1st Practical Applications of NMR in Industry Conference 2012 (PANIC)

Symposium Co-Chairs:

Darón Freedberg, *CBER, FDA*
Joseph Ray, *Baxter Healthcare Corporation*

October 15-17, 2012
Hyatt Regency Hotel
Schaumburg, IL USA

Organized by

CASSS

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Welcome to the 1st Practical Applications of NMR in Industry Conference (PANIC)

Our main objective is to bring cutting-edge technological developments in NMR to Industry so they can be implemented to solve real life problems we encounter daily in laboratories that are not involved with long-term research, typical of academic research groups. The highly interactive atmosphere of the meeting will provide opportunities to discuss a wide range of topics from small molecules to polymers and their broad applications in materials, biologics and pharmaceuticals, as well as the regulatory agencies impact on these methods. The presentations and workshops will be catalysts to spark discussions to address practical concerns with experts in the NMR community and foster a cooperative network with colleagues who share your problems. The symposium will encourage open participation of all attendees to discuss issues that are important to laboratories that must handle a wide range of problems that must be solved in a short amount of time.

Two sessions and one workshop will be solely devoted to the quantitative power of NMR. In addition, we will explore the applications of new emerging technologies, applications to raw materials, uses of in-line and on-line NMR as well as a session on the impact of regulatory agencies.

This conference will provide perspective to the Industrial community encouraging the application of novel and emerging technologies to “real-life” challenges.

We would like to thank the speakers who are generously giving their time and resources and also you for your attendance, which will make this endeavor a success.

We gratefully acknowledge the generosity of our exhibitors and program partners: Agilent Technologies, Inc., ACD/Labs, Analytical Methods, Baxter Healthcare Corporation, Bruker BioSpin, Cambridge Isotope Labs, Doty Scientific, Inc., EMD Millipore, Genentech, a Member of the Roche Group, JEOL USA, Mestrelab Research, Pfizer, Inc., Technology Networks, Ltd., and Spectroscopy.

Acknowledgements

Symposium Co-Chairs:
Joseph Ray, *Baxter Healthcare Corporation*
Darón Freedberg, *CBER, FDA*

Scientific Program Committee:
Carlos Amezcua, *Baxter Healthcare Corporation*
Yves Aubin, *Health Canada*
Molly Bohlen, *Proctor and Gamble, Inc.*
John Edwards, *Process NMR Associates, LLC*
Kathleen Farley, *Pfizer, Inc.*
Gonzalo Hernandez, *Vis Magnetica*
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CASSS Practical Applications of NMR in Industry Conference (PANIC) Student Travel Grants

CASSS is pleased to provide a limited number of student travel grants for PhD students and post-docs who present applicable posters at the 1st Practical Applications of NMR in Industry Conference 2012 (PANIC). PhD students or post-doctoral fellows conducting research at academia throughout the world are eligible.

Why you should apply:
This conference provides an interactive forum for discussion of the latest developments in the use of NMR for applications to real problems faced by scientists in industry and research institutions including quantitation, molecular structure characterization, trace component and mixture analysis, product support, polymers, and biosimilars. As a participant, you will have an excellent opportunity to meet, network and participate in exchanging knowledge for mutual education with other NMR practitioners.

Requirements are:

- Present a poster on a NMR topic
- Proof of studentship/post-doc status
- Recommendation from the supervisor/advisor
- An abstract submission
- A CV for the candidate

CASSS would like to gratefully acknowledge the Suraj Manrao Student Travel Science Fund for their contribution to subsidize one additional science student to attend the PANIC Conference.

This year's winners include:

Lingyu Chi
Missouri University of Science and Technology, USA
Quantitative HNMR Tests for Determining the Mass Percentage of Small Molecules in Biomass Conversion Reactions

Derek Langeslay
University of California, Riverside, USA
Analysis Heparin and Heparan Sulfate by 1H-15N HSQC NMR

Fabíola Manhas Verbi Pereira
Empresa Brasileira de Pesquisa Agropecuária (Embrapa) – Instrumentação, Brasil
Evaluation of Raw Beef Quality with Time-domain Nuclear Magnetic Resonance

Maiara da Silva Santos
Instituto de Química de São Carlos, Universidade de São Paulo, Brasil
Analysis of Forumulated Products by qNMR Using Filter Diagonalization Method

1st Practical Applications of NMR in Industry Conference 2012 (PANIC)

Monday, October 15, 2012

07:00 – 17:45 **Registration** in Mahogany Ballroom Foyer

07:30 – 08:30 **Continental Breakfast** in Mahogany Ballroom 5, 6 and 7

08:30 – 08:45 **CASSS Welcome and Introductory Comments** in the Mahogany Ballroom 1, 2, and 3
Edwin Moore, *Baxter Healthcare Corporation, Round Lake, IL USA*
Darón Freedberg, *CBER, FDA, Rockville, MD USA*

Quantitation Applications 1
Plenary Session in Mahogany Ballroom 1, 2 and 3
Session Chairs: Kathleen Farley, *Pfizer, Inc.* and Gonzalo Hernandez, *Vis Magnetica*

08:45 – 09:10 **Yesterday, Today and Tomorrow in NMR**
George Gray, *Agilent Technologies, Inc., Santa Clara, CA USA*

09:10 – 09:35 **Quantitative NMR Applications Across a Product Life Cycle**
Christina Szabo, *Baxter Healthcare Corporation, Round Lake, IL USA*

09:35 – 10:00 **Universal qNMR: Concentration Referencing without Adding Standards**
Huaping Mo, *Purdue University, West Lafayette, IN USA*

10:00 – 10:15 Discussion – Questions and Answers

10:15 – 10:45 AM Break – Visit the Exhibits and Posters in Mahogany Ballroom 5, 6 and 7

Analysis of Raw Material and Nutritional Supplements
Plenary Session in Mahogany Ballroom 1, 2 and 3
Session Chairs: Carlos Amezcua, *Baxter Healthcare Corporation* and
Molly Bohlen, *Procter & Gamble, Inc.*

10:45 – 11:10 **Analysis of "Equivalent" Raw Materials Used in Polyurethane Synthesis**
James DeFelippis, *The Dow Chemical Company, Spring House, PA USA*

11:10 – 11:35 **Innovative Quantitative NMR for Natural Product Standards and Botanical Standardization**
Tanja Gödecke, *University of Illinois at Chicago, Chicago, IL USA*

11:35 – 12:00 **Aloe Vera: Quantification of Key Metabolites for Identity and Quality Assessment**
Kim Colson, *Bruker BioSpin, Billerica, MA USA*

Monday, October 15, 2012 continued…

12:00 – 12:15 Discussion – Questions and Answers

12:15 – 13:45 Hosted Lunch

12:45 – 13:45 **Technical Luncheon Seminar**

New Insights into Quality Control of Food, Beverages and Dietary Supplements by NMR Based Screening

Kimberly L. Colson[1], Manfred Spraul[2], Birk Schuetz[2], Hartmut Schaefer[2], Fang Fang[2], Eberhard Humpfer[2], Jimmy Yuk[1], Christian Fischer[2]

[1]Bruker BioSpin, Billerica, MA, USA; [2]Bruker BioSpin GmbH, Rheinstetten, Germany

Sponsored by Bruker BioSpin Mahogany Ballroom 1, 2 and 3

13:45 – 14:00 Break

Quantitation Applications 2
Plenary Session in Mahogany Ballroom 1, 2 and 3
Session Chairs: John Edwards, *Process NMR Associates, LLC* and Kathleen Farley, *Pfizer, Inc.*

14:00 – 14:25 **High Precision Purity Determination by qNMR - How to Achieve an Uncertainty of Measurement of 0.15%?**
Torsten Schonberger, *Federal Criminal Police Office, Wiesbaden, Germany*

14:25 – 14:50 **Process Analytical Applications of Quantitative Online NMR Spectroscopy**
Michael Maiwald, *BAM, Federal Institute for Materials Research and Testing, Berlin, Germany*

14:50 – 15:05 Discussion – Questions and Answers

15:05 – 15:35 PM Break – Visit the Exhibits and Posters in Mahogany 5, 6, and 7

15:35 – 16:35 **Workshop I**: **Quantitative NMR: How Good Can We Be, and How Good Do We Need to Be?**
In Mahogany Ballroom 1, 2 and 3

Workshop Chairs: John Marino, *NIST* and Joseph Ray, *Baxter Healthcare Corporation*

16:35 –16:45 Mini-Break

Monday, October 15, 2012 continued…

16:45 – 17:45 **Technical Seminar**

Applications of Quantitation in NMR Post-processing
Sergey Golotvin; Patrick Wheeler; Ryan Sasaki

ACD/Labs, Toronto, ON, Canada

Sponsored by ACD/Labs Mahogany Ballroom 1, 2 and 3

17:45 – 19:00 Exhibitor and Poster Reception in Mahogany 5, 6, and 7

19:00 Adjourn Day One

Tuesday, October 16, 2012

07:30 – 17:30 **Registration** in Mahogany Ballroom Foyer

07:30 – 08:30 **Continental Breakfast** in Mahogany Ballroom 5, 6 and 7

08:30 – 08:45 **Announcements** in Mahogany 1, 2 and 3
Joseph Ray, *Baxter Healthcare Corporation*

Emerging Measurement Science and Technologies
Plenary Session in Mahogany Ballroom 1, 2 and 3
Session Chairs: Michael Shapiro, *Pfizer, Inc. and* Edward R. Zartler, *Quantum Tessera Consulting*

08:45 – 09:10 **DOSY NMR – Techniques and Applications**
Paul Williard, *Brown University, Providence, RI USA*

09:10 – 09:35 **High-Resolution NMR Spectroscopy on a Chip for Metabolomic Applications**
Marcel Utz, *University of Southhampton, Southhampton, United Kingdom*

09:35 – 10:00 **Dissolution Dynamic Nuclear Polarization Studies of Biological Molecules and their Interactions**
Christian Hilty, *Texas A&M University, College Station, TX USA*

10:00 – 10:15 Discussion – Questions and Answers

10:15 – 10:45 AM Break – Visit the Exhibits and Posters in Mahogany Ballroom 5, 6 and 7

10:45 – 11:45 **Workshop II: Making the Best Use of Your Data?**
in Mahogany Ballroom 1, 2 and 3

Workshop Chairs: Carlos Amezcua, *Baxter Healthcare Corporation and*
Edward R. Zartler, *Quantum Tessera Consulting*

11:45 – 13:30 Hosted Lunch

12:15 – 13:15 **Technical Luncheon Seminar**

The Application of Non-Uniform Sampling (NUS) Techniques to Routine Small Molecule NMR Measurements
Paul J. Bowyer, *Agilent Technologies, Inc., Santa Clara, CA, USA*

NMR in Big Business: Working in the Intersection of Chemistry, Physics and Time Driven Projects
Don Eldred, *Dow Corning, Auburn, MI USA*

Sponsored by Agilent Technologies, Inc. Mahogany Ballroom 1, 2 and 3

Tuesday, October 16, 2012 continued…

13:15 – 13:30 Mini-Break

At-Line/In-Line NMR and Time-Domain NMR
Plenary Session in Mahogany Ballroom 1, 2 and 3
Session Chairs: John Edwards, *Process NMR Associates* and Mark Zell, *Pfizer, Inc.*

13:30 – 13:55 **On-Line Applications of 60 MHz High-Resolution NMR Systems in Industry: Direct Measurements, Chemometric Correlations and Multiple Spectroscopy Data Fusion**
John Edwards, *Process NMR Associates, Danbury, CT USA*

13:55 – 14:20 **Online NMR Reaction Monitoring in Pharmaceutical Process Development**
David Foley, *Pfizer, Inc., Groton, CT USA*

14:20 – 14:45 **Beyond Hammers in Search of Nails: An Instrument Technologist's Perspective on Developing Miniaturized NMR Spectrometers for New Applications**
Andrew McDowell, *ABQMR, Inc., Albuquerque, NM USA*

14:45 – 15:00 Discussion – Questions and Answers

15:00 – 16:00 **Poster Session** – Visit Posters in Mahogany 5, 6 and 7

16:00 – 16:25 **LF-NMR Studies of Mechanically Induced Gel Syneresis in Cheese**
Søren Engelsen, *University of Copenhagen, Frederiksberg, Denmark*

16:25 – 16:50 **NMR Quantification of Structural Features in Food Science and Technology**
John van Duynhoven, *Unilever, Vlaardingen, The Netherlands*

16:50 – 17:15 **Rheological Measurements on Non-Newtonian Fluids Using a Process Compatible MRI**
Emilio Tozzi, *Aspect Imaging, Davis, CA USA*

17:15 – 17:30 Discussion – Questions and Answers

17:30 Adjourn Day Two

Wednesday, October 17, 2012

07:30 – 13:00 Registration in Mahogany Ballroom Foyer

07:30 – 08:30 Continental Breakfast in Mahogany Ballroom 5, 6 and 7

08:30 – 08:45 Announcements in Mahogany 1, 2 and 3
Joseph Ray, *Baxter Healthcare Corporation*

Regulatory Topics
Plenary Session in Mahogany Ballroom 1, 2, and 3
Session Chair: John Marino, *NIST*

08:45 – 09:10 **Assessment of the Three-Dimensional Structure of Recombinant Protein Therapeutics at High Resolution Isotopic Enrichment is Not Required**
Yves Aubin, *Health Canada, Ottawa, ON Canada*

09:10 – 09:35 **Practical Aspects of Incorporating NMR Based Methods in CMC Strategy of Vaccines**
C. Abeygunawardana, *Merck & Co., Inc, North Wales, PA USA*

09:35 – 10:00 **Roles for NMR in Assessing Biosimilars**
Darón Freedberg, *CBER, FDA, Rockville, MD USA*

10:00 – 10:15 Discussion – Questions and Answers

10:15 – 10:45 AM Break – Visit the Exhibits and Posters in Mahogany 5, 6 and 7

10:45 – 11:10 **NMR for Assessment of Drug Product Quality: Examples from FDA Studies**
David Keire, *CDER, FDA, St. Louis, MO USA*

11:10 – 11:35 **Qualitative and Quantitative Biophysical Comparability Studies on Protein Biopharmaceuticals Using 1D ^{1}H NMR**
Julie Wei, *Biogen Idec Inc., Cambridge, MA USA*

11:35 – 12:00 **NMR for the Quantitation of Trace Small Molecules in the Presence of Protein: Applications for Process Development and Process Validation**
Ken Skidmore, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

12:00 – 12:15 Discussion – Questions and Answers

12:15 – 12:30 **Closing Comments** – Joseph Ray, *Baxter Healthcare Corporation*

Quantitation Applications 1
Plenary Session Abstract

Session Co-Chairs:

Kathleen Farley, *Pfizer, Inc., Groton, CT USA*
Gonzalo Hernandez, *Vis Magnetica, Montevideo, Uruguary*

The increased role of NMR as a tool for quantitation is highlighted by two sessions of the conference devoted to this topic. These sessions will review the history of the field, regulatory considerations, and compare the different types of standards (internal, ERETIC, solvent, etc). In addition, several speakers will discuss the applications of NMR quantitation during a products life cycle or in reaction monitoring. These talks will illustrate practical applications of setting up the NMR instrument as well as parameter optimization. Furthermore, the accuracy, precision, limits of detection, limits of quantitation and reproducibility of this technique will be discussed.

NOTES:

Quantitation Applications 1
Oral Session Abstracts

Yesterday, Today and Tomorrow in NMR

George Gray

Agilent Technologies, Inc., Santa Clara, CA USA

NMR has been the most successful instrumental technique in chemistry and biochemistry. There are fundamental reasons for this fact, the most important of which is that experimenters can control the Hamiltonian by selection of the combination of delays, pulses, phases and gradients. Since the interactions measured by NMR are weak on the scale of electronic and nuclear interactions, the time scales are longer and this gives us the ability to affect the Hamiltonian.

In this talk I will try to review how the success of NMR came about and the major milestones along the way. I've been around long enough to have seen most of them realized. NMR today is a well-established technique used by in a wide variety of applications. The lack of intrinsic sensitivity has pushed innovation in many ways. The central need (even in the name!) for the technique is the magnet. I'll try to address the perennial "price/value" question that is even more relevant as we move to the GHz regime. At the same time how it is possible to make (relatively) inexpensive, high performance spectrometers that most users can visualize actually buying.

Tomorrow is always dangerous territory. I will try to give some idea of the challenges facing both instrument companies and individual scientists in the quest for higher, better, faster and cheaper!

NOTES:

Quantitative NMR Applications across a Product Life Cycle

Christina Szabo

Baxter Healthcare Corporation, Round Lake, IL USA

A survey of quantitative NMR applications across product life cycle will be presented. To identify a new lead compound at the exploratory stage, a direct ^{13}C method was developed to determine the composition of a non-anticoagulant, sulfated polysaccharide and measure natural as well as process impurities. From a single ^{13}C NMR spectrum, we measured: 1) main monosaccharide levels (related to purity and homogeneity of the polysaccharide drug), 2) naturally-occurring impurity levels (related to presence of an inactive component), and 3) other impurities, including a process impurity, in the complex polysaccharide. Methods were established to achieve ruggedness in the form of analyst-to-analyst repeatability.

For an anticoagulant small molecule drug, various quantitative NMR methods were applied to concept stage, process development and final formulation upon filing for a new drug formulation. At the concept stage, NMR was used to identify the sites of *in situ* salt formation, which explained the improved solubility characteristics of this drug in a new acetic acid formulation. For process development, quantitative NMR was used to analyze a reactive process impurity using External Referencing to access In vivo Concentrations (ERETIC). Subsequently, a comparison was made between internal, external, and ERETIC methods for general quantitation. NMR was then applied to the final formulation to demonstrate the absence of certain impurities.

Finally, an example of how NMR is used to support existing products is discussed. In this example, NMR is applied to extracts from a diverse set of filters used in manufacturing as part of filter qualification. LC-MS, GC-MS, and total organic carbon are routinely used to identify and quantify the extractable materials. We utilize 1D- and 2D-NMR spectroscopy to identify the extractable components that escape LC- and GC-MS and use a semi-quantitative methodology to determine the carbon concentration based on observed proton signals.

NOTES:

Universal qNMR: Concentration Referencing without Adding Standards

Huaping Mo

Purdue University, West Lafayette, IN USA

We have shown that the concentration of solvent water and/or receiving efficiency can be readily utilized for universal qNMR without adding concentration standards. As a cross-validation, we can use water as an external reference and “measure” the concentration of a number of routinely used solvents in the lab. We demonstrate that the difference between expected concentration and NMR measured solvent concentration is less than 2%. As a result, any solvent can be potentially used for qNMR without significant risk of losing accuracy.

NOTES:

Analysis of Raw Materials and Nutritional Supplements
Plenary Session Abstract

Session Co-Chairs:

Carlos Amezcua, *Baxter Healthcare Corporation, Round Lake, IL, USA*
Molly Bohlen, *Procter & Gamble, Inc., Cincinnati, OH USA*

With the ever increasing globalization in the chemical, pharmaceutical, nutritional supplements, and health and beauty industry, the need for multiple sourcing of raw materials is critical. It becomes necessary to insure raw materials from different suppliers are truly "equivalent". Manufacturers must also ensure identity, purity, strength and composition to guarantee the quality and safety of health-related products. NMR plays an important role in the analysis and characterization of raw materials, nutritional supplements, and health and beauty products. This session will discuss applications of NMR to the detection of structural and reactive functional group differences in raw materials from alternate suppliers, the development of qualitative and quantitative NMR characterization methods for routine analysis of nutritional supplements reference materials, and the development of a fully automated NMR analysis for the characterization of *Aloe vera* samples.

NOTES:

Analysis of Raw Materials and Nutritional Supplements
ORAL SESSION ABSTRACTS

Analysis of "Equivalent" Raw Materials Used in Polyurethane Synthesis

Jim DeFelippis

The Dow Chemical Company, Spring House, PA USA

With the ever increasing globalization in the chemical industry, the need for multiple sourcing of raw materials is critical. Compositional differences in "equivalent" raw materials from alternate suppliers can lead to final products with different performance characteristics. Therefore, insuring that raw materials from different suppliers are truly "like for like" replacements is critical. The application of NMR to detect differences in oligomer structure and reactive functional groups in raw materials from alternate suppliers will be discussed.

NOTES:

Innovative Quantitative NMR for Natural Product Standards and Botanical Standardization

Tanja Gödecke, Shao-Nong Chen, Birgit U. Jaki, David C. Lankin, James B. McAlpine, José G. Napolitano, Kuan-Wei Peng, María F. Rodríguez-Brasco, Guido F. Pauli

University of Illinois at Chicago, Chicago, IL USA

Botanical dietary supplements are popular alternatives for the alleviation and treatment of a variety of health conditions. Numerous studies have demonstrated that frequently multiple phyto-constituents present in an herbal preparation are responsible for beneficial health effects. This generates growing interest in the development of new analytical methods for the qualitative and quantitative characterization of multiple chemical markers, i.e., *multi-target standardization*, of botanicals.

As part of a program aimed at the spectrometric validation of botanical reference materials, a total of 160 natural product reference standards from ten of the U.S. top-selling botanicals were systematically analyzed by 1D/2D NMR and tandem LC-MS techniques for comparison. Their purities and detailed composition profiles were determined and quantitative ^{1}H NMR (qHNMR) measurements played a critical role in the simultaneous identification and quantification of the individual phyto-constituents especially for profiling of impurities such as adulterants, and residual organic solvents. The high field ^{1}H NMR data acquired as part of this project also formed the basis for an ongoing project to build a ^{1}H NMR natural product database, which will aid future dereplication efforts of isolates as well as permit the direct qualitative and quantitative characterization of known plant constituents in complex fractions or herbal preparations.

This report discusses examples that illustrate the utility of qHNMR as a fast, reliable tool for routine analysis of botanical reference materials especially with respect to building simple and robust analytical methods for the standardization of botanical dietary supplements.

Acknowledgements: The present research work was financially supported by NIH/NCCAM through grant RC2 AT005899. T.G. was partially supported as recipient of a generous USP Fellowship. The construction of the UIC Center for Structural Biology (CSB) was funded by NIH/NIGMS grant P41 GM068944.

NOTES:

Aloe Vera: Quantification of Key Metabolites for Identity and Quality Assessment

Michelle Markus[1]; Kimberly Colson[1]; Christian Fischer[2]; Marc Wolff[2]; Stefan Gafner[3]

[1]Bruker BioSpin, Billerica, MA USA [2]Bruker BioSpin GmbH, Rheinstetten, Germany; [3]Tom's of Maine, Kennebunk, ME USA

Aloe vera is a medicinal plant with a wide range of uses from topical application to soothe burns to oral consumption to aid digestion. It is added to a wide range of health and beauty products. The quality and safety of dietary supplements has been emphasized since the 2007 FDA cGMP ruling, which states that manufacturers must ensure identity, purity, strength, and composition of their products. Botanical material is highly variable depending on species and growing conditions which make evaluation of these materials challenging. For analyzing *Aloe vera* extract in detail, components include glucose, acetylated mannose polymers, and malic acid. As the material ages, degradation products include acetic acid, lactic acid, formic acid, and fumaric acid. Common additives include the preservatives sodium benzoate and potassium sorbate. Depending on the formulation, other additives such as glycerol may be present. Nuclear magnetic resonance spectroscopy provides an effective means of evaluating botanical material as a result of its ability to be used as (1) a fingerprinting tool and (2) for quantitative analysis. Presented here is the implementation of a 1H-NMR spectroscopy-based method [1] to evaluate *Aloe vera* in the Assure-RMS software package to provide a fully automated analysis of *Aloe vera* samples.

The automated analysis will be described, first presenting the readily quantitated components, emphasizing the features of the spectra of these components that lend themselves to robust analysis. Then more problematic components will be examined. Strategies to improve the quantitation, including additional data and more sophisticated analysis, will be discussed.

References: [1] Jiao et al. (2010) J. of the AOAC International, Vol. 93, p 842-848.

NOTES:

Quantitation Application 2
Plenary Session Abstract

Session Co-Chairs:

John Edwards, *Process NMR Associates, Danbury CT USA*
Kathleen Farley, *Pfizer, Inc., Groton, CT USA*

The increased role of NMR as a tool for quantitation is highlighted by two sessions of the conference devoted to this topic. These sessions will review the history of the field, regulatory considerations, and compare the different types of standards (internal, ERETIC, solvent, etc). In addition, several speakers will discuss the applications of NMR quantitation during a products life cycle or in reaction monitoring. These talks will illustrate practical applications of setting up the NMR instrument as well as parameter optimization. Furthermore, the accuracy, precision, limits of detection, limits of quantitation and reproducibility of this technique will be discussed.

NOTES:

Quantitation Application 2
Oral Session Abstracts

High Precision Purity Determination by qNMR – How to Achieve an Uncertainty of Measurement of 0.15%?

Torsten Schonberger

Federal Criminal Police Office, Wiesbaden, Germany

The method described was developed for the purity determination of analytical standards. Due to the great variability qNMR is perfectly suited for this application.

But what about the precision? Considering various precautions qNMR can achieve highest metrological quality. The most important aspects will be discussed in the lecture. These include the sample preparation as well as the relevant parameters for acquisition, processing, and spectra evaluation. The reference materials used as internal standards for qNMR have to fulfil special requirements.

The major obstacle of the method is the possible overlap of the evaluated signals by impurity signals. Some solutions for this will be shown.

The uncertainty of measurement was determined by using two different approaches (GUM[1] and Nordtest) with very similar results. An overview of the partial contributions to the uncertainty will also be given. 1 Guide to the Expression of Uncertainty in Measurement.

NOTES:

Process Analytical Applications of Quantitative Online NMR Spectroscopy

Michael Maiwald

BAM, Federal Institute for Materials Research and Testing, Berlin, Germany

There is a considerable trend to recognize online techniques as potentially useful tools for chemical production and manufacture in general as a need to study complex multi-component mixtures and to gain insight into their behavior in the real process. Therefore the industry increasingly benefits from reliable online analytical technology for production control and preventive assurance of the demanded product quality with an optimum use of equipment, raw materials, and energy. At the same time process analytical devices allow to permanently improve processes, devices and safety.

Due to its extreme specifity online NMR spectroscopy is the method of choice for the investigation of complex fluid mixtures with analytically challenging compounds, where other analytical in situ methods suffer from insufficient differentiation of components. NMR spectroscopy provides valuable information on chemical structure as well as accurate quantitation in complex reacting multi-component mixtures for equilibrium or reaction kinetic studies. A major advantage of NMR spectroscopy is that no calibration is needed for quantification in most cases, and the method features a high linearity between absolute signal area and sample concentration, also for boundary areas in concentration. Furthermore, online NMR spectroscopy allows investigations under elevated pressures, e.g., to prevent the solutions from boiling, or for studies under process conditions.

Different set-ups for direct and non-invasive coupling of reaction and separation equipment with online NMR spectroscopy were realized using commercial NMR probes for elevated temperatures and pressures. Experimental set-up and acquisition parameters were studied and optimized to allow reliable quantitative NMR experiments on real technical samples. Several applications are discussed.

NOTES:

Emerging Measurement Science and Technologies
Plenary Session Abstract

Session Co-Chairs:

Michael Shapiro, *Pfizer, Inc., Groton, CT USA*
Edward R. Zartler, *Quantum Tessera Consulting, Collegeville, PA USA*

The variety of applications of NMR to solve difficult problems in chemistry is extraordinary and the rate at which new concepts being developed is daunting. In an attempt to exemplify some of this diversity, this session will include talks by leaders in their respective fields of NMR. The talks will show how an "old" technology, diffusion NMR, can be applied to "new" problems; a novel and exciting application of using chips a conduit to Metabolomic NMR studies; and a demonstration of the very HOT area of DNP in NMR as applied to the study of bio-molecules.

NOTES:

Emerging Measurement Science and Technologies
Oral Session Abstracts

DOSY NMR – Techniques and Applications

Paul Williard

Brown University, Providence, RI USA

A brief background highlighting key developments of the theory and some applications of pulsed gradient spin echo (PGSE) and diffusion NMR (DOSY) techniques will be discussed. I will present a survey of the use of these to research projects as diverse as the determination of polymer homogeneity, epitope mapping, small molecule formula weight determination, and addition of PGSE sequences into two dimensional correlation experiments. I will conclude with a brief outline of some applications from my lab in correlating DOSY results with x-ray diffraction analyses of reactive organometallic intermediates.

NOTES:

High-Resolution NMR Spectroscopy on a Chip for Metabolomic Applications

Marcel Utz

University of Southampton, Southampton, United Kingdom

Nuclear magnetic resonance spectroscopy is an ideal tool for metabolomic studies, due to its universality and its linear response. Combining NMR spectroscopy with microfluidic technology opens many possibilities for the combination of separation with NMR-based detection. In particular, we are interested in direct metabolomic observation of on-chip cell populations in response to external stimuli such as changes in nutrient levels, temperature, addition of drugs, infection, etc. In order to harness the full potential of NMR spectroscopy, a number of technical issues must be solved.

Small sample sizes require the design of optimized, micro-fabricated radio frequency resonator structures for good sensitivity. At the same time, magnetic susceptibility-induced line broadening must be avoided. In this contribution, recent advances on those fronts will be presented. Challenges and opportunities of this approach in the context of analytical chemistry, medical diagnostics, biology, as well as drug development and drug safety testing will be discussed.

NOTES:

Dissolution Dynamic Nuclear Polarization Studies of Biological Molecules and their Interactions

Christian Hilty

Texas A&M University, College Station, TX USA

Dissolution dynamic nuclear polarization (DNP) affords a sensitivity gain sufficient to enable single scan acquisition of NMR spectra at low concentration, of insensitive nuclei or of nuclei with low natural abundance. Here, several applications of potential interest for biomedical investigations, and in particular, drug discovery are presented. Various spin-1/2 nuclei in small molecules are readily hyperpolarized to yield NMR signals three or four orders of magnitude beyond thermal polarization. Combined with stopped-flow mixing, interactions of such small molecule ligands with proteins can be investigated by observing the effect of binding on the ligand signals.

Apart from the detection of binding over a wide range of affinity by relaxation based methods, dissociation constants can be determined. It is further possible to transfer polarization from a hyperpolarized ligand to a protein through the Nuclear Overhauser effect. The efficiency of intermolecular polarization transfer is determined by various parameters, including the kinetics of binding, cross-relaxation rates and spin diffusion within the protein. Using appropriate mathematical models allows the determination of some of these parameters from experimental data. Protein signals in the resulting spectra are selectively enhanced in the vicinity of the binding site, which offers potential benefits for reducing spectral complexity especially for large proteins.

Finally, proteins can also be directly hyperpolarized using dissolution DNP. Here, the enhanced signal enables the rapid acquisition of a series of spectra in time for the observation of protein folding.

NOTES:

At-Line/In-Line NMR and Time-Domain NMR
Plenary Session Abstract

Session Co-Chairs:

John Edwards, *Process NMR Associates, Danbury, CT USA*
Mark Zell, *Pfizer, Inc., Groton, CT, USA*

The quantitative nature of NMR spectroscopy data combined with the specificity and orthogonality of the molecular information observed in the NMR spectrum makes it an ideal candidate for on-line and at-line process control or quality control. Traditional approaches such as simple integration of relevant NMR signals can be used to identify or quantify individual components or an entire complex mixture. Alternatively, powerful multivariate statistical approaches (chemometrics) can utilize the entire spectrum to obtain chemical and physical properties of samples being analyzed, or to classify them in a manner that allows a quality control measure or specification to be applied to the sample. The applications involve the analysis of industrial samples in an NMR tube or samples passed through a flow cell attached to a sampling slipstream from a reaction vessel - that vessel can be a round bottomed flask in a fume hood, a batch reactor in a pharmaceutical manufacturing plant, or a product emerging from a 10 story crude distillation tower. The papers presented in this session will provide a taste of the diverse applications of NMR across industry and will present examples developed on superconducting systems in the 7-14 Tesla range (300-600 MHz for 1H) as well as cryogen-free permanent magnet NMR systems operating at or below 1.4 Tesla (60 MHz for 1H).

NOTES:

At-Line/In-Line NMR and Time-Domain NMR
Oral Session Abstracts

On-Line Applications of 60 MHz High-Resolution NMR Systems in Industry: Direct Measurements, Chemometric Correlations, and Multiple Spectroscopy Data Fusion

John Edwards

Process NMR Associates, LLC, Danbury, CT USA

For the past two decades high resolution ^{1}H NMR systems combined with chemometric analyses have been utilized in refineries and chemical plants to predict the chemical and physical properties of process streams and finished products. The ability to perform these analyses with on-line NMR instrumentation has allowed tighter control and optimization of the plant to obtain margin improvement, reduced reworking of off-specification materials, and higher yields of finished products. Examples of refinery and petrochemical applications will be given along with some examples of multinuclear NMR applications utilizing ^{31}P and ^{19}F NMR. The permanent magnet based 1.5 Tesla NMR instruments will be described along with a description of how these compact, cryogen-free NMR systems can be utilized on the bench-top or in the fume-hood as continuous or stop-flow chemistry sensors for reaction monitoring, mixing/dilution monitoring, or purity/conversion monitoring. Food applications will also be described such as dairy (butter, cream cheese) and edible or essential oil analysis. Finally, the ability to improve the quality of the correlations derived in the chemometric modelling by "fusing" NMR data with spectral information from other spectroscopies (NIR, Mid-IR) will be discussed.

NOTES:

Online NMR Reaction Monitoring in Pharmaceutical Process Development

David Foley

Pfizer, Inc., Groton, CT USA

The use of online NMR technology in the development of organic reaction processes provides information rich data, which can be used to provide a deeper understanding of the process under investigation. When coupled with other PAT techniques, it allows chemists and engineers to make informed decisions based on a suite of analytical information, ultimately leading to more robust processes as part of a quality by design (QbD) strategy. The development of an NMR reaction monitoring platform will be discussed, including a novel flow cell designed for increased flexibility of use, compared to fixed flow cells. The implementation of this technology will also be demonstrated; citing specific examples of reactions developed and optimized employing online NMR.

NOTES:

Beyond Hammers in Search of Nails: An Instrument Technologist's Perspective on Developing Miniaturized NMR Spectrometers for New Applications

Andrew McDowell

ABQMR, Inc., Albuquerque, NM USA

Academic physics and physical chemistry groups have spawned a small but active community of non-traditional NMR instrument developers who have produced remarkable devices that overcome the practical limitations of traditional NMR instrumentation. However, this community has a hard time finding nails for their hammers. Potential end users can be very hard to find within companies, and too little is known about the total context in which the devices must operate. The users who are found can have expectations based on high-field and high-res instruments, and may not see how a lower-power instrument could solve some important problems. Managers may have a vision of NMR as being expensive and complex.

Often, companies are reluctant to divulge information regarding problems that need to be solved. A common response of the device developer is to build a general purpose instrument, aimed at mimicking the performance of traditional NMR systems. This “build it and they will buy” strategy runs the risk that the chosen device specifications may not be a good match to any particular application.

We would prefer to develop hammer & nail as a unit. Non-traditional devices necessarily have performance limitations that must be balanced by their unique advantages. The difficulty of such an approach is that it requires close collaboration with the elusive end user. Furthermore, it requires that the end user become comfortable with a lower level of performance. The approach combines two major business challenges: the introduction of a new version of a technology and a new manner of its use, making for a risky business plan.

In the projects we have pursued to-date, we have attempted to mover from developer-driven to user-driven device designs.

NOTES:

LF-NMR Studies of Mechanically Induced Gel Syneresis in Cheese

Christian Lyndgaard Hansen, Frans van den Berg & Søren Balling Engelsen

University of Copenhagen, Frederiksberg, Denmark

In cheese manufacture the milk gel formation and syneresis processes are of major importance for the water content, texture, and flavor properties of the final cheese. In this study gel formation and syneresis was monitored by low field nuclear magnetic resonance (LF-NMR) and rheological measurements in an experimental design of 20 experiments with three factors (1) pH, (2) temperature and (3) gel firmness at cutting time [1].

A major challenge with low field NMR data analysis is the estimation of the number of exponential components. In this work we will demonstrate how PARAFAC-core-consistency using the so-called Double-Slicing method [2] can help to unambiguously determine the number of exponential components.

[1] C.L. Hansen, Å. Rinnan, S.B. Engelsen, T. Janhøj, E. Micklander, U. Andersen & F. van den Berg, Effect of Gel Firmness at Cutting Time, pH and Temperature on Rennet Coagulation and Syneresis – An in situ 1H NMR Relaxation Study, *Journal of Agricultural and Food Chemistry* (2010), 58(1), 513-519.

[2] L. Andrade, E. Micklander, I.A. Farhat, R. Bro & S.B. Engelsen, DoubleSlicing: a non-iterative single profile multi-exponential curve resolution procedure. Application to time-domain NMR transverse relaxation data, *Journal of Magnetic Resonance* (2007), 189(2), 286-292.

NOTES:

NMR Quantification of Structural Features in Food Science and Technology

John Van Duynhoven[1, 2]; Gert-Jan Goudappel[1]; Adrian Voda[1]; Frank Vergeldt[2]; Henk Van As[2]

[1]Unilever, Vlaardingen, The Netherlands; [2]Wageningen University, Wageningen, The Netherlands

Within the foods industry, Time-Domain (TD)NMR methods have acquired a strong position as structural assessment tools, with applications ranging from explorative research to product development and manufacturing. Within food research, TDNMR is typically used within the context of 'deductive' strategies to derive structure-property relationships. Here TDNMR methods are deployed to quantify structural features between the meso- en microscale. The hypothesis-driven measurement approaches operate on time-scales which often do not match with the currently required innovation pace. As an alternative 'inductive' approach we present a multivariate modelling approach to associate food microstructure with functionality. Within product development and manufacturing, TDNMR is routinely deployed to measure phase-compositional and microstructural features to predict or define product (prototype) quality. Userfriendly automation can make benchtop NMR equipment directly and safely available to large communities of non-trained users, providing them with (semi-)solid contents and water and oil droplet size distributions in high throughput and with short response times. Although these automated benchtop TDNMR NMR methods are fast, precise and accurate, they still require sampling of products into NMR tubes. Thus hand-held NMR sensors have gained interest due to their promise for non-invasive product inspection in the food supply chain. In a feasibility study the joint deployment of a NMR sensor and multi-variate calibration enabled assessment of the microstructural quality of a food emulsion in a non-invasive ('through package') manner.

NOTES:

Rheological Measurements on Non-Newtonian Fluids Using a Process Compatible MRI

Michael McCarthy[1]; Kathryn McCarthy[1]; Emilio Tozzi[2]; Robert Powell[1]

[1]University of California at Davis, Davis, CA; [2]Aspect Imaging AI, Davis, CA

Magnetic resonance flow imaging allows spatially resolved measurement of fluid motion in pipe flow. The velocimetry data generated in flow imaging contains information on shear rates at different radial positions in the pipe as well as other characteristics of the flow such as the presence of plug regions, wall slip, settling and turbulence.The imaging parameters can be varied over wide ranges to access different levels of resolution in both spatial and time scales, making it a particularly versatile technique.

A flow image and a single pressure drop measurement provide information sufficient to construct a rheogram. Apparent yield stress and apparent slip velocity, which frequently occur in complex fluids such as food or personal care products can be directly observed. Flow imaging can also provide information on phase separation, of interest in systems where settling of heavy particles or rising of a gas phase may occur and needs to be detected and or quantified. This rheological method is well suited for either in-line or on-line process control.

NOTES:

Regulatory Topics
Plenary Session Abstract

Session Chair:

John Marino, *NIST, Rockville, MD USA*

High-resolution nuclear magnetic resonance (NMR) spectroscopy can provide important tools to establish product consistency in drug manufacturing, to detect changes in the drug substance resulting from modifications in the manufacturing process, to compare the quality attributes of a biosimilar to an innovator reference product, and to detect trace impurities. In this session, we will discuss high-resolution NMR techniques that provide simple, robust spectroscopic approaches for obtaining information on the composition and structure of the bioactive form of protein therapeutics and vaccines at atomic resolution, as well as determining the composition of multi-component mixtures and detecting trace impurities. The session will include speakers from regulatory agencies and companies and will address practical experimental issues related to the implementation of NMR approaches and how the latter can support drug development, manufacturing and regulation.

NOTES:

Regulatory Topics
Oral Session Abstracts

Assessment of the Three-Dimensional Structure of Recombinant Protein Therapeutics at High Resolution – Isotopic Enrichment is Not Required

Yves Aubin

Health Canada, Ottawa, ON Canada

The current perception of nuclear magnetic resonance (NMR) spectroscopy applied to proteins is most often characterized with the words complicated, time-consuming and expensive, to name only these. In this paper, we will show through a case study, that a simple NMR method can provide a wealth of information on the three-dimensional structure of protein therapeutics.

Filgrastim is the generic name for recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF). It is produced in *Escherichia coli* (*E. coli*) in a non-glycosylated form. Filgrastim is marketed under the brand name Neupogen® by Amgen. Since this product has lost patent protection, many subsequent entry versions have been approved or are in the process of filing for market authorization throughout the world, including Canada. In order to be authorized as a subsequent entry product, the sponsors must demonstrate similarity with an approved product in Canada via an appropriate comparability exercise. Here we show that the NMR fingerprint assay can be used to assess the three-dimensional structure of the active ingredient in the formulated product from two different sources as well as a comparator, the approved product Neupogen®.

Recombinant metHuG-CSF was prepared in *E. coli* and isotopically enriched with ^{13}C and ^{15}N isotopes. Samples were analysed by NMR to study the effects of varying the pH, the concentration of excipients (sorbitol and polysorbate-80), the ionic strengths with several salts, and co-solutes. Spectra of mutants have been recorded to assess the sensitivity of the method to small structural changes. Finally, NMR spectra were recorded for Neupogen®, purchased at a local pharmacy, and a chemical reference standard from the European Directorate for Quality Medicine (EDQM).

The NMR fingerprint assay applied to Filgrastim provided residue specific information of the structure of the active ingredient of a product. In addition to current methods, the ability to assess the conformation with a high degree of resolution can greatly facilitate the comparability exercise.

NOTES:

Practical Aspects of Incorporating NMR Based Methods in CMC Strategy of Vaccines

C. Abeygunawardana

Merck & Co, Inc., North Wales, PA USA

Bacterial infections due to *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and *Salmonella enterica* (Typhi) continue to be a major cause of death and morbidity in children and older adults worldwide. Vaccines containing cell-surface polysaccharides isolated from these organisms have long been shown to be effective in preventing infections caused by type specific bacteria. At present, several vaccine products containing purified polysaccharides or polysaccharide-protein conjugates are licensed and many are under development. These products in general contain a mixture of antigens (multi-valent vaccine) to maximize serotype coverage and thus involve complex manufacturing and quality control processes. NMR spectroscopy is considered to be the most useful analytical tool currently available for the analysis of bacterial polysaccharides.

Incorporation of NMR analyses in the development stages of polysaccharide-containing vaccines provides a powerful analytical tool to support process validation, product characterization, formulation, and streamlined quality control of the commercial product. QNMR analyses of various manufacturing stages of the product provide an efficient evaluation of product/process consistency with regard to polysaccharide as well as potential residual components related to the manufacturing process and residuals of bacterial origin. Similarly, chemical modifications of the polysaccharides, whether due to purposeful change (e.g. activation of polysaccharides for use in conjugate vaccine manufacture) or due to time-dependent changes (i.e. stability), can be monitored. As a result, several QNMR based methods have been developed, validated and implemented in testing of multiple vaccine products. Practical aspects of some of this work will be presented.

NOTES:

Roles for NMR in Assessing Biosimilars

Darón Freedberg

CBER, FDA, Rockville, MD USA

Early in 2012, the FDA released a draft Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product. This draft guidance document describes the factors to consider when demonstrating that a proposed protein product is highly similar to a reference product and provides recommendations to applicants on the scientific and technical information of the chemistry and manufacturing (CMC) section of a marketing application for a proposed biosimilar product. The guidance document stresses that analytical methods should be used in comparing the biosimilar protein product to the innovator product, but does not specify which analytical methods should be used because each product is unique and may require different methods to demonstrate that the biosimilar is indeed highly similar to the reference product. In this talk, I will outline the analytical portion of this guidance document and discuss the potential for NMR as an analytical method in assessing protein similarity.

NOTES:

NMR for Assessment of Drug Quality: Examples from FDA Studies

David Keire

CDER, FDA, St. Louis, MO USA

Application of NMR methods for testing drug quality is relatively sparse in the United States Pharmacopeia (USP) or European Union pharmacopeias monographs where HPLC methods predominate. Although a robust tool, HPLC has limitations as a test to evaluate the structure and composition of complex biologically derived drugs, including; poor resolution of structurally similar products, variability due to buffer composition and column life cycle, and no direct assessment of the tertiary structure of the drug. By contrast, nuclear magnetic resonance (NMR) spectroscopy has demonstrated utility in the identification of drugs, determination of isomeric composition or tertiary structure, determination of the composition of multi-component mixtures and measurement of impurity levels and elucidation of their structures. Therefore, as part of an effort to modernize the USP monograph with current analytical tests for complex drugs, the agency has been exploring the use of NMR spectroscopy and mass spectrometry (MS) as candidates for the next generation of USP monograph assays. For example, the FDA has applied these modern analytics to the study of heparin sodium, low molecular weight heparins, glatiramer acetate, protamine sulfate and other compounds.

NOTES:

Qualitative and Quantitative Biophysical Comparability Studies on Protein Biopharmaceuticals Using 1D ^{1}H NMR

Julie Wei; Steven Berkowitz

Biogen Idec Inc, Cambridge, MA USA

Nuclear Magnetic Resonance (NMR) is a well-known elucidation tool of the three-dimensional structure of proteins. It provides a map of the protein structure at atomic resolution. Each protein processes a set of NMR signals that can be used for the detection of batch-to-batch variations in the structure of drug substances, thereby ensuring production consistency. Typically, one-dimensional spectra are used for ~ 10 kDa proteins. For large proteins, the fingerprint map is obtained either from 15N-HSQC, which requires 15N isotope labeling that is impossible in process development. The natural abundance of 15N and 13C are too low and too insensitive to be practical in throughput. This is the reasons that pharmaceutical companies that produce large monoclonal antibodies tend to shy away from this analytic tool. At Biogenidec, a quick and high-throughput method requiring only naturally abundant 1H isotopes has been developed that is based on the quantitative analysis of a set of one- and two-dimensional NMR spectral intensities using statistics, including chemometrics. This method shows applicability to the higher-order structural assessment of biopharmaceutical products that are >10 kDa in molecular weight.

NOTES:

NMR for the Quantitation of Trace Small Molecules in the Presence of Protein: Applications for Process Development and Process Validation

Ken Skidmore

Genentech, a Member of the Roche Group, South San Francisco, CA USA

Detection of a broad range of impurities in protein containing process pools can be challenging. Interference from large amounts of protein, buffer components, and water can blanket ^{1}H NMR spectra with signals that are orders of magnitude larger than those from the analytes of interest. Furthermore, these interfering signals may cover nearly the entire spectral window. Under these conditions, accurate interpretation and quantification of data for the purposes of process validation can be difficult.

However, a standard Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (with solvent suppression) provides a robust, practical method for detecting and quantifying trace impurities in the presence of large amounts of protein drug. With proper parameter selection, resonances from high molecular weight protein drug are essentially eliminated, while signals from smaller molecules remain and allow for accurate quantification. The data indicate that NMR spectra obtained through this approach can be used to quantitate a range of impurities, from small molecule components to higher molecular weight leachables. Furthermore, quantitation of impurities by NMR is reliable and accurate enough for biopharmaceutical process validation, even for extractables whose structures are not precisely known. The use of a CPMG sequence allows quantitation of these components without protein removal, mitigating the risk of contamination or loss of analyte due to sample handling. Advantages of the NMR method include the ability to: (1) detect all soluble proton containing substances; (2) achieve practical detection limits of 1 ug/mL or less; and (3) identify and quantify many different components in solution based on unique chemical shift and resonance multiplicity resulting from different molecular structures. We have routinely applied this method in process validation studies, using both direct and surrogate standards, to demonstrate that no unacceptable levels of small molecule impurities are present in the bulk materials.

NOTES:

Workshop Abstracts

Workshop I
Monday, October 15, 2012
15:30 – 16:30

In Mahogany Ballroom 1, 2 and 3

Quantitative NMR: How Good Can We Be, and How Good Do We Need to Be?

John Marino, *NIST, Rockville, MD USA*
Joseph Ray, *Baxter Healthcare Corporation, Round Lake, IL USA*

This workshop is designed to be a participative discussion among the conference attendees, where we will investigate the best practices of this very important application of NMR. Attendee participation will be a must if this workshop is to succeed, and we encourage attendees to bring electronic examples of their data to share during the discussion.

Topics that we intend to explore are:

- What NMR method is best for quantitation - internal, external or electronic references – what are the pluses and minuses of each of these techniques?
- Do calibration standards available from organization such as NIST meet the needs of the NMR analysis – would other standards be more suitable?
- How quantitative can NMR be - what are the practical limits (e.g. signal to noise, spectral complexity, baseline irregularities, etc.)?
- How quantitative does NMR have to be - what are the problems/questions?
- What factors are beyond our control?

NOTES:

Workshop Abstracts

Workshop II
Tuesday, October 16, 2012
10:45 – 11:45

In Mahogany Ballroom 1, 2 and 3

Making the Best Use of Your Data?

Carlos Amezcua, *Baxter Healthcare Corporation, Round Lake, IL USA*
Edward R. Zartler, *Quantum Tessera Consulting, Collegeville, PA USA*

The quality of a spectrum impacts the quality of the analysis. This is even more important when analyzing large numbers of spectra. This workshop will discuss issues that arise when acquiring a single spectrum, *e.g*. lineshape, baseline issues, *etc*. Issues that arise when analyzing large numbers of spectra, *e.g*. acquired as part of raw material analysis, will also be discussed. It is the aim of this workshop to discuss practical solutions to these issues and share best practices. We encourage attendees to bring electronic examples of their data to share during the discussion.

Topics for discussion:

- Quality attributes of a spectrum

  How to deal with bad baselines?, what is the best way to correct the slope/bias of integrals?, what are the best practices for post-acquisition processing?, what signal to noise is sufficient?, how important are optimal tuning and shimming?

- Quality attributes of multiple spectra

  What are the effects of changes in field, spectrometer manufacturer, and/or probe sensitivity?, how to handle impurities?, what is the typical sample to sample heterogeneity?, what are the best methods for spectral comparison?

NOTES:

Technical Seminar Abstract

Monday, October 15, 2012
12:45 – 13:45

In Mahogany Ballroom 1, 2 and 3
Sponsored by Bruker BioSpin

New Insights into Quality Control of Food, Beverages, and Dietary Supplements by NMR Based Screening

Kimberly L. Colson[1], Manfred Spraul[2], Birk Schuetz[2], Hartmut Schaefer[2], Fang Fang[2], Eberhard Humpfer[2], Jimmy Yuk[1], Christian Fischer[2]

[1]Bruker BioSpin, Billerica, MA, USA; [2]Bruker BioSpin GmbH, Rheinstetten, Germany

Applying metabolic profiling based routines to investigate food, beverage and dietary supplement samples by high resolution NMR is a completely new procedure for quality control of these products. This goes far beyond the ability of time domain NMR, which has been used for many years in food analysis, to typically deliver one parameter per sample like the fat content or the moisture content. The suitability of NMR for food/beverage/dietary supplement quality control relies on several advantages of the technology:

- Minimized sample preparation requirements
- Highest reproducibility and transferability of the NMR procedure
- Low cost per sample and even lower cost per parameter, as multiple parameters are accessible with one measurement
- Targeted and non-targeted analysis with one measurement

High resolution NMR screening allows the identification and quantification of many compounds within a single measurement. Importantly, the compound specific nature of NMR allows for the detection of 'unknown, unknowns' for the rapid identification of adulterated products.

Applications to Wine, Fruit Juice, Ginseng as well as other materials will be presented.

NOTES:

Technical Seminar Abstract

Monday, October 15, 2012
16:45 – 17:45

In Plaza Mahogany Ballroom 1, 2 and 3
Sponsored by ACD/Labs, Toronto, Canada

Applications of Quantitation in NMR Post-processing

Sergey Golotvin; Patrick Wheeler; Ryan Sasaki

ACD/Labs, Toronto, ON, Canada

Quantitative applications of NMR spectroscopy are almost as old as the NMR technique itself, and quantitative techniques are widely applied in the field. Because of the broad utility of the experiment, there is a constant need for better, faster, more efficient and accurate processing and reporting of quantitative information. We will present on the techniques that have been developed to better support quantitation in post-processing of NMR data, including concentration determination and reaction monitoring. We describe how accurate, intelligent structure verification is an aid to effective automated quantitation activities.

NOTES:

Technical Seminar Abstract

Tuesday, October 16, 2012
12:15 – 13:15

In Plaza Mahogany Ballroom 1, 2 and 3
Sponsored By Agilent Technologies, Inc.

The Application of Non-Uniform Sampling (NUS) Techniques to Routine Small Molecule NMR Measurements

Paul J. Bowyer

Agilent Technologies, Inc., Santa Clara, CA, USA

Recently, non-uniform sampling (NUS) methods have come to prominence in NMR spectroscopy as a way of obtaining multidimensional spectra in significantly less time, or obtaining higher resolution spectra in the same time, compared to conventional, uniform sampling methods. NUS data require the application of special processing techniques to retrieve a spectrum from the raw FID data. One such technique, CLEAN, has been shown to yield very high quality spectra with low levels of artefacts. A key advantage of CLEAN over several other NUS processing techniques is its relative speed: processing of a typical 2D dataset takes only a few seconds, thus making it highly amenable to the routine, automated measurement of these data. An introduction to NUS methods will be given, and their application to the quantitative analysis of soy-based dietary supplements will be demonstrated.

NMR in Big Business: Working in the Intersection of Chemistry, Physics and Time Driven Projects

Don Eldred

Dow Corning, Auburn, MI USA

Dow Corning is a specialty chemicals business offering over 7000 products and earning > $6B per year. We produce textiles, lubricants, anti-foams, surfactants, release agents, coupling agents, cosmetics, resins, waveguide materials, solids, liquids and much more. Dow Corning's Global Analytical Sciences Department is charged with product and R&D support. Molecular characterization through NMR plays a key role in supporting these initiatives. Supporting such a broad portfolio requires command of NMR experimental capabilities, intimacy with the chemistry toolset of the corporation, and creativity/innovation in coupling analytical capabilities to any given problem. We will discuss various innovative solutions which the Dow Corning Analytical Sciences NMR group has applied to drive key understandings and support Dow Corning's scientific portfolio.

NOTES:

Poster Abstracts

P-001

Analysis of Formulated Products by qNMR Using Filter Diagonalization Method

Maiara Santos[1]; Tiago Moraes[2]; Luiz Alberto Colnago[3]

[1]Instituto de Química de São Carlos, São Carlos, Brazil; [2]Instituto de Física de São Carlos, São Carlos, Brazil; [3]Embrapa Instrumentação, São Carlos, Brazil

NMR spectroscopy has been widely used in quantitative analyses (qNMR) due to its several advantages. However, the spectra of complex samples, such as formulated products, may contain broad and intense signals which may overlap analyte signals. Thus, the quantitative measurement is impracticable because there is not selectivity. To resolve this problem, the present work proposes the use of Filter Diagonalization Method (FDM) to process spectra containing signals overlapping that interfere in the quantification. The ¹H NMR spectra were acquired in a Varian INOVA 400 spectrometer using a validated method with pulse width of 10.5 μs (90°), 6 s acquisition time, 30 s for recycle delay and 8 scans. The samples were standard paracetamol, a medicine containing caffeine and commercial glyphosate. The internal standard was dimethylsulfone and the solvent DMSO-d_6. FDM was performed in homemade software using 2500 data points. For the regularization, we have use the method named "pseudo-noise averaging" (q=0.01 and 10 averages), as it do not cause distortions in the line shape and remove efficiently the noise artifacts. The efficiency of the FDM processing was verified from the correlation between the concentrations of standard sample processed via FT (Fourier Transform) and FDM (r^2 = 0.9986). Then, this processing method was also applied to FID of complex samples. In the spectrum of the medicine, the FDM was employed to exclude the water residual signal, which overlapped with methyl group of the caffeine. In the spectrum of formulated glyphosate, the signals excluded were of the formulation components, that overlapping with the singlet, referent to methylene group of the glyphosate. Both results agreed with the measurements carried out from other signals, which were not overlapping and were processed by FT. Thus, it was concluded that the method presented could be useful for the quantification of complex spectra.

P-002

Quantitative HNMR Tests for Determining the Mass Percentage of Small Molecules in Biomass Conversion Reactions

Lingyu Chi; Klaus Woelk; Rex E., II Gerald

Missouri S&T, Rolla, MO USA

Hydrothermal biomass-to-fuel reactions are a viable pathway for generating liquid biofuels. Newly developed, quantitative HNMR techniques are employed to identify reaction intermediates, understand the mechanisms, and study the kinetics of hydrothermal biomass reactions. The investigations are carried out in a 5-mm NMR tube with a 1-mm capillary tube insert filled with an integration reference standard, so that the aqueous sample drawn from the reactive solution is isolated from the D2O lock and the external integration and chemical-shift reference. The external integration reference standard can be

used to accurately report mass percentages of biomass reaction products. This report will identify some of the practical aspects pertaining to HNMR-based quantitative analysis and highlights some of the limitations, uncertainties, and applications of this particular approach to the analyses of biomass reaction products. A salient feature we developed is to measure the volume factor that is needed to calculate reaction product concentrations from the concentration and integrated signal intensity of an external reference sample. To do this we filled both the 5-mm and concentric 1-mm NMR tubes with the same stock solution (1.00 M maleic anhydride in D2O). The overlapping signals from the 5-mm and 1-mm sample tubes were separated by carefully dissolving small, individual single crystals of copper sulfate pentahydrate in the 5-mm-tube solution until two signals could easily be deconvoluted and integrated. From the signal integration ratios of these two signals, we determined a volume factor of 26±0.3.

NOTES:

P-003

Quantitative NMR for Low Level Impurity Determination in Pharmaceutical Samples

Joan Malmstrøm; Henrik Olsen

Novo Nordisk A/S, Måløv, Denmark

Identification and characterization of low level impurities in pharmaceutical samples (active pharmaceutical ingredients, excipients and raw materials) can be a demanding task and often an interdisciplinary approach is required. Thus disciplines like isolation of impurities; MS and NMR analysis (hyphenated or stand-alone) are most often needed when working with small molecule samples (M_r below 1000-1500 Da). In most cases examination of samples by LC-MS and LC-MS/MS analysis is straightforward and will be sufficient to describe the impurities present. However, in some cases the LC method is not compatible with a MS detector and then the handle of the impurity (known retention time) is lost. In cases like that a combination of getting an UV spectrum of the impurity and combine the analysis with $^{1}H/^{13}C$ NMR spectroscopy can lead to structural elucidation without the need of MS data. When NMR spectroscopy has been included in the structural elucidation work a quantitative determination of the amount of impurity present is easily reached. To assist the qNMR determination of low-level impurities it can be an advantage to use the ^{13}C satellite signal of the main compound for the calculations, as the area of this signal will be in the same range as the area of the impurity. When the impurity is available as reference material it can be convenient to use the NMR solvent peak as an internal standard and quantitate the analyte by an external standard method. The strategy described above has been applied to several cases implying identification of low-level impurities observed in pharmaceutical samples.

P-004

qNMR Assessment of Polysaccharides in Complex Food Matrices

Ewoud van Velzen[2]; Niels de Roo[2]; Christian Grun[2]; Ruud Poort[2]; Linda van Adrichem[2]; Kommer Brunt[3]; Henk Schols[4]; Yvonne Westphal[2]; John Van Duynhoven[1]

[1]Unilever & Wageningen University, Vlaardingen, Netherlands; [2]Unilever R&D, Vlaardingen, Netherlands; [3]Eurofins, Heerenveen, Netherlands; [4]Wageningen University, Wageningen, Netherlands

Polysaccharides are common ingredients for providing texture and shelf-life to a wide range of food products. Despite their extensive use as food ingredients, the analytical toolkit to identify and quantify such polysaccharides in complex food products is surprisingly small. This can be attributed to low formulation levels, the structural complexity of typical polysaccharides used as ingredients and their interactions with other components. NMR has proven itself as a tool for unraveling the overall structure of intact polysaccharides and this encouraged us to explore its potential for quantitative assessment of polysaccharides in complex food matrices. A critical first step was the isolation of polysaccharides from the food matrix. Here we relied upon enzymatic treatments, dialysis and solvent extractions to remove protein, salt, lipids and (semi-) polar low molecular weight species. Next we optimized the experimental conditions for obtaining well resolved high-resolution NMR spectra at 600 and 900 MHz using a cryoprobe. Within the acquired 1D and 2D HSQC NMR spectra mixtures of up to 4 polysaccharides of

different structural classes (galactomannans, carageenans, gellans) could be resolved. By making use of spectral assignments from the literature and a home-built (1D/2D) NMR spectral library a quantitative assessment of the identified polysaccharides could be made. Whereas for relatively simple compositions we could rely upon interactive (Chenomx) 1D spectral fitting, we needed to deploy quantitative 2D NMR approaches for more complex ones. The ability of qNMR to identify and semi-quantify polysaccharides in complex food matrices makes it a complementary technique in the current toolkit for analytical assessment of polysaccharide mixtures in food matrices.

NOTES:

P-005

A Comparison of Quantitative NMR Techniques: Internal Reference, External Reference and ERETIC

Christopher Cullen; Christina Szabo; Joseph Ray

Baxter Healthcare Corporation, Round Lake, IL USA

The performance of three quantitative nuclear magnetic resonance (NMR) methods was compared in terms of short and long-term precision and accuracy, robustness, linear range, and general applicability. The Internal Reference method employs a reference material co-dissolved with analyte; the External Reference method employs a reference material contained in a separate solution; and the third method, known as ERETIC (Electronic REference To access In-vivo Concentrations) [1], employs an externally calibrated digital reference peak. The Internal Reference method results were the most precise and remained stable within 0.1% over four weeks. The results from the External Reference and ERETIC methods were practically equivalent to each other during this time. These methods exhibited a small bias and slightly lower precision, establishing them as practical alternatives to the Internal Reference method. In contrast to the Internal Reference method, the External Reference and ERETIC methods possess several advantages that address peak overlap and flexibility of calibration. The study was designed such that each spectrum contained the information needed to compare the three methods while all other variables were kept constant. Applicability of pulse width compensation is addressed. [2] ERETIC software compensation and minor adjustments to 90° pulse width were concluded to be unnecessary for this system. Although chemical purity values were calculated and compared for each of the methods, this evaluation applies generally to absolute quantitation by NMR.

1 L. Barantin, A. Pape, S. Akoka, Magn. Reson. Med. 1997, 38, 179-182.
2 G. Wider and L Drier, J. Am. Chem. Soc. 2006, 128, 2571-2576.

P-006

Calculation of Average Molecular Descriptions of Heavy Petroleum Hydrocarbons by Combined Analysis by Quantitative 13C and DEPT-45 NMR Experiments

John Edwards

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Much debate has centered around the validity and accuracy of NMR measurements to accurately describe the sample chemistry of heavy petroleum materials. Of particular issue has been the calculated size of aromatic ring systems that in general seem to be underestimated in size by NMR methods. This underestimation is principally caused by variance in chemical shift ranges used by researchers to define the aromatic carbon types observed in the 13C NMR spectrum, in particular the bridgehead aromatic carbons that can be shown to overlap strongly with the protonated aromatic carbons. The ability to discern between bridgehead aromatic carbons and protonated carbons in the 108-129.5 ppm region of the spectrum is key in the derivation of molecular parameters that describe the "molecular average" present in the sample. Utilizing methodologies developed by Pugmire and Solum for the solid-state 13C NMR analysis of coals and other carbonaceous solids we have developed a new liquid-state 13C NMR

method that allows the relative quantification of overlapping protonated and bridgehead aromatic carbon signals to be determined. The NMR experiments involve the combined analysis of both quantitative 13C single pulse excitation which observes all carbons quantitatively, and a DEPT45 polarization transfer which observes only the protonated carbons in the sample. Though the DEPT45 results are not quantitative across all carbon types (CH, CH2, and CH3) due to polarization transfer differences, the technique is well enough understood that simple multiplication factors allow the relative intensities of the different carbons to be determined. The average ring system sizes derived from these NMR experiments tend to be several ring systems larger than has been calculated in previous studies. In heavy petroleum asphaltenes the average aromatic ring system is 5-7 rings in size which is in agreement with FTICR-MS and fluorescence measurements, rather than the 3-4 rings previously reported.

NOTES:

P-007

Response Factor Variation in Quantitative ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy

Michele DeRider; Charlotte Carroll

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Nuclear Magnetic Resonance spectroscopy is routinely utilized for quantitative applications. Quantitative carbon NMR data collection is significantly more complex than quantitative proton NMR data collection due to various factors such as low magnetic response, NOE effects, and slow relaxation times. A model system was utilized to quantitate the impact various NMR parameters had on relative response factors in 1D ^{13}C spectroscopy. The model system contained an internal standard and a model drug substance prepared in deuterated DMSO. The parameters were deliberately modified and the resulting variation in the response factors compiled for different carbon moieties. The parameters included acquisition time, relaxation properties, and digital resolution.

A case study described here is the application of a quantitative carbon method for the determination of the relative ratio of two individual components in a formulated drug product. The formulation did not allow conventional approaches to be utilized in part due to the presence of excipients. A quantitative ^{13}C NMR method was developed to correlate to the ratio of the two components. As part of the method development, formulations of known ratios of the two components were evaluated. The analysis of the relative integration versus the molar ratio was found to be linear (R^2 of 0.99). Although the two components were of similar molecular weight and the two carbons were both carbonyl carbons, the response factors in the quantitative spectrum varied significantly for the two components. The resulting correlation factor was 1.33.

P-009

Reaction Monitoring for Fun and Profit

Mike Bernstein; Santiago Dominguez; Manuel Pérez Pacheco; Carlos Cobas; Isaac Iglesias

Mestrelab Research, Santiago de Compostela, Spain

There is tremendous demand for robust analytical techniques that provide information and insight on chemical reactions. This might be driven by a fundamental interest in reaction mechanisms, or requirements to meet strict regulatory requirements for pharmaceutical ingredient production. Whilst vibrational spectroscopy and LC are often used, the uptake of NMR in this area is surprisingly low.

We will describe the advanced tools available in Mnova to work with arrayed NMR data possibly collected whilst a chemical reaction is occurring. The software offers the choice to perform the analysis *post facto*, or in real time whilst the reaction is occurring. Analysis requires that any necessary data manipulation occurs, and peak or multiplet areas are determined either using traditional “summation” methods, peak fitting, or peaks found in spectrum deconvolution (GSD) [1].

Since the analysis ideally requires quantitation, we have included mechanisms to simply produce plots of Concentration v time for any species that has an NMR-active and observed nucleus. These can be further manipulated and be fit against exponentials, etc. The software therefore provides a clean and complete environment in which to perform the analysis of NMR kinetic data.

The “listener” functionality has tremendous potential in production environments, following well characterized reactions. This allows the collection and processing of NMR data “on the fly”. The kinetics project is updated each time a new spectrum is added, giving access to real-time data on important chemical species. Critically, alert criteria can be defined, and these can be used to automatically trigger new events, such as the closing of a valve, or starting/ending a solvent or compound addition.

Reaction Monitoring using NMR is greatly facilitated by highly competent and feature rich software to process and use it, as will be demonstrated in this poster.

1. Cobas, C.; Seoane, F.; Domínguez, S.; Sýkora, S., Spectroscopy Europe, 23(1), 25-30, 2010

NOTES:

P-010

qNMR for All Occasions

Mike Bernstein; Manuel Pérez; Chen Peng; Carlos Cobas; and Agustín Barba

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It has long been recognised that NMR is particularly well suited to compound quantitation [1,2] and has established itself as the "gold standard". We wish to descibe qNMR analysis software that has considerable flexibility and functionality to be used either manually or under full automation. At its core are algorithms to rank the contender multiplets for suitability for use in the final compound concentration calculation.

When analysing ^{1}H NMR spectra the initial requirement is that the peaks have been picked, multiplets identified, and the number of nuclides (NN) per multiplet determined. This process may rely on (a) standard peak picking, (b) the very powerful Global Spectrum Deconvolution (GSD) and its associated peak classification [3], or (c) regular line fitting. The second step, multiplet identification, may use long-standing algorithms in the software, the output from Automated Assignments [4], or Automated Structure Verification [5]. The user may then choose whether to convert integrals to concentration either using an absolute area conversion factor, or an internal reference signal of specified concentration. Choosing between these options will be predicated largely on the data quality and type, and performance requirements.

At this point the software will compute the concentration of the analyte based on every available multiplet. The next important step is to select amongst the available multiplets the ones that are likely to provide the best overall estimate of compound concentration. We achieve this programatically using a number of approaches, and suitability again depends on the data. Finally, reporting is done in conventional ways.

We have tested the process and found it to typically operate as well as a manual quantitation.

Batch operation poses some design challenges, but the potential rewards can be huge. For example, results must compensate for changes in spectral acquisition parameters. With the core qNMR functionality available as a bolt-on option, users can perform batch qNMR alone or at the same time as verification. This will be of particular interest to those doing quality assurance on compound libraries. To further leverage the core functionality in highly automated environments we have added the capability to perform NMR quantitation at the same time as Verification (ASV). And finally, we have a "listener" functionality which gathers and processes data as they are produced, making it another key component to a highly automated environment.

1. Lane, S.; Boughtflower, B.; Mutton, I.; Patterson, C.; Farrant, D.;Taylor, N.; Blaxill, Z.; Carmody, C.; Borman, P., Anal. Chem., 77, 4354−4365, 2005
2. Barding, G.A. Jr; Salditos, R.; Larive, C.K., Anal Bioanal Chem, Online First, 6 July 2012, DOI: http://dx.doi.org/10.1007/s00216-012-6188-z
3. Cobas, C.; Seoane, F.; Domínguez, S.; Sýkora, S., Spectroscopy Europe, 23(1), 25-30, 2010

4. Cobas, C.; Bernstein, M; Vaz, E.; Seoane, F.; Sordo, M.; Domínguez, S.; Pérez, M.; Sýkora, S, "An Expert System for the Automatic Assignment of 1H NMR Spectra of Small Molecules". Poster at 53rd ENC, Miami, FL. April 15-20, 2012
5. Web content: http://mestrelab.com/blog/topic/automatic-structure-verification/

NOTES:

P-011

Structure Verification in the Real World

Mike Bernstein[1], Santiago Dominguez[1], Manuel Pérez Pacheco[1], Felipe Seoane[1], Carlos Cobas[1], and Stan Sýkora[2]

[1]Mestrelab Research, Santiago de Compostela, SPAIN [2]Extra Byte, Castano Primo, Italy

The development of software for the automatic verification of chemical structure identity using NMR and MS data has been an arduous project. At the heart of the procedure for a ^{1}H spectrum is a deceptively simple work-flow based around detection of peaks from the compound, deriving multiplets, comparing these with predicted values, and reporting. But each of these stages is beset with significant challenges. Our capability to simultaneously quantitate [1] these samples is a considerable asset.

Despite these challenges, we have brought our ASV approach to a point where it is consistently showing encouraging results in customer field tests, and we have confidence that a very usable product now exists [2].

In this poster we wish to explain the ways the software can be used (a) in an open-access environment for chemists, and (b) in situations where unattended usage is required. In all cases we have put significant effort to make the software as "user friendly" as possible by incorporating a flexible feedback functionality. Each scenario has the potential for quite different implementation, and our modular approach leads to great flexibility in deployment. In addition to the core Verification functionality, we have had to take extra steps to allow its user interaction in a variety of highly automated ways. This includes batch operation on a local PC, and working in an environment without a Graphical User Interface (GUI). The latter has the advantage of making the software amenable to customer-specific environments that may make use of Web services, and automated environments such as Pipeline Pilot or Knime.

1. Bernstein M.A, et al, "qNMR for All Samples". Poster at SMASH 2012, Providence, RI, September 10 – 12, 2012.
2. Web content: http://mestrelab.com/blog/topic/automatic-structure-verification/

P-012

Estimating Metabolite Concentrations Using a New Deconvolution Routine

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[1]ACD/Labs, Inc, Toronto, Canada; [2]ACD/Labs, Inc., Moscow, Russia; [3]UNC-Hamner Metabolomics Laboratory, Chapel Hill, NC USA; [4]Liposcience Inc., Raleigh, NC USA

An NMR metabolomics study begins with the collection of biofluid spectra. Because these spectra have many thousands of peaks, the data are often simplified by dividing each spectrum into small integral regions called "bucket". While there are many robust approaches to bucket integration, they typically do not solve the problem of peak overlap in complex biofluid spectra. For this issue, peak deconvolution algorithms have the potential to provide better quality integration data for metabolomics.This study will

highlight a new algorithm to perform automatic deconvolution on an entire spectrum. It will include the results of the automatic deconvolution of a spectrum from a liver extract sample to illustrate the algorithm's ability to handle high levels of spectral complexity.

NOTES:

P-013

Purity Determination of Reference Standards by NMR

Catherine Quinn; Christopher Cullen; Christina Szabo

Baxter Healthcare Corporation, Round Lake, IL USA

Baxter Healthcare Corporation qualifies reference standards for product testing and R&D applications. NMR is one technique for structural identification and purity determination. For purity determination, NMR methods commonly involve absolute quantitation by direct measurement of the analyte versus a reference standard. This methodology has the advantage over other techniques in that we can account for other impurities such as inorganics and water even though we do not directly detect them. The European Pharmacopeia calculates reference standard purity by applying the principle of mass balance and purity by an absolute method for confirmation of the primary purity value. Therefore, the absolute NMR purity can be used for confirmation. Since NMR is used to qualify new lots, we have developed novel strategies for determining linearity and accuracy for validation of the NMR methods.

P-014

1H qNMR Determination of Acetylated Polysaccharides, Glucose, Maltodextrin, Isocitrate, Degradation Products, Preservatives and Additives in Aloe Vera Leaf Juice

John Edwards

Process NMR Associates, LLC, Danbury, CT USA

Aloe Vera is a botanical component that is used widely in the cosmetic, natural product, herbal supplement, and pharmaceutical industries. The widespread use of Aloe Vera has lead to the need to adequately analyze the authenticity, quality, and quantity of the various components present in this material. The ^{1}H qNMR method described here was developed and validated by Process NMR Associates for a number of NMR service customers and the method will be included in an upcoming Monograph on Aloe Vera published by the American Herbal Pharmacopoeia. The method can be used for the detection and quantitation of the primary components of interest in Aloe Vera juice products and raw materials for compliance with IASC (International Aloe Science Council) certification requirements, specifically, for determination of the content of acetylated polysaccharides, the presence of glucose, the presence and content of maltodextrin, and the content of isocitrate. Additionally, for meeting quality control specifications beyond IASC requirements, the presence and content of the following groups of compounds can be determined: degradation products (e.g., lactic acid, pyruvic acid, succinic acid, fumaric acid, acetic acid, formic acid, and ethanol), preservatives (e.g., potassium sorbate, sodium benzoate, and citric acid/citrate), and other atypical impurities, additives, or adulterants (e.g., methanol, glycine, glycerol, sucrose, maltodextrin, flavorants (propylene glycol/ethanol)). We will describe a common internal-standard NMR methodology that does not require additional equipment or advanced automation software. The method is applicable to a number of different Aloe Vera raw materials and products, including liquid and dried juices. In aloe vera finished products the method is only applicable when the observable aloe vera constituents are present at a high enough concentration to be observed and are not obscured by additional product ingredients with signals in overlapping areas.

NOTES:

P-015

Compact, Cryogen-Free, High-Resolution 60 MHz Permanent Magnet NMR Systems for Reaction Monitoring and On-Line/At-Line Process Control Observing 1H, 19F, 31P

John Edwards[1]; Tal Cohen[2]; Paul Giammatteo[1]

[1]Process NMR Associates, LLC, Danbury, CT USA; [2]Aspect AI, Shoham, Israel

A compact high resolution NMR system will be described that can be situated on the bench-top or in the fume hood to be used as a continuous or stop-flow detector and/or an “in-situ” reaction monitoring system. The same system can be fully integrated into on-line shelters for on-line process control or utilized by engineers and technicians in an "at-line" environment. The system uses a unique 1.5 Tesla permanent magnet that can accommodate sample tube diameters of 3-10 mm with half-height spectral resolution (water resonance) approaching 1-3 Hz depending on the sample volume size and with excellent single pulse sensitivity. These systems can be utilized in a traditional NMR methodology approach or combined with chemometric approaches that allow NMR data to predict chemical and physical properties of materials via regression analyses that establish correlations between observed spectral variability and sample-to-sample property variance [1].

1) “Process NMR Spectroscopy: Technology and On-line Applications” John C. Edwards, and Paul J. Giammatteo, in Process Analytical Technology: Spectroscopic Tools and Implementation Strategies for the Chemical and Pharmaceutical Industries, 2nd Ed., Editor Katherine Bakeev, Blackwell-Wiley, 2010

P-016

Evaluation of Raw Beef Quality with Time-Domain Nuclear Magnetic Resonance

Fabiola Pereira

Embrapa Instrumentation, São Carlos, Brazil

Sensory parameters (flavor, juiciness and tenderness) and physicochemical parameters, such as cooking loss, fat and moisture content and instrumental tenderness using Warner Bratzler shear force were evaluated for samples of sixty-one Bonsmara heifers. This sampling was separated into five groups based on genetic (breeding composition) and feed system (grain and grass feed). Time-domain nuclear magnetic resonance (TD-NMR) relaxometry measurements were also performed to same samples.

The profiles of the time-domain nuclear magnetic resonance (TD-NMR) relaxometry were dependent on water content and its distribution in raw beef samples. Then, the relaxometry studies were performed using a standard Carr-Purcell-Meiboom-Gill (CPMG) sequence to measure the T_2 values and the sequence known as Continuous Wave-Free Precession (CWFP) to measure longitudinal relaxation time (T_1) and T_2 in a single and fast experiment. The TD-NMR signals obtained from the sequences were used to develop linear models to predict these aforementioned seven properties. The main objective was to speed up the sensory tests and to improve the quality control.

The best time (independent variable) for each parameter was selected by means of computed F-test at confidence interval of 95%. The predictive ability of the method was evaluated using the root mean square error (RMSE) for the calibration (RMSEC) and validation (RMSEP) data sets. The reference and predicted values showed no significant differences at a 95% confidence level.

NOTES:

P-017

Approaches to Speed TD-NMR Data Processing

Bruce Campbell; Catherine Shawl

Kraft Foods Group, Glenview, IL USA

Efficient data handling can be a challenge with time-domain NMR measurements. T2 relaxation rates are typically calculated by CONTIN or other data fitting algorithms on decay curves. When multiple data sets are collected for a single sample type to best represent sample heterogeneity, those data require normalization and averaging prior to fit. Software strategies for handling the data in a batch-wise mode with macros and automation to speed processing were devised. These include an interface between the vendor's data acquisition software and a standard spreadsheet application. Advantages and cautions to this approach will be outlined.

P-018

CapPack: A New Device for Testing Solvent-Signal Suppression Sequences

Annalise Pfaff

Missouri S&T, Rolla, MO USA

In performing NMR analysis of biomass-to-fuel reactions in proteo-solvents, it is often necessary to employ a solvent-signal suppression pulse sequence because large solvent signals prevent the resolution of analyte signals. The water-signal suppression sequence used in our laboratory to circumvent these issues is known as EXCEPT-20, a sequence developed to mitigate variations in suppression performance due to changes in sample T_1s.

In order to test the effectiveness of EXCEPT-20 against other known water suppression sequences, such as WET and WATERGATE, we have developed devices known as "Gradient CapPack" and "T_1 CapPack." The Gradient CapPack consists of 1) a 5-mm NMR tube containing an array of capillary tubes, 2) a 0.5 M maleic anhydride in acetone-d_6 integration standard with Cr $(acac)_3$ relaxation agent in a sealed 1-mm NMR tube, and 3) a series of nine 325-µm o.d., 25-µm i.d. capillary tubes filled with the same concentration of $CuSO_4$ solution arranged across the diameter of the 5-mm tube. When a constant magnetic field gradient is applied perpendicular to the long axis of the sample tube the protons in the individual capillary tubes produce nine similar signals at regular intervals over a range of approximately 1 ppm. The T_1 CapPack uses the same integration standard and 5 mm outer tube, but the inner diameter of the capillary tubes is changed to 75 µm, and each of the six capillary tubes are filled with a different concentration of $CuSO_4$ solution (0.1M-0.00156 M). A different concentration of the relaxing agent $CuSO_4$ in each of the solutions produces a 1 ppm chemical shift distribution of proton signals from the six water samples that also have different T_1s. With these devices, the signal reduction, dispersion, and T_1 insensitivity of a water suppression pulse sequence can be tested in a consistent and reproducible manner.

NOTES:

P-019

Use of Time Domain NMR to Measure Temperature in Intact Seeds and Seeds Inside Soils

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[1]Institute of Chemistry of São Carlos, São Carlos, Brazil; [2]EMBRAPA Instrumentation, São Carlos, Brazil

Soil temperature is important in agriculture and affects directly the seed germination. Some seeds may survive in high temperature environment for days, but the seedling may die in few hours.The current methods to measure soil temperature are based on conventional thermometry but they don't provide information of seed's temperature. Here, we are showing that it's possible to use Time Domain NMR to measure oilseeds temperature. It was used oilseeds as a sensor and Carr-Purcell (CP) sequences with low refocusing flip angles, named CP-CWFP (Carr-Purcell Continuous Wave Free Precession). Therefore, CP-CWFP sequence can be a useful method in Time Domain NMR, widely used in agriculture and food industry, because the samples tend to have similar relaxation times in low magnetic field. The measurement is based on the T_2 dependence of oil viscosity and temperature. The oilseeds used were Macadamia integrifoli and peanut. It was observed that the relaxation times (T*, T_1 and T_2) increase with increase of temperature. As a result, in 29°C the T_2, T_1 and T* values were 0.09s, 0.09s and 0.10s; respectively. In 60°C the T_2, T_1 and T* values were 0.18s, 0.21s and 0.23s; respectively. Thereat, it was possible to predict how the heat was transferred inside the soil and the oilseed in function of time. Although soil type doesn't influence directly on the signal acquisition, the seed will heat more or less depending on the soil type. This method allows calculating thermal properties like thermal diffusivity (D) and for macadamia nut D=2.31.10-8 m^2s^{-1}. Also, it was important to store and to dry seeds. This study shows that the NMR-TD can be use like a new method to measure seeds and soil temperatures. It is possible to predict how they will behave in temperature changes. Finally, it is possible to obtain information quickly compared with other techniques.

P-020

Analysis of Complicated Model T_1 Curves

Rex Gerald; Ming Huang; Klaus Woelk

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The analysis of complicated T_1 and T_2 distributions obtained from heterogeneous materials is important to the petrochemical industry. To better understand how well various commercial programs are able to analyze time-domain NMR relaxation data, we developed devices and methodologies that discretize the problem. In one of our previous approaches, we reconfigured a Varian Inova 400-MHz NMR spectrometer to operate with a Bruker MiniSpec 20-MHz NMR magnet to acquire nuclear spin relaxation data of groups of discrete samples contained in up to seven 5-mm NMR tubes. Each sample tube contained the same nominal concentration of water but a different amount of a nuclear spin relaxation agent ($CuSO_4$). The poor resolution afforded by the low-field magnet resulted in the overlap of the NMR signals from all six samples. Complicated inversion-recovery relaxation curves were fitted to a discrete sum of exponential terms. By combining samples into various groups bimodal, symmetric

and skewed distributions were mimicked. We have extended our studies by performing similar experiments at high field employing groups of discrete capillary-tube samples (25-μm i.d., 350-μm o.d.) that can be accommodated inside a 5-mm NMR tube. The high resolution spectrum of a capillary-tube sample group reveals a discrete dispersion of NMR signals. The dispersed signals can be collapsed into a single peak by applying a transverse magnetic field gradient to an appropriately ordered spatial arrangement of the capillary-tube samples in the group. Analysis of complicated inversion recovery curves for various model groups of capillary-tube samples will be presented.

NOTES:

P-021

Analysis of Heparin and Heparan Sulfate by 1H-15N HSQC NMR

Derek Langeslay; Consuelo Beecher; Cynthia Larive

University of California, Riverside, Riverside, CA USA

Heparin is a widely prescribed pharmaceutical drug with antithrombotic activity. It exists as a heterogeneous mixture of polydisperse linear oligosaccharides and is exclusively isolated from animal tissue. It is manufactured as both unfractionated heparin as well as low molecular weight heparin. Low molecular weight heparins have more desirable PKPD properties, but have additional structural diversity based on the depolymerization method employed. The need for analytical methods for compositional analysis and fingerprinting were blatantly illustrated by the heparin contamination crisis of 2008 which resulted in numerous deaths worldwide after lots of heparin were purposely adulterated by over-sulfated chondroitin sulfate. The complexity of heparin makes it difficult to analyze by chromatographic methods, but NMR has shown great promise. Since the 2008 crisis, many methods have been developed to characterize heparin via 1H and 13C NMR. These methods have proven valuable, but spectra can be complicated due to the abundance of these nuclei in heparin.

In order to simplify the NMR analysis of heparin, we have turned to the otherwise untapped potential of 15N NMR for aminosugar analysis. With this approach we are able to focus specifically on N-sulfo- and N-acetyl- glucosamine residues and their local environments through the use of the 1H-15N HSQC experiment. Our goal in this project was to assign the observed correlations in the 1H-15N HSQC spectra to elements of structure and then to use this method to evaluate different heparin preparations. This is accomplished through a combination of the analysis of isolated heparin-derived oligosaccharides, uniformly modified intact heparins as well as an in-depth probe using 1H-15N HSQC-TOCSY. Assigning these spectra will provide a foundation for the development of routine quality control as well as in-depth investigations of heparin and related glycosaminoglycans.

P-022

Susceptibility-matched Multiwell Plates for High-throughput Screening by Magnetic Resonance Imaging and Spectroscopy

Kenneth Fishbein

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Multiwell assay plates are used in a wide variety of high-throughput measurements in clinical chemistry and immunology as well as in drug discovery, combinatorial chemistry and other research applications. MRI of multiwell plates offers the possibility of performing new kinds of high-throughput assays, including the detection of targeted magnetic nanoparticles attached to or within cells. Moreover, MRI-guided localized NMR spectroscopy could be used to perform detailed analysis of metabolites not possible by any other common analytical technique. Best of all, MRI techniques exist which permit all samples in one or more plates to be analyzed at once. While localized spectra have been obtained using bundles of glass capillaries, attempts to resolve spectra from individual wells of a conventional plate have been unsuccessful. This poor resolution is largely due to the fact that commercial plates, formed

from polystyrene or polypropylene and having shallow, open wells, provide inadequate matching of magnetic susceptibility, X, between the samples, plate, and surrounding air. This results in B_0 field distortion and reduces the sensitivity and resolution of NMR spectra.

I will present a new multiwell plate design incorporating one-piece polyetherimide (ULTEM®) construction for improved X-matching for aqueous samples. Further gains in X-matching can be made by adding ULTEM plugs to each well to displace the air-water interface (meniscus) above the plane of the plate. These plugs can be combined in a single "cap mat" or inserted individually. These designs are compatible with the same robotic equipment currently used to handle standard well plates. The new multiwell plate/plug design reduces magnetic field distortions and should dramatically improve spectral resolution and sensitivity for NMR and MRI-based high-throughput screening. By eliminating the need to transfer samples to NMR tubes or flow cells, body fluids, cells and other materials may be rapidly scanned without fear of contamination or sample loss.

NOTES:

P-023

NMR Metabolomics Applied to Characterization and Detection of Breast Cancer Cell

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The tumor biology of breast cancer is complex, patients respond differently and, often, poorly to treatment. In this case, the transforming process from normal to malignant cells is associated with profound metabolic disturbances. Metabolomics is a science that provides a dynamic portrait of metabolic status inside the cell. In vitro analysis of tissue extracts have been used to study metabolic profile, but this process is also laborious and direct comparison to histopathology is impossible. In this work, we discuss in vitro analyses of intact breast cancer cells, using high-resolution magic angle spinning (HR-MAS). Human breast cancer cell line, MCF-7 and MDA-MB-231, was maintained in DMEM supplemented with 10% fetal bovine serum. After centrifugation, the pellet was ressuspended in D_2O and centrifugated for further NMR analysis. ^1H HR-MAS spectroscopy was performed at 9.4 T and 5 KHz of spinning rate using DRX 400 BRUKER NMR spectrometer. To analyze the metabolic profile of each strain of breast cancer, we obtained the HR-MAS spectra in triplicate, that demonstrated signals only in the region 0.9 to 4.5ppm. The sample signals were very similar with little variation in proportion of their constituents. However, the differences in metabolic products were observed between different breast cancer cell lines, as the absence of the metabolite acetate and alanine in MDA-MB-231 and significant variation in the proportion of constituents. Probably, it can be correlated to metabolic mechanisms that occur within the cells. The HR-MAS analysis of breast cancer cell improved resolution and reproducibility of metabolomic profiling. The ^1H NMR metabolomics has the potential to influence clinical oncology and probable future use as a biomarker for early cancer diagnosis and treatment efficacy.

P-024

Validation of qNMR with applications to Certified Reference Materials

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Industrial applications of NMR include among others structural elucidation, purity assessment, process control and some aspect of quantitation. The latter has gained widespread application in the recent years with various implementations that include signal injection methods such as ERETIC and the use of internal and external standards.

This poster concerns the use of NMR for quantitation in general, but in particular the use of external standards for the quantitative certification of reference materials. Perhaps one of the most significant properties that allow quantitative NMR (qNMR) to be the method of choice for this application is its uniform molar response for a given nucleus. For a given spectrometer hardware setup, the method permits determination of molar responses via independent external standards which is important in the quantification of reference materials that may not be amenable to internal standards.

Previous work has determined the conditions that have an effect on the quality of the data that can be acquired by NMR for quantitative purposes. These conditions affect the robustness of the method. The extent to which parameters such as tuning and matching, pulse width calibration, inter-pulse delay, receiver gain as well as dummy and acquisition scans affect the robustness has been determined, and will be shown. Further, the variability in sample tube diameter contributes to the error in quantitation. This work also presents the results of validation of the qNMR method in a certified reference materials environment. Properties of the method such as sensitivity, selectivity, accuracy and precision have been evaluated in this context. Unusual effects of solvent pre-saturation on the relative molar response have been found and will be discussed.

NOTES:

P-025

Profiling Whole-cell Biomass by High Resolution-Magic Angle Spinning Spectroscopy (HR-MAS)

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It is advantageous to study intact tissues, cells, raw materials or product formulations, or metabolites in their native environment without disrupting/degrading extractions, or chemical modifications. Solution NMR is both non-destructive and quantitative but it is not suitable for semi solid materials due to restricted mobility of the matrix. High resolution magic angle spinning (HR-MAS) spectroscopy on the other hand is a specialized NMR technique that can be used to overcome the severe line broadening as a result of restricted mobility in semi-solid material. HR-MAS can be applied to many fields including natural products, metabolic changes in diseased and treated tissues, combinatorial chemistry, and whole cells. In this study HR-MAS was used to observe the macromolecular assemblies from whole-cell algae and yeast for profiling and quantitation of lipid and polysaccharide content. Samples of freeze dried biomass were accurately weighed, hydrated with 100% D_2O and transferred into Teflon HR-MAS disposable inserts and 1-D NMR spectra were recorded under quantitative conditions. Acquisition of a series of 1-D HR-MAS spectra over 14 hours revealed that lipid decomposition occurred at 298K, this decomposition was retarded by both hydrating samples and acquiring spectra at 277K. 2-D HR-MAS experiments were analyzed to identify carbohydrate components and lipid classes in the biomass. Accurate profiles from three different types of biomass, *N. granulata*, *B. Braunii* a high lipid producing algae strain, and a marine yeast *S. roseus* were compared. This rapid and robust technique is being extended to quantitatively profile protein, metabolite and inorganic material in an effort to establish methodologies for quality control and quality assurance (QA/QC) for cultivation of various marine organisms.

P-026

Higher Order Protein Structure Comparability Assessment using ECHOS-NMR

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An NMR method for quantitative assessment of higher order structure (HOS) comparability of protein-based biopharmaceutical products, named ECHOS-NMR (Easy Comparability of Higher Order Structure by NMR), has been developed. ECHOS-NMR uses the correlation coefficient derived from linear regression analysis of binned NMR spectra to estimate the similarity between samples. The method was successfully applied to 1D- and 2D-NMR spectra of non-glycosylated proteins spanning a molecular weight range of 6-67 KDa. It will facilitate and increase the use of NMR as an orthogonal analytical technique in comparability exercises for product development and submission to regulatory agencies.

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P-027

NMR Fingerprint Assay of Recombinant Glycoprotein Therapeutics

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The assessment of a subsequent entry biologic (SEB) (a SEB is a biologic drug that enters the market subsequent to a version previously authorized in Canada, see "Guidance for Sponsors: Information and Submission Requirements for Subsequent Entry Biologics") requires a comparability exercise with a recognized reference product. For this purpose, our laboratory has developed a method that utilizes nuclear magnetic resonance spectroscopy to assess the structure of the active ingredient of SEB. The application of the method was limited to the assessment of non-glycosylated proteins. However, a number of products contain recombinant glycoproteins, such as erythropoietin and interferon beta, as active ingredients. These therapeutics present a higher level of difficulty in their characterization resulting from the presence of sugar chains of various lengths and compositions. Here we present the progress made toward the extension of this methodology to recombinant glycoprotein therapeutics.

The development of NMR-based methods requires the production of labelled glycoproteins with NMR-detectable isotopes such as carbon-13 and nitrogen-15. Labelled samples allow optimisation of NMR parameters for data acquisition used for the characterisation. It thus becomes possible to analyse the structure of the glycans ($^{1}H,^{13}C$-2D-HSQC experiments) and the polypeptide chain ($^{1}H,^{15}N$-2D-HSQC experiments) in a non-destructive manner. The yeast-based expression system *Pichia pastoris* can produce labelled glycosylated proteins in high yields and at affordable prices. It uses ^{13}C-labelled glucose and methanol, and ^{15}N-ammonium salt as sole sources of carbon and nitrogen, respectively. We have optimised the expression and purification of labelled N-glycosylated-GM-CSF with stable NMR active isotopes. Analysis of NMR spectra allows the comparison of the conformation of the protein in the presence and absence of glycans. For GM-CSF, the overall fold of the protein is maintained.

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