a superfamily of aromatic amino acid hydroxylases that all contain a catalytic mononuclear non-heme iron. These enzymes show extensive sequence similarity at the catalytic domains, and are believed to have the same reaction mechanism. The low abundance and the unstability of the isolated TPH have previously hindered studies with this enzyme. We have know expressed and isolated in large scale a stable truncated form of recombinant human TPH (ΔN90-TPH). The crystal structures of TH and PAH have recently been determined and, based on the sequence and structural similarities of these enzymes, we have created a 3D-structural model of ΔN90-TPH. In order to get further insights on the catalytic mechanism for TPH, we seek to determine the conformation of Trp and the pterin cofactor when bound to this truncated form. By measuring the paramagnetic effect of the enzyme bound Fe(III) on the longitudinal relaxation rates ($1/T_1$) of proton resonances of Trp and of L-erythro-7,8-dihydrobiopterin (BH_2), an inactive analogue competitive to BH_4, we have estimated the distances from these ligands to the catalytic iron. The distance between the hydroxylation site in Trp, i.e. the C5 atom (about 4.2-5.0 Å) is in agreement with a role of the iron both in the binding and activation of dioxygen and in the hydroxylation reaction. In addition, the interproton distances in the enzyme bound Trp and BH_2 have been determined by transferred NOESY spectra taken at different mixing times and enzymes concentrations. Based on the distance constraints obtained by NMR complemented by distance geometry calculations, we have determined the conformation of Trp and BH_2 bound to TPH. The resulting bound conformers of both ligands have been fitted into the modelled 3D-structure of TPH by molecular docking and results will be presented.

Acknowledgments. The work was supported by the Research Council of Norway.

METALLO-SUPRAMOLECULAR BIOLOGY.
Interaction between supramolecular cylinders and DNA

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Mike Hannon, Department of Chemistry, University of Warwick.

Supramolecular chemistry has enhanced our understanding of how simple interactions between molecules can control structure and function. The concept underlying the presentation will be the possibility of applying supramolecular principles within biological systems [1]. Protein-DNA recognition is often mediated by binding of zinc finger to the major groove of DNA. By contrast , small molecule DNA binding is often associated the minor groove. We have prepared metallo-supramolecular triple helices which are of appropriate size to slot into the major groove, but too large for the minor groove. These cylindrical cations binds effectively to DNA in solution.

The interaction between a double-helical DNA oligonucleotide, 5´-d(GACGGCCGTC)$_2$,and a dinuclear iron(II) triple helical supramolecule, $[Fe_2(C_{25}H_{20}N_4)_3]^{4-}$, has been sudied by 1D and 2D NMR spectroscopy. Preliminary results of these studies will be presented.

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PHYSICAL PROPERTIES OF THE TRANSMEMBRANE SIGNAL MOLECULE, *sn*-1-STEAROYL 2-ARACHIDONOYL GLYCEROL.

Acyl chain segregation and its biochemical implications.[¶]

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ABSTRACT

sn-1,2-diacylglycerol (DAG), a key intermediate in lipid metabolism, activates protein kinase C and is a fusogen. Phosphoinositides, the main sources of DAG in cell signalling, contain mostly stearoyl and arachidonoyl in the *sn*-1 and -2 positions, respectively. The polymorphic behavior of *sn*-1-stearoyl-2-arachidonoyl-glycerol (SAG) was studied by differential scanning calorimetry, X-ray powder diffraction and solid state magic angle spinning (MAS) ^{13}C NMR. Three α-phases were found in the dry state. X-ray diffraction indicated that the acyl chains packed in a hexagonal array in the α phase while the two sub-α phases packed with pseudo-hexagonal symmetry. In the narrow angle range strong diffractions of ~31 and ~62 Å were present. High power proton decoupled MAS ^{13}C NMR of isotropic SAG gave 16 distinct resonances of the 20 arachidonoyl carbons and 5 distinct resonances of the 18 stearoyl carbons. Upon cooling, all resonances of stearoyl weakened and vanished in the sub-α_2 phase while arachidonoyl carbons from 8/9 to 20 gave distinct resonances in the frozen phases. Remarkably, the ω-carbon of the two acyl chains had different chemical shifts in α, sub-α_1, and sub-α_2 phases. Large differences in spin-lattice relaxation of the stearoyl and arachidonoyl methene and methyl-groups were demonstrated by contact time (CP) MAS ^{13}C NMR experiments in the solid phases □□, sub-α_1 and sub-α_2. This shows that stearoyl and arachidonoyl in SAG have different environments in the solid states (α, sub-α_1, and sub-α_2 phases) and may segregate during cooling. The NMR and long spacing X-ray diffraction results suggest that SAG does not pack in a conventional double layer with the two acyls in a hairpin fashion. Our findings thus provide a physico-chemical basis for DAG hexagonal phase domain separation within membrane bilayers; such hexagonal domains are postulated to be responsible for the various biological actions such as membrane destabilization, enzyme activation and fusion events promoted by SAG or other 1-saturated-2-polyunsaturated-glycerols.

Supported by NIH Grants #TG5T32HL-07291 and 5 PO1HL26335 (DMS) and EU BIOMED 2 grant EC BMH4-97-2609.

Diffusion measurements at long observation times in the presence of internal magnetic field gradients

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Diffusion measurements in heterogeneous media may contain a significant source of error, the influence of internal magnetic field gradients on the attenuation of the NMR signal. The strength of these gradients depends on the magnetic susceptibility difference when going from one phase to another in the heterogenous sample, on the strength of the operating magnetic field, and on the different geometries of phases within the sample (1). Due to the heterogeneity of the sample, the internal gradients may consist of a large distribution of values with both polarities (2,3).

Usually this error has been assigned to the use of monopolar pulsed field gradient experiment where the error originates from the coupling between the applied and internal magnetic field gradients (4). Depending on the relative strength between the internal gradients and the applied ones, the measured diffusivity may therefore not correspond to the actual diffusion coefficient of the probing molecules within the heterogeneous sample (2).

The application of bipolar magnetic field gradients has been introduced to suppress this error. One has then been able to perform reliable diffusion measurements in the presence of internal gradients of the same strength as the applied gradients (5-7).

The basic assumption for the sucsessfull removal of the cross terms between the applied and the internal gradients in the attenuation of the NMR-signal is that the diffusing molecules are experiencing a constant internal gradient during the experiment (6). The consequense is that the root of the mean squared displacement must not exceed the distance in which it is likely that an internal gradient will change its polarity or its strength significantly.

In the study presented here it will be provided theoretical and experimental evidences that the application of bipolar magnetic field gradients may fail to suppress the effect from all the cross terms between internal and applied gradients effectively at long observation times.

By comparing diffusion measurements performed in randomly packed spheres of polystyrene with a corresponding system of glass spheres, it is shown experimentally that a sucessfull suppression of the cross terms is strongly dependent on the observation time, and on the τ-value in the bipolar pulsed field gradient stimulated echo experiment. It is shown that this τ-dependency can be used for an estimation of the true diffusivity at long observation times.

Also diffusion measurements performed in porous polyethylene particles show that the effect of internal magnetic field gradients is not totally suppressed at long observation times even if bipolar gradients are applied. It is therefore important to keep the τ-value low, or, if possible, use the τ-dependency to estimate the true value of the diffusion coefficient, when performing diffusion measurements in such systems.

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NMR-studier av antocyaner i tulipan

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I denne presentasjonen vil det gjennomgås hvordan følgende NMR-teknikker ble benyttet i strukturoppklaring av fargestoffer (antocyaner) isolert fra to tulipankultivarer: ^{1}H, ^{13}C SEFT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HSQC og ^{1}H-^{1}H TOCSY. De fire hovedantocyanene ble fullstendig strukturoppklart til å være 3-*O*-[6''-*O*-(2'''-*O*-acetyl-α-rhamnopyranosyl)-β-glucopyranosid] og 3-*O*-(6''-*O*-α-rhamnopyranosyl-β-glucopyranosid) av pelargonidin og cyanidin. De to første pigmentene er tidligere ikke funnet i naturen. Det forekommer heller ikke tidligere publikasjoner hvor en acylgruppe i et antocyan er i en aksial sukkerposisjon.

NMR as a tool for optimization of high quality baccalao production.

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Abstract.

Baccalao is produced by rehydrating of cod fillet after drying it under special conditions. The quality of the final product is strongly affected by what kind of treatment the raw fish fillet has undergone during transportation and storage. Effects of freezing-thawing and salting of cod fillet on the baccalao quality was of particular interest. In this work several NMR techniques have been combined in order to study a number of important characteristics of cod fillet directly or indirectly related to the quality of raw material used for baccalao production.

We have used low field NMR for studying of spin-spin (T_2) and spin-lattice (T_1) relaxation times as well as for measuring diffusion constants in cod fillet samples subjected to different treatments.

High field NMR imager equipped with an in-house made Na^+ probe has been used for studying of cod fillet salting process in real time. The obtained sets of images produce interesting information about the rate and inhomogeneity of salt impregnation into a fish fillet. The same Na^+ probe was utilized for bulk measurements of total salt content in differently treated cod fillet.

Based on the obtained results, we have proposed a qualitative model, which describes structural changes in fish fillet under freezing-thawing-salting processes.

Sammensetning av brystkreftvev analysert med mikro MRI og høyoppløsning MAS spektroskopi

Beathe Sitter, Trond E. Singstad, Hans Fjøsne, Kjell Arne Kvistad og Ingrid S. Gribbestad

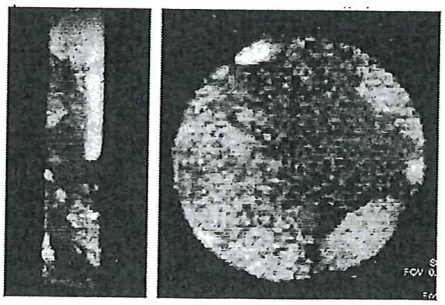
Bakgrunn

Brystkreft er en av de hyppigste dødsårsaker hos kvinner i den vestlige verden. Informasjon om endrede biokjemiske prosesser i kreftceller, muligens detekterbare før morfologiske forandringer, vil kunne bidra til både økt forståelse for kreft og som et verktøy i tillegg til histopatologi i diagnose og vurdering av behandlingseffekter. Konvensjonell MR spektroskopi av vevsprøver har vist høy spesifisitet og sensitivitet for differensiering mellom maligne og benigne kreftsvulster (1) og høyoppløsning proton MAS spekter av intakt vev fra brystkreftpasienter korrelerer godt med histopatologiske funn (2). Det er også velkjent at den metabolske profilen varierer betydelig mellom prøver (3). Ulikt bidrag av tumor vev og annen type vev antas å bidra til disse variasjonene.

Materialer og metode

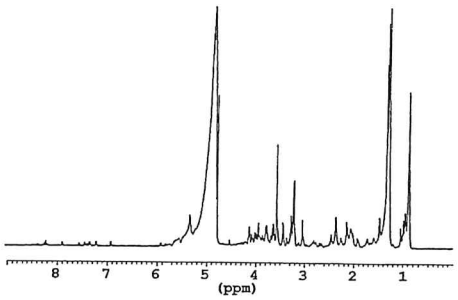
Biopsier fra brystkreftvev ble lagt i flytende nitrogen etter operasjon og lagret frem til MR analysene. Vevsprøvene ble tilskjært for å passe en 4 mm rotor tilsatt tungtvann. Analyser ble gjort i henhold til følgende protokoll: mikroMRI – MAS – mikroMRI – histologi. Mikro MR bildene ble tatt opp ved et BRUKER AVANCE DMX 200. Biopsier ble avbildet med 7 transversale snitt, 1 mm i tykkelse, ingen avstand mellom snittene. Mikro MR bilder ble tatt opp med en T_1-vektet SE sekvens og en T_2-vektet MSME sekvens over 6 mm FOV. Høyoppløsning MAS analyser ble gjort ved et BRUKER AVANCE DRX 600 spektrometer. Prøvene ble spunnet ved 8 kHz og spekter tatt opp med formetning av vannsignalet og T_2-filtrering med effektive ekkotider, 102, 270 og 580 ms. Alle forsøk ble utført ved romtemperatur.

Resultater



Figur 1 Mikro MR bilder (oversiktsbilde til venstre, T1-vektet til høyre) av biopsi fra brystvev.

Et mikro MR bilde av biopsi fra brystvev er vist i figur 1 og HR MAS spekter av en annen biopsi i figur 2. Bildene (Fig. 1) viser tilnærmet 50% fett i prøven, som korrelerer med histopatologiske analyser av samme prøve. Høyoppløsnings MAS spektra (Fig. 2) viser tilnærmet samme oppløsning som høyoppløsnings spekter av PCA ekstrakter.



Figur 2 HR MAS spekter av infiltrert ductalt carcinom. T_2-filtrert spinn ekko sekvens, effektiv ekkotid: 290 ms.

Konklusjon

Høyoppløsning MAS spektra har potensiale til å bli et verktøy innen kreftdiagnostikk og for identifisering av metabolitter som kan være av betydning ved vurdering av behandlingsregimer. Mikro MRI av vevsprøver kan gi en rask vurdering av vevs-sammensetning før eller etter MAS eksperimenter. Variasjoner i sammensetning av prøver bør vurderes hvis metabolsk profil anvendes i kreftdiagnostikk.

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POSTERE

Postere

Navn	E-postadresse	Tittel
Aksnes, Dagfinn, Gjerdåker, Lars and Kimtys, Liudvikas	@kj.uib.no	NMR investigations of tert-butyl cyanide in bulk and in porous silica
Sørland, G.H., Seland, J.G., Krane, J. and Anthonsen, H.W.	@unimed.sintef.no	Improved Convection Compensating Pulsed Field Gradient Spin-Echo and Stimulated-Echo Methods
Arstad, Bjørnar, Kolboe, Stein og Pedersen, Bjørn	@kjemi.uio.no	A study of coke depositions in SAPO34 with NMR and GC/MS
Hassel, Bjørnar and Bråthe, Anders	@kjemi.uio.no	Cerebral metabolism of lactate *in vivo*.Evidence for neuronal CO_2 fixation.
Dao, Kim Khanh, Teigen, Knut, Haavik, Jan, Knappskog, Per M., Frøystein, Nils Åge and Marti´nez, Aurora	@pki.uib.no	The structural basis of the recognition of the cofactor tetrahydrobiopterin by aromatic amino acid hydroxylases studied by 1H NMR spectroscopy.
Dudin, Khaled I., Frøystein, Nils Åge and Songstad, Jon	@kj.uib.no	Alkylselenosubstituted Propanoic Acids, $RSeCH_2CH_2C(O)OH$. Compounds with Collapsing AA'BB'^{1}H Spin Systems for the -CH_2CH_2-Fragment.
Aksnes, Dagfinn W., Gjerdåker, Lars and Kimtys, Liudvikas	@kj.uib.no	NMR investigations of *tert*-butyl cyanide in bulk and in porous silica
Aksnes, Dagfinn W., Gjerdåker, Lars and Kimtys, Liudvikas	@kj.uib.no	A high-field NMR study of pivalic acid in porous silica.
Halskau, Ø, Døskeland, A.P., Muga, A., Martinez, A. and Frøystein, N.Å.	@kj.uib.no	Conformation of alpha-lactalbumin bound to model membranes studied by NMR-monitored hydrogen exchange
Liu, Yangzhong and Sletten, Einar	@kj.uib.no	Interaction between DNA nucleotides and nickel complexes
Liu, Zheng, Holmsen, Holm and Nerdal, Willy		Chlorpromazine Interaction with Glycerophospholipid Liposomes Studied by Magic Angle Spinning Solid State ^{13}C- and ^{31}P-NMR

Netland, Kjetil Andreas, Gundersen, Lise-Lotte and Rise, Frode	@kjemi.uio.no	^{13}C assignment of cyclopropenone resonances
Optun, Odd Inge, Schult, Tove and Anthonsen, Henrik W.	@unimed.sintef.no	Molecular Weight Characterization of Diffusion Separated Pullulans by Pulsed Field Gradient NMR
Spilsberg, Bjørn, Rise, Frode, Petersen, Dirk, Rønning, Øystein W. and Nissen-Meyer, Jon	biokjemi.uio.no	The low-molecular-weight "growth inhibitory factor" produced by hybridoma cell cultures identified as thymidine by NMR-spectrometry
Doudin, Khaled I., Frøystein, Nils Åge and Songstad, Jon	@kj.uib.no	Ditetradecyl Selenid $(C_{14}H_{29})_2Se$, a Convenient Reference Compound in ^{77}Se NMR
Vinje, Jo, Sletten, Einar, Nakatani, Kazuhiko and Saito, Isato	@kj.uib.no	Interactions between Anthraquinone derivatives and DNA Oligomers.
Østby, Ole Benny, Rise, Frode and Gundersen, Lise-Lotte	@kjemi.uio.no	Assignment of 13C Resonnances of Indolizines using Optimized COLOC, HMBC and INADEQUATE NMR."

NMR investigations of *tert*-butyl cyanide in bulk and in porous silica

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The phase behaviour and molecular dynamics of *tert*-butyl cyanide (TBCN) confined within silica pores with nominal diameter 6 and 20 nm were studied as a function of temperature by measuring the ^{1}H and ^{13}C NMR line-widths and relaxation times as well as the diffusivity, and the results are compared with new results obtained for the bulk material. In the bulk sample, the uniaxial molecular reorientation of the *tert*-butyl group is faster than the methyl group reorientation in both solid phases.

The confinement in the pores gives rise to substantial changes in the phase behaviour and the rotational and translational dynamics. The ^{1}H line-shape observations reveal a narrow line superimposed on a broad resonance at temperatures below the freezing region. This observation is also reflected in the T_2 data shown in Fig. 1. In the two-component region, the narrow and broad lines are attributed to the surface layer and the crystalline phase at the interior of the pores, respectively. The relatively mobile surface component exhibits a remarkable high diffusion rate, even at temperatures far below the transition temperature of the bulk material, as demonstrated in Fig. 1.

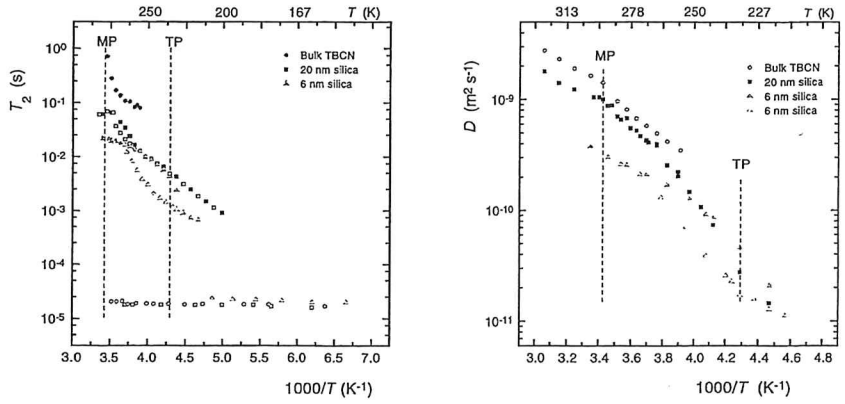

Fig. 1. Temperature dependence of T_2 and the diffusivity of TBCN in bulk and in porous silica.

Improved Convection Compensating Pulsed Field Gradient Spin-Echo and Stimulated-Echo Methods

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Abstract

The need for convection compensating methods in NMR has been manifested through an increasing number of publications related to the subject over the past few years (1-5). When performing measurements at elevated temperature, small convection currents may give rise to erroneous values of the diffusion coefficient. In work with high resolution NMR spectroscopy, the application of magnetic field gradients also introduces an eddy current magnetic field which may result in errors in phase and baseline in the FFT-spectra. The eddy current field has been greatly suppressed by the application of bipolar magnetic field gradients (6). However, when introducing bipolar magnetic field gradients, the pulse sequence is lengthened significantly. This has recently been pointed out as a major drawback because of the loss of coherence and NMR-signal due to transverse relaxation processes (7). Here we present modified convection compensating pulsed field gradient double spin-echo and double stimulated-echo sequences which suppress the eddy current magnetic field without increasing the duration of the pulse sequences.

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A high-field NMR study of pivalic acid in porous silica

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It is well known that a substance confined in a porous medium exhibits very different thermal and dynamic properties compared to the bulk material. NMR is a well-established method for characterising porous materials and for studying the behaviour of adsorbed specie.[1] In particular, NMR relaxation and self-diffusion measurements may provide valuable information on pore geometries and on the important process of mass transport.

In this work, the hydrogen bonding and molecular dynamics of pivalic acid ($(CH_3)_3CCOOH$) (PA) confined within silica pores of nominal diameter 6 and 20 nm were studied using high field (9.4 T) NMR. The ^1H, ^2H and ^{13}C line-widths, relaxation times (T_1, T_2), and diffusivities are reported as a function of temperature, and the results are compared to the data obtained for the bulk material as demonstrated in Fig. 1.[2]

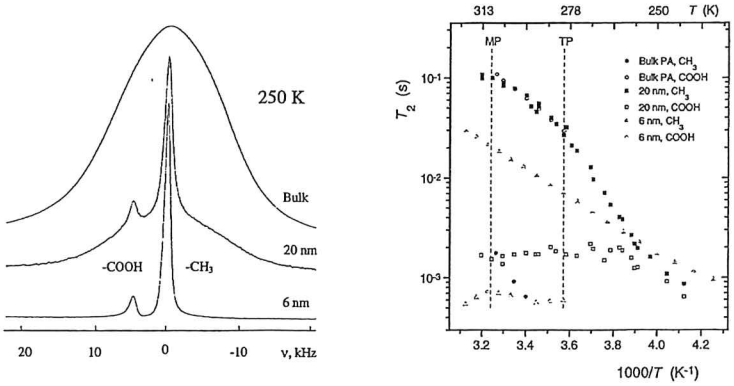

Fig. 1. ^1H NMR line-shapes and T_2 values of PA in bulk and in porous silica.

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A study of coke depositions in SAPO34 with NMR and GC/MS

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We have investigated the depositions (coke) in and on the zeotype material SAPO-34 when used as a catalyst to convert methanol to hydrocarbons. We have used solid state ^{13}C-MAS-NMR on untreated coked samples and GC/MS on extracts from dissolved coked samples to investigate the coke composition.

The samples have been prepared in a fixed-bed reactor. Time on stream has been varied between 2 and 18 hours. The temperature ranged between 300^{0}C and 425^{0}C. The samples were dissolved in 1M HCl in a closed system with He-atmoshere. The gas over the dissolved sample was analysed with GC/MS. The organic compounds in the water phase were extracted with CCl_4. Some of the samples were also studied by thermogravimetry.

The coke is shown to have a very complex composition. A NMR-spektrum of the solid consists of an alifatic region and an aromatic region, making it possible to study the change from alifatic to aromatic hydrocarbons as a function of temperature and time on stream. From the GC/MS spectra it is possible to give a more detailed description of the composition of the volatile and soluble part of the depositions.

The combination of methods used, has a potential to probe which species that affect the formation of the products. The conclusions that can be drawn on basis of only one of the methods are shown to be incomplete. The complexity of the coke depositions, and the reactions taking place in the depositions may only be described adequately by using different methods.

Cerebral metabolism of lactate *in vivo*. Evidence for neuronal CO_2 fixation.

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Lactate is of interest as a possible energy substrate for the brain. During hypoglycemia lactate can sustain cerebral function,[1] and *in vitro* studies suggest that lactate is an important energy source for neurons after cerebral hypoxia.[2]

We injected [3-^{13}C]lactate or [1-^{13}C]glucose i.v. into awake mice and analyzed brain and blood extracts by ^{13}C NMR spectroscopy. Labeling of lactate C-2 from [3-^{13}C]lactate, which was not seen with [1-^{13}C]glucose and which has not been seen previously with [2-^{13}C]acetate, made us hypothesize that [3-^{13}C]lactate to large extent is metabolized via pyruvate carboxylation in neurons.

Intrastriatal injection of [1-^{14}C]pyruvate conclusively demonstrated neuronal pyruvate carboxylation.

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The structural basis of the recognition of the cofactor tetrahydrobiopterin by aromatic amino acid hydroxylases studied by ^{1}H NMR spectroscopy.

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Phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) are tetrahydrobiopterin (BH_4)-dependent enzymes that catalyze the hydroxylation of the respective aromatic amino acids, using dioxygen as additional substrate. These enzymes constitute the superfamily of aromatic amino acid hydroxylases and they show extensive sequence similarity at the catalytic domains. They all contain a catalytic mononuclear non-heme iron and are believed to have the same reaction mechanism. The crystal structure of TH and PAH has recently been determined and, based on the sequence and structural similarities of these enzymes, we have created a 3D-structural model of human TPH. In order to get further insights on the catalytic mechanism and regulation of these hydroxylases by the tetrahydrobiopterin cofactor (BH_4), we seek to determine the conformation of the bound cofactor and the conformational changes induced by binding. The interproton distances in BH_4 when bound to recombinant human PAH, TH and TPH have been estimated by transferred NOESY spectra taken at different mixing times and different enzyme subunit concentration. Spectra are taken in the presence of 10 mM dithiothreitol (DTT) added to avoid the oxidation of the cofactor. Based on the distance constraints obtained by NMR complemented by distance geometry calculations and preliminary docking of the conformers into the crystal structure of the enzymes, we have determined the conformation of BH_4 bound to each of the hydroxylases. As previously found for dihydrobiopterin (BH_2) when bound to PAH [1], the hydroxyl groups at the dihydroxypropyl side chain at C6 in BH_4 seem to adopt a *cis* conformation when bound to the three hydroxylases. However, a more extended conformation of this side chain is found in TPH-bound BH_4 than in TH- or PAH-bound cofactor. These differences in conformation may be related to the different regulatory properties elicited by the cofactor in the three hydroxylases.

Acknowledgments. The work was supported by the Research Council of Norway and L. Meltzer høyskolefond.

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Alkylselenosubstituted Propanoic Acids, $RSeCH_2CH_2C(O)OH$. Compounds with Collapsing AA´BB´ ^1H Spin Systems for the $-CH_2CH_2-$ Fragment.

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The ^1H NMR spectra of 4-selenaoctadecanoic acid, 5-(2-naphthyl)-4-selenapentanoic acid and selenodipropanoic acid in $CDCl_3$ reveal some interesting features. These compounds may in principle be considered as 1,2-disubstituted ethanes, ACH_2CH_2B, with two distinctly different substituents, RSe- and -C(O)OH. A 600 MHz ^1H NMR study in $CDCl_3$ and CCl_4 has revealed that these compounds display a singlet for the CH_2 protons integrating for four protons. The corresponding sulfur compounds, however, show the expected doublet of triplets at 600 MHz but not at 200 MHz where a multiplet is observed. Only in C_6D_6, and partly in $(CD_3)_2SO$, are the signals due to the 2- and 3-CH_2 protons of 4-selenaoctadecanoic acid sufficiently separated to give two triplets. The particular form of the ^1H NMR spectra in $CDCl_3$ and CCl_4 was found to be independent upon the concentration; *i.e.* the monomer-dimer ratio. A temperature study has revealed that while the signal due to the 3-CH_2 protons is essentially independent of the temperature the 2-CH_2 proton signal is downfield shifted with decreasing temperature causing the close-lying signals to be indistinguishable around room temperature.

The spectra of selenodipropanoic acid in $CDCl_3$ as a function of temperature were simulated using the SWANMR program. The appearance of the spectra did not seem to be affected by variations in the geminal coupling constants but only by the vicinal ones. At the collapsing temperature the spectrum was difficult to simulate.

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Conformation of α-lactalbumin bound to model membranes studied by NMR-monitored hydrogen exchange.

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Knowledge on how proteins interact with membranes is essential to understand many biological processes, including cellular communication. There are certain soluble proteins that during the course of their action bind and insert into plasma- or intracellular membranes. It is believed that the soluble, native protein, undergoes destabilizing conformational changes resulting in the exposure of hydrophobic patches at the surface, triggering its partition into the membrane. However, the mechanism of the interaction and the details of the structural changes are not known. α-lactalbumin is a well characterized 123-residue protein with known crystallographic structure. Although water-soluble, α-lactalbumin is a secretory protein that exerts its function in a membrane environment and it is able to interact with membranes (liposomes) in a pH-controlled manner [1]. The study of amide hydrogen exchange rates in proteins is a useful probe to study the structure and conformational changes of proteins in different environments. In order to identify regions of the protein involved in the binding to membranes, we have studied the exchanging rate of backbone amines in soluble and liposome-associated forms of α-lactalbumin. In order to detect and perform the sequence specific assignment of amide protons, NOESY- and TOCSY-type spectra with good water suppression using pulsed field gradients [2,3] are required. Spectra are also recorded for protein samples allowed to exchange with D_2O at increasing periods of time (from 0 to 10 days) in the presence and the absence of membranes (liposomes). We have implemented and improved upon NMR methods to acquire these 2D-spectra with reasonable resolution and good signal-to-noise ratio on dilute samples (1mM) of the protein within an hour. The method chosen is the band-selective TOCSY experiment with homonuclear decoupling during evolution [3]. Preliminary data on the assignment of the detected amide signals and the differences encountered in the hydrogen exchange rate for membrane-free and membrane-associated α-lactalbumin will be presented.

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Interaction between DNA nucleotides and nickel complexes

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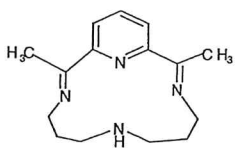
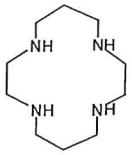
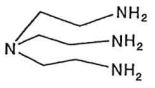
Macrocyclic nickel complexes are of interest because of their special functions in reacting with DNA. $Ni^{III}CR$, for example, can cleave nucleotides at unpaired guanine residues exclusively. This property was suggested in probing the structure of native nucleotides. Its sequence-selectivity was confirmed in the reaction with $tRNA^{Phe}$ as shown in a 3-dimensional structure determination[1]. Some other complexes, *e.g.*, Ni^{III}(cyclam) can convert DNA from *B*—*Z* form[2].



CR



cyclam



tren

The reactions of nucleotide monomers, AMP, GMP and a dimer ApG with different nickel complexes, NiCR, Ni(cyclam) and Ni(tren), have been studied by NMR and electronic spectroscopy. ^{1}H NMR 1D, T_1 titration and ^{15}N HMBC were carried out. Results show that divalent nickel complexes have different reactivity in binding to nucleotides in the order of Ni(tren)>>Ni(cyclam)≈NiCR. This disparity results from their different coordination geometry. Oxidant $KHSO_5$ can promote the binding ability of Ni(cyclam) much more than that of NiCR. $Ni^{III}CR$ is more reactive in oxidizing nucleotides than Ni^{III}(cyclam) since the redox potential of NiCR is higher than that of Ni(cyclam). GMP is easier oxidized by $Ni^{III}CR$ than AMP. This feature probably causes the selective cleavage of DNA by $Ni^{III}CR$ at guanine residues. The binding abilities in these nucleotides is G-N7>>A-N7> others.

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Chlorpromazine Interaction with Glycerophospholipid Liposomes Studied by Magic Angle Spinning Solid State ^{13}C- and ^{31}P-NMR.

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ABSTRACT

sn--stearoyl-sn-2oleoyl-phosphatidylserine (SOPS) and synthetic dipalmitoylphosphatidylcholine (DPPC) were used to make DPPC/DMPC and DPPC/SOPS large unilamellar liposomes with a diameter of ~1 μm. ChlorpromazineHCl (CPZ), an amphipathic cationic psychotropic drug of the phenothiazine group, is known to partition into lipid bilayer membranes of liposomes with partition coefficients depending on the acyl chain length and to alter the bilayer structure in a manner depending on the phospholipid headgroups. The effects of adding CPZ to these membranes were studied by solid state magic angle spinning ^{13}C and ^{31}P nuclear magnetic resonance spectroscopy on the DPPC(60%)/SOPS(40%) and the DPPC(54%)/SOPS(36%)/CPZ(10%) liposomes.

This work is supported by EU BIOMED 2 grant EC BMH4-97-2609 from the European Union (EU).

^{13}C ASSIGNMENT OF CYCLOPROPENONE RESONANCES

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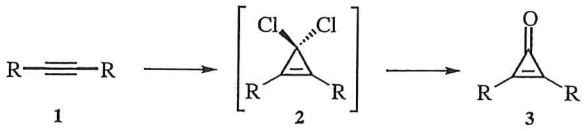
The main goal of our project was development of efficient preparative methods for synthesis of cyclopropenones **3** (**Scheme 1**).[1]



Scheme 1

When we wanted to assign ^{13}C signals of cyclopropenones, we found that few structures have been published with complete ^{13}C assignment. In the end, we solved the problem by integration of the ^{13}C resonances.

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Molecular Weight Characterization of Diffusion Separated Pullulans by Pulsed Field Gradient NMR

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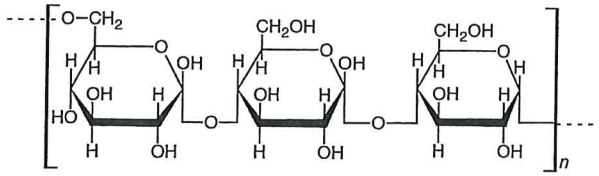
ABSTRACT

Polysaccharides such as dextrans, pullulans and cellulose are of great importance in the medical and pharmaceutical fields as well as in food, pulp and paper, and textile industries, among others [1]. They are essential to virtually all biological systems. Optimizing the functions these compounds perform is directly dependent upon knowledge of their structure and molecular weight distribution (MWD) and the extent to which these affect polymer behaviour. This can be achieved through a technique such as size exclusion chromatography (SEC) in combination with light scattering (LS) that can quantify these parameters to establish effective comparisons among different polysaccharides. This method detects transport rates that depend on molecular size and require calibration or data transformation to obtain MWD.



In this work, diffusion ordered NMR spectroscopy (DOSY, [2]), a technique based on pulsed field gradient NMR (PFGNMR), was used to obtain MWD for samples of pullulan in D_2O. The distribution of diffusion coefficients *G(D)* was achieved by analysis of PFGNMR data using constrained regularization (CONTIN, [3]) and direct exponential curve resolution algorithm (DECRA, [4]). The estimated distribution was then converted to the mass weighted distribution of molecular weight by means of the relation obtained from experiments on monodisperse reference standards. A comparison of the molecular weight data obtained by NMR is made to the LS method.

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The low-molecular-weight "growth inhibitory factor" produced by hybridoma cell cultures identified as thymidine by NMR-spectrometry

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Myeloma and hybridoma cells have been reported to secrete a low-molecular-weight growth inhibitory factor (1, 2). This factor was isolated to homogeneity from the media (4.0 liters) of hybridoma cell cultures by ultrafiltration, "dialysis concentration" and reverse phase chromatography under acidic and neutral conditions. The inhibitory factor was identified as thymidine by NMR spectroscopy.

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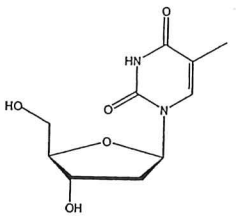

Thymidine

Ditetradecyl Selenide, $(C_{14}H_{29})_2Se$, a Convenient Reference Compound in ^{77}Se NMR

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In ^{77}Se NMR a 60% solution of Me_2Se in $CDCl_3$ has become the most widely used reference compound. However, the low boiling point combined with its obnoxious odour and high toxicity limits its use, particularly when a reference solution of known concentration has to be made; the chemical shift of this compound is known to vary by ~ 9 ppm from neat Me_2Se to a dilute solution in $CDCl_3$. Of this reason a 60% solution of Me_2Se in $CDCl_3$ is generally used as external standard and is defined as 0.0 ppm.

In this poster we want to report on our experience with the title compound as reference compound; 162.2 ppm downfield from a 0.25 M solution of Me_2Se in $CDCl_3$ (-5.0 ppm).

1. $(C_{14}H_{29})_2Se$ is a non-odourous, colourless and crystalline compound, m.p. 48 °C, which is stable for long periods, particularly when direct sunlight is avoided.
2. The compound can be handled without any particular precautions and can be used as both internal and external standard.
3. The compound is readily prepared from commercial 1-bromotetradecane and elemental selenium and is easily purified.
4. The compound is soluble in all the usual solvents except water; the solubility in acetonitrile and dimethyl sulfoxide, however, is somewhat limited requiring temperatures slightly above room temperature.
5. The ^{77}Se chemical shift of $(C_{14}H_{29})_2Se$ is independent of the concentration in the 0.01 – 0.50 M range.
6. The chemical shift in $CDCl_3$ varies approximately linearly with the temperature in the 270 – 345 K range with a temperature coefficient of ~0.11 ppmK^{-1}, slightly higher than for Me_2Se.

Interactions between Anthraquinone derivatives and DNA Oligomers.

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Many anti-cancer drugs consisting of an aromatic chromophore react with double helical DNA by an intercalating mode [1-3] where the drug is placing the aromatic chromophore between two adjacent base pairs. The major stabilising factor is the stacking between the aromatic chromophore on the drug and the adjacent base pairs. This can bee seen as an interaction between the LUMO of the aromatic chromophore and the HOMO of the adjacent base pairs [4]. From this point of view intercalators should selectively intercalate between GG/CC since this sequence has shown to have the lowest ionization potential i.e. highest energetical HOMO orbital (Koopman's theorem) of the possible stacking of two nucleobasepairs [5]. For intercalators bearing large substituents the intercalation site is also determined by these substituents. To clarify the molecular basis for the suggested GG/CC sequence selectivity of intercalators consisting of an aromatic chromophore and not carrying bulky substituents we have examined the adduct formation between double-helical deoxynucleotide sequences, and different simple synthetic anthraquinone derivatives. The systems studied include 5´-d(ATGGGTACCCAT)$_2$ and 5´-d(GACGGCCGTC)$_2$, and adducts of neutral, cationic and anionic anthraquinone derivatives, respectively. ^{1}H and ^{31}P NMR spectroscopy were used to characterize the adduct formation. The results indicate that the cationic derivative show specific intercalation while the neutral and anionic derivatives tend to associate on the outside of the helix.

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Assignment of ^{13}C Resonances of Indolizines Using Optimised COLOC, HMBC and INADEQUATE NMR.

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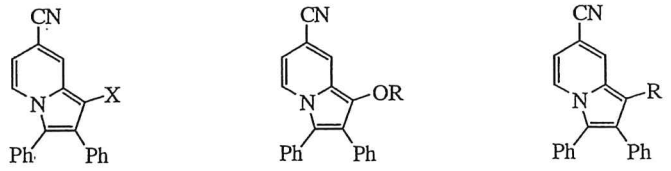
1-Indolizinols[1] and several O-protected 1-indolizinols[2] have shown antioxidant/radical scavenging properties and might have a therapeutic value in treatment of several diseases. Inspired by this, we have synthesised several 1-substituted 7-cyano-2,3-diphenylindolizines with structures like **1**, **2** and **3** (Figure 1). The ^{13}C resonances in compounds like **1**, **2** and **3** have close chemical shifts, and not all expected C,H correlations are observed when COLOC and HMBC experiments are acquired with standard parameters. We have tried to assign the ^{13}C resonances by optimising the COLOC and HMBC experiment. We have also acquired several 2D INADEQUATE experiments, and improved the signal to noise ratio for quartenary carbons by adding "artificial blood" and oxygen to the sample.[3]



1 **2** **3**

Figure 1

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