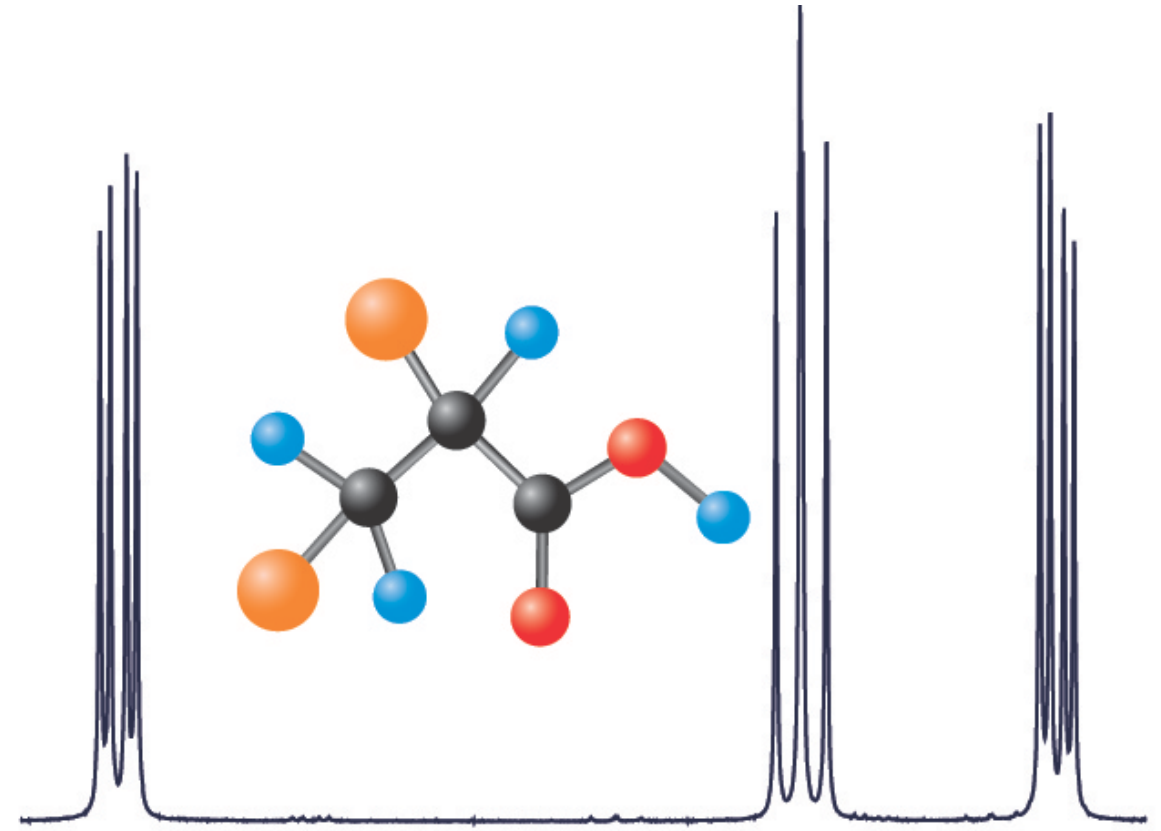

Conference Program

September 9th-12th, 2012
Providence, Rhode Island

SMASH 2012 NMR Conference

Dear SMASH 2012 Attendees,

We want to welcome you to the 2012 **S**mall **M**olecules **A**re **S**till **H**ot NMR conference in Providence, Rhode Island, The Ocean State. The conference will be held this year in the Rhode Island Convention Center, which is located in downtown Providence and connected via a sky-bridge to the Conference Hotel (Providence Westin), the Providence Place Mall, and the Dunkin' Donuts Center. Numerous restaurants and historical attractions are within walking distance of the conference site. Providence is located at the north end of Narragansett Bay about 10 miles north of the airport and is an easy drive to Newport and Jamestown for those who rent a car. The weather is typically dry and sunny with high temperatures of approximately 70 °F (21 °C).

We are excited to announce that we will be co-hosting the conference with CoSMoS, the **Co**nference on **S**mall **Mo**lecule **S**cience. Each conference will have their own oral sessions and workshops on Monday and Tuesday, and then combine for joint sessions on Wednesday. Since we wanted to facilitate interaction between the two groups, we will share all meals, breaks, poster sessions, and our after dinner speaker. More importantly, your SMASH registration will allow you to attend any CoSMoS session/workshop, and you'll also see CoSMoS attendees in our sessions. This conference combination highlights the interdisciplinary nature of the work that many of us are doing. This year's program for Monday and Tuesday has oral sessions titled, *"Calculations of NMR Parameters. Applications to Constitutional and Configurational and Conformational Analysis", "Student and Post-Doctoral Presentations", "Beyond Conventional NMR Experiments for Determination of Configuration", "Solid-State NMR & Pharmaceuticals, It's Just What the Doctor Ordered", "J-Based Analysis and Measurement of Heteronuclear Couplings", "New Developments in Ultrawideband Pulses"*. This year's workshops include, *"Automatic Structure Verification," "Pulse Programming Tutorial", "Teaching Mass Spectrometry to NMR Spectroscopists", "NMR of Other Nuclei"*. On Wednesday, just as we did in Portland in 2010, we are having a day of joint activities with CoSMoS that include oral sessions titled *"Potential Genotoxic Impurities" and "Predictive Quantitation", and the workshop "Wisdom of the Crowd"*. Right after the closing remarks of the conference on Wednesday afternoon SMASH/CoSMoS will be hosting a unique *Career Development Panel Discussion and Mixer*. A panel designed primarily for early career scientists and students, but open to everyone. Please register for FREE at the SMASH website.

While posters will be up the entire conference, those with even numbers will present on Monday and odd numbers on Tuesday. After dinner mixers on both evenings in the poster area will allow for discussions to continue.

The Monday keynote after dinner speaker is Koji Nakanishi, of Columbia University. He will be talking about "*Bioorganic Studies of Vision and Ginkolides."* Professor Nakanishi is also an accomplished magician and rumor has it that there may be a little magic performed during the evening.

On behalf of the Organizing Committee, we want to extend a warm welcome and thank you for attending SMASH 2012.

Sincerely,

Roberto Gil & Gary Martin
Co-Chairs, SMASH 2012 NMR Conference

SMASH 2012 NMR Conference Program

Sunday, September 9th

04:30 PM - 06:00 PM Registration

06:00 PM - 08:00 PM Dinner

08:00 PM - 11:00 PM Mixer

Monday, September 10th

07:30 AM - 08:45 AM Breakfast

08:45 AM - 09:00 AM Opening Remarks

09:00 AM - 10:30 AM **Calculations of NMR Parameters. Applications to Constitutional, Configurational and Conformational Analysis**
Chair: Armando Navarro-Vazquez, *University of Vigo*, Spain

Small Molecule Structures: How Can Calculations Help Experiments?
Jonathan Goodman, Unilever Centre for Molecular Science Informatics, Department of Chemistry, Cambridge, UK

Quantum Mechanical Approaches to Chemical Shift Prediction
Michael Lodewyk, Department of Chemistry, University of California, Davis, Davis, CA, USA

***Ab initio* Chemical Shift Calculations as a Tool within Pharmaceutical Research**
Gary Sharman, *Eli Lilly*, Guildford, UK

Statistical Filtering for NMR Based Structure Generation
Jochen Junker, INCT-IDN & CDTS/FioCruz, Rio de Janeiro, Brazil

10:30 AM - 11:00 AM Break

11:00 AM - 12:30 PM **Students and Postdocs**
Chair: Nicole Trease, *Stony Brook University, USA*

^{1}H NMR Based Metabolomics and Assessment of Antiproliferative Constituents of Black Raspberry
Liladhar Paudel, *The University of Akron*, Akron, OH, USA

Advanced qNMR Methods in the Analysis of Natural Products
Feng Qiu, University *of Illinois College of Medicine at Chicago,* USA

The Through Space Transmission of the ^{19}F-^{15}N Coupling Constant
Denize Favaro, University of Campinas, Campinas, SP, Brazil

Structural Diversity in Enamines and Iminium Ions Revealed by NMR
Michael Hammer, Chemistry Department, *University of Regensburg*, Germany

12:30 PM - 01:30 PM Lunch, Free Time & Vendor Discussions

01:30 PM - 03:00 PM **Beyond Conventional NMR Experiments for Determination of Configuration**
Chair: Roberto R. Gil, *Carnegie Mellon University*, USA

NMR, Chiroptics, X-ray and Synthesis for Small Molecule Relative and Absolute Configuration
Christian Griesinger, *Max Planck Institute for Biophysical Chemistry*, Goettingen, Germany

Helically Chiral Polyacetylenes as Enantiodifferentiating Alignment Media
Michael Reggelin, Clemens-Schopf-Institute for Organic Chemistry and Biochemistry, *Technical University of Darmstadt, Darmstadt*, Germany

Accurate NOE-Derived Interproton Distances - Fact or Fiction?
Catharine Jones, Department of Chemistry, *University of Bristol*, Bristol, UK

Fast Access to Residual Dipolar Couplings by Single-Scan 2D NMR in Oriented Media
Patrick Giraudeau, *Université de Nantes*, CNRS, Nantes, France

03:00 PM - 03:30 PM Break

03:30 PM - 05:00 PM Workshop/Tutorial (concurrent)

Automatic Structural Verification
Co-chairs: John Hollerton, *GlaxoSmithKline, UK;*
Gary Sharman, *Eli Lilly and Company Ltd, UK*

Pulse Programming Tutorial
Co-chairs: Krish Krishnamurthy, *Agilent, USA;*
Clemens Anklin, *Bruker BioSpin, USA*

05:00 PM - 06:30 PM **Poster Session**
Chair: Mike Bernstein, *Mestrelab Research, UK*

07:00 PM - 11:00 PM Dinner/After Dinner Speaker/Social Hour

Bioorganic Studies on Vision and Ginkgolides
Professor Koji Nakanishi, *Columbia University*, NY, USA

Tuesday, September 11th

07:30 AM - 08:45 AM Breakfast

09:00 AM - 10:30 AM **Solid-State NMR & Pharmaceuticals, It's Just What the Doctor Ordered**
Chair: Jochem Struppe, *Bruker BioSpin, USA*

^{51}V Solid-State NMR and Density Functional Theory: A Powerful Approach to Probe Geometry and Electronic Structure in Bioinorganic Molecules
Tatyana Polenova, Department of Chemistry and Biochemistry, *University of Delaware*, Newark, DE, USA

Amorphous Quantitation of Active Pharmaceutical Ingredients in Drug Products
Heather Frericks-Schmidt, *Pfizer*, Groton, CT, USA

Solid State NMR: an Essential Tool for Pharmaceutical Development
George Crull, *Bristol-Myer Squibb*, New Brunswick, NJ, USA

Crystal Structure Determination from High-Resolution Proton NMR and Crystal Structure Prediction
Maria Baias, *Université de Lyon*, Centre de RMN à Très Hauts Champs

10:30 AM - 11:00 AM Break

11:00 AM - 12:30 PM **J-Based Analysis and Measurement of Heteronuclear Couplings**
Chair: Thomas Williamson, *Merck, USA*

J-Based Configuration Analysis: Basic Concepts and Applications
Nobuaki Matsumori, Department of Chemistry, Graduate School of Science, *Osaka University*, Osaka, Japan

Quantum Chemical Calculation of *J* Coupling Constant in the Stereochemical Determination of Organic Compounds
Giuseppe Bifulco, Department of Pharmaceutical and Biomedical Sciences, *University of Salerno*, Salerno, Italy

Combining *J*-Based Configuration Analysis, Chemical Derivatization Studies and Organic Synthesis to Determine the Relative and Absolute Configurations of Complex Natural Products from Marine and Freshwater Algae
Alban R. Pereira, *Scripps Institution of Oceanography, University of California, San Diego/Gilead Sciences, Inc.*, CA, USA

Configuration and Conformation from fc-rDG/DDD: all in one shot
Matthias Köck, *Alfred-Wegener-Institute for Marine and Polar Research*, Germany

12:30 PM - 01:30 PM Lunch, Free Time & Vendor Discussions

01:30 PM - 03:00 PM **New Developments in Ultrawideband Pulses**
Chair: Steve F. Cheatham, *DuPont, USA*

Limits of Universal Rotation Pulses and Ultrabroadband NMR Spectroscopy
Burkhard Luy, *Karlsruhe Institute of Technology*, Karlsruhe, Germany

Constant Amplitude Broadband Refocusing Pulses from Numerical Optimization
Douglas Brown, *Indiana University*, Bloomington, Indiana, USA

Composite Pulses and Spin Echoes: The Effect of Symmetry
Stephen Wimperis, *University of Glasgow*, Glasgow, UK

Ultrafast NMR for Dummies
Meerakhan Pathan, *Université de Nantes*, France

03:00 PM - 03:30 PM Break

03:30 PM - 05:00 PM Workshop/Tutorial (concurrent)

Teaching Mass Spectrometry to NMR Spectroscopists
Chair: Charles Ross, *Merck*, West Point, USA

NMR of Other Nuclei
Co-chairs: Steve Cheatham, *DuPont*, USA
Gary Martin, *Merck*, USA

05:00 PM - 06:30 PM **Poster Session**
Chair: Mike Bernstein, *Mestrelab Research*, UK

07:00 PM - 11:00 PM Dinner/Awards/Social Hour

Wednesday, September 12th

07:30 AM - 08:45 AM Breakfast

09:00 AM - 10:30 AM **Potential Genotoxic Impurities**
Co-chairs: Karen Alsante, *Pfizer*, USA
Roberto R. Gil, *Carnegie Mellon University*, USA

The Use of NMR as an Efficient Tool for the Analysis of Potential Genotoxic Impurities
Andrew Phillips, *AstraZeneca*, UK

Evaluation of Residual 2-Vinylpyridine Levels in Axitinib
Greg Sluggett, *Pfizer*, CT, USA

10:30 AM - 11:00 AM Break

11:00 AM - 12:30 PM **Wisdom of the Crowd**
Chair: Bill Farrell, *Pfizer*, USA

12:30 PM - 01:30 PM Lunch, Free Time & Vendor Discussions

01:30 PM - 03:00 PM **Predictive Quantitation**
Co-chairs: Karen Alsante, *Pfizer*, USA
Bill Farrell, *Pfizer*, USA
Roberto R. Gil, *Carnegie Mellon University*, USA

Quantitative Structure Retention Relationship Modeling (QSRR): Comparative Investigation of the Predictive Capability of an L-PLS Model versus ACD Labs™ Commercial Software using a Typical Pharmaceutical Compound
Jim Morgado, *Pfizer*, Groton, CT, USA

From Understanding Organic Reactions to Speeding Up High Resolution MS Interpretation: A Multinuclear $^{31}P^{1}HU$- and $^{19}F^{1}HU$-qNMR Approach
Jonas Buser, *Eli Lilly and Company*, Indianapolis, IN, USA.

Integrated Spectrometric Analysis for the Quantitation of Complex Mixtures Exemplified with Natural Products
Jose G. Napolitano, *University of Illinois at Chicago*, IL, USA

03:00 PM - 03:15 PM Closing Remarks

03:30 PM - 05:30 PM **Career Development Workshop (Mixer)**
Co-chairs: Jeffrey Standish, *ACS*, USA
Betsy McCord, *E. I. du Pont de Nemours and Co.*, USA
Doug Kiehl, *Eli Lilly*, USA

Brian Stockman, *Adelphi University*, NY, USA

Aaron Wheeler, *University of Toronto*, Canada

Dorothy Phillips, *Waters Corporation*, USA

SMASH 2012 Scholarship Recipients

A Name You Can Trust

The following students received a scholarship to attend SMASH 2012

- **Cristhian Cañari,** Pontificia Universidad Católica del Perú, Perú
- **Christopher Connors,** Rensselaer Polytechnic Institute, United States
- **Christian Feldmeier,** University of Regensburg, Germany
- **Daniel Finkelstein-Shapiro,** Northwestern University, United States
- **Michael Hammer,** Universtiy of Regensburg, Germany
- **Erich Hellemann,** Universidad Nacional Autónoma de México, México
- **Alexis Krupp,** Technische Universität Darmstadt, Germany
- **Vanessa Leyva,** Pontificia Universidad Católica del Perú, Perú
- **Damjan Makuc,** National Institute of Chemistry, Slovenia
- **Beau Martini,** University of California at Irvine, United States
- **Nils-Christopher Meyer,** TU Darmstadt, Germany, Germany
- **Meerakhan Pathan,** University of Nantes, France
- **Pothual Reddy,** Indian Institute of Chemical Technology, India
- **Stefanie Sippl,** University of Wisconsin - La Crosse, United States
- **Eduardo Troche-Pesqueira,** Universidade de Vigo, Spain

Thanks to our scholarship sponsors for their generous support.

SMASH 2012 NMR Conference Acknowledgements

The SMASH 2012 Conference gratefully acknowledges the support provided by the following companies:

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Christina Thiele
Technische Universitat Darmstadt

Martin Will
Sanofi-Aventis

Monday, September 10th
9:00 AM - 10:30 AM

Calculations of NMR Parameters. Applications to Constitutional, Configurational and Conformational Analysis

Chair: Armando Navarro-Vazquez

Speakers:

Jonathan Goodman
Unilever Centre for Molecular Science Informatics, Cambridge, UK

Michael Lodewyk
UC Davis, Davis, CA, USA

Gary Sharman
Eli Lilly, Guildford, UK

Jochen Junker*
INCT-IDN & CDTS/FioCruz, Rio de Janeiro, Brazil

* Upgraded Poster

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Small Molecule Structures: How Can Calculations Help Experiments?

Jonathan M Goodman

Unilever Centre for Molecular Science Informatics, Department of Chemistry, University of Cambridge, Cambridge, UK

Calculating NMR spectra for small molecules is fairly routine; interpreting the results is not. The spectra of similar molecules are usually rather similar; calculated spectra for the same molecules will also be rather similar, but different to all of the experimental spectra. Our Bayesian-approach, DP4, gives a measure of the certainty of assignments and so both increases and quantifies the amount of useful information which can be obtained by combining experimental and simulated NMR spectra[1]. The method has been applied to a wide variety of similar molecules and has successfully distinguished many similar diastereoisomers and isomers.

1. Steven G. Smith, Jonathan M Goodman *J. Am. Chem. Soc.* **132**, 12946-12959, 2010.

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Quantum Mechanical Approaches to Chemical Shift Prediction

Michael W. Lodewyk and Dean J. Tantillo

University of California, Davis; Davis, CA, USA

With appropriate techniques and considerations, high accuracy chemical shift calculations are both feasible and readily affordable, and can be applied to a variety of molecules, including complex natural products.[1] Common applications include structure determination (confirming expected structures, elucidating unknown structures, and correcting mis-assigned structures), as well as spectra assignment, conformational analysis, and characterization of reactive intermediates. In many cases, very closely related diastereomers or regioisomers can be analyzed and distinguished using these techniques. Recent examples applied to natural products include nobilisitine A,[2,3] welwitindolines,[4] and yohimbinoids.[5]

While quantum mechanical NMR calculations often suffer from a significant amount of inherent error, several techniques allow for the efficient reduction of this error. These techniques include solvent modeling, conformational averaging, empirical correction, and use of alternate reference compounds. When used in conjunction, these techniques often reduce the error to near-negligible amounts (sometimes on the order of experimental uncertainty), and allow affordable calculations to be applied successfully to a variety of systems of interest, including closely related isomers.

The application of quantum mechanical NMR chemical shift calculations to natural product and other small molecule systems will be presented. The techniques listed above will be discussed in the context of specific examples from our research. Discussion of the theoretical basis of these calculations will be limited to the points which apply directly to improvement of results.

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Ab initio Chemical Shift Calculations as a Tool within Pharmaceutical Research

Gary J. Sharman[1], Paul Tan[1], James Tunaley[2], and Juliet Morgan[1]

1. Eli Lilly & Co., Erl Wood Manor, Windlesham, Surrey, UK
2. Chemistry Department, University of York, UK

NMR chemical shift prediction via databases has long been a useful tool to elucidate or verify chemical structures from experimental NMR data. However in recent years, advances in computing power and software have made the alternative approach of *ab initio* prediction much more feasible. This approach has the advantage that it does not require a reference database and as such can be particular useful for novel structural motifs. This talk will cover validation and determination of the accuracy of the technique, as knowing the accuracy of calculations is key to determining if differences are significant or not. It will also cover examples of structure elucidation and problem solving from a pharmaceutical research enviroment for a variety of nuclei.

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Statistical Filtering for NMR Based Structure Generation

Jochen Junker

INCT-IDN & CDTS/FioCruz, Rio de Janeiro, Brazil

Nuclear Magnetic Resonance is the most common tool used for the structure elucidation of new compounds. The used 2D NMR experiments like COSY, HSQC, and ^{13}C-HMBC deliver correlation information between atoms that can be translated into connectivity information. Out of these, correlation information from COSY and HSQC experiments can be transcribed directly into connectivity between atoms. But the ^{13}C-HMBC correlations need more attention because of their ambiguity and complexity. Hence the difficulty of the structure elucidation problem depends more on the type of the investigated molecule than on its size. This ambiguity has driven the development of different software packages like COCON to aid in the interpretation of the ^{13}C-HMBC correlation data [1-19] as much as the development of additional correlation experiments [20,21].

In the case of unsaturated molecules COCON [3, 21-24]Â will usually generate a very large number of possible solutions. Since the solutions will then have to be checked manually for their chemical feasibility and sense, different efforts have been made to reduce the number solutions. The statistical filter shown compares the suggested constitutions against existing molecules, like the ones contained in the PubChem database. For each Cocon suggested constitution all 1 sphere elements of the constitutions are checked for corresponding elements in PubChem. This comparison is done indirectly, by generating molecular dynamics parameters in smi23d, which has been used to generate 3D coordinates for almost 13M compounds contained in PubChem and succeeded on generating coordinates for 99.6% of the molecules contained in the Database. The filtering application eliminates those constitutions for which smi23d fails because of lacking MD parameters.

Since smi23d has successfully been used on so many well known compounds, this means that the structural element for which parameters were missing has hardly ever been observed and therefore might not exist in natural products. Due to the nature of the filter, no ranking of the remaining constitutions is carried out and further methods might be necessary to improve the results.

The filter has been used with several different molecules on the WebCocon Server, and a webpage with some results has been made available on the server: http://cocon.nmr.de/StatisticalFilter/. Several molecules have been analized, for some, like Ascomycin, the filter showed no reduction in the number of solutions. For others, like Aflatoxin B1, the number of suggested constitutions when using only COSY and ^{13}C-HMBC data, dropped from 970 to 539, a reduction of 45%.

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Monday, September 10th
11:00 AM - 12:30 PM

Students and Postdocs

Chair: Nicole Trease

Speakers:

Liladhar Paudel
The University of Akron, OH, USA

Feng Qiu
College of Pharmacy, University of Illinois at Chicago, IL, USA

Denize Favaro
University of Campinas, Campinas, SP, Brazil

Michael Hammer*
Chemistry Department, University of Regensburg, Germany

* Upgraded Poster

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^{1}H NMR Based Metabolomics and Assessment of Antiproliferative Constituents of Black Raspberry

Liladhar Paudel[1], Peter L. Rinaldi[1], Faith J. Wyzgoski[2], Joseph C. Scheerens[3], R. Neil Reese[4], M. Monica Giusti[5], Jodee Johnson[5], Ann M. Chanon[3] , James K. Hardy[1], Joshua Bomser[6], Danijela Smiljanic[1], Chrys Wesdemiotis[1], A. Raymond Miller[3], and Artemio Z. Tulio Jr.[7]

1. Department of Chemistry, The University of Akron, Akron, OH, USA
2. Department of Chemistry, The Ohio State University-Mansfield, Mansfield, OH, USA
3. Department of Horticulture and Crop Science, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH, USA
4. Department of Biology and Microbiology, South Dakota State University, Brookings, SD, USA
5. Department of Food Science and Technology, The Ohio State University, Columbus, OH, USA
6. Department of Human Nutrition, The Ohio State University, Columbus, OH, USA
7. U. S. Food and Drug Administration, Summit-Argo, IL, USA

The classical biochemical and pharmaceutical methods of identifying active components from natural sources rely on bioassay guided reductionist approaches. Use of these methods to develop novel drug formulations is very difficult, expensive and time-consuming. Moreover, these methods may have significant limitations to elucidate synergisms of activity [1], which is more likely the cause of higher efficacy of whole fruits as compared to a single fraction or compound [2].

Studies using animal and human subjects [3,4] have shown that black raspberries (*Rubus Occidentalis* L.) have chemopreventive properties, most notably with regard to aero-digestive cancer. Seeking to better understand the health benefits of these berries, a NMR-based metabolomics model was initially developed to determine active chemical constituents of the berry fruits and to discern the relationships between the constituents with respect to total monomeric-anthocyanin content (TMA), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays using partial least squares (PLS) regression analysis [5]. Through this model active components were ascertained and their contributions were analyzed with regard to assay type and sample variation. Results showed that anthocyanins, cyanidin 3-O-rutinoside, cyanidin 3-O-xylosylrutinoside and cyanidin 3-O-glucoside were significant contributors to the variability in assay results. Many other statistically important bins from minor components were common among assay models indicating further investigation using a more rigorous model system.

To allow more efficient studies of biologically active constituents of foods and natural product pharmaceuticals, a metabolomics approach comparing the ^{1}H-NMR spectra of relatively crude black raspberry fruit extracts against HT-29 colon cancer cell proliferation have been developed. This approach takes advantage of genetically and environmentally induced variation in secondary metabolites [6] and results in the description of biological activities of specific compounds, anthocyanins, as well as, nonanthocyanins. After the ^{1}H NMR regions responsible for these activities had been identified by the model, advanced 1D and 2D NMR techniques were used to verify molecular structures of the compounds. To unequivocally identify minor nonanthocyanin components, black raspberry fractions rich in these compounds were collected by semi-preparative HPLC then subjected to advanced 1D, and 2D-NMR, HPLC-ESI-MS and MS/MS analyses. When all of these analyses of the fractions

were combined with our metabolomics model, in addition to anthocyanins, a variety of nonanthocyanin compounds, including a citrate type moiety, salicylic acid glucosyl ester, benzoic acid glucosyl ester, salicylic acid derivatives without attached sugar, quercetin 3-glucoside, quercetin 3-rutinoside, *p*-coumaric acid, epicatechin, *trans*-piceid, and methyl ellagic derivatives, were found to be important contributors to the antiproliferative activities of black raspberries. Moreover, our research demonstrates how NMR and mass spectral techniques can be used to analyze highly variable fruit extracts and provide valuable information when assessing their phytomedical and nutraceutical benefits.

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NMR Pattern Recognition Enables Dereplication of Natural Products

Feng Qiu[1], Ayano Imai[1], James B. McAlpine[1], David C. Lankin[1], Ian Burton2 Tobias Karakach[2], Norman R. Farnsworth[1,3], Shao-Nong Chen[1] and Guido F. Pauli[1]

1. Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA
2. Institute for Marine Biosciences, National Research Council, Halifax, Nova Scotia, Canada
3. Deceased on September 10, 2011

The rapid identification of known natural products, a process known as dereplication, is important for targeting isolation of compounds of interest from natural resources. While ^{1}H NMR has received much attention for the analysis of complex natural products mixtures, the interpretation of spectra remains a rather challenging task due to severe peak overlap. The structural characteristics of any single chemical entity (SCE) are represented by a unique pattern of signals in the 1D/2D ^{1}H NMR spectra. Much like biometric recognition, a sub-portion of these complex spectroscopic patterns might be sufficient to distinguish the different chemical entities. Imitating modern biometric systems, novel computer-aided dereplication (CAD) tools using 1D/2D (q)HNMR combined with pattern recognition techniques have been recently developed and validated in our lab for qualitative and quantitative identification of natural products in mixtures. This talk exemplifies practical applications of these tools in the dereplication of cycloartane triterpenes from Actaea plants. While Actaea triterpenes are major bioactive constituents of complementary and alternative medicines, their structural similarity creates a major dereplication problem. Based on an in-house NMR database of these triterpenes, two computational models using canonical discriminate analysis (CDA) and classification binary trees (CBTs) were developed for in silico determination of the aglycone types. These two tools utilize the Me ^{1}H NMR chemical shifts as partial structural indicators and have high accuracy in structural dereplication and prediction of Actaea triterpenes. Moreover, taking advantage of the enhanced LOD of a 700 MHz 1.7 mm cryo-microprobe spectrometer, an HMBC-based approach was developed to identify minor constituents in mass-limited samples. This method is based on computer-assisted pattern recognition of characteristic HMBC correlations of the methyl protons. The power of this tool is exemplified by the simultaneous identification of five co-occurring minor constituents, belonging to four different triterpene skeleton types, in a 0.4 mg sample. The concept of NMR pattern recognition has great potential to be applicable to other natural products in structural dereplication as well as metabolomic profiling.

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The Through Space Transmission of ^{19}F-^{15}N Coupling Constant

Denize C. Favaro and Cláudio F. Tormena

Chemistry Institute, State University of Campinas, Brazil

Indirect spin–spin coupling constants (SSCCs) has an important role in the structural studies of molecules, independently of the nature (organic or organ metallic) or length (small molecules, peptides or proteins). Until 1970's decade were believed that the transmission of the scalar coupling could occur just through electrons of the covalent bonds.[1] However, a great number of experimental and theoretical studies have been showed that the transmission of the nuclear information can occur *"Through Space (^{TS}J)"*. These couplings cover an extensive range of values (4 Hz until 800 Hz), depending of the nucleus involved.[2,3]

Fluorine nucleus (^{19}F) is a spin-½ species, which exits in 100% natural abundance and possesses the second greatest magnetogyric ratio, excluding tritium, this high sensitivity makes ^{19}F a probe in a large number of studies involving protein structure, dynamics, and function. While traditional ^{1}H, ^{13}C, and ^{15}N solution state NMR protocols provide a wealth of data on protein structure and dynamics, ^{19}F provides a unique perspective of conformation and topology.[4] With this in mind, we focus our attention in rationalizing the mechanisms involved in the transmission of the coupling between ^{19}F-^{15}N using like model compounds fluoroximes rigid. Mallory et al [3] studied these couplings however any detailed rationalization was assessed. In this way, we obtained theoretically the most stable geometries and also analyzing the molecular electronic structure through, Natural Bond Orbitals (NBO) and the Quantum Theory of Atoms in Molecules (QTAIM) methodologies. Several compounds was taking into account in the present studied, such as 2-fluorobenzaldehyde oxime, (E)-8-fluoro-5-methyl-3,4-dihydronaphthalen-1(2H)-one oxime, (E)-7-fluoro-4-methyl-2,3-dihydroinden-1-one oxime and (E)-9-fluoro-4-methyl-2,3-dihydrocyclopenta[α]naphthalen-1-one oxime. It has been observed in the present study that J_{FN} coupling constant is dependent not only for the distance between coupled nuclei as suggested by Mallory [3], but the most important effect involved in the transmission of J_{FN} coupling is related to the overlap between LP(F) and LP(N), which is dependent of the orientation of fluorine and nitrogen. In the case where FC term is the main mechanism involving into transmission the % s character of LP(F) is also important.

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Structural Diversity in Enamines and Iminium Ions Revealed by NMR

Michael Hammer and Ruth M. Gschwind

Chemistry Department, University of Regensburg, Germany

The knowledge of structural features of intermediates is crucial to the understanding of the outcome of most stereoselective chemical reactions.[1-5] Structural information of intermediates in a variety of organocatalytic transformations have been gained using a broad scope of NMR methods. With the aid of espeacially NOESY spectroscopy surprising[6] structural diversity has been revealed in a huge number of investigated intermediates. A broad substrate scope (carbonyl compounds) as well as a broad catalyst scope (mainly proline based secondary amines [7]) was investigated in a variety of solvents. Temperature dependency as well as concentration dependency and the influence of additional water present in the reaction mixture was studied intensively. We found that enamines derived from proline-based catalysts and α-unbranched aldehydes show, depending on the catalyst structure, either a high conformational lock or a high conformational diversity. Prolinol (ether) catalysts exhibit strong conformational lock[8] whereas proline, a proline tetrazole catalyst[9] and prolinol show strong stuctural fluctuations wihtin the investigated, aldehyde derived, enamines. We found almost uniform population of the s-cis as well as the s-trans isomer (concerning the newly formed N-C bond) of aldehyde derived enamines with these catalysts as well as strong fluctuations concerning the puckering of the five-membered ring in the intermediate structure. Investigations concerning ketones as carbonyl species yielded iminium ions which were investigated thouroughly for the first time in solution. The E/Z ration was determined and correlated with the structure of the substrate as well as the catalyst. Kinetic investigations of the iminium-ion formation were done via a rapid-injection NMR technique and the relative reactivity of these iminium ions in the classical aldol reaction was investigated. In the case of a α-cyclic aldehyde, namely cyclopropanecarboxyaldehyde unprecedented free rotation around the C=N(+) bond was revealed within the iminium species.

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Monday, September 10th
1:30 PM - 3:00 PM

Beyond Conventional NMR Experiments for Determination of Configuration

Chair: Roberto R. Gil

Speakers:

Christian Griesinger
Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

Michael Reggelin
Clemens-Schopf-Institute for Organic Chemistry and Biochemistry, Technical University of Darmstadt, Darmstadt, Germany

Catharine Jones
Department of Chemistry, University of Bristol, Bristol, UK

Patrick Giraudeau*
Université de Nantes, CNRS, Nantes, France

* Upgraded Poster

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NMR, Chiroptics, X-ray and Synthesis for Small Molecule Relative and Absolute Configuration

Han Sun[1], Manuel Schmidt[1], Edward d'Auvergne[1], Michael Müller[1], Matthias Köck[2], Armando Navarro-Vázquez[3], Jikai Liu[4], Andrei Leonov[1], Uwe M. Reinscheid[1], and **C. Griesinger[1]**

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3. Organic Chemistry Department, Universidade de Vigo, Vigo, Spain
4. Kunming Institute of Botanic, Chinese Academy of Sciences, Kunming, China

The combination of NMR spectroscopy with chiroptical methods is especially powerful for the elucidation of the relative and absolute configuration of flexible molecules summarized with published examples from my laboratory in ref. [1]. For mefloquine, the anti-malarial drug Lariam administered as racemate, it has been shown that the (+)-enantiomer has less dramatic side effects compared to the (-)-enantiomer, leading to a patent filed [2]. However, there are two enantioselective syntheses [3] which came to the opposite conclusion as a study based on anomalous X-ray diffraction [4] as well as the combination of residual dipolar coupling enhanced NMR with chiroptical methods [5]. Since physical methods (NMR & chiroptics as well as X-ray) delivered the same answer but opposite to two syntheses, we derivatized mefloquine with Mosher ester and conducted an enantioselective synthesis ourselves confirming the NMR & chiroptics as well as X-ray assignment. The example is interesting since enantioselective syntheses even with established reactions may not deliver the correct configuration of a molecule. Further examples: LLG1 and Comp450a will be given. Finally, a medium for chiral distinction of amines is shown.

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Helically Chiral Polyacetylenes as Enantiodifferentiating Alignment Media

Michael Reggelin, Nils-Christopher Meyer and Alexis Krupp

Technische Universität Darmstadt, Clemens Schöpf Institut für Organische Chemie und Biochemie, D-64287 Darmstadt, Germany

Lyotropic liquid crystalline phases of helically chiral polyacetylenes[1, 2] containing amino acid moieties (valine and phenylalanine decylester) in the repeating units are introduced as new enantiodifferentiating alignment media. Both phases are suited to align small molecules like isopinocampheol (IPC) and strychnine in $CDCl_3$ and toluene solutions to such an extent that the extraction of CH-one-bond couplings in the range of ±15 Hz is possible using F2-coupled HSCQ-type Experiments.[3] Moreover, the alignment tensors calculated for (+)- and (-)-IPC are very different as judged from the small value (down to 0.07) of the generalized cosine β between them.[4] Based on the unusual temperature dependence of the quadrupolar splitting of the ^{2}H-signal of the solvent ($CDCl_3$), observed with the phenylalanine containing polymer, three distinct states of the LLC-phase with different orientational properties can be identified. This offers the opportunity to measure multiple alignment data sets without changing the sample.

Unexpectedly, the largest difference in the orientation of the enantiomers of IPC was found in the high temperature domain of the phase which is even more astonishing, because at temperatures above approximately 10°C the helical structure of the polymer backbone breaks down leaving only the stereogenic centers of the amino acid moieties in the repeating units as reason for the enantiodifferentiation. Finally, at the same temperature, the orientation induced by the two polymers is very different for a given analyte, allowing the preparation of a set of independently aligning media just by a simple exchange of the amino acid used for the synthesis of the monomer.

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Accurate NOE-Derived Interproton Distances – Fact or Fiction?

Catharine R. Jones and Craig P. Butts

School of Chemistry, University of Bristol, Bristol, UK

The determination of accurate interproton distances in solution using nuclear Overhauser effect (NOE) data is an area of significant interest and complexity – the large majority of approaches rely on full relaxation matrix analysis of these data. We present a much simpler method that can be used to derive accurate interproton distances from within rigid small molecule systems using 1D or 2D NOESY data.

Establishing an Initial Level of Accuracy

Strychnine is used as a model system to test the validity of this method. A comparison between the 1D NOE-derived distances and the best solvent-corrected gas-phase structure of strychnine [1] produces a mean absolute error of only 2.3% (0.07Å) [2].

Low Level Conformer Identification

A second low-level conformer of strychnine is subsequently identified experimentally by the NOE data and confirmed by computation, thereby demonstrating the potential of accurate NOE measurements to determine minute contributions to structure ensembles in solution [3].

Stereochemical Determination

The relative configuration of the doubly quaternary epoxy functional group in a rigid marine natural product, conicasterol F, is determined using a quantitative NOE analysis [4]. This problem is particularly challenging for stereochemical determination due to the five contiguous, quaternary stereogenic centres around the epoxy group, and as such, classical NMR data analyses ($^3J_{HH}$, qualitative NOEs) fail to provide unequivocal results.

This technique is further applied to the conformationally-flexible example of arugosin C. An accurate NOE-distance treatment coupled with conformational analysis enables the successful assignment of the relative configuration of this natural product [5].

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Fast Access to Residual Dipolar Couplings by Single-Scan 2D NMR in Oriented Media

Patrick Giraudeau[1], Tobias Montag[2], Benoît Charrier[1] and Christina M. Thiele[2]

1. Université de Nantes, CNRS, CEISAM UMR 6230, Nantes, France
2. Technische Universität Darmstadt, Clemens Schöpf Institut für Organische Chemie und Biochemie, Darmstadt, Germany

In the last 20 years, NMR spectroscopists have designed numerous approaches dealing with the time drawback of multi-dimensional NMR experiments. One of the most impressive and efficient is probably the “ultrafast” (UF) 2D NMR methodology [1], capable of providing a complete 2D correlation in a single scan. In the last six years, we have significantly improved the performances of this approach [2,3], thus highly increasing its range of applications. Recent examples will be shown, illustrating the variety of small molecule applications where UF NMR can be useful (study of fast reactions [4], coupling with HPLC [5], metabolic studies [6], etc.)

This presentation will particularly focus on an original NMR method that we recently developed to measure Residual Dipolar Couplings in a very short time, by UF 2D NMR experiments performed for the first time in oriented media. The utility of residual dipolar couplings for determining the structure of small organic molecules is increasingly recognized [7], however their measurement suffers from the long experiment times characterizing 2D NMR experiments. If one would want to investigate transient species, these measurement times need to be reduced significantly.

In this study, we applied the UF methodology to isotropic and anisotropic isopinocampheol (IPC) samples. In the former case, the 2D HSQC spectrum was recorded in a single scan, while for the anisotropic sample, signal averaging was performed to obtain an optimum sensitivity, resulting in a 60 seconds total experiment time, which is still two orders of magnitude lower than the duration of the conventional experiments generally used to measure RDCs. The dipolar couplings extracted from these experiments are in very good agreement with those obtained from conventional experiments. These results highlight the potentialities of ultrafast 2D NMR as a tool for determining the configuration of organic compounds, and open promising perspectives in the field of small molecule NMR analysis.

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Monday, September 10th
3:30 PM – 5:00 PM

Workshop: Automated Structure Verification

John Hollerton, GlaxoSmithKline, UK
Gary Sharman, Eli Lilly and Company, UK

Tutorial: Pulse Programming Tutorial

Krish Krishnamurthy, Agilent Technologies, USA
Clemens Anklin, Bruker BioSpin, USA

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Automated Structure Verification

John Hollerton[1] and Gary Sharman[2]

1. GlaxoSmithKline, UK
2. Eli Lilly and Company, UK

There has been significant interest in using computational means to verify that NMR data is consistent with a proposed chemical structure. This has been (possibly inaccurately) called Automated Structure Verification or "ASV". There are a number of commercial packages available to attempt this. There will be technical representatives from the major players in this area, together with people who have a long history of work in ASV.

This workshop looks at the challenges associated with ASV and will allow a full discussion with the software vendors. We will look at issues such as the acceptable levels of false negative and false positive results for different uses of ASV and what outcomes people expect from such software.

Expect this to be a lively debate (judging by the previous SMASH workshop on this topic!).

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Pulse Programming Tutorial

Krish Krishnamurthy[1] and Clemens Anklin[2]

1. Agilent Technologies, Inc., Santa Clara CA
2. Bruker BioSpin Corp., Billerica MA

This workshop will provide an overview of pulse sequence architecture in both Bruker and Agilent software environments along with a discussion of tools and functions available to modify existing and/or write new pulse sequences. The workshop will have short presentations as introduction from scientists from both applications labs and will be primarily driven by Q&A.

Participants are encouraged to submit suggestions for specific topcis or questions to the workshop chairs prior to the conference.

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Tuesday, September 11th
9:00 AM - 10:30 AM

Solid-State NMR & Pharmaceuticals, It's Just What the Doctor Ordered

Chair: Jochem Struppe

Speakers:

Tatyana Polenova
Department of Chemistry and Biochemistry, University of Delaware, DE, USA

Heather Frericks-Schmidt
Pfizer Inc., CT, USA

George Crull
Bristol-Myer Squibb, NJ, USA

Maria Baias*
Université de Lyon, Centre de RMN à Très Hauts Champs

* Upgraded Poster

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^{51}V Solid-State NMR and Density Functional Theory: A Powerful Approach to Probe Geometry and Electronic Structure in Bioinorganic Molecules

Tatyana Polenova

University of Delaware, Department of Chemistry and Biochemistry, Newark, DE, USA

In this presentation, I will give an overview of a hybrid ^{51}V solid-state NMR spectroscopy / Density Functional theory approach for probing geometry and electronic structure in vanadium (V) containing bioinorganic molecules. I will discuss the exquisite sensitivity of the anisotropic NMR parameters, electric field gradient (EFG) and chemical shift anisotropy (CSA) tensors, to the local environment [1], and illustrate how the combined use of experiment and theory yields in-depth information about the salient structural and electronic features determining chemical reactivity in a wide array of vanadium (V) complexes exhibiting interesting functions ranging from catalysis to anti-diabetic activity. This synergistic approach provides geometric and electronic-structure information inaccessible from single crystal X-ray diffraction studies, such as protonation states of the metal and the ligands, and reveals how the ligand and counter ion environments modulate the molecular orbital energies [2-5]. I will introduce experiments that permit recording internuclear distances between the half-integer quadrupolar vanadium (I=7/2) and the ligand atoms (such as ^{15}N) [6], providing additional structural constraints particularly important in noncrystalline systems. I will describe how to derive detailed structural information in the mixed-valence V(V)/V(IV) molecules containing sparsely dispersed paramagnetic sites, using electron-nuclear dipolar interaction [7-9].

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Amorphous Quantitation of Active Pharmaceutical Ingredients in Drug Products

Heather Frericks-Schmidt

Pfizer, Inc. Research and Development, Groton, CT, USA

The bioavailability, bioequivalence, chemical stability and physical stability of active pharmaceutical ingredient (API) are dependent on the physical solid form. API and drug product processing steps can convert crystalline solids to another crystalline form, or more commonly to an amorphous form. Amorphous API content can be generated during milling, spray drying, wet granulation, compaction, etc. Consequently, regulatory agencies require proof that the physical solid form is controlled in the final drug product. Solid state NMR can identify the solid form in the drug product and with careful consideration, quantify the content. I will present carbon and fluorine ssNMR methods to determine the relative quantity of amorphous and crystalline API in drug products. Using fluorine ssNMR the physical stability of an amorphous API-polymer dispersion was studied as a function of the formulation components. Proton decoupled fluorine magic angle spinning (MAS) experiments and spectral deconvolution were used to quantify crystalline growth (down to 1% crystalline API) in temperature and humidity stressed samples. These results identified the most physically stable formulation. In the second study carbon ssNMR methods verified Raman results of the amorphous content generated during wet granulation and spray coating processes in drug products containing 4.8% and 6.1% API loading. Carbon cross polarization MAS spectra, optimized for sensitivity, were acquired on calibration standards, process mapping tablets and stability tablets. The spectra were analyzed by three quantitative methods, with chemometric modeling having the best linearity, lowest error and best agreement with Raman results.

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Solid State NMR: an Essential Tool for Pharmaceutical Development

George B. Crull

Drug Product Science and Technology, Bristol-Myers Squibb, New Brunswick, NJ, USA

When one considers the tools required to select and manufacture a Pharmaceutical several come to mind, e.g. pXRD, HPLC, or Dissolution, but this presentation will highlight the importance of ssNMR in: the selection of Form, selection of state (crystalline vs. amorphous), the control of polymorphs, the application to formulated materials, and support for stability studies and marketed materials. Regulatory and patent agencies are requesting better characterization of the specific polymorph and conformation of salt form prior to approval. These details are frequently only available from ssNMR studies due to the low drug loading and complex formulations typical of new pharmaceuticals. ssNMR of amorphous pharmaceuticals is of growing importance as many compounds have low solubility. Because NMR is inherently quantitative if is frequently applied as the Primary method to support Secondary techniques, e.g. IR, NIR or Raman. This application of ssNMR continues to grow. Specific examples using ssNMR of H-2, C-13, F-19, N-15 and P-31 will be presented and where possible supported by pXRD or optical spectroscopy. No other tool contributes to as many aspects of the Pharmaceutical Development process as ssNMR.

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Crystal Structure Determination from High-Resolution Proton NMR and Crystal Structure Prediction

Maria Baias[1], Jean-Nicolas Dumez[1], Graeme M. Day[2], Elodie Salager[1], Sonia Augado[1], Jerome Canivet[3], Virginie Moizan-Basle[4], David Farrusseng[3], Anne Lesage[1], and Lyndon Emsley[1]

1. Université de Lyon, Centre de RMN à Très Hauts Champs, Villeurbanne, France
2. Department of Chemistry, University of Cambridge, Cambridge, UK
3. IRCELYON, Institut de Recherches sur la Catalyse et l'Environnement de Lyon, Villeurbanne, France
4. IFP Energies nouvelles, Solaize Cedex, France

A protocol for ab initio structure determination of powdered solids at natural isotopic abundance by combining solid-state NMR spectroscopy, crystal structure prediction and DFT chemical shift calculations is used to determine the crystal structures of a series of drug molecules: flutamide, flufenamic acid, cocaine and theophylline. For flutamide and flufenamic acid we find that assigned ^{1}H isotropic chemical shifts provide sufficient discrimination so as to determine the correct structures from a set of predicted structures using the root-mean-square deviation between experimentally determined and calculated chemical shifts. In both cases neither unassigned ^{1}H shifts nor ^{13}C shifts could be used to determine the structures. The correct structures were determined to within an atomic rmsd of less than 0.12 Ã… with respect to the known structures. For theophylline the NMR spectrum is too simple to allow unambiguous structure selection. We show how the information contained in the NMR chemical shifts is complementary (orthogonal) to PXRD. Combining ^{1}H NMR chemical shift, CSP/DFT and PXRD leads to a very robust structure determination protocol, with no hypothesis, and no bias. We also use this procedure to determine for the first time the crystal structure of a pharmaceutically pertinent molecule with previously unknown structure, where previous attempts by X-ray diffraction alone had not been successful.

Combining ^{1}H solid-state NMR spectroscopy with first-principles calculations can also be applied to the crystal structure determination of metal organic frameworks. We demonstrate this by reporting the discovery of the previously unknown crystal structure of a novel porous imidazolate substituted metal organic framework with possible applications as a H2 storage material. ^{1}H NMR experiments provided a description of the proton environments within the MOF, which in combination with DFT chemical shift calculations and PXRD led to the elucidation of the complete crystal structure.

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Tuesday, September 11th
11:00 AM - 12:30 PM

J-Based Analysis and Measurement of Heteronuclear Couplings

Chair: Thomas Williamson

Speakers:

Nobuaki Matsumori
Department of Chemistry, Graduate School of Science, Osaka University, Osaka, Japan

Giuseppe Bifulco
Department of Pharmaceutical and Biomedical Sciences, University of Salerno, Salerno, Italy

Alban R. Pereira
Scripps Institution of Oceanography, UCSD/Gilead Sciences, Inc., USA

Matthias Köck*
Alfred-Wegener-Institute for Marine and Polar Research, Bremerhaven, Germany

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J-Based Configuration Analysis: Basic Concepts and Applications

Nobuaki Matsumori and Michio Murata

Department of Chemistry, Osaka University, Osaka, Japan

Since the *J*-based configuration analysis (JBCA) method was reported in 1999 [1], the analysis has become a standard method widely used for the relative configuration determination of polysubstituted acyclic natural products [2]. The JBCA is based on the dihedral angle dependence of spin-coupling constants including germinal and vicinal carbon-proton couplings ($^{2,3}J_{C,H}$). This method assumes that conformations of each C-C bond can be represented by three staggered rotamers. This seems reasonable for acyclic carbon chains with hydroxy and methyl substitutions, which, unlike bulky substituents, usually cause no significant deviations from the staggered conformations. In the case of 1,2-dimethine system, the JBCA method can specify a single rotamer out of six staggered rotamers possible in *threo* and *erythro* diastereomers, thus leading to the assignment of relative stereochemistry. Similarly, the relative configuration of 1,3-dimethine system can be established by the JBCA method via stereospecific assignment of diastereotopic methylene protons. The JBCA method is also applicable to multiple conformation system. In most cases, the multiple conformation equilibrium is described by the interconversion between two staggered rotamers of each C-C bond, and this method can identify the two equilibrating rotamers.

This method was first applied to maitotoxin [3], the largest secondary metabolite known to date, dysiherbaine [4], and amphidinol 3 [5]. Since the method was systematized in 1999 [1], this method has been used for structure determination of wide variety of natural products [2]. During the course of its application, the method has been modified according to each compound. For example, the application of the *J*-based method had extended from stereocenters bearing methyl and hydroxy groups to those having amines or chlorides [2]. However, we have also made some mistakes in stereochemical assignments by the JBCA method [6], largely because the assignments were made on the basis of insufficient *J*-coupling information. In this context, introduction of vicinal carbon-carbon coupling constants ($^{3}J_{C,C}$) is an effective measure to make this method more robust and to extend its applicability to asymmetric quaternary carbon centers.

Besides, around the same time, Kishi et al reported universal NMR database method, which provided another tool for determining relative stereochemistry of natural products [7]. Combined use of the *J*-based method and universal NMR database surely provides a more practical way for stereochemical analysis of natural products.

In this presentation, the basic concepts and applications of the *J*-based configuration analysis will be overviewed

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Quantum Chemical Calculation of *J* Coupling Constant in the Stereochemical Determination of Organic Compounds

Giuseppe Bifulco

Department of Pharmaceutical and Biomedical Sciences, University of Salerno, Salerno, ITALY

The correct assignment of the configurational pattern in chiral organic compounds, containing more than one stereocenter, is undoubtedly a key step of the structure elucidation process. The stereochemical knowledge, in fact, is essential in several fields including the total synthesis of the molecules under investigation, and the understanding, at the molecular level, of the biological mechanism of active natural compounds. In this field, our research group has developed an approach based on the comparison between the experimental and quantum mechanical (QM) calculated NMR parameters for the structural studies of natural products [1, 2]. In particular, this approach can be used for the full prediction of the common 2D ^{1}H–^{1}H COSY, 2D ^{1}H–^{13}C HSQC, and 2D ^{1}H–^{13}C HMBC NMR spectra [3], and such spectra may be helpful in addressing different issues regarding the conformational and/or configurational analysis of organic compounds. Furthermore, we have developed a QM/NMR approach inspired by the original *J*-based method proposed by Murata [4] which has been shown to be very helpful for the relative configurational assignment of different natural products [5]. In the course of the last decade, in fact, this method was used in the structure elucidation of organic molecules with different chemical frameworks such as marine peptides (callipeltins [6], celebesides [7], perthamides [8] and mutremdamide A [9]), plant glycosides (stemmosides [10]), marine triterpenoids (aplysiols [11]), pyrrole-imidazole alkaloid (palau'amine [12]) and antibiotics belonging to the enediyne class (kedarcidin chromophore [12]). More recently, we have developed, by means of experimental and quantum chemical calculated coupling constants, a general carbon-proton vicinal coupling constant ($^{3}J_{C\text{-}H}$) prediction equation [13] including the electronegativity effect of the substituents attached to the ^{13}C-C-C-^{1}H fragment and the dihedral (Φ) dependence of the heteronuclear spin-coupling.

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Combining *J*-Based Configuration Analysis, Chemical Derivatization Studies and Organic Synthesis to Determine the Relative and Absolute Configurations of Complex Natural Products from Marine and Freshwater Algae

Alban R. Pereira[1], Christopher D. Vanderwal[2] and William H. Gerwick[1]

1. Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA, USA
2. Department of Chemistry, University of California, Irvine, CA, USA

Natural products play a dominant role in the discovery of leads for drug development. Roughly 70% of all approved therapeutic agents in the last 30 years are somehow inspired by a natural product [1]. Within this field, marine and freshwater-derived chemical entities play an increasingly important role in the treatment of human disease [2,3]. *J*-based configuration analysis was used in combination with traditional derivatization studies and organic synthesis to determine the relative and absolute configurations of two classes of highly bioactive natural products. Hoiamide A [4], the first member of a family of mixed polyketide/non-ribosomal peptide macrocycles isolated from marine cyanobacteria, is a partial agonist of the voltage-gated sodium channels. Danicalipin A [5] and Malhamensilipin A [6,7], chlorosulfolipids isolated from freshwater chrysophytes, captured the attention of synthetic chemists in the past decade as members of this compound class appeared to be causative agents of Diarrhetic Shellfish Poisoning. These small molecules represent promising drug leads and/or useful cell biology tools for further pharmacological examination. A summary of experimental observations and conclusions associated with the configurational analyses of these complex natural products will be presented.

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Configuration and Conformation from fc-rDG/DDD: all in One Shot

Matthias Köck

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The determination of the relative and absolute configuration of natural products is essential to understand their interactions with biological targets on a molecular level and to allow their procurement through total synthesis. Methods such as X-ray crystallography require crystalline products, and chemical synthesis is usually very time consuming and not always definitive. The structural elucidation of amorphous molecules with several unknown stereogenic centers would benefit greatly from a method that could simultaneously analyze all configurations. Here we discuss how effective NOE/ROE effects in combination with computational methods can be used for this purpose.

The NOE/ROE restraints may be used in a qualitative way or as distance restraints in EM or MD simulations. These approaches are problematic with a large number of unknown stereogenic centers. Therefore, a method is required which allows the determination of all unknown stereogenic centers simultaneously and without the necessity of crystalline products. The fc-rDG/DDD method (floating chirality restrained DG/DDD) [1] is a combination of distance geometry (DG) [2] and distance-bounds driven dynamics (DDD) calculations [3] using interproton distances and floating chirality [4] in order to determine the relative configuration of small molecules (including low molecular-weight natural products).

Recently, the fc-rDG/DDD method was applied to structurally very complex members of the pyrrole-imidazole alkaloid family [5]. These dimeric pyrrole-imidazole alkaloids have all eight stereogenic centers in common, necessitating a method which allows a simultaneous determination of all unknown centers. The results of the fc-rDG/DDD calculations on tetrabromostyloguanidine revised the relative configuration of the palau'amine congeners in 2007 [6].

The investigation were expanded to the dimeric pyrrole-imidazole alkaloids axinellamine A [7] and 3,7-*epi* massadine chloride [8]. Here the originally proposed configuration was confirmed by the fc-rDG/DDD calculations [9]. Out of the 128 possible diastereomeres of axinellamine only four were generated in the fc-rDG/DDD calculations. One of them was generated by more than 90% which is the same diastereomer originally published for axinellamine.

Another example is an intermediate in the total synthesis of palau'amine, 20-deoxymacropalau'amine azide [10] which will be the main focus of this contribution. This compound has a very interesting structure with a 9-membered ring. Besides the configurational assignment of the five stereogenic centers, a detailed conformational analysis was carried out. This is of special importance because macro-palau'amine is the direct precursor of palau'amine in its total synthesis. The final step in this synthesis is a transannular ring closure of macro-palau'amine to palau'amine [11].

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Tuesday, September 11th
1:30 PM - 3:00 PM

New Developments in Ultrawideband Pulses

Chair: Steve F. Cheatham

Speakers:

Burkhard Luy
Karlsruhe Institute of Technology, Karlsruhe, Germany

Douglas Brown
Indiana University, Bloomington, IN, USA

Stephen Wimperis
University of Glasgow, Glasgow, UK

Meerakhan Pathan*
Université de Nantes, France

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Limits of Universal Rotation Pulses and Ultrabroadband NMR Spectroscopy

Burkhard Luy

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In contrast to point-to-point (PP) pulses, like excitation or inversion pulses which are optimized to only transfer a single magnetization component, universal rotation (UR) pulses act like a perfect hard pulse for a defined offset range with all three magnetization components well defined. The most frequently used UR pulses are 90° and 180° pulses, where the latter ones are also known as refocusing pulses.

Following the fundamental work on exploring the physical limits of broadband excitation and inversion pulses [1,2], we will present data for lower bounds of corresponding broadband 90° and 180° UR pulses. The corresponding pulse shape families, which we termed BURBOP-90 and BURBOP-180, were obtained with amplitude-restricted optimal control-derived algortihms and result mostly in two types of symmetries, which were not imposed on the optimizations and where one symmetry type represents the construction scheme introduced earlier [3]. The pulse shapes, their performance, and a comparison to existing broadband UR pulses will be outlined. We will also give an exemplary answer to the question, if a single 180° UR pulse or a pair of inversion pulses will give better performance in INEPT-type transfer elements. As an extension to conventional UR pulses we will also introduce the BUBI pulse, which represents a concurrent shape optimized for a ^{1}H UR-180 and ^{13}C inversion that is compensated for J-coupling evolution. The outstanding performance of the pulse sandwich is demonstrated experimentally and incorporated into the COB-INEPT transfer which is optimized for ultimate robustness [4].

In the second part of the talk, a novel concept for obtaining ultrabroadband NMR spectra is introduced. By using xyBEBOP pulses optimized for maximum transfer of z-magnetization into the xy-plane, 2D spectra can be obtained for offset ranges of 200 kHz and more using conventional probeheads. Several examples are given ranging from ^{19}F to ^{15}N and ^{195}Pt correlation spectroscopy.

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Constant Amplitude Broadband Refocusing Pulses from Numerical Optimization

Douglas Brown

Indiana University, Bloomington, Indiana, USA

After success exploring the limits of direct numerical optimization of shaped pulses to create efficient MICA broadband inversion pulses from pure Bloch simulations, the same method was pursued for refocusing pulses. Again the primary goal was to create minimal duration pulses with 99% conversion of suitable power limits and bandwidths for high field broadband ^{13}C applications. Initial results with simple target functions proved that this would be a much more difficult problem than inversion pulses so the fairly unexplored method of Luy et al. of point to point transformations was pursued. They described that a 90x pulse followed by its time reversed, phase reversed transformation yields a universal rotation pulse that can be used for refocusing. With this method a broadband 90x pulse (M_x=1, M_y=0, M_z=0) is created. The optimizations for these pulses are much more difficult than for inversion pulses but proved to be achievable to create CAR refocusing pulses at an 18 kHz field of 200 to 400 µs durations with bandwidths of 40 to 80 kHz. It is also notable that these optimizations are much more difficult than the Luy et al. pulse conditions of 1 ms duration, 40 kHz bandwidth and 20 kHz maximum field. In addition, unlike the Luy et al. result, constant amplitude (i.e. full power) were found to always give the most efficient pulses in terms of conversion versus duration. To test CAR pulses, the demanding experiment 1,1-ADEQUATE was run on strychnine with MICA inversion and CAR refocusing ^{13}C pulses and produced much improved results versus the hard pulse version. More recently, attention has been returned to creating refocusing pulses directly from Bloch simulations without the need for the 90x intermediate condition and these promising results will be presented.

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2. D. Brown, Constant amplitude broadband refocusing pulses from numerical optimization, Magn. Reson. Chem., 49 (2011), pp. 705–709.

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Composite Pulses and Spin Echoes: The Effect of Symmetry

Stephen Wimperis[1], Smita Odedra[1] and Michael J. Thrippleton[2]

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2. Medical Physics, University of Edinburgh, Edinburgh, UK

The radiofrequency pulses used in NMR are subject to a number of imperfections such as those caused by inhomogeneity of the radiofrequency (B1) field and an offset of the transmitter frequency from precise resonance. The effect of these pulse imperfections upon a refocusing pulse in a spin-echo experiment can be severe. Many of the worst effects, those that distort the phase of the spin echo, can be removed completely by selecting the echo coherence pathway using either the "Exorcycle" phase cycle [1] or magnetic field gradients. It is then tempting to go further and try to improve the amplitude of the spin-echo signal by replacing the simple refocusing pulse with a broadband composite 180° pulse that compensates for the relevant pulse imperfection.

We show here that all composite pulses with a symmetric or asymmetric phase shift scheme will reintroduce phase distortions into the spin echo, despite the selection of the echo coherence pathway. In contrast, all antisymmetric composite pulses yield no phase distortion whatsoever, both on and off resonance, and are therefore the correct symmetry of composite refocusing pulse to use [2].

Our conclusions are put to use in a novel method designed to suppress the broad "background" signal often found in ^{1}H MAS NMR. This unwanted signal arises from ^{1}H nuclei that are outside the MAS rotor and radiofrequency coil, probably located on the surfaces of the static components of the probehead. For many years a popular method of background suppression has been the "depth pulse" experiment [3, 4], consisting of a 90° pulse followed by one or two 180° pulses that are phase cycled according to the Exorcycle scheme, which removes signal associated with imperfect 180° pulses. Consequently, only spins in the center of the radiofrequency coil contribute to the ^{1}H MAS spectrum, while those experiencing a low B1 field outside the coil are suppressed. Although very effective at removing background signal from the spectrum, one drawback with this approach is that significant loss of the desired signal from the sample also occurs. Here, we demonstrate the use of novel antisymmetric passband composite pulses to replace the simple pulses in a depth pulse experiment [5]. We show that it is possible to improve the intensity of the ^{1}H signals of interest while still maintaining fully effective background suppression.

There are very few antisymmetric broadband composite 180° pulses in the literature, particularly for compensation of B1 inhomogeneity. Therefore, novel antisymmetric composite 180° pulses are designed for use in NMR and verified experimentally in ^{1}H solution-state NMR [6]. The new pulses are simultaneously broadband with respect to both inhomogeneity of the B1 field and resonance offset and, as a result of their antisymmetric phase schemes, can be used to form spin echoes without the introduction of a phase error. These dual-compensated pulses are designed analytically, using symmetry arguments and a graphical interpretation of average Hamiltonian theory. Two families of composite refocusing pulses are presented. There are an infinite number of sequences in each family owing to a free phase variable in the solution to the average Hamiltonian

equations and this allows selection of individual composite pulses with particular properties, including ones with a passband profile.

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6. S. Odedra, M.J. Thrippleton, S. Wimperis, in preparation.

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Ultrafast NMR for Dummies

Meerakhan Pathan, Jean-Bruno Mougel, Benoit Charrier, Illa Tea, Serge Akoka and Patrick Giraudeau

Université de Nantes, France

Ultrafast (UF) 2D NMR is a very promising methodology enabling the acquisition of 2D spectra in a single scan [1]. The analytical performances of UF 2D NMR have been highly increased in the last few years, thus increasing its range of applications [2,3]. However, its implementation and use by non-specialists are far from being straightforward, due to the specific acquisition and processing procedures and parameters characterizing UF NMR. Moreover, a major limitation affecting these experiments is their inability to cover large spectral widths without losing resolution. To make this methodology implementable and applicable by non-specialists, we developed a simple algorithm capable of translating the conventional parameters (spectral widths, transmitter frequencies) into specific UF parameters (gradients and chirp pulse parameters).

By defining simple parameters equivalent to those of conventional 2D NMR experiments, the user can obtain all UF-specific parameters without prior knowledge of this methodology. Moreover, our algorithm also takes into account the possibility of folding a user-defined region of the spectrum into the main observation window along the spatially-encoded dimension [4].

This algorithm was implemented in a webpage which will be soon available for external users. Its direct implementation in the commercial software is also being developed. The algorithm was designed for two widely used 2D experiments: COSY and HSQC, but can be easily extended for any other pulse sequence. This approach was tested successfully on a variety of small molecules.

We hope that this tool will remove much of the mystery surrounding ultrafast 2D NMR and will make the technique usable by a wider audience of organic and analytical chemists.

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Tuesday, September 11th
3:30 PM – 5:00 PM

Workshop: Teaching Mass Spectrometry to NMR Spectroscopists

Charles Ross, Merck, USA

Tutorial: NMR of Other Nuclei

Steve Cheatham, DuPont, USA
Gary Martin, Merck, USA

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Teaching Mass Spectrometry to NMR Spectroscopists

Charles W. Ross

Merck Research Laboratories, Process & Analytical Chemistry - Structure Elucidation Group
West Point, PA, USA

The workshop will provide a brief review of ionization methods and detectors in mass spectrometry. The main discussion will focus on why accurate mass measurement is used, what it provides compared to nominal mass measurements, and how it can be used in collaboration with NMR. Analysis of multicomponent samples and structural characterization will also be examined.

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NMR of Other Nuclei

Steve Cheatham[1] and Gary Martin[2]

1. DuPont, USA
2. Merck, USA

^{19}F NMR Techniques:

Three main topics will be discussed in the workshop.

1. Basic ^{19}F acquisition including the best methods for performing sample quantification using ^{19}F.
2. Two-dimensional homonuclear (^{19}F-^{19}F) and ^{1}H-^{19}F {^{1}H} acquisition. Examples, instrument setup and hardware requirements.
3. Heteronuclear ^{19}F-X acquisition including triple resonance. Examples, instrument setup and hardware requirements.

Nitrogen – That "Other" Nucleus:

Nitrogen has two nuclides, ^{14}N and ^{15}N. Very early work was generally done with ^{14}N because of its high natural abundance (vs. 0.037% for ^{15}N). Unfortunately most of the early ^{14}N data is of little utility as compared to ^{15}N data which are now routinely acquired.

There are numerous problems inherent to ^{15}N that have made the direct acquisition of ^{15}N spectra a challenge. The relative sensitivity is about 2% of that of ^{13}C. ^{15}N has a negative gyromagnetic ratio which is disadvantageous insofar as the nuclear Overhauser effect; the signal can completely disappearing in unfavorable cases. For direct observation, generally large samples (molar) have been employed in conjunction with large diameter tubes and the use of INEPT or DEPT sequences.

In contrast, via indirect detection 2D NMR methods, ^{15}N is a very useful structural probe and has been the subject of a number of reviews. Considerations implicit in the acquisition and utilization of ^{15}N data can be discussed. Facets of the discussion may include ^{15}N 90^{o} pulse width implications, pulse sequences, ^{15}N chemical shift ranges, sample sizes, and strategies for employing ^{15}N data in structural studies of impurities and degradants of pharmaceuticals, alkaloids, and as input for computer-assisted structure elucidation programs. What is discussed will depend on the interests of those participating in the workshop.

Chapters and Reviews of Applications of ^{15}N NMR by Direct and Inverse-Detection:

1. S. Berger, S. Braun, H.-O. Kalinowski, "^{15}N NMR", in NMR Spectroscopy of the Non-Metallic Elements, John Wiley & Sons, New York, English translation, 1997, pp. 111-318.
2. G.E. Martin and C.E. Hadden, *J. Nat. Prod.*, **65**, 543-585 (2000).
3. R. Marek and A. Lycka, *Curr. Org. Chem.*, **6**, 35-66 (2002).
4. G.E. Martin and A.J. Williams, "Long-range ^{1}H-^{15}N 2D NMR Methods," in G.A. Webb, Ed., *Ann. Rep. NMR Spectrosc.*, **55**, Elsevier, Amsterdam, 2005, pp. 1-119.
5. R. Marek, A. Lycka, E. Kolehmainen, E. Sievanen, and J. Tousek, *Curr. Org. Chem.*, **11**, 1154-1205 (2007).
6. G.E. Martin, M. Solntseva, and A.J. Williams, "Applications of ^{15}N NMR Spectroscopy in Alkaloid Chemistry," in Modern Alkaloids, Structure, Isolation, Synthesis and Biology, E. Fattorusso and O. taglialatela-Scafati, Eds., Wiley-VCH, New York, 2008, pp. 409-472.

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Wednesday, September 12th
9:00 AM - 10:30 AM

Potential Genotoxic Impurities

Co-chairs: Karen Alsante and Roberto R. Gil

Speakers:

Andrew Phillips
AstraZeneca, UK

Greg Sluggett
Pfizer Inc., CT, USA

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The Use of NMR as an Efficient Tool for the Analysis of Potential Genotoxic Impurities

Andrew R. Phillips, Ian C. Jones and Steve Coombes

Pharmaceutical Development, AstraZeneca, Macclesfield UK

NMR is a well-established quantitative technique for looking at relatively small amounts of material – for example residual solvents within an active pharmaceutical ingredient down to levels of 0.1% w/w.

Recently there has been considerable concern from pharmaceutical regulatory agencies over the control of potential genotoxic impurities (PGI) in medicinal products. The Threshold of Toxicological Concern (TTC) for PGIs in commercial products is 1.5 μg/day, or single-figure ppm with respect to a typical drug substance. Consequently, methods for the measurement of impurities in the single-ppm range are required – presenting a significant analytical challenge.

We have recently shown that NMR can detect down to these low level often with significant advantages over other more traditional techniques in terms of method development, sample preparation and experiment time. [1]

The key to the success of NMR is overcoming the inherent lack of sensitivity so this presentation will focus on some of the important factors that determine the level of detection, such as the performance of the NMR system, substrate concentration, linewidth, resolution and dynamic range. This will include how new state of the art equipment in our laboratory has been utilised to overcome some of these challenges.

In addition a number of new genotoxic impurities examples analysing both pure compounds and formulated products will be presented. Furthermore it will shown that the use of NMR can be expanded to other trace analysis problems such as cleaning validation: confirming vessels used for chemical reactions are not contaminated with material from previous experiments.

1. Andrew R. Phillips ‘Analysis of Genotoxic Impurities by Nuclear Magnetic Resonance Spectroscopy’ in ‘Genotoxic Impurities: Strategies for Identification and Control edited by Andrew Teasdale, 2011, Wiley-Blackwell

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Evaluation of Residual 2-Vinylpyridine Levels in Axitinib

Gregory W. Sluggett

Analytical Research & Development, Pfizer Inc., Groton, CT, USA

2-Vinylpyridine (**2-VP**), a starting material for the synthesis of axitinib, is reported to be Ames positive and, therefore, its residual levels in axitinib have been evaluated. Three different methods for **2-VP** determination (GC-FID, headspace GC-FID and HPLC with UV detection) were developed and validation data will be presented. Batch analysis and spiking studies have demonstrated that the synthetic process has an extremely high capability to purge **2-VP** and consistently delivers axitinib with undetectable **2-VP** levels well below the 1.5 µg/day Threshold of Toxicological Concern (75 ppm).

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Wednesday, September 12th
11:00 AM - 12:30 PM

Wisdom of the Crowd
Chair: Bill Farrell

- An interactive, problem-solving session where academic presentations on current research problems are discussed in order to access the advice, guidance, and suggestions of conference participants… literally tapping into the "wisdom of the crowd."
- This session highlights the benefits of attending CoSMoS and places the conference squarely at the crossroads between academia and industry. Everyone benefits from the open discussion format

  "…This was a useful session from my perspective. The audience enjoyed it because it was a different type of session compared to the rest of the meeting and compared to almost any other scientific meeting."

  "…Everyone at the meeting is a good problem solver and there is an extraordinary breadth of experience within the members of the group. These folks enjoy solving problems and are prepared to provide their comments openly. This is a relatively rare combination."
- Abstracts provided for current ongoing research are reviewed in 1-5 slides to provide background of the problem, summarize the key issues and solicit audience comments.

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Wednesday, September 12th
1:30 PM – 3:00 PM

Predictive Quantitation
Co-chairs: Karen Alsante, Bill Farrell and Roberto Gil

Speakers:

Jim Morgado
Pfizer Inc., CT, USA

Jonas Buser
Eli Lilly and Company, IN, USA.

Jose G. Napolitano
University of Illinois at Chicago, IL, USA

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Quantitative Structure Retention Relationship Modeling (QSRR): Comparative Investigation of the Predictive Capability of an L-PLS Model versus ACD Labs™ Commercial Software using a Typical Pharmaceutical Compound

Koji Muteki and **James E. Morgado**

Pfizer Global R&D, Groton, CT

Quantitative Structure Retention Relationship (QSRR) models may afford an opportunity to enhance the speed of chromatographic method development and reduce the scope of screening approaches employed for identification of acceptable chromatographic separations. A comparative analysis of the predictive results of a QSRR model generated using an L-PLS strategy and that obtained from commercially available software from ACD Labs will be presented. The effectiveness and/or limitations of the proposed methods to predict the retention of potential degradation products will be demonstrated through a practical pharmaceutical example. Opportunities for improvements and the potential long term benefits of such approaches will also be presented for consideration.

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From Understanding Organic Reactions to Speeding Up High Resolution MS Interpretation: A Multinuclear $^{31}P^{1}HU$- and $^{19}F^{1}HU$-qNMR Approach

Jonas Y. Buser

Eli Lilly and Company, Indianapolis, Indiana, USA.

Many active pharmaceutical ingredients (API) or reagents used in the reactions to generate the API's contain one or more fluorine or phosphorus atoms. These nuclei provide an uncrowded spectral landscape that can be highly advantageous for NMR spectroscopists when elucidating impurity structures or monitoring reactions during mechanistic studies. For the mass spectrometrist interpreting high resolution mass spectrometry data, however, the presence of one or more fluorine or phosphorus nuclei can often add a great deal of difficulty in narrowing down the potential molecular formula. This presentation will detail a simple and unique way of utilizing a multinuclear ^{1}H-^{19}F/^{31}P quantitative NMR approach to assist in the MS formula determination of species containing fluorine and/or phosphorus nuclei, in addition to aiding the NMR spectroscopist in structure elucidation and reaction characterization analyses.

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Integrated Spectrometric Analysis for the Quantitation of Complex Mixtures Exemplified with Natural Products

Jose G. Napolitano and Guido F. Pauli

Institute for Tuberculosis Research and Department of Medicinal Chemistry & Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA

The development of analytical methods for the qualitative and quantitative assessment of complex, metabolomic mixtures, such as botanicals and other natural products (NPs), remains a challenge. Given the popularity of dietary supplements in the U.S. and worldwide, analytical methods that can guarantee authenticity, quality, safety, and efficacy of NPs, are in high demand. Due to its high sensitivity, **LC-MS/MS** has found widespread application in food, environmental, clinical, forensic, and NP analysis, and is primarily used for the quantification of specific marker compounds. Due to its nature as a primary analytical method and its unique quantitative capabilities, quantitative ^{1}H NMR (**qHNMR**) has recently been recognized as another powerful tool for mixture analysis, and is increasingly applied in NP QC and metabolomic research.

The present study explored the development of integrated spectrometric methods using both qHNMR and LC-MS/MS, focusing on the analysis of the top-10 selling U.S. botanicals. The main objective of this orthogonal approach is to identify strengths and weaknesses of these complementary techniques, but also to implement new qHNMR methodology to improve performance towards an overall determination of botanical product integrity.

To this end, we have developed an analytical method based on ^{1}H iterative Full Spin Analysis (**HiFSA**). This computer-assisted approach can overcome the common problem of signal overlap in 1D ^{1}H NMR spectra and enable parallel identification and quantification of target analytes. The process involves the generation of highly-reproducible, field-independent ^{1}H NMR fingerprints, and allows accurate quantitation without the need for identical reference materials for calibration purposes. Another aspect addressed by this study is the quality control of the reference standards and their suitability for LC-based analysis. Here, qNMR takes an important role for the detection and quantitation of impurities, whereas the application of LC-MS/MS is essential for the investigation of samples with relatively high *residual complexity*, e.g., NPs containing many structurally related compounds. Finally, using Ginkgo and Green Tea as examples, results from the cross-evaluation of qNMR and LC-MS/MS analysis will be presented, emphasizing the importance of multi-technique characterization of complex mixtures of NPs.

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Wednesday, September 12th
1:30 PM – 3:00 PM

Career Development Workshop (Mixer)

Co-chairs: Jeffrey Standish, Betsy McCord and Doug Kiehl

Panelists:

Brian Stockman
Adelphi University, NY, USA

Aaron Wheeler
University of Toronto, Toronto, Canada

Dorothy Phillips
Waters Corporation, USA

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Career Development Workshop

Brian Stockman
Aaron Wheeler
Dorothy Phillips

Adelphi University, NY, USA
University of Toronto, Toronto, Canada
Waters Corporation, USA

- Academic Career versus Industry Career: How interchangeable are they?
- Are degreed students prepared for careers in industry?
- Can an industry scientist transition back to academia?
- What training and tools exist to help bridge the gap between academia and industry?

These are just some of the questions that will be addressed during the joint SMASH/CoSMoS panel discussion. Subject matter experts from both academia and industry will provide short vignettes and case studies to help stimulate interaction. This panel discussion is designed primarily for early career scientists and students, but is open to everyone.

Monday, September 10th
5:00 PM – 6:30 PM

Tuesday, September 11th
5:00 PM – 6:30 PM

Poster Sessions

Co-Chairs: Mike Bernstein and Daneen Angwin

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1 Role of Viral-envelope Glycans in Immune Response against HIV

Syed Shahzad-ul-Hussan[1,2], Mallika Sastry[1], Marie Pancera[1], Ling Xu[1], Youngping Yang[1], Baoshan Zhang[1], Gary J. Nabel[1] Carole A. Bewley[2] and Peter D. Kwong[1]

1. Vaccine Research Center, NIAID
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Roughly 50% mass of the envelope glycoprotein, gp120 of HIV is carbohydrates. These carbohydrates are thought to provide a shield to HIV against the immune system of the host. However, recently several broadly HIV-neutralizing antibodies have been isolated from the sera of HIV patients that neutralize in a carbohydrate dependent manner. PG9, PG16 and PGT128 are examples of such potent anti-HIV antibodies.

We employed ligand-based NMR techniques to understand the atomic level structural basis of glycan recognition by these antibodies and whether direct antibody-glycan interaction is necessary for the neutralization.

Our results showed that PG9 and PG16 specifically recognized Man5-GlcNAc2 and hybrid-type glycan, respectively. The different carbohydrate specificity of the two homologous antibodies could be the result of different evolutionary pathways of these antibodies. We determined the bioactive conformation of Man9 in PGT128 bound state by applying 3-dimensional NMR techniques after labeling Man9 with ^{13}C isotope. This is the first example of successful carbohydrate labeling by the recombinant technique.

2 Fast access to Residual Dipolar Couplings by Single-Scan 2D NMR in Oriented Media

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The utility of residual dipolar couplings for determining the structure of small organic molecules is increasingly recognized [1]. The most frequently used RDCs are 1DCH, which can be measured either by F2- or F1-coupled HSQC experiments. While the efficiency of RDCs is highly recognized, these approaches suffer from the long experiment times characterizing 2D NMR experiments, due to the need to record numerous t1 increments to sample the indirect dimension. If one would want to investigate transient species, these measurement times need to be reduced significantly.

NMR spectroscopists have designed several approaches dealing with the time drawback of multi-dimensional NMR experiments. One of the most impressive and efficient is probably the “ultrafast” 2D NMR methodology, recently proposed by L. Frydman and co-workers [2], capable of providing a complete 2D correlation in a single scan, ie in a fraction of a second. In the last five years, we have significantly improved the performances (sensitivity, resolution, lineshape, etc.) of this approach [3,4], thus increasing its range of applications [5]. Here, we present an original NMR method to measure Residual Dipolar Couplings in a very short time, by ultrafast 2D NMR experiments performed for the first time in oriented media.

We applied this methodology to isotropic and anisotropic isopinocampheol (IPC) samples. In the former case, the 2D HSQC spectrum was recorded in a single scan, while for the anisotropic sample, signal averaging was performed to obtain an optimum sensitivity, resulting in a 60 seconds total experiment time, which is still two orders of magnitude lower than the duration of the conventional experiments generally used to measure RDCs. The dipolar couplings extracted from these experiments are in very good agreement with those obtained from conventional experiments. These results highlight the potentialities of ultrafast 2D NMR as a tool for determining the configuration of organic compounds, and open promising perspectives in the field of small molecule NMR analysis.

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3 Structural Diversity in Enamines and Iminium Ions Revealed by NMR

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The knowledge of structural features of intermediates is crucial to the understanding of the outcome of most stereoselective chemical reactions.[1-5] Structural information of intermediates in a variety of organocatalytic transformations have been gained using a broad scope of NMR methods. With the aid of espeacially NOESY spectroscopy surprising[6] structural diversity has been revealed in a huge number of investigated intermediates. A broad substrate scope (carbonyl compounds) as well as a broad catalyst scope (mainly proline based secondary amines [7]) was investigated in a variety of solvents. Temperature dependency as well as concentration dependency and the influence of additional water present in the reaction mixture was studied intensively. We found that enamines derived from proline-based catalysts and ?-unbranched aldehydes show, depending on the catalyst structure, either a high conformational lock or a high conformational diversity. Prolinol (ether) catalysts exhibit strong conformational lock[8] whereas proline, a proline tetrazole catalyst[9] and prolinol show strong stuctural fluctuations wihtin the investigated, aldehyde derived, enamines. We found almost uniform population of the s-cis as well as the s-trans isomer (concerning the newly formed N-C bond) of aldehyde derived enamines with these catalysts as well as strong fluctuations concerning the puckering of the five-membered ring in the intermediate structure. Investigations concerning ketones as carbonyl species yielded iminium ions which were investigated thouroughly for the first time in solution. The E/Z ration was determined and correlated with the structure of the substrate as well as the catalyst. Kinetic investigations of the iminium-ion formation were done via a rapid-injection NMR technique and the relative reactivity of these iminium ions in the classical aldol reaction was investigated. In the case of a ?-cyclic aldehyde, namely cyclopropanecarboxyaldehyde unprecedented free rotation around the C=N(+) bond was revealed within the iminium species.

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4 Ultrafast NMR for Dummies

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Ultrafast (UF) 2D NMR is a very promising methodology enabling the acquisition of 2D spectra in a single scan [1]. The analytical performances of UF 2D NMR have been highly increased in the last few years, thus increasing its range of applications [2,3]. However, its implementation and use by non-specialists are far from being straightforward, due to the specific acquisition and processing procedures and parameters characterizing UF NMR. Moreover, a major limitation affecting these experiments is their inability to cover large spectral widths without losing resolution. To make this methodology implementable and applicable by non-specialists, we developed a simple algorithm capable of translating the conventional parameters (spectral widths, transmitter frequencies) into specific UF parameters (gradients and chirp pulse parameters).

By defining simple parameters equivalent to those of conventional 2D NMR experiments, the user can obtain all UF-specific parameters without prior knowledge of this methodology. Moreover, our algorithm also takes into account the possibility of folding a user-defined region of the spectrum into the main observation window along the spatially-encoded dimension [4].

This algorithm was implemented in a webpage which will be soon available for external users. Its direct implementation in the commercial software is also being developed. The algorithm was designed for two widely used 2D experiments: COSY and HSQC, but can be easily extended for any other pulse sequence. This approach was tested successfully on a variety of small molecules.

We hope that this tool will remove much of the mystery surrounding ultrafast 2D NMR and will make the technique usable by a wider audience of organic and analytical chemists.

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5 Crystal Structure Determination from High-Resolution Proton NMR and Crystal Structure Prediction

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A protocol for *ab initio* structure determination of powdered solids at natural isotopic abundance by combining solid-state NMR spectroscopy, crystal structure prediction and DFT chemical shift calculations is used to determine the crystal structures of a series of drug molecules: flutamide, flufenamic acid, cocaine and theophylline. For flutamide and flufenamic acid we find that assigned ^{1}H isotropic chemical shifts provide sufficient discrimination so as to determine the correct structures from a set of predicted structures using the root-mean-square deviation between experimentally determined and calculated chemical shifts. In both cases neither unassigned ^{1}H shifts nor ^{13}C shifts could be used to determine the structures. The correct structures were determined to within an atomic rmsd of less than 0.12 Å with respect to the known structures. For theophylline the NMR spectrum is too simple to allow unambiguous structure selection. We show how the information contained in the NMR chemical shifts is complementary (orthogonal) to PXRD. Combining ^{1}H NMR chemical shift, CSP/DFT and PXRD leads to a very robust structure determination protocol, with no hypothesis, and no bias. We also use this procedure to determine for the first time the crystal structure of a pharmaceutically pertinent molecule with previously unknown structure, where previous attempts by X-ray diffraction alone had not been successful.

Combining ^{1}H solid-state NMR spectroscopy with first-principles calculations can also be applied to the crystal structure determination of metal organic frameworks. We demonstrate this by reporting the discovery of the previously unknown crystal structure of a novel porous imidazolate substituted metal organic framework with possible applications as a H2 storage material. ^{1}H NMR experiments provided a description of the proton environments within the MOF, which in combination with DFT chemical shift calculations and PXRD led to the elucidation of the complete crystal structure.

6 Poly(phenylacetylenes) as Chiral Alignment-Media

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The application of RDCs to solve conformational und configurational problems of (small) organic molecules in solution is a field of NMR spectroscopy of increasing interest [1,2]. A precondition for the measurements of these global anisotropic NMR-parameters containing distance and angle information, is to partially orient the analyte molecules with respect to the magnetic field. This can be done either by streched polymer gels (SAG – strain induced alignment in a gel) or by dissolving the analyte in a lyotropic liquid crystalline phase (LLC-phase).

If the goal of the investigation of a chiral, non-racemic molecule is the absolute configuration, the basic requirement is the orientation of the analyte in an enantiodifferentiating manner, which requires the application of a chiral and uniformly configured alignment medium. The number of chiral alignment media, compatible with common organic NMR-solvents, is still very small [3,4]. Therefore, we started to work on the development of new chiral alignment media and found helically chiral amino acids-based poly(phenylacetylenes) [5,6] to be very well suited for this purpose.

Herein we would like to describe the application of a new poly(phenylacetylene) poly-1, available in four steps from the natural amino acid phenylalanine, as chiral LLC-alignment medium. Poly-1 forms stable LLC-phases in $CDCl_3$ starting at a concentration as low as 10% (w/w). Moreover these phases show a very pronounced enantiodiscrimination with (+)/(-)-IPC and (-)-strychnine. Interestingly, these LLC-phases display an unusual temperatur-behaviour of the quadrupolar splitting, indicating depending on the temperature the existance of three different phases. This, in fact allows us to determine three different alignment-tensors in one nmr-sample. Finally, we were able to demonstrate that the three phases are characterized by three different degrees of enantiodiscriminations as was shown with IPC.

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7 Combined Computational and Experimental NMR: Applications in Drug Discovery

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The combination of computational methods with NMR data to determine solution conformation, connectivity, and relative stereochemistry has most often been applied to the field of natural products. We decided to apply some of combined computational and experimental NMR techniques to medicinal chemistry problems. Specifically, we implemented NAMFIS[1] and determining relative stereochemistry by NMR chemical shift calculation using the DP4 probability method introduce by Smith and Goodman[2]. NAMFIS was used to attempt to explain an observed dramatic increase in potency that resulted from forming a macrocycle[3]. The hypothesis was that the potency increase was the result of restricting the inhibitor's conformations such that it favors the conformation necessary for binding the target. The DP4 probability method was used to assist determination of relative stereochemistry of medicinal chemistry samples. The poster will describe how well the DP4 probability was able to determine the correct relative stereochemistry.

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8 A Novel Approach Towards False Positive Reduction in Automated Structure Verification

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Automated structure verification using ^{1}H NMR data or a combination of ^{1}H and HSQC NMR data is gradually gaining more interest as a routine application for qualitative evaluation of large compound libraries produced by synthetic chemistry. The goal of this automated software method is to identify a manageable subset of compounds and data that require human expertise and review. In practice, the automated method will flag structure and data combinations that exhibit some inconsistency (i.e. strange chemical shifts, conflicts in multiplicity, or over and underestimated integral values) and validate those that appear consistent. One drawback of this approach is that no automated system can guarantee that all passing structures are indeed correct structures. The major reason for this is that approaches using only ^{1}H or even ^{1}H and HSQC spectra often do not provide sufficient information to properly distinguish between similar structures. Therefore current implementations of automated structure verification systems allow, in principle, false positive results.

In this work, a novel method for fully automated structure verification by NMR has been described that dramatically reduces the probability of false positives [1]. The software method automatically generates and evaluates a series of similar compounds against 1D ^{1}H and 2D HSQC spectra to determine the ambiguous nature of the data provided. A mechanism for the automated generation of negative control challenge structures as well as discussion of the impact this approach has on false negatives will also be examined.

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9 Filter Diagonalization Method for Processing PFG NMR Data

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Diffusion-ordered spectroscopy (DOSY) is an NMR technique used to characterize mixtures in solutions by isolating NMR signals from individual components of the mixture, or to determine diffusion coefficients for these individual components (see [1] and references therein). The diffusion information is encoded, e.g. in a 2D diffusion data set, as a multi-exponential decay with the different decay constants associated with diffusion coefficients of the mixture components.

Therefore, the major challenge associated with DOSY is the data inversion, which requires implementation of either an Inverse Laplace Transform (ILT) along the diffusion dimension or a related multi-exponential fit, both being notoriously difficult problems of numerical analysis. The difficulties in solving these ill-posed problems has lead to the development of a number of data inversion techniques, each with its own shortcomings begging to be addressed. Many of the existing techniques factorize the 2D inversion problem into that of the 1D inverse Fourier Transform (FT) applied to 1D slices of the 2D data along the acquisition dimension, followed by a 1D ILT applied to the 1D slices along the diffusion dimension of the FT-processed data. This factorization inevitably leads to a loss of information which manifests itself as difficulties associated with the ILT of a short and noisy 1D data set. Our approach to DOSY is based on utilizing the filter diagonalization method (FDM), which is a truly multivariate method that utilizes the entire DOSY data set, and as such avoids factorization issue mentioned above. Compared to a related method, the Regularized Resolvent Transform (RRT), which was developed previously [2], FDM uses a somewhat different numerical procedure, based on solving ill-conditioned generalized eigenvalue problems (rather than ill-conditioned linear systems), and is much faster. FDM-DOSY code for Matlab was developed as a module for DOSYToolbox, a widely distributed PGF NMR signal processing software package written by Mathias Nilsson [3], and is being included with the current release.

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10 Challenges in the Automation of NMR Methods for Raw Material Screening: Heparin and Aloe vera

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NMR spectroscopy is a powerful tool for identifying and quantifying compounds in mixtures. The signal in an NMR experiment is proportional to the amount of material present. For some materials, standard analytical methods based on NMR have been established. We would like to encourage the widespread adoption of these methods and the development of methods for additional materials by automating the NMR analysis. Toward that end, we have developed the Assure-RMS software. We report on our experiences implementing the analyses for heparin and Aloe vera.

Heparin is a highly sulfated glycosaminoglycan used as an anticoagulant. In 2008, heparin contaminated with "oversulfated" chondroitin sulfate (OSCS) led to 81 deaths before an FDA recall prevented further tragedy. NMR is an attractive method to screen for OSCS because it contains signals at unique ^{1}H chemical shifts compared to native heparin, and a method has been established [1]. The established method is essentially graphical, comparing the heights of know peaks from heparin to the peak heights in regions where unwanted contaminants occur. Automation requires measurements of peak heights, which depends critically upon the definition of the baseline.

Aloe vera is a plant used therapeutically to soothe burns and aid digestion. It is included in a wide variety of products, for both topical and oral use. The published NMR method [2] quantitates components of the Aloe vera mixture, including glucose, malic acid, lactic acid, acetic aid, and acetylated polysaccharides. For accurate quantitation, we have employed lineshape fitting. The challenge here is setting lineshapes specific enough to correctly identify the components of interest yet flexible enough to identify peaks which may shift due to acid concentrations or broaden due to sample viscosity. We validated the quantification of glucose using lineshape fitting by building a PLS model based on glucose samples of different concentrations and using that model to quantify Aloe vera samples. The correlation between the values is quite good with a correlation coefficient (R^2) of 0.9848.

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11 Mixtures of Pharmaceutical Polymorphs: Quantitative Solid-State NMR Analysis

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Solid-state NMR is a commonly used technique for the analysis of pharmaceutical polymorphs. Carbon chemical shift is a sensitive probe for the crystal packing and even simple ^{13}C CP-MAS spectra provides enough information for identification and discrimination of different crystal forms in mixtures. However, the quantitative analysis of the mixtures is difficult due to insufficient resolution of solid-state NMR spectra and non-quantitative nature of the CP-MAS experiment. We propose a new quantitative analysis strategy for polymorphs mixtures based on the assumption that CP-MAS spectrum of the mixture is a linear combination of spectra obtained from the individual polymorphic forms under the same CP-MAS conditions. Direct fitting of the mixture spectra by spectra of individual components should provide quantitative results. To avoid mathematical complications of fitting using frequency-domain NMR spectra, the time-domain NMR signal was used instead. The two pure isomorphs of manidipine (α and ß) and their mixture have been studied and analysed using proposed technique. The results demonstrate that the proposed analysis strategy provides reliable quantitative results for mixture analysis of pharmaceutical polymorphs.

12 Monitoring Chemical Reactions by Low Res Benchtop NMR

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Monitoring chemical reactions by NMR is a common practice in the pharmaceutical industry. High resolution NMR instruments are mainly used for this purpose. Two approaches have been pursued, tube and tubeless NMR. Tube NMR gives an initial understanding on process and mechanism of reactions using deuterated or non-deuterated solvents [1]. Tubeless NMR where the chemical reactor is directly connected to the NMR flow cell through tubing is an emerging field to monitor reactions in situ, understand kinetics and speciation [2]. However, high resolution NMR instruments are expensive, require high maintenance costs, and need analysts with expertise in NMR to operate them. In addition, high resolution NMR instruments are not straight forward to develop as Process Analytical Technology (PAT) tools. An alternative is low resolution benchtop NMR instruments. They are inexpensive, do not require cryogenics, have low maintenance costs, small footprint, and have a potential to be PAT tools. Benchtop NMR instruments mainly acquire 1D ^{1}H NMR experiment, which is the most common and simple experiment used to monitor reactions.

To explore the potentials and limitations of monitoring chemical reactions by low resolution NMR, a 45 MHz picoSpin-45 benchtop NMR instrument has been evaluated. Two reaction types have been carried out for this evaluation, the Fischer esterification and the Suzuki coupling. The Fischer esterification is a classic example of a carboxylic acid and an alcohol catalyzed with sulfuric acid to form an ester. In this particular case, methanol and acetic acid were used to follow the reaction by the changes in the areas of the methoxy groups from the alcohol and the ester over time. However, in real cases, chemical reactions occur under solvents where the reactants and the products may be soluble. The Suzuki coupling reaction [3], an important building block in synthetic chemistry schemes, was chosen because it is a cross-coupling reaction of organoboranes commonly used to form C-C bonds. This reaction has been carried out to evaluate the role of solvents, their concentration dynamic ranges related to the solutes (reactants and products), the potential overlapping of their proton signals with the solutes, and more. In this work, we present our preliminary conclusions on the use of a 45 MHz benchtop NMR instrument for reaction monitoring in chemistry.

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13 With NMR Towards New Diagnostic Methods for Dengue Fever

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Dengue is a viral disease that is quite common in tropical countries. The diagnosis of Dengue is done by eliminating other possible causes of the observed symptoms, a direct antibody diagnosis becomes possible only after several days into the disease. Hence, for the first days, a patient is either not treated (not strong enough symptoms) or treated "on suspicion". A method for early identification of Dengue is desired.

The blood is the main means of transport inside the human body, not only for proteins (like antibodies), but also for small molecules. Due to changes in their metabolism, infected cell could be producing different secondary metabolites, which will show up in the blood plasma. This changes should appear quickly, faster then antibodies. Blood plasma has been extensively studied by NMR [1, 2] and many small mole-cules can be associated to certain NMR signals.

In our study we have analyzed blood plasma from health and infected subjects using proton NMR. The samples were all filtered with 3kDa filters, freeze-dried and re-suspended in D_2O. The resulting 1D NOE spectra [3] were then subjected to an extensive Principal Components Analysis (PCA) [4,5,6], in order to obtain a differentiation. Signal ranges that showed significant changes within the same group were excluded for the PCA, as well as the glucose signals were excluded.

The resulting PCA model was applied to the classification of additional samples. With the exception of one sample, all of them were correctly classified; the wrong one was actually marked as being "out of the model", meaning that a proper classification was impossible.

It turns out that PC2 is the most important component for the differentiation be-tween healthy and infected subjects, whereas PC1, PC3 and PC4 do not contribute to the differentiation. From the PCA model we can identify the most important regions responsible for that result, mainly around 1.92 ppm and 3.38 ppm.

According to assignments published in the literature for blood plasma we can associate the varying signals to a series of compounds: proline, lysine, arginine, acetate, treonine, ß-glucose, pyruvate, citroline, glutamine, isoleucine and other smaller ones. Currently we are trying to establish a connection between the observed signal changes and known body reactions.

The first change that we can assign is the change in the arginine signal. Arginine is associated with the normal immune response of the body[7]. Other changes that were identified are in phenyalanine, citrulline and proline levels. Not all differences found in the NMR spectra could yet be associated to

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14 Statistical Filtering for NMR Based Structure Generation

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Nuclear Magnetic Resonance is the most common tool used for the structure elucidation of new compounds. The used 2D NMR experiments like COSY, HSQC, and ^{13}C–HMBC deliver correlation information between atoms that can be translated into connectivity information. Out of these, correlation information from COSY and HSQC experiments can be transcribed directly into connectivity between atoms. But the ^{13}C–HMBC correlations need more attention because of their ambiguity and complexity. Hence the difficulty of the structure elucidation problem depends more on the type of the investigated molecule than on its size. This ambiguity has driven the development of different software packages like COCON to aid in the interpretation of the ^{13}C–HMBC correlation data [1–19] as much as the development of additional correlation experiments [20,21].

In the case of unsaturated molecules COCON [3, 21-24] will usually generate a very large number of possible solutions. Since the solutions will then have to be checked manually for their chemical feasibility and sense, different efforts have been made to reduce the number solutions. The statistical filter shown compares the suggested constitutions against existing molecules, like the ones contained in the PubChem database. For each Cocon suggested constitution all 1 sphere elements of the constitutions are checked for corresponding elements in PubChem. This comparison is done indirectly, by generating molecular dynam- ics parameters in smi23d, which has been used to generate 3D coordinates for almost 13M compounds contained in PubChem and succeeded on generating coordinates for 99.6% of the molecules contained in the Database. The filtering application eliminates those constitutions for which smi23d fails because of lacking MD parameters.

Since smi23d has successfully been used on so many well known compounds, this means that the structural element for which parameters were missing has hardly ever been observed and therefore might not exist in natural products. Due to the nature of the filter, no ranking of the remaining constitutions is carried out and further methods might be necessary to improve the results.

The filter has been used with several different molecules on the WebCocon Server, and a webpage with some results has been made available on the server: http://cocon.nmr.de/StatisticalFilter/ . Several molecules have been analized, for some, like Ascomycin, the filter showed no reduction in the number of solutions. For others, like Aflatoxin B1, the number of suggested constitutions when using only COSY and 13C-HMBC data, dropped from 970 to 539, a reduction of 45%.

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

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Multiwell assay plates are used in a wide variety of high-throughput measurements, particularly in clinical assays, combinatorial chemistry and drug discovery and are amenable to robotic handling. Image-guided NMR spectroscopy could be used to rapidly obtain spectra of many samples contained in such plates and thereby dramatically increase throughput in NMR assays of small molecules requiring moderate spectral resolution and sensitivity. Unfortunately, attempts to obtain spectra from samples in conventional well plates have been frustrated by broad linewidths arising from poor matching of magnetic susceptibility between the plate, samples, and their surroundings. The short liquid column height in each well, in particular, makes samples in conventional multiwell plates very difficult to shim. Here, we present a new design in which wells of standard size and spacing are formed in a block of susceptibility-matched plastic. Together with susceptibility-matched well caps, these plates dramatically improve shimming and thus spectral resolution and sensitivity. Data obtained using the PRESS pulse sequence on a 7 Tesla 30 cm bore NMR spectrometer demonstrate that highly-resolved spectra of small molecules in aqueous solution loaded into a 96-well susceptibility-matched multiwell plate can be obtained with no detectable crosstalk between samples in adjacent wells. These novel, NMR-compatible multiwell plates should therefore find wide applicability for high-throughput screening in drug discovery, pharmaceutical and chemical manufacturing and quality control measurements, especially where transfer of samples to NMR tubes or flow probes is difficult, unsafe or otherwise undesirable.

16 Search for New Helical Folds in Peptides from α-Amino Acids with Alternating Chirality

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The peptides with an alternation of D- and L-amino acids have been shown to fold as a ß-Helix or a mixed helix[1-2]. Such helices contain intertwined H-bonds, both in the forward and backward direction. In the family of a-peptides, derived from a-amino acids, a 20/22-mixed helix (observed in Gramicidin A), containing intertwined N-H(i+1)•••O=C(i+6) and NH(i+7)•••O=C(i) H-bonds (i+1—> i+6 / i i+4 / i i+2 / i i+2 / i i+2 / i i+2 / i nds in a-peptides in apolar solvents.

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17 NMR Studies of Indole-based Anion Receptors and Anion Thioureas as Transmembrane Transporters

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The conformational preferences of indole-based receptors have been studied by a combination of NMR spectroscopy and ab initio calculations. In the first group of receptors, a sole indole scaffold, which has been functionalized with a variety of amide, urea and thiourea moieties at C2 and C7 [1–3]. Anion–receptor interactions were evaluated through ^{1}H and ^{15}N chemical shift changes. NOE enhancements showed that anti–anti conformation along C2–C2a and C7–N7a bonds is preferred in an acetone-d6 solution in the absence of anions. Upon anion binding to receptors, syn–syn conformation becomes prevalent. The second group of receptors exhibited an additional indole group, which led to diindolyl(thio)ureas [4]. NOE experiments showed that the anti–anti conformer along the C7–N7a bonds was favored in DMSO-d6 solution in the absence of anions. Anion-induced ^{1}H and ^{15}N chemical shift changes suggested weak binding of chloride anions and negligible conformational changes. Strong deshielding of the ureido protons and moderate deshielding of the indole NH has been observed upon the addition of oxoanions, which indicates that the predominant hydrogen bond interactions occurred at urea donor groups. Binding of oxoanions caused remarkable conformational changes along the C7–N7a bonds and the syn–syn conformer was predominant for anion–receptor complexes. The conformational changes in functionalized indoles and diindolyl(thio)ureas upon anion binding are in good agreement with the energy preferences established by ab quantum mechanical calculations.

Tripodal anion transporters containing urea and thiourea groups are capable of chloride/bicarbonate transport through a lipid bilayer and as such represent an interesting class of bicarbonate transport agent [5]. Bicarbonate transport properties of selected novel anion transporters were studied by ^{13}C NMR spectroscopy.

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18 Structure-Based Drug Discovery for Amyloid-ß Peptide: A Novel Example of Rational Screening for Interactions with Intrinsically Disordered Proteins

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Alzheimer's disease (AD) accounts for 60 to 80 percent of all dementia cases, with as many as 5.3 million people suffering from AD in the US [1]. A pathological hallmark of AD is formation of senile plaques, which are primarily composed of aggregated, insoluble amyloid-beta peptide (Aß). The most common forms of Aß are 40 and 42 amino acid residues in length and are called Aß40 and Aß42, respectively. Aß is an intrinsically disordered protein (IDP), which constantly sample a large number of conformations and are prone to aggregation, often causing to abnormal conditions in the cell. While considered benign in its monomeric form [2], Aß aggregates (Aß fibrils [3] and oligomers [4]) are toxic to neurons in culture and in vivo. This has led to the proposition of the amyloid cascade hypothesis, which is now widely accepted as central to AD pathogenesis [5].

There is currently no cure or effective treatment of AD and identification of potential drug interactions with Aß, both attenuating and exacerbating AD pathogenesis, is necessary for a better understanding of future treatments. This study uses a combined in silico and in vitro approach that is novel for use in IDPs. Screening of compounds was accomplished using molecular docking of REMD simulated centroid structures of Aß to a database of small molecules. Targeted compounds were selected based on their theoretically binding affinities for NMR chemical shift perturbation (CSP) and saturation transfer difference experiments were then used to determine experimental interactions between Aß and the small molecule binder. ThT assays, quantifying fibril formation, and dot blots with A11 antibody, measuring oligomer presence, were utilized to determine the effects of binding on Aß aggregation. Such a multifaceted approach will be able to be applicable in the future to other disease-causing IDPs.

Tranilast is the first novel Aß interaction that has been determined using this approach. It is a small molecule drug whose use in the treatment of neurodegenerative conditions, including AD, are currently under patent, having been shown to have anti-apoptotic effect on neurons and increased neurogenesis in neuronal stem cells, both in a dose dependent manner [7]. Despite this, its direct interaction with Aß was previously unknown. By comparing CSP due to tranilast binding to theoretical contact and binding data from the molecular docking, we were able to both correctly infer that tranilast would bind to Aß40 with greater affinity than to Aß42 and predict the modes of binding with reasonable accuracy. Further, our screening method reproduced other, known Aß binders, validating our approach. We have also demonstrated that, despite a poor experimental binding affinity, tranilast significantly increases fibril formation in a dose dependent manner for both Aß40 and 42, with greater effect on Aß40, These results are consistent with the aggregation effect hypothesized from the molecular docking. The initial success of this methodology in identifying novel Aß interactions is just a first step in our goal of identifying

novel compounds that prevent Aß aggregation.

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19 Understanding Relaxation in Solid Pharmaceutical Formulations: Causes, Uses, and Ways to Overcome Long Relaxation Times

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The ability to effectively deliver solid pharmaceuticals is directly related to the form of the drug in the solid state. This is important because more than 70% of all pharmaceuticals are formulated as solids. Drugs may be formulated in several different states, including amorphous, crystalline, or diluted with excipients. In addition, many drugs exhibit polymorphism, or the ability to exist in two or more crystalline phases that differ in the arrangement or conformation of the molecules in the crystal lattice. We are developing solid-state NMR spectroscopy as a technique for the analysis of pharmaceuticals. We are particularly interested in characterizing the effects of formlation on the properties of pharmaceutical solids.

Relaxation times, specifically ^{1}H T1 relaxation times, can be quite long in crystalline pharmaceutical solids. This plays a critical role in the acquisition of CPMAS NMR spectra of drug formulations in the solid state, where the recycle delay for cross polarization experiments from ^{1}H to dilute nuclei such as ^{13}C or ^{15}N depends upon the ^{1}H T1 relaxation time. We have found that it is common to have ^{1}H T1 relaxation times that are tens of seconds at 300 MHz, and that some compounds, such as salicylic acid, can have relaxation times that exceed 5000 s. A typical characteristic of these compounds is that there is little motion occurring at high frequencies, such as methyl group rotation. In these compounds the relaxation time depends upon the distance that the magnetization has to travel to find a relaxation sink.

The most common relaxation sinks for these compounds are either the surface of the crystal or a crystal defect. When the crystals have no defects, we have found that particle size of the drug compounds can be correlated back to the NMR relaxation time. For compounds with no internal relaxation sinks, the particle size that can be measured may be as large as several tens of microns. When particles are micronized, both particle size is reduced and crystal defects are introduced. Crystal defects correspond to high-energy sites within the crystal that are also high mobility, and can relax the particles. Crystal defects are also the nucleation site for either polymorph conversion or chemical degradation. We have found that gabapentin relaxation times correlate well with the chemical degradation rates to the lactam. The advantage of measuring relaxation times over other techniques such as surface area is that relaxation times can be measured in the final dosage form, such as a tablet.

A down side of long relaxing samples is that it may take days to acquire a spectrum. We have been developing a new probe design that facilitates high-throughput solid-state NMR. One fundamental problem with the probe design is providing isolation on the proton side between channels. Even a small amount of cross talk during decoupling of one module can saturate the signal on the other module. We will show how an active feedback circuit can essentially eliminate crosstalk.

20 Conformational Analysis of A Secondary Hydroxamic Acid by NOE Spectroscopy

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Background: Because of their structure, hydroxamic acids (HA's) have an efficient metal-binding ability, especially for ferric iron, and thus function as naturally occurring metal ligands. HA's are used in several settings including pharmaceuticals and metal extractions, and may also have additional applications in medicine and industry [1-3, 4b,5,12]. This is due to their presence in microbial molecules called siderophores, which bacteria and fungi utilize to obtain and bind iron as an elemental nutrient in metabolism [6]. Many microbes that use these iron-binding molecules are pathogenic, causing illnesses such as cholera, the plague, whooping cough, and meningitis, and some examples include e. coli, salmonella, staphylococcus, and streptococcus [6]. Also, as cancer cells also have an extreme demand for iron, there is interest in developing siderophores as anti-cancer agents [1,7]. The relevance of hydroxamic acids to medicine, microbial iron harvesting, and industrial metal-binding applications has prompted the development of various model systems [1-13].

Hydroxamic acids exist in two conformations, E and Z (anti- and synperiplanar) and must be in Z conformation to bind iron [13]. Assignment of E and Z forms has been made using NMR chemical shift trends and NOE studies have been used to assign conformations [4,13-15, 10]. But inconsistencies exist; E and Z have each been assigned as predominant for N-alkyl hydroxamic acids in water in different studies [5, 14, 15].

We are studying NMHA, which is a hydroxamic acid that is very similar in structure to native siderophores. Through these studies we hope to understand this compound's conformational preferences as a function of solvent polarity and concentration. This knowledge is necessary for the creation and design of synthetic HA's that behave in the same manner as those found in nature, which will allow for development in industry and medicine.

Methods: A synthesis of hydroxamic acids reported by Lee and Miller was modified to make NMHA in three reactions [16]. In the first reaction, paraformaldehyde was stirred with O-benzylhydroxylamine at a pH of ~7, then extracted and dried to isolate the oxime intermediate. Next, in the second reaction, using oven-dried glass ware, the oxime was dissolved in glacial acetic acid, followed by additions of acetic anhydride and then cyanoborohydride. The mixture was then extracted, dried, evaporated, and purified by column chromatography to isolate O-benzyl N-methyl acetohydroxamic acid. In the final reaction, using acid washed glassware, the O-benzyl N-methyl acetohydroxamic acid was hydrogenated for 45 minutes, then centrifuged and evaporated to yield the NMHA product.

NMR studies were done using a Bruker Avance III 400 MHz spectrometer running Topspin 3.1 software. NMHA was dissolved in low-paramagnetic D2O (Cambridge Isotopes, used without further purification) to a concentration of 0.035M, and a second sample was prepared at 0.0035M by dilution of the first sample. The specimen used for NOE studies (0.035M) was septum sealed and degassed using

three freeze/pump/thaw cycles. D2O spectra were externally referenced to 25mM sodium 3-(trimethylsilyl)-1-propanesulfonate in D2O. NMR temperature was controlled for all data collection. Both 1-Dimensional proton spectra and NOE spectra were collected, with the mixing times for the NOE spectra ranging from .2 to 1 sec. Data were collected at 281K to obtain maximum signal resolution and minimize exchange. For NOE experiments, irradiation at various mixing times was centered on the major or minor N-methyl frequency.

Results: Analysis by NOE spectroscopy was complicated by conformational interconversion, because irradiation can be transferred from one form to another via chemical exchange. Thus, an NOE build-up curve was used to diagnose and differentiate direct versus relayed NOE's. Interpretation was further complicated by weak long range coupling between acetyl and N-methyl protons in both conformations, especially in very short mixing times. Evidence of long-range coupling will be presented. A clear positive peak was obtained at mixing times =0.2s, which defined mixing times used for studies. In the growth curve for responses to irradiation of the minor N-methyl resonance, the intensity of the first response can be extrapolated back to t˜0, indicating a direct NOE between the minor N-methyl and minor acetyl signals. By contrast, the NOE build-up curve for the major peak appears to begin at a time after 0 sec, which is consistent with a relayed NOE due to conformational interconversion. These data are supportive of a favored conformation of E for NMHA in D2O. The minor species is thus assigned as the Z conformation.

From our findings, the conformational preferences of N-methyl acetohydroxamic acid (NMHA), based on NOE data in D2O at 281 K, is that NMHA exists in the expected states in a 76:24 ratio, but favors the E conformation, which is different from prior report [5]. Further behavior of NMHA in other solvent systems is currently being studied, with comparisons to existing literature where possible. These studies are done to assemble a detailed picture of the conformational behavior of the prototypical N-alkyl hydroxamic acid as a function of solvent polarity. These results have implications for the understanding of native siderophore function, as well as for the design of novel hydroxamic acids for specific purposes.

Discussion: Results of the NOE studies conclusively identify the minor conformation of NMHA at 281K in D2O as Z. The presence of long range coupling in both conformations is also demonstrated. These data help clarify the structural preference of NMHA, which has been reported to favor the E conformation in all solvents except DMSO [4b]. Additional conformational analysis of NMHA and homologs will aid in effective hydroxamic acids for medicinal and commercial applications.

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21 Segmented Flow Into LC Probes Doubles Flow-NMR Sensitivity and Throughput

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* Maintains uniform original concentration, for ligand-binding or qNMR studies.
* Confines sample in NMR coil volume, for optimal sensitivity.
* Improves throughput by 10 min/sample; reduces solvent consumption 5-fold.
* Applicable to cryo-flow for trace sensitivity, or old LC probe for high-throughput.

Flow-injection NMR (FIA-NMR) has proven valuable for acquiring spectra directly from vials or 96-well plates, avoiding the time and expense of preparing samples in tubes, and specialized automation to load tubes. Extending our previous work in segmented flow loading of microcoil probes ("microdroplet NMR") [1], we here show how segmented flow (SFA) loading solves several problems seen with conventional flow-NMR into LC probes (60 uL or larger).

Segmented flow loads samples without dispersion or dilution -- the sample is transported like a drop of water in oil. The flow system is filled with an immiscible fluid (fluorocarbon FC43) so the sample moves as a droplet or, in narrow tubing, as an elongated plug with a sharp boundary and with uniform (undiluted) concentration throughout. FC43 has magnetic susceptibility matched to D2O, and functions as "Shigemi tubes for a flow system" to confine the entire sample within the observed volume.

To implement segmented flow, use of a fluorinated fluid in Teflon tubing is ideal; fluoro-silanized glass surfaces also work well. The data presented were obtained by using a stock commercial flow-NMR platform (Protasis One-Minute NMR) with a Teflon sample loop and transfer line. SFA and FIA were compared in in video of injected dye, and in on-flow and stopped-flow NMR spectra of a standard (20 mg/mL caffeine in D2O).

Results: SFA loading was explored in a cryo-flow system with a 60 uL nominal NMR observed volume (120 uL flowcell volume; 150 uL dead vol to center of V-obs). SFA loading 70 uL of the standard gave twice (2x) the NMR signal intensity as FIA loading; which was 95% the signal intensity of a flowcell filled with the standard. A 50 uL sample could be shimmed to the same lineshape as 70 uL or larger, but accordingly shows only 70% of the signal strength (50 uL/70 uL = 71%).

Video of dye injections by SFA and FIA provide a concrete picture. Samples loaded with SFA move into the detected volume as a discrete object, like a car into a garage; accordingly, on-flow NMR signal strength rises linearly from first light to 100% as the leading edge of sample fills the coil volume; plateaus at 100% while the coil is filled, then linearly decreases to 0 as the trailing boundary of the sample passes out of the detector region. FIA samples, in contrast, arrive like the hot water at a distant tap: signal intensity increases more slowly with a slope projecting to 100% after 0.7 dead volumes, but cresting and tailing for smaller samples. Parabolic flow in the flowcell produced large concentration

gradients both radially and axially, reflected in the lineshape (30 Hz) of the caffeine methyl resonances, which are concentration-sensitive. When flushing samples, the tailing in FIA required 300 uL (nearly 3 flowcell volumes) to bring residual signal below 2%.

Applications: Loading samples at a defined and uniform concentration enables ligand-binding or qNMR studies. Confining samples into the observed volume gives the theoretical optimum sensitivity for a probe, under unattended automation. SFA-NMR with cryoprobes gives high sensitivity; an old LC probe would serve for high throughput of routine chemistry samples.

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22 Searching for the Source of Enantiodifferenting Alignment in Lytropic Liquid Cristalline Phase of Helically Chiral Polyacetylenes

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The determination of conformations and relative configurations by nuclear magnetic resonance usually involves distances from the NOE and dihedral angles from ^{3}J couplings. It is, however, often complicated by either absence of NOE data and/or ^{3}J coupling data, remoteness of the stereocenters or conformational equilibria. The recently reintroduced residual dipolar couplings (RDCs)[1] provide complementary information to these conventional NMR restraints.

RDCs belong to the class of anisotropic NMR parameters and therefore the compound in question needs to be oriented with respect to the magnetic field in order to be able to observe them. The only known class of chiral orienting media for organic solvents are the lyotropic liquid crystalline (LC) phases of rigid polymers like homopolypeptides[2] and as first non peptidic polymer, helical chiral polyguanidines[3].

We recently presented enantiodifferenting alignment media based on helically chiral polyacetylenes[4]. These polyacetylenes based lyotropic liquid crystalline phases showed some beneficial properties, like weak alignment, small polymer residue signal in the spectra, simple phase preparation and the highest degree of enantiodifferenting known today for any alignment media in organic solvents. This new system has been validated for chiral analytes.

The mechanism of enantiodiscrimination alignment is for today still unknown, this lack of knowledge is one of the key drawbacks in our aim to determine the absolute configuration of unknown compound by the use of RDCs.

In this work we tried to figure out how enantiodifferenting alignment comes into effect. In a helically chiral polyacetylenes are two kind stereogenic information, on the one hand the stereocenter of the alpha amino-acid in the sidechain of the monomers and on the other hand the helical superstructure. The superstructure of the helically chiral polyacetylenes is stabilized by hydrogen-bonds between neighboring side-chains. This hydrogen bond network can easily be alter this also an huge effect of the persistence length of corresponding helix. This is important due to fact, that these helices are the mesogenes forming the lyotropic liquid crystalline phase (LLC), therefore by removing the helices, you would destroy the LLC.

Based on the work of Yashima[5] we are utilizing the dynamic nature of the polyacetylenes to form sergeant and soldier copolymers from achiral amino-acid 2-aminoisobutylic acid and a small amounts of chiral monomer. LLCs based on this co-Polymer, which forms helices similar to the homopolymer, have

an reduced number of chiral centers in the side-chain. If we find the same amount enantiodifferenting alignment in this phase it is a strong evidence that the source enantiodifferenting alignment is originated in the helically superstructure of the polymers.

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23 ADEQUATE – the B Sides

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The structure elucidation of natural products is particularly challenging because of the novel or non-standard structural features they often contain. Usually, if a crystal structure is not accessible, the molecular constitution of a new natural product is elucidated by the application of different spectroscopic (IR, NMR, and UV) and spectrometric (MS) methods. In the course of the analysis, NMR spectroscopy has a special importance, as it is the only out of the four methods which delivers the structure in atomic resolution, i.e. constitution, configuration, and conformation.

Standard NMR approaches for the constitutional assignment of natural products uses HSQC, COSY, and HMBC experiments. If the structure cannot be unambiguously solved with this approach, further methods such as $^{13}C,^{13}C$ correlations may help to elucidate the molecular constitution. The classic experiment for homonuclear carbon correlations, the INADEQUATE (Incredible Natural Abundance Double Quantum Transfer) is usually too insensitive to be of practical use for natural product samples since often only milligram quantities (or even less) are available.

The ADEQUATE experiments represent a complete methodology to observe C,C correlations over two to six bonds in non-labeled organic molecules. The most sensitive experiment of this series is the 1,1-ADEQUATE which is a valuable method in gaining unambiguous constitutional information. The 1,n- and n,1-ADEQUATE experiments which require substantially more measuring time give the possibility “to see” one atom further than the HMBC experiment.

The direct translation of proton/carbon correlations, i.e. connectivities, observed in the HMBC spectrum into bonding information is hampered by the fact that correlations between protons and carbons separated by 2, 3 or more bonds cannot be distinguished. For proton-poor compounds with additional heteroatoms (non-carbon atoms), e.g. alkaloids, this will result in a large quantity of possible molecular constitutions. The application of the 1,1-ADEQUATE experiment allows to differentiate between two- and three-bond correlations in the HMBC spectrum.

The use of an 1,1-ADEQUATE experiment definitely reduces the risk to determine the constitution of a natural product wrongly. We have shown several times that the incorporation of 1,1-ADEQUATE data into a structure generator (e.g. COCON) dramatically reduces the number of structural proposals.

Recent developments in NMR spectroscopy with respect to sensitivity, such as cryogenic probes and micro probes (1, 1.7, and 3 mm), have dramatically decreased the required amount of material for structure elucidations to a few micrograms for proton spectra and to a few 100 micrograms for proton/carbon correlation experiments. Especially the combination of both developments allow the use of $^{13}C,^{13}C$ correlations as standard experiments if only a few milligrams of a compound are available. Therefore, the 1,1-ADEQUATE experiment is becoming a standard tool in the structure elucidation of

natural products.

In order to support the last sentence several, so far unpublished, applications of the ADEQUATE experiment will be discussed. A special focus is given on measuremets with the 1.7 mm cryo probe which allows to get this kind of correlations with sample amounts of 2 mg.

24 Configuration and Conformation from fc-rDG/DDD: All in One Shot

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The determination of the relative and absolute configuration of natural products is essential to understand their interactions with biological targets on a molecular level and to allow their procurement through total synthesis. Methods such as X-ray crystallography require crystalline products, and chemical synthesis is usually very time consuming and not always definitive. The structural elucidation of amorphous molecules with several unknown stereogenic centers would benefit greatly from a method that could simultaneously analyze all configurations. Here we discuss how effective NOE/ROE effects in combination with computational methods can be used for this purpose.

The NOE/ROE restraints may be used in a qualitative way or as distance restraints in EM or MD simulations. These approaches are problematic with a large number of unknown stereogenic centers. Therefore, a method is required which allows the determination of all unknown stereogenic centers simultaneously and without the necessity of crystalline products. The fc-rDG/DDD method (floating chirality res-trained DG/DDD) [1] is a combination of distance geometry (DG) [2] and distance-bounds driven dynamics (DDD) calculations [3] using interproton distances and floating chirality [4] in order to determine the relative configuration of small molecules (including low molecular-weight natural products).

Recently, the fc-rDG/DDD method was applied to structurally very complex members of the pyrrole-imidazole alkaloid family [5]. These dimeric pyrrole-imidazole alkaloids have all eight stereogenic centers in common, necessitating a method which allows a simultaneous determination of all unknown centers. The results of the fc-rDG/DDD calculations on tetrabromostyloguanidine revised the relative configuration of the palau'amine congeners in 2007 [6].

The investigation were expanded to the dimeric pyrrole-imidazole alkaloids axinell-amine A [7] and 3,7-epi massadine chloride [8]. Here the originally proposed configuration was confirmed by the fc-rDG/DDD calculations [9]. Out of the 128 possible diastereomeres of axinellamine only four were generated in the fc-rDG/DDD calculations. One of them was generated by more than 90% which is the same diastereomer originally published for axinellamine.

Another example is an intermediate in the total synthesis of palau'amine, 20-deoxy-macropalau'amine azide [10] which will be the main focus of this contribution. This compound has a very interesting structure with a 9-membered ring. Besides the configurational assignment of the five stereogenic centers, a detailed conformational analysis was carried out. This is of special importance because macro-palau'amine is the direct precursor of palau'amine in its total synthesis. The final step in this synthesis is a transannular ring closure of macro-palau'amine to palau'amine [11].

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25 Conformational Properties of Poly-Alcohols Using Hydroxy Protons as Structural Probes

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Due to high flexibility and tendency to form hydrogen bonding networks, conformational study of poly-alcohols (polyols or sugar alcohols) is a troublesome task. Furthermore, conformations of polyols are known to play a significant role at molecular level of sweet taste reception. The aim of this work is to elucidate topological features of polyols by employing somewhat unusual NMR techniques. Polyols investigated in this work are glycol, glycerol, erythritol, xylitol, mannitol and sorbitol. The method involves assignment of each sugar alcohol by 1D ^{1}H and ^{13}C, 2D homonuclear NOESY, ROESY, TOCSY, and 2D heteronuclear HSQC NMR spectra. More importantly, hydrogen bonding properties were tracked by ^{1}H chemical shifts (delta), vicinal coupling constants (^{3}J), temperature coefficients (d(delta)/dT) and exchange rates (k(ex)) of hydroxy protons. The results has been clarified hydrogen bonding attributes, showing that despite of their flexibility, polyols of interest tend to have distinct conformations in aqueous solution at 263 K. The outcomes of this work could be exploited to reveal geometrical trends of sugar alcohols and, subsequently, larger sweet-tasting molecule structures in aqueous environments.

26 Towards a Better Understanding of 1D ^{1}H NMR Spectra: Application of ^{1}H Iterative Full Spin Analysis

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NMR spectroscopy is one of the most powerful and versatile tools to obtain structural information at the molecular level. After more than 50 years of continuous development, solution-state NMR has gradually evolved into a cornerstone technique for the analysis of a wide range of materials. Still, the in-depth interpretation of complex resonance patterns observed in 1D ^{1}H NMR spectra typically remains a challenging task. In the case of small molecules, three main factors contribute most to the interpretation challenge: signal overlap due to the narrow 1H domain, higher-order coupling effects, and sometimes unexpected long-range coupling. Although these challenges can be overcome by the use of NMR simulation and deconvolution tools, these computational methods are still underutilized and possibly undervalued.

This report describes the development of an standardized workflow for ^{1}H iterative Full Spin Analysis (HiFSA), allowing the complete description of 1H NMR signal patterns. This methodology involves (a) the prediction of basic NMR parameters (dH, $J_{H,H}$) using a 3D model of the analyte, (b) the simulation of a ^{1}H NMR spectrum using the predicted parameters; and (c) the iterative optimization of the calculated parameters by comparison with experimental ^{1}H NMR data. The dH, $J_{H,H}$ and line widths values are refined until an excellent agreement with the experimental observations is reached, thereby obtaining a highly detailed, reproducible ^{1}H NMR profile, i.e., a ^{1}H fingerprint. Furthermore, as the HiFSA-generated ^{1}H NMR profiles are composed of field-independent parameters, the ^{1}H fingerprints can mimic NMR spectra recorded at any magnetic field. Presented examples of ^{1}H fingerprints of small molecules, particularly natural products, and their generation by HiFSA will demonstrate the feasibility of the HiFSA workflow for regular application in NMR spectral analysis.

27 Study of Alignment Media Based of Polyacrylamide Gels and Applications

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Residual dipolar couplings (RDCs) have had a great impact on structure calculations of small molecules and bio-molecules in the past decade and have proven to be very efficient to solve stereochemical problems in small molecules. They hold the promise of the determination of stereochemistry even for non-rigid molecules, making it possible to provide extremely efficient and powerful structural information in the analysis of small molecules [1-5].

Since RDCs cannot be directly observed in conventional liquid state NMR experiment (isotropic conditions), the sample needs to be exposed to an anisotropic medium to observe them. In this context, it's fundamental to use an alignment media that is compatible with organic solvents and shows repeatability of alignment [1,5,6].

It is well known that RDC's give information on the orientation of internuclear vectors. But, furthermore, residual chemical shift anisotropy (RCSAs) of the chemical shielding tensors of each atom in the molecule can also be observed, in other words, RCSAs also can be used to determine configuration of molecules [7, 9].

The determination of anisotropic NMR parameters such as RDCs and RCSAs is the goal of this work. Our group has developed different polyacrylamide gels as alignment media mainly for applications in DMSO [8-10].

This work evaluates the effect of the concentration of the cross-linker (N, N'-Methylenebisacrylamide) in the polyacrylamide gel. We obtained gels with repeatability of alignment; it was observed that the alignment is dependent on the concentration of the cross-linker in the gel composition. We also obtained an increase in alignment with increase of cross-linker concentrations. Finally, we achieve different states of alignment with the different gels, which can also be used to also measure RCSA.

Preparation of the confined cross-linked polymers (gels), sample preparation, NMR experiments for the acquisition of RDCs in aligned media, as well as few examples of the comparison of different alignment tensors obtained with each gel using 11R,12R (-)-threo-mefloquine HCl as test molecule will be discussed.

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28 Design, Development and Application of an Online NMR Reaction Monitoring Platform

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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. It allows chemists and engineers to make informed decisions based on qualitative and quantitative information, ultimately leading to more robust processes as part of a quality by design (QbD) strategy. An overview of an NMR reaction monitoring platform will be outlined, and the implementation of this technology will also be demonstrated; citing specific examples of reactions developed and optimized employing online NMR reaction monitoring.

29 qNMR for All Samples

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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 describe 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.

Performance expectations such as the acceptable error must be underpinned by adequate data quality, where typical considerations include shimming, signal-to-noise (S:N), and time between pulses relative to compound relaxation (T1) times. Further complications can arise when the sample is impure.

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 one(s) that are likely to provide the best overall compound concentration. We achieve this programmatically using a number of approaches, and suitability again depends on the data. A relatively simple option is to choose multiplets that deliver the lowest RMSD, but other selection criteria can be selected that bias choices to the most favourable multiplets on the basis that their integration exclusively represents the analyte. Finally, reporting is done in conventional ways.

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.

We have designed a powerful, versatile tool for qNMR. A more detailed A more explanation of the workflow and performance will be described in the poster.

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30 Automated Structure Verification and Assignment- From ^{1}H and HSQC to Global Multi-Spectral Auto-Assignment

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Automated structure verification software methods have mostly surrounded the use of ^{1}H NMR data or a combination of ^{1}H and HSQC NMR data [1]. While these methods have produced reasonable results for evaluating the consistency of proposed chemical structures, there is significant interest in the accurate automatic atomic assignments of ^{1}H and ^{13}C chemical shifts. A previous publication has shown that the inclusion of COSY connectivity information does improve the auto-assignment accuracy as well as the false positive rate [2]. Presented in this current work is the expansion of our current auto-assignment algorithm to a collection of NMR spectra that also include long-range correlation information from COSY and HMBC experiments.

This method has been tested on spectral collections (including ^{1}H, HSQC, COSY, and HMBC) for several commercially available compounds. The resultant assignment was compared to that obtained by our current combined verification approach (based on ^{1}H and HSQC data). In addition, for each proposed structure, several isomers were generated with maximal NMR-HSQC similarity to compare this new approach against the standard combined verification approach.

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31 Structure Revision of Asperjinone using Computer-Assisted Structure Elucidation (CASE) Applications

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Computer-Assisted Structure Elucidation (CASE) applications are widely used today to characterize chemical structures ranging from natural products to synthetic products. In the past decade, multiple comparisons have been published to illustrate that ACD/Structure Elucidator [1,2].

The development of ACD/Structure Elucidator is derived primarily by continuously challenging the application with new structural problems described in the literature. Within this study we utilized the analytical data reported by Liao et al. to deduce the chemical structure of a new natural product known as Asperjinone [3]. Based on the results of the CASE application, the chemical structure of Asperjinone was revised. This work will highlight the differences between the original and revised structures and discuss the methods employed for the structure identification.

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32 Experimental and Theoretical Investigation of $^{1}J_{CC}$ and $^{n}J_{CC}$ Coupling Constants in Strychnine

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A relatively unexplored and unexploited means of establishing stereochemistry and probing vicinal bond angles is through the use of long-range ^{13}C-^{13}C coupling constants. In the past, the primary approach to measuring long-range carbon-carbon homonuclear coupling constants was through the synthesis of isotopically labeled compounds or the acquisition of time-consuming heteronuclear detected experiments like INADEQUATE requiring large amounts of sample. The development of cryogenic NMR probes has facilitated the measurement of $^{1}J_{CC}$ coupling constants through the use of the ^{1}H detected J-modulated ADEQUATE experiment. The next logical step, the measurement of the stereochemically diagnostic $^{n}J_{CC}$ couplings, has not been reported on sample amounts that are practical for the practicing organic chemist. We describe here a generalized protocol for the measurement of $^{1}J_{CC}$ and $^{3}J_{CC}$ couplings and the first results obtained using strychnine as a model compound. We also demonstrate the utility of DFT calculations for the calculation of these useful molecular descriptors. Comparison of the experimentally measured $^{1}J_{CC}$ coupling constants with those calculated using DFT methods showed an r2 > 0.99 (rmsd = 1.35Hz); for the $^{3}J_{CC}$ couplings measured, excellent agreement was also obtained with r2 > 0.94 (rmsd = 0.43 Hz).

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33 Selective Excitation 1D-NMR Experiments for the Assignment of the Absolute Configuration of Secondary Alcohols

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Routine selective excitation experiments, easy to set up on modern NMR spectrometers, allow for the determination of the absolute configuration of chiral secondary alcohols by double derivatization directly in the NMR tube. As a general method, TOCSY1D with selective excitation of the alpha proton in the MPA esters and with a short mixing time reveals only the nearby protons in the coupling network. Typically, the analysis takes less than 30 minutes. Longer mixing time, selective excitation of other signals, or NOESY1D experiments can be used for measuring Delta(delta)RS of other protons.

34 Determination of Amino Acid Chirality in Natural Peptides using Marfey's Method: Solving the Second Chiral Center by HPLC-SPE-NMR

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Natural non-ribosomal peptides may be comprised of both proteinogenic and non-proteinogenic amino acids with L- or D- configuration. Regarding the structural elucidation of these compounds, once the sequence and planar structure of a natural peptide has been established, determination of its amino acid chirality is typically carried out by application of Marfey's method [1]. This consists in a complete acid hydrolysis of the peptide and a subsequent derivatization of the resulting amino acid pool with a chiral reagent [1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA, Marfey's reagent)] which carries a strong chromophore. Derivatization of enantiomeric L- and D- amino acids with the chiral reagent converts them into diastereomeric pairs which can be resolved by chromatography using conventional reversed-phase HPLC. Comparison with the corresponding derivatized amino acid standards allows unambiguous establishment of chirality at Ca. The use of LC-MS and ion extraction processing greatly facilitate such analyses especially for those cases where peak overlap among different amino acid derivatives occurs. Nevertheless, amino acids carrying a second chiral center may be problematic. Marfey's derivatives of epimeric amino acids at the second chiral center are very difficult or impossible to resolve by reversed-phase HPLC and thus the establishment of absolute configuration at this second chiral center is not trivial. Such is the case of isoleucine (Ile) and threonine (Thr) which can be found in many natural peptides also as their allo-forms, or 3- and 4-hydroxyproline (Hyp) which may occur in nature with both cis or trans configurations. We propose a new approach based on the use of Marfey's method combined with HPLC-SPE-NMR to sort out this problem. This approach is based on the fact that Marfey's derivatives of epimeric amino acids display different chemical shifts. Thus, simple comparison of trapped HPLC peak 1H NMR spectra with the corresponding spectra of standards allows unambiguous assignment of the absolute configuration at the second chiral center in such cases. The use of a low volume microcryoprobe facilitates the analysis of the very minor amounts of Marfey´s derivatives usually obtained after the hydrolysis of a peptide at a sub-milligram scale. This novel approach is illustrated for the pair L-Ile/L-allo-Ile and applied to a natural cyclopeptide recently isolated from the fungus Onychocola sclerotica [2].

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35 Inverted $^{1}J_{CC}$ 1,n-ADEQUATE: Spectral Editing of a Long-Range Carbon-Carbon Correlation Experiment for Structure Elucidation

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ADEQUATE experiments are a type of out-and-back 2D NMR correlation experiment that begins with a $^{1}J_{CH}$ transfer followed by either a $^{1}J_{CC}$ transfer (1,1-ADEQUATE) or a $^{n}J_{CC}$ transfer (1,n-ADEQUATE) [1]. Magnetization is then transferred back along the same pathway to the starting proton. Correlations to adjacent ($^{1}J_{CC}$) or long-range ($^{n}J_{CC}$) carbons are observed at the proton shift of the starting proton, and the carbon shift of the remote carbon. The transfer pathways are shown schematically below. These experiments are useful and can be performed on 1 mg or less of material using MicroCryoProbe NMR at 600 MHz [2,3].

When acquiring 1,n-ADEQUATE spectra, because of the oscillatory frequencies of the $^{1}J_{CC}$ and $^{n}J_{CC}$ couplings, there is always unavoidable leakage of $^{1}J_{CC}$ correlation information into a 1,n-ADEQUATE spectrum. Rather than viewing these correlations as a nuisance, we elected instead to develop a pulse sequence that inverts them, rendering them readily differentiated from the desired $^{n}J_{CC}$ correlations. Furthermore, with the $^{1}J_{CC}$ readily identified, in some cases it becomes possible to avoid even acquiring a 1,1-ADEQUATE spectrum.

The alkaloid retrorsine was used as a model compound for the development of the new inverted $^{1}J_{CC}$ 1,n-ADEQUATE experiment. Compared to the original 1,n-ADEQUATE, the new experiment has a cleaner noise floor and is also amenable to covariance processing [4].

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36 $^{1}J_{CC}$-Edited HSQC-1,n-ADEQUATE: A New Paradigm for Establishing Molecular Structure

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Undesired $^{1}J_{CC}$ correlations unavoidably "leak" into 1,n-ADEQUATE spectra because of the oscillatory nature of the amplitude transfer functions of the $^{1}J_{CC}$ and $^{n}J_{CC}$ coupling constants. Consequently, the combination of 1,n-ADEQUATE spectra with GHSQC spectra using unsymmetrical indirect covariance (UIC) or generalized indirect covariance (GIC) processing yields a spectrum in which $^{1}J_{CC}$ correlations are indistinguishable from $^{n}J_{CC}$ correlations [1,2].

However, generalized indirect covariance (GIC) processing of a non-edited GHSQC spectrum with an inverted $^{1}J_{CC}$ 1,n-ADEQUATE [3] spectrum affords a $^{1}J_{CC}$-Edited HSQC-1,n-ADEQUATE spectrum [4]. Direct ($^{1}J_{CC}$) correlations between pairs of protonated carbons are diagonally symmetric and have negative phase; $^{1}J_{CC}$ correlations between protonated and adjacent non-protonated carbons are diagonally asymmetric and negative; long-range (typically *via* $^{3}J_{CC}$) correlations between pairs of protonated carbons are diagonally symmetric and positive; long-range correlations between protonated and non-protonated carbons are diagonally asymmetric and positive. A hypothetical structural fragment is shown below followed by a schematic representation of the types of correlations observed in the spectrum.

Strychnine is used as a model compound to illustrate the results obtained. The resulting $^{1}J_{CC}$-Edited HSQC-1,n-ADEQUATE spectrum provides unequivocal access to $^{1}J_{CC}$ correlation data equivalent to $^{2}J_{CH}$ correlations in a GHMBC and predominantly $^{3}J_{CC}$ long-range correlations equivalent to $^{4}J_{CH}$ GHMBC correlations. When used to complement a readily acquired GHMBC spectrum, the synergy of these data provides a powerful new paradigm for establishing molecular structures.

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37 Automatic Spectra Assignment Using Human Logic Emulation

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The assignment of NMR spectral signals to given molecular features of a sample is a very sophisticated task performed by the analyst in order to determine the consistency between the spectrum and the given structure. A structure is considered to be in consistence with the spectrum, only if all peaks in the spectrum can be fully explained logically and by taking into account all available information with all spectroscopically relevant features of the molecule or with “artifacts” arising from solvent signals or impurities.

Therefore the whole spectrum or all available spectra and the complete molecule are taken into account as well as information from sample workup and synthesis.

Here we present latest results on our new computer science approach based on “human logic emulation” (HLE) where we developed a sophisticated algorithm which does exactly this. The results of this fully automatic data analysis and spectra interpretation can either be used “as is” or can be used as a very good starting point for further expert refinements of the interpretation.

Our algorithm analyzes standard 1D 1H NMR spectra and can also handle additional information from other spectra such as 2D HSQC. The analysis can either be started manually after processing of the NMR data off-line in a separate program (CMC-assist). The algorithm also may run directly on the NMR instrument, providing the user with an interpreted and analyzed spectrum directly.

The HLE algorithm uses all available information provided by the user (the structure itself, 1D or 2D spectra as well as information on solvents and possible impurities). It builds up interpretation hypothesis spaces for structure and spectra and calculates a wealth of possible assignment solutions, which it ranks by probability based on a multitude of chemical and spectroscopic rules. The rules themselves are also weighted and range from “very fuzzy” and “nice-if-obeyed” to very strict “killer-rules” which could rule out the consistency between spectrum and structure. The HLE algorithm therefore takes into account all spectroscopic features such as signal integrals and shapes, multiplicities and possible coupling constants or chemical shift ranges.

The algorithm yields a statement about the consistency between spectrum and structure. It also provides a fully explained spectrum where all signals are assigned either to structural features or solvent or impurities. In addition, a purity estimation is provided which puts impurity signals and compound signals into relation. And, if the spectrometer was calibrated, the algorithm also offers an automatically determined concentration of the compound.

38 Evaluating the Use of ASAP-HMQC and Sensitivity-Edited HSQC Spectra Acquired with Non-Uniform Sampling for Rapid Screening of Organic Samples

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There has been increasing interest in using edited HSQC spectra in combination with ^{1}H and ^{13}C data sets for screening organic compounds. However, the time and/or sample concentration still inhibits this approach unless one has access to a cryoprobe. An alternative would be to use the ASAP-HMQC sequence of Freeman and Kupce, which allows for faster acquisition but does not provide spectral editing and gives slightly poorer ^{13}C resolution. However, significant further improvements are possible with the availability of software for acquiring and processing non-uniformed sampled (NUS) 2D data. We have investigated this possibility using an Agilent DD2-600 NMR spectrometer equipped with a OneNMR (room temperature) probe. Using 10mM (ca 2 mg) solutions of representative organic compounds, we find that, with the aid of NUS, it is feasible to obtain high quality 2D shift-correlated spectra in l.5 minutes or less with ASAP-HMQC and as little as 3 minutes with the sensitivity-enhanced CRISIS2-gHSQC sequence. In each case, the time to acquire a spectrum is less than the 'overhead' time required for a sample changer to replace one sample with another, followed by locking, tuning and shimming. Thus, there would be limited advantage in further decreasing acquisition times, although a cryoprobe would allow one to further decrease the sample concentration requirements.

39 Distingushing the Different Ontario Ginseng Landraces and Asian Ginseng Species using NMR-based Metabolomics

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The use of NMR to distinguish and identify unique markers of five Ontario ginseng land races and comparison of two ginseng species were evaluated. North American P. quinquefolius was distinguishable from Asian P. ginseng by PCA-ANOVA analysis utilizing a 1D-CPMG experiment. Of the five P. quinquefolius land races,. the PCA-ANOVA analysis showed that landraces (2 and 5) differ significantly from each other and to the remaining three land races (ps and demonstrates the potential for this technique to be used to identify ginseng land races to the individual farm level. The specific differences between each farm will likely be more apparent over time as land races drift further apart.

Background

Ginseng cultivation in Ontario began over 100 years ago when the seed was cultivated from wild ginseng plants. Today, Ontario ginseng (Panax quinquefolius) comprises several unimproved land races. A consistent seed stock has been maintained in these land races without mixing or introducing new seeds. In a previous HPLC-DAD study, ginsenoside content within and between Ontario ginseng land races was examined using 6 major ginsenosides (Rg1, Re, Rb1, Rc, Rb2, and Rd) as markers [1]. Using this method, it was clearly shown that significant variation in ginsenoside content does occur between land races, however, unique characteristics that would rapidly identify each land race based on these markers could not be identified. There is interest in identifying unique characteristics of land races as the differences in phytochemical characteristics could lead to the development of unique cultivars with distinct activities. Furthermore, developing techniques to distinguish land races has standardization and quality control implications as this would display the possibility of verifying the cultivation location for individual farms.

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40 Applications of NMR Spectroscopy in the Detection, Characterization, and Quantitation of Drug Metabolites

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Unambiguous structural characterization of chemically reactive and potentially toxic metabolites is on the critical path to any successful lead optimization program. Current MIST guidelines require complete toxicological evaluation, identification and characterization of all significant metabolites (>10% of parent) observed in human as well as unique human metabolites not observed in other species. Drug metabolites are usually isolated in very small quantities (µg) from in vivo biological matrices and can contain endogenous impurities which often co-elute by HPLC along with metabolites of interest. Sensitivity limitations have typically limited the use of NMR to later stage drug candidates where sufficient (low mg to high µg) quantities of the metabolite are available for detailed structural studies. Additionally, endogenous impurities (such as plasma proteins, bile salts) can complicate if not prevent NMR spectral assignments and may require re-purification of metabolite resulting in further loss of material. Here we will illustrate how complete NMR dataset including 1D, 2D, homo and heteronuclear experiments can be obtained on low nanomoles of material through the use of 3 mm MicroSample cold probe, enabling complete structural characterization. Using NMR mixture analysis tricks such as selective excitaton, diffusion-editing and spectral deconvolution, metabolites can also be detected, identified and quantitated from biological matrices without need for re-purification and some of these strategies will be also be illustrated.

While used most often for structural analysis, NMR is increasingly considered a critical quantitative tool, and with advances in probe technologies, NMR is very relevant for quantitation studies of drug metabolites in biological matrices.1 This is especially relevant in the current regulatory climate and provides the opportunity to be able to quantify significant circulating drug metabolites from biological matrices where no reference standards may exist. Absolute quantitation by NMR or qNMR available with the VNMR J3 software package uses external standards and does not require reference standards of the metabolite you are trying to quantify. Periodic calibration with an external standard can deliver accuracy as high as 99.9% and precision of 0.59%, while performing calibration with each study can result in accuracy and precision as high as 100% and 0.35%, respectively. Using this methodology we were able to quantify tetracycline with an LOQ of two nanomoles and excellent linearity of data (R2 = 0.99) was observed up to four orders of magnitude limited only by concentrations analyzed.

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41 Length Scale of Disorder: One State vs. Two States Models and Implications for Solid State Method Development

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In this study we are trying to understand the effect of length scale of sample domains on the ability of different analytical techniques to characterize (and quantify) disorder. We are presenting comparison of PXRD, DSC and SSNMR data on several model pharmaceutical compounds subjected to simulated process induced disorder by cryogrinding them for different times.

Many techniques have been successfully applied to differentiate and quantify crystalline and amorphous phases. A partial list includes PXRD, DSC, ITC, TSC, DVS, IGC, vibrational spectroscopies, DES, SSNMR, and dissolution tests. The actual mechanisms behind their selectivity are as diverse as the techniques themselves, but they share a common thread: almost all require pure crystalline and amorphous reference standards to exist and the quantification is based on calibration curve based on a series of binary mixtures of variable crystalline/ amorphous content. It is, therefore, assumed that the ‘two states’ model is applicable, i.e. any sample can be described by linear combination of the pure standards. However, each technique may exhibit a potentially strong sensitivity to physical properties of the analyte such as particle size distribution, crystalline phase defects, type of preparation, the extent of structural relaxation and domain sizes. For these reasons, even well calibrated techniques may differ substantially in quantitative estimates of crystallinity.

The disorder in the cryomilled model compounds studied could not be modeled completely by either the 'two states' or 'one state' models. We are suggesting an explanation for the apparently different behavior of each technique in terms of their different length-scale dependences. Implications for solid state method development are also discussed.

42 Spin-Spin Coupling Constants in 2-Fluorobenzamides and Derivatives

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Nuclear Magnetic Resonance Spectroscopy (NMR) is one of the most powerful tools for identification, characterization and structure elucidation of organic and organometallic compounds. At the same time, the nuclear spin-spin coupling constants (SSCCs) are of substantial interest due to its application on several stereochemical aspects.

The 2-Fluorobenzamide and its derivatives, such as N-methyl and N,N-dimethylfluorobenzamides, shown an interesting behavior which respect to coupling constants $^{3}J_{FC}$, $^{4}J_{NF}$ and $^{5}J_{FH}$ [1]. Moreover, the fluorine compounds have received considerable attention in the last years because their pivotal role in recent pharmaceutical, agrochemical and materials science.

In the present work we analyzed the mechanisms involved on transmission couplings of 2-Fluorobenzamides and its derivatives by the use of a new and efficient methodology dubbed Fermi contact coupling pathways in terms of canonical molecular orbitals (FCCP-CMO) [2]. Quantum Theory of Atoms in Molecules (QTAIM) analysis was used to verify the intramolecular C-F…H-N hydrogen bond.

The 2-Fluorobenzamides and its derivatives were synthesized and the NMR spectra were obtained. The experimental values were compared to theoretical data in order to understand the pathway transmission of SSCCs and the influence of the intramolecular hydrogen bond in these couplings. The structures were optimized at the B3Lyp/epr-iii level using the Gaussian03 program. Coupling constants and all four terms (FC, SD, PSO and DSC) were calculated using epr-iii basis set. The CMO information was obtained as CMOs expanded in NBOs basis. QTAIM analysis was performed using the AIMALL program. The dates show that depending on the substituents on the nitrogen, the coupling transmission have an important component occurring through space (TS).

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43 RI-DFT Fast Calculation of Chemical Shifts

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DFT based prediction of chemical shifts is becoming more and more an important tool in the determination of relative configuration of small organic compounds.[1] Predictions based on non-hybrid GGAs approach in many cases the quality of the more expensive MPW1PW91 or PBE0 computations. [2] Exploiting this fact and the high-speed gains which can be obtained in DFT non-hybrid-GGA computations through the use of the resolution of identity (RI) Coulomb energy approximation we present here a protocol based on the use of IGLO RI-DFT chemical shift computations as implemented in the ORCA package, non-hybrid GGA functionals, and the family of pcS-x NMR specialized basis sets . The performance of this protocol has been tested in a series of natural compounds.Very important savings in computer time are observed without loss of accuracy respect to non-RI GIAO computations. This makes possible to compute chemical shifts for molecules of 300-500 in 5-10 minutes using a commodity computer (one quad processor) with fair accuracy.

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44 NMR Metabolic Profiling of Two Exotic Native Fruits from Peru: Vanilla Pompona ssp Grandiflora and Physalis Peruviana L

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NMR based metabolomics has become a widely applied analytical tool for the identification and discrimination of fruits and other agricultural products according to their origin, ripeness, species and possible adulteration. NMR offers unique advantages compared to other analytical methods (e.g. GC-MS, LC-MS). In particular, it is nondestructive, non - biased, quantifiable, requires little or no separation, permits the identification of novel compounds, is capable of relatively high - throughput automated analyses, and needs no chemical derivatization.

As part of a continuous effort to promote and support Peruvian food industry, we present here NMR - based studies of two exotic native fruits from Peru: Cape gooseberry (Physalis peruviana L.) and Vanilla pompona spp. grandiflora (Lindl.) Soto-Arenas.

Wild V. pompona ssp. grandiflora (Orchidaceae) recently discovered in the Amazonian wetland ecosystems in Madre de Dios - Peru, yield large and fragrant fruits after nine months of hand pollination. The main constituents of these fruits are phenolic compounds which exist mainly in their glycoside form. The 1H-NMR profiles of V. pompona spp. grandiflora fruits - crude and ß-glycosidase-treated extracts - are presented. NMR assignments of metabolites were assessed by 1D and 2D NMR experiments such as COSY, TOCSY, J-Resolved and HSQC. In addition, the NMR profiles of Vanilla pompona ssp. grandiflora and Vanilla planifolia Andrews fruits were compared. A noticeable difference in phenolic composition between these two species was observed.

Cape gooseberry (Physalis peruviana L.) is a native plant from the Andes. It grows in subtropical zones in a wide range of altitude from 3300 m above sea level. We report here a 1H-NMR analysis of its juicy berry. The NMR metabolic profile contains signals which correspond to sugars, organic acids and amino acids. In addition, a chemometric approach of the 1H-NMR spectra allows distinguishing among four different ecotypes of Physalis peruviana L.: Villa Andina, San Marcos, Cumbico and Encañada. 1D and 2D NMR experiments were used for further assignment of the metabolites found.

45 Estimating Metabolite Concentrations Using a New Deconvolution Routine

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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.

46 NMR Characterization of Specialty Coffee, Key Agricultural Product in the Peruvian Export Food Industry

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The Peruvian coffee is well appreciated worldwide for its unique flavor and aroma. The privileged environmental conditions throughout different regions of Peru -one of the twelve megadiverse countries in the world- is responsible for the various types of specialty coffee beans known to exist in this country. Currently, Peru is considered the third principal producer of coffee in South America, and the sixth worldwide. Exports have progressively increased from $ 450 millions in 2007 to $ 883 millions in 2010. Most of the coffee is exported as green beans due in part to the fact that the local quality control of the roasting process is not yet standardized.

The advantages offered by NMR -direct, quick and comprehensive multicomponent analysis- are here used to analyze complex mixtures obtained from different Peruvian special roasted coffee beans.

One and two dimensional NMR techniques such as ^{1}H-NMR, edited HSQC, TOCSY, selective TOCSY, COSY and J-Resolved as well as 1D CPMG allowed the simultaneous identification of ten compounds known to be associated with flavor and aroma of coffee. In addition, a new chemical protocol was developed for the simultaneous quantification of trigonelline, caffeine, among other key compounds in coffee. The quantitation was performed using ERETIC2 -based on PULCON [1]- as the referencing tool. The differences among the ^{1}H-NMR profiles of the various brands of coffee studied can be related with the quality of the roasting process.

These results indicate that NMR can be used as a quality control tool to optimize the roasting conditions of Peruvian special coffee. This work is part of a continuous effort being conducted to implement methodology that will allow local Peruvian coffee farmers improve their roasting protocols.

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47 Correlating the ^{7}Li NMR Shift to Microstructure Growth in Li Metal Batteries

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Although, Li metal is the lightest and most electropositive anode material, giving the greatest capacity and voltage for a battery, commercial anode materials are most commonly made of carbon, due to safety issues associated with dendritic lithium formation. Dendrites can grow on the lithium metal surface that penetrate the separator leading to a short-circuiting of the battery. Dendrites can also break, producing ”free” lithium in the cell, which leads to a decrease in battery life as well as overall performance. An understanding of the electrochemical conditions under which dendrites form will aid in the development of commercially available Li metal batteries. Here we utilize in situ ^{7}Li NMR, in situ ^{7}Li MRI, and SEM techniques to develop an understanding of the electrochemical conditions that lead to the formation of Li dendrites.

Previous in situ ^{7}Li NMR studies utilized the bulk magnetic susceptibility and the skin depth effects of Li metal to quantitatively study Li microstructure growth [1]. These studies, however, could not distinguish between dendritic or mossy Li growth. Recently we have used ^{7}Li MRI/CSI (chemical shift imaging) to visualize where microstructures grow in a Li metal cell [2]. Utilizing in situ ^{7}Li NMR and CSI, and SEM techniques to study Li metal electrodes, we have further defined correlations between the NMR shift and type of Li microstructures formed. Correlating the ^{7}Li NMR shift to SEM images allows one to assign a range of shift to the topology of the microstructure formed. Analysis of ^{7}Li CSI images further allows one to assign correlations between the shift and spatial formation of Li microstructures within the cell. These correlations between NMR shift and type of microstructure provides the foundation for the development of NMR as a powerful tool to gain further insight into the mechanism by which dendrites form in Li metal batteries.

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48 NMR Spectral Alignment Approaches

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Spectra recorded on similar but non-identical samples, the same sample over time, or in an experiment where pulse-program conditions vary, can show changes in peak positions, and these small changes in signal position can significantly disrupt data analyses. Previous to any form of analysis which involves the comparison across different spectra, the one step which is crucial and often overlooked is the alignment of peaks in these similar spectra. In this poster we will review our own experience using different algorithms and workflows to combat this difficulty and present solutions to common problems that are based on sophisticated algorithms

Depending on the nature of the data to be analyzed as well as on the type of analysis to be performed or even if this is of manual or an automatic approach, the choice of alignment strategy may change.

We describe three distinct methods for the alignment of NMR data, of the many which can be implemented [1], and which situations we find them to be of most use. These are:

- Absolute referencing
- Auto-Tuning [2]
- Global alignment

The correct application of the algorithms to the data being analysed, whether these aremetabolomics[3], ligand screening, reaction monitoring or any other set of experiments, will be one of the critical factors for the rigorous analysis of the data, and certainly the first condition which has to be met to facilitate the automatic analysis of experiments. Ultimately, the pre-processing of data so all are correctly aligned, when required, is critical to the analysis of groups of data acquired through any analytical technique [4].

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49 Improvements in Flow-Injection NMR as a Tool for High-Throughput Sample Analysis

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Recently, dramatic improvements in S/N have been obtained using customized flow-NMR instrumentation by incorporating a small volume flow-cell (10-60 uL) with a high-sensitivity 600 MHz Bruker 5-mm TCI-cryoprobe and a Protasis sample delivery system. The large sensitivity gain provided by cryoprobe technology (as much as 20X increase compared to a micro-coil 500 MHz RT flow-probe) has dramatically reduced data collection times to a point where routine ^{1}H and g-HSQCdept spectra can be collected on small samples (25 uL at 20 mM) in under 10 minutes. These hardware improvements have allowed implementation of rapid quantitative-NMR analysis for HTPP (High Throughput Purification Process) samples using Digital-Eretic referencing as an integral calibration technique. In addition, new flow-cell inserts have been designed which easily can be interchanged to alter sample volume or switched from flow to tube based operations in just a few minutes with minimal setup time. The concept of tailoring the flow-cell volume to match that of the intended sample stream (with an active volume of 10, 30 or 60 uL) using a standard Bruker pass-through cryoprobe, will be discussed as part of our continuing effort to improve NMR sampling efficiency. The Protasis sample delivery station and operating system (One-Minute NMR) provides a flexible and convenient platform for submitting samples in 96-well plate format, or as single samples in walk-up mode. Automated data processing using ACD Automation Server provides a reliable solution for archiving and distributing the large amounts of processed data that are generated from plate-base HTPP submissions. In addition, segmented-flow NMR methods (SFA), using a susceptibility matched push-solvent (FC-43) to limit sample dispersion in the flow cell, are also being investigated in an effort to improve S/N with mass-limited samples. The initial results of these studies, which show up to a two-fold improvement in S/N vs. single-solvent push techniques, will be presented.

50 NMR of Novel Inorganic Anticancer Agents

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A series of novel anticancer agents based on the elements platinum and arsenic has been synthesized and characterized by NMR. These compounds combine structural and compositional motifs from different therapeutics that are already known to be effective in fighting cancer, and preliminary evidence shows that the hybrid derivatives exhibit greater potency than the parent compounds that inspired them.

Characterization of the compounds by NMR included acquisition of a number of heteronuclear spectra, and many interesting chemical shifts and scalar couplings were observed. These include ^{195}Pt, ^{15}N and ^{13}C 1D spectra in which $^{1}J_{195Pt\text{-}15N}$ and $^{1}J_{15N\text{-}13C}$ couplings are apparent. One compound includes a thiocyanate group, and we determined that it bound to one of the metal centers by two means: one with an M-N bond, and one with an M-S bond. A version of this compound was prepared with isotopically-labeled $S^{13}C^{15}N^{-}$, and was investigated by direct-observe ^{15}N and ^{13}C NMR, enabling us to assign the resonances of the N-bound and S-bound isomers. Variable temperature ^{1}H NMR of the SCN-containing compound was employed to determine the thermodynamically favored isomer and obtain thermodynamic parameters $\Delta G°$, $\Delta H°$, and $\Delta S°$ for the isomeric equilibrium.

51 Elucidating Binding of Visible-Light Sensitizers on TiO_2 Nanoparticles using Pulsed-field Gradient NMR and Relaxometry Measurements

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The investigation of ligand binding to nanoparticles has mainly relied on the measurement of free ligand or on photometric techniques when the associated complex has either a different extinction coefficient than the free form or when its fluorescence can be quenched by the surface. Pulsed-field Gradient (PFG) diffusion measurements can be used to give the hydrodynamic radius of the ligand and thus directly measure its association constant. Relaxometry studies obtained using NMR provide complementary information on a different length and timescale and can be used to infer the motion as well as lateral interactions of the ligands. Although these techniques have been used extensively in the field of biochemistry, their application to small molecules on nanoparticles is only recently emerging and has its specific challenges. In this work, we present the application of PFG and relaxometry in the case of dopamine on TiO_2 nanoparticles. We find that independent measurements of the association constant using NMR and photometric techniques give differing answers, providing a new insight on the binding sites for these molecules and the requirements for forming charge-transfer complexes. A more thorough picture of the structures necessary for interfacial charge transfer will have a direct impact in solar energy technologies, for example dye-sensitized solar cells.

52 The Role of the Receptor in Rational Drug Design

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Nowadays most of pharmaceutical efforts in drug design are focused on the discovery, development and optimization of new ligands that efficiently bind to receptors involved in human disease. Even though traditional computational techniques are able to find small molecules that maximize binding free energy, they are not able to predict how small differences in ligand binding could reflect into structural and functional response of the receptor. For this reason, receptors that undergo a substantial conformational transition upon ligand binding should be precluded from structure based drug design.

Here we present a case study on Integrin avb3. One major problem with integrin ligands is their potential to activate conformational changes, which can initiate unwanted signals. Neglecting this aspect could lead to the development of drugs that induce agonist-like activities and adverse paradoxical effects. In this context, we exploited a combination of computational and biochemical studies to determine the biologically active conformation of a small library of cyclopeptides, to discriminate in silico binders from non-binders, to understand at molecular level integrin allosteric changes induced by ligand binding and to identify real antagonist among the selected binders. Our study reveals the importance of ligand-receptor complex dynamics in drug design studies aiming at developing new real integrin antagonists, confirming that ligand induced conformational change of the receptor and its structure-function relationship should play a crucial step in common drug design strategies.

53 Mechanistic Studies of Photocatalytic Reactions by NMR Spectroscopy

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The use of visible light as a sustainable energy source for synthetic applications has gained rising interest in recent years. Despite their huge importance, many of the photocatalytic reactions lack a detailed understanding of the underlying mechanisms. Using NMR spectroscopy we study the mechanism of photooxidations of benzyl alcohols to the corresponding aldehydes catalyzed by different flavin derivatives [1,2]. Furthermore ruthenium and eosin Y catalyzed conversion of tertiary amines to iminium ions and subsequent trapping by nucleophiles are investigated [3,4]. Another system is the photocatalytic asymmetric alkylation of aldehydes catalyzed by the third generation MacMillan organocatalyst and a photoactive catalyst [5-7].

In order to be able to investigate these photochemically catalyzed reactions by means of NMR the spectrometer was supplemented by an illumination device consisting of pulsed LEDs and an optical fiber guiding light directly into the NMR sample and illuminating it from the inside out [8,9]. With this setup well established NMR techniques are supplemented by detailed kinetics of the photochemical reactions and illusive intermediates are detected and characterized. The interplay of the nuclear spins and the intersystem crossing of radical pairs in photocatalytic reactions in the magnetic field causes Photo-Chemically Induced Dynamic Nuclear Polarization (Photo-CIDNP). This effect results in anomalous polarizations in the NMR spectra and is caused by radical intermediates [9]. This way short lived radical intermediates were detected in flavin catalyzed photooxidations of benzyl alcohols to the corresponding aldehydes by difference spectroscopy between the spectra of the dark sample and the sample illuminated with 440 nm light. Well defined light pulses implemented into pulse sequences allowed the distinction between polarization caused by Photo-CIDNP and secondary polarization through cross relaxation. This way the radical key intermediates were further characterized.

The riboflavin tetraacetat (RFT) catalyzed photooxidation of benzyl alcohols to the corresponding aldehydes turned out to be much more efficient in acetonitrile/water mixture than in pure acetonitrile [1,2]. ^{1}H-DOSY measurements [10] were performed to investigate the aggregation behavior of the photocatalyst in both solvents. By calculating the hydrodynamic volume of RFT from its experimental diffusion coefficient and comparing it to theoretical values RFT was found to form aggregates in acetonitrile which break up with the addition of water. Furthermore the aggregation of 10-arylisoalloxazines as possibly not aggregating flavin catalysts was investigated in different solvents by 1H-DOSY NMR. The aggregation trends of the different flavin derivatives in solution were compared to each other and to their respective crystal structures. This way Π-Π stacking was found to be the dominating intermolecular interaction causing the aggregation of the flavins in acetonitrile solution.

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54 Quantitative ^{1}H qNMR Method for Complex Mixture Analysis: Determination of Acetylated Polysaccharides, Glucose, Maltodextrin, Isocitrate, Preservatives, Additives and Degradation Products in Aloe Vera Leaf Juice - Raw Material and Consumer Products

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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 qNMR method described here was developed and validated by Process NMR Associates (Danbury, CT) and is similar to an independently validated method developed by Jiao et al [1]. The method described is to 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.

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55 Practical Applications of Compact, Cryogen-Free High-Resolution 60 MHz Permanent Magnet NMR Systems for Reaction Monitoring and Online/At-Line Process Control

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For the past two decades high resolution ^{1}H NMR at 60 MHz has been utilized to monitor the chemical physical properties of refinery and petrochemical feedstreams and products1. These approaches involve the use of partial least squares regression modelling to correlate NMR spectral variability with ASTM and other official test methods, allowing the NMR to predict results of physical property tests or GC analysis. The analysis is performed in a stop flow environment where solenoid valves are closed at the beginning of the NMR experiment. This approach allows up to 5 or 6 different sample streams to be sent to the sample in order to maximize the impact of the instrument. The current work with these permanent magnet NMR systems is to utilize them as chemistry detectors for bench-top reaction monitoring, mixing monitoring, dilution monitoring, or conversion monitoring. In the past use of NMR for these applications has been limited by the need to bring the "reaction" to the typical "superconducting" NMR lab. 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 system uses a unique 1.5 Tesla permanent magnet that can accommodate sample diameters of 3-10 mm with half-height resolution approaching 1-3 Hz (depending on the sample size) and excellent single pulse sensitivity. Reaction monitoring can be performed using a simple flow cell analyzing total system volumes of 2 to 5 mL depending on the length and diameter of the transfer tubing. Further, detection limits of analytes in the 200+ ppm range are possible without the use of typical deuterated NMR solvents. Analysis times of 5 to 20 seconds are also possible at flow rates of 5 to 20+ ml/minute. Reaction monitoring directly in standard 5-10 mm NMR tubes using conventional (non-deuterated) reactants, solvents and analytes will also be described. Examples of ^{1}H, ^{19}F and ^{31}P analyses will be described.

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

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Over the years 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 ^{13}C 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 properly describe the "molecular average" present in the sample. Utilizing methodologies developed by Pugmire and Solum [1] for the solid-state ^{13}C NMR analysis of coals and other carbonaceous solids we have developed a new liquid-state ^{13}C NMR method that allows the relative quantification of overlapping protonated and bridgehead aromatic carbon signals to be determined [2]. The NMR experiments involve the combined analysis of both quantitative ^{13}C single pulse excitation which observes "all carbons in the sample" and DEPT-45 polarization transfer which observes only the protonated carbons in the sample. Though the DEPT45 results are not quantitative across all carbon types (CH, CH_2, and CH_3) 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. An additional aspect of the experiments is the addition of a standard material (PEG polymer) that allows the calculation of the absolute percentage of the carbons observed by the NMR technique. This allows the relative amount of bridgehead carbon to be calculated by direct comparison of the aromatic region with the standard signal intensity. 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 asphaltenes for example the ring systems are 5-7 rings in size rather than the 3-4 rings reported previously. The ring sizes determined by this new combined NMR method are in agreement with FTICR-MS and fluorescence measurements.

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57 ^{35}Cl Solid-State NMR of Active Pharmaceutical Ingredients: Pure Samples, Polymorphs and Pills

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The differentiation of polymorphs of active pharmaceutical ingredients (API) is of great importance in the pharmaceutical industry, since distinct polymorphs can exhibit different physical properties such as bioavailability, shelf life, stability, etc., and each unique polymorph is patentable.[1] Since most APIs are prepared as solids (in many cases packed, shipped and added to excipients as solids), it is crucial to structurally characterize all of their possible solid forms, including hydrates, solvates, and solid forms contained within excipient materials.

Polymorphs are commonly differentiated using X-ray diffraction (XRD) and 13C SSNMR techniques.[2] The former is restricted to crystalline samples (i.e., no amorphous solid phases). Different powder XRD (pXRD) patterns can be used to distinguish polymorphs, but provide little insight into the electronic environments of the individual atoms. ^{13}C SSNMR can be applied to both crystalline and non-crystalline samples, but in many instances, it not a reliable fingerprinting technique (i.e., 1D and 2D NMR spectra of two distinct polymorphs are often nearly indistinguishable). Since ca. 50% of APIs are prepared as HCl salts, ^{35}Cl SSNMR can provide a useful structural probe which addresses both the issues of spectral fingerprinting and intermolecular interactions.[3]

To this end, we will present a comprehensive structural study of a variety of HCl pharmaceuticals and several polymorphs using 35Cl SSNMR (9.4 T and 21.1 T), pXRD and first-principles density functional theory (DFT) calculations. The combination of ^{35}Cl SSNMR data and DFT calculations allows the NMR parameters CQ and ?Q to be determined for each crystallographically distinct chlorine site, and enables us to explore their relationships to the number and spatial arrangement of short Cl-H contacts. We will also discuss applications of ^{35}Cl for (i) rapid differentiation of API polymorphs and (ii) comparison of pure bulk APIs and APIs contained within tablets, both of which may have potential for general use in quality and assurance.

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58 Comparison of the Performance of Quantum Mechanical, Topological and SDAR Descriptor Pools for Modeling the Binding Affinity of 130 Estrogen Disruptors

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The performance of eight commonly used descriptor classes (atomic type, molecular type, bond type, constitutional, geometrical, topological, charge related and semi-empirical) was compared to that of a novel abstract descriptor space based on pairs of ^{13}C-NMR chemical shifts and associated inter-atomic distances (3D-QSDAR). A PLS algorithm written in Matlab was employed to relate all descriptor types to the logarithm of the relative binding affinity of 130 estrogen receptor binders. Each of the models based on descriptors of different nature was validated using 100 randomly generated training/test set partitions. After every 100 runs the random number generator was re-initialized in order to reproduce an identical training/test sequence, thus assuring fair comparisons. A scrambling procedure was used to assess the quality of all models. The 3D-QSDAR abstract space was partitioned into congruent "3D bins" ranging in size from 2 ppm x 2 ppm x 0.5 Å to 20 ppm x 20 ppm x 2.5 Å1. CODESSA was used to calculate a total number of 585 descriptors (those with variance < 10-6 by default were automatically excluded by CODESSA). In terms of predictive power (R2test), every model based on 3D-SDAR descriptors, regardless of the grid size, performed better than any based on CODESSA descriptors.

59 Comparison of Tandem and High-Resolution Mass Spectrometry for the Analysis of Nerve Agent Metabolites in Urine

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The use of a high-resolution accurate mass spectrometer (HR-MS) was evaluated for the quantitation of nerve agent metabolites in urine. The results obtained using a mass resolution of 50,000 and a mass extraction window of 10 ppm around the theoretical m/z were used for quantitation of each metabolite. The precision, accuracy, linearity and sensitivity were compared with the current selected reaction mode triple quadrupole tandem mass spectrometry [1]. This study included five hydrolysis metabolites in urine, used to determine human exposure to VX, RVX, GB, GD, and GF nerve agents from 1 – 200 ng/mL. The application of HR-MS operating in full scan mode allows the detection of other similar compounds while simultaneously quantitating the known metabolites. This is particularly useful for the identification of exposure to nerve agents not currently included in this panel. Initial HRMS results indicated comparable precision and accuracy of standards and quality control samples to the tandem MS method.

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60 Detection of Fentanyl Analogs and Metabolites in Human Urine Utilizing Online-SPE Liquid Chromatography Tandem Mass Spectrometry

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Fentanyls are powerful narcotic analgesics that are used to treat breakthrough pain, for palliative care, and as an anesthetic. Multiple analogues have been identified since fentanyl was first synthesized in 1960; some so powerful they are used only for large game. These drugs have a rapid onset and short duration of action, but have significant side effects and are highly addictive; hence they are often sold and used illicitly. Because of their potential for abuse, a method to rapidly detect and quantify multiple fentanyl analogs and their major metabolites in urine is desired. A method using liquid chromatography and mass spectrometry was developed to quantitate 10 fentanyl analogs and 3 metabolites. Select deuterated internal standards for three fentanyls and three metabolites were used to improve accuracy and precision. To enhance sensitivity and reduce ion suppression encountered in previous methods, both reversed phase and HILIC chromatographic separations were evaluated along with online solid phase extraction (SPE). Initial investigations of online-SPE coupled with HILIC chromatography showed significant reductions of ion suppression. Implementation of online-SPE techniques will simplify the process, minimize analyst exposure to these compounds and utilize novel instrumentation while reducing preparation time; thus providing a method to identify exposure to various fentanyl analogs.

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61 Identification of Two Photodegradants of an 11-Beta-Hydroxysteroid Dehydrogenase Inhibitor

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An 11-beta-Hydroxysteroid Dehydrogenase (HSD) Type 1 Inhibitor is under investigation as active pharmaceutical ingredient (API) for the treatment of type 2 diabetes. During a photostability study, two major degradants were observed by analytical HPLC. LC/MS analysis of the photoreaction mixture revealed that the degradants were the isomers of the API, but yielded enhanced responses under ESI (-) mode suggesting the formation of an acidic moiety such as a phenol. Cleavage of one of the carbon - oxygen ether bonds (A or B) and subsequent formation of a new carbon-carbon bond could produce the required acid moiety in the form of a phenol. Further characterization was required for unambiguous identification of the degradant structures since it was not possible to determine the precise location of the new carbon - carbon bond from the MS data and mechanistically there were several reasonable possibilities. Therefore, the degradants were isolated via preparative HPLC and their structures were characterized by interpretation of 1D & 2D NMR data leading to assignment of structures 1 and 2. It is clear that the photo-induced homolysis of carbon-oxygen bond was through B and degradants 1 and 2 were formed via the Fries Rearrangement mechanism. Details of isolation and structure elucidation of these photodegradants and the rationale for their formation will be presented.

62 Bioelectronics: From Novel Concepts to Practical Applications

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Novel biosensors digitally process multiple biochemical signals through Boolean logic networks of coupled biomolecular reactions and produce output in the form of YES/NO response. Compared to traditional single-analyte sensing devices, biocomputing approach enables a high-fidelity multi-analyte biosen-sing, particularly beneficial for biomedical applications. Multi-signal digital bio-sensors thus promise advances in rapid diagnosis and treatment of diseases by processing complex patterns of physiological biomarkers. Specifically, they can provide timely detection and alert to medical emergencies, along with an immediate therapeutic intervention. Application of biocomputing concepts has been successfully demonstrated for systems for logic analysis of biomarkers of different injuries, particularly exemplified for liver injury. Wide ranging applications of multi-analyte digital biosensors in medicine, environmental monitoring and homeland security are anticipated.

63 Robust LC/MS Application for Bioanalysis of Pain Management Drugs

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For efficient therapeutic drug monitoring, it is important for clinicians to have access to fast/ robust analytical methods for accurate assessment of drug efficacy. Industrial trends toward highly specific LC/MS applications over traditional ELISA type immunoassay has resulted in the need for high-speed chromatographic assays along with simplified sample preparation methods. Often the limitation of a bioanalytical technique is based upon the effectiveness of the sample preparation technique. Plasma and serum samples are often suspect to assay irregularities due to matrix induced interferences. In this study a combination of fast chromatographic separation along with a novel phospholipid depletion platform is utilized for the analysis of pain management drug tapentadol and associated metabolites.

Methods:

Here a bioanlaytical assay for tapentadol and associated metabolites is demonstrated using a combination of HILIC chromatographic separation along with a novel phospholipid depletion device for efficient analysis of plasma samples. Targeted depletion of phospholipid matrix interference was achieved using novel HybridSPE-Phospholipid 96well plate. Plasma samples spiked with tapentadol and associated metabolites were prepared using the HybridSPE-Phospholipid sample prep technique and assayed using HILIC conditions, resulting in a fast and accurate bioanalytical assay method. Absolute recoveries along with CV values are demonstrated using the described technique.

Results:

The novel sample prep technique of the HybridSPE-Phospholipid enabled a simplified sample processing with highly efficient phospholipid matrix depletion. Processed samples were then analyzed directed on an Ascentis Express HILIC phase without the need for evaporation of solvent exchange. By depleting the phospholipid matrix from the sample prior to LC/MS analysis, the resulting method demonstrated no matrix interference as compared to that of the standard protein precipitation techniques. The combination of the simple and efficient sample technique along with the unique selectivity of the HILIC chromatographic method resulted in a fast and robust bioanlaytical technique for pain management drug tapentadol and associated metabolites.

64 Developing a Suitable Analytical method for Detecting Trace Amount of Formic Acid in Pharmaceutical Excipients

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Formic acid is present in many pharmaceutical excipients in trace levels. As formic acid is considered as non-toxic, its presence and levels in these excipients are usually not tested. However, formic acid is capable of reacting with some functional groups in many APIs. Therefore, an accurate and sensitive analytical method is needed to monitor its levels in various pharmaceutical excipients. When a GC-MS method was used to analyze the formic acid content in 500 mg croscamellose sodium, low recoveries were observed. This suggested that 1% p-toluenesulfonic acid as the acid catalyst in the method might not be sufficient to get a quantitative derivatization for a strong basic excipient like croscarmellose sodium. When a stronger acid such as sulfuric acid was used, the recoveries were much better, but serious sample solution stability was discovered – the prepared standard solutions at both 5 and 10 ppm levels were losing area counts by 40% after 4 hours. Several means were used trying to solve the stability issue but not successful. Therefore, alternative analytical technique, ion exchange chromatography, was explored for the analysis. By adding a few percent of acetonitrile as a co-solvent, the insolubility/wettability of some pharmaceutical excipients was solved. Experiments were also performed to prove that there is no impact of co-solvent to the analysis. During the experimental work, it's found that some of glassware has significant levels of formic acid, so it is critical to use the formic acid free glassware for this analysis. Using the developed ion exchange chromatographic method, a series of pharmaceutical exicpients were analyzed for the contents of formic acid. The method is accurate and robust…

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65 Real-Time Assessment using DART (Direct Analysis in Real Time)-Enabled Derivitization and Chemical Analysis

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The rapid sampling of DART-MS permits monitoring of reaction products in seconds per sample using only a few microliters of sample. The utility of this method for quality control experiments will be discussed using determination of fatty acids from edible oil. Chemical derivitization can be a time-consuming process and often requires the use of noxious reagents. Previously we described the co-mixing of derivatizing reagents and analyte on a surface suitable for desorption and use of heated ionizing gas exiting a DART source to produce stable derivatives for MS. In order to leverage this in-situ-DART derivatization strategy it is necessary to implement a protected atmosphere for the reaction to proceed with minimal environmental exposure. We describe an enclosure designed to eliminate the gases these reactions produce, while providing a stable environment for ionization and ion transfer. Reagents and conditions for oxidation reactions for profiling fatty acids in oils and methylation reactions used for the analysis of complex carbohydrates are investigated.

An enclosure was constructed that fits tightly around the ionization region of a DART source. Sample was applied to a metal mesh substrate and a small amount of a chemical reagent was applied on top of the aliquot. The card was inserted into the enclosure and a power supply was activated to drive current through the screen, catalyzing the reaction and vaporizing the product into the gas phase.

66 Simultaneous, Fast Analysis of Melamine and Analogues in Pharmaceutical Components Using Q Exactive - Benchtop Orbitrap LC-MS/MS

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Potential drug contamination by melamine and its analogues remain a major concern by FDA. We present a workflow for analysis of melamine and its analogues in at-risk pharmaceutical components using high resolution benchtop Orbitrap LC-MS/MS: Q Exactive. Simultaneous, fast screening and quantitation for melamine and its analogues in complex matrices were achieved by HRAM full scan, ms/ms in a data-dependent fashion with polarity switching. Comparing with commonly used Triple Quadrupole MS method, this workflow allows for added post analysis flexibility, it avoids the upfront selection of specific compound masses pertaining to SRM methods. Confident identification is achieved by accurate mass measurement of both precursor and fragment ions, as well as the fine isotope pattern of ^{13}C and ^{15}N.

67 Metabolic Stability Screening Workflow using a Second Generation High Resolution Accurate Mass Benchtop Instrument

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In vitro metabolic stability screening performs a very important role in the drug discovery stage for compound selection in pharmaceutical companies. The screening is primarily supported by LCMS, which involves the monitoring of the disappearance of parent compounds, using selected reaction monitoring (SRM) on triple-quadrupole instruments. If moderate to high turnover is observed, separate metabolite identification experiments are then conducted to characterize the biotransformation products. In this study, we present a novel workflow using a high resolution accurate-mass benchtop Mass spectrometer, Exactive. This workflow combines relative metabolic stability and initial metabolite information from the same analysis. The high mass resolution with high scan speed data acquisition is compatible with UHPLC for high throughput screening.

68 Isolation of Pharmaceutical Degradents Using SFC

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Chromatographic isolation of degradents, whether from stressed lots of pharmaceutical compounds or directly from the API, can be a painstaking process for the analytical laboratory. Peaks of interest must selected based on chromatographic signatures and often must be profiled by varying degradation conditions, and the degradent peaks – which may be trace components in the mixture – isolated in sufficient quantity for structure elucidation by MS/MS and NMR. Generally a suite of analytical instrumentation and multiple chromatographic methods are required, and the final isolation can be a slow process of accumulating one fraction over many chromatographic injections. We demonstrate that using Supercritical Fluid Chromatography (SFC) in place of traditional reversed-phase and normal phase HPLC timelines can be greatly reduced by leveraging the rapid method development cycle of SFC and the high efficiency of preparative separations. In addition, the lability of degradents during the isolation process may be minimized in common SFC solvent systems and by the collection of highly concentrated fractions.

69 Towards the Optimum Technique for Maximizing the Ratio of Peak Capacity to Analysis Time in One-dimensional Liquid Chromatography

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The common goal of most chromatographers is to separate all substances of a sample in the shortest possible time with appropriate resolution. This becomes even more relevant with increasing sample complexity. One way to achieve this goal is to maximize the peak capacity to analysis time ratio. Maximizing this ratio commonly implies using smaller diameter porous particles in columns of extended length or even coupled columns, but such setups obviously result in substantially elevated column head pressures. Another approach is to improve separation efficiency at moderate pressure levels with monolithic or superficially porous particle phases. The highest possible peak capacities can be achieved by employing an additional chromatographic dimension, but this requires dedicated instrumentation and extensive method development for the proper combination of separation chemistries. The theoretical basis for all these considerations has been summarized in a review by Guiochon 1.

In this work, experimental results from the use of different stationary phase architectures in column lengths of 150 mm to 500 mm and at pressures up to 120 MPa are discussed and an evaluation is made as to which approach provides the best peak capacity to analysis time ratio for various applications. The impact of instrumental parameters such as flow, column temperature and detection specifics influencing the use of different column concepts to maximize possible performance, is evaluated. A brief discussion as to how the single-dimension approach competes with 2D-LC will be presented. In short, we provide a road map to chromatographers about which methodology separates complex mixtures in the shortest possible time to fully exploit modern UHPLC technology.

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70 Can High Peak Capacity and Universal Detection Solve the Challenges in LC Characterization of Botanicals and Natural Products?

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Natural products, with a pronunciation on botanical sources, undergo revived interest in targeted drug development. In particular traditional medicines from various geographies provide a truly global offering for promising leads. Related samples exhibit a significant complexity and many of their ingredients do not contain a chromophore or cannot readily be ionized. Hence they imply challenging separations with often limited use of UV absorbance and mass spectrometry detection.

High resolution UHPLC, using long columns packed with small or core enhanced particles and shallow gradients, is a very practical way to boost peak capacity. With the related increase in resolution, small next to large size peaks can be more accurately integrated and characterization quality of complex mixtures is improved. This can be critical when a universal monitor like the charged aerosol detector (CAD) is applied. By selected examples of herbal medicines form around the world, we demonstrate the effectiveness of this long column approach in combination with a wide range of detection techniques (ECD, CAD, UV/VIS and MS).

71 Sensitive Analysis of Underivatized Amino Acids and Peptides Using UHPLC with Charged Aerosol Detection

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The quantification of derivatized amino acids by (U)HPLC with UV detection is one of the most widely used methods to date. Unfortunately, separation and detection of underivatized amino acids is difficult as many of them are similar in structure, and few possess adequate chromophores. Derivatizing agents, either pre- or post-column, may overcome some of these issues, but can add time and complexity to the method. Presented here is a simple UHPLC gradient method using a new RSLC 2.2 µm 2.1 x 250 mm column with charged aerosol detection for the measurement of 20 underivatized amino acids. Retention of hydrophilic amino acids was achieved by the addition of a volatile ion-pairing agent heptafluorobutyric acid (HFBA) to the mobile phase. All analytes were resolved in less than 10 minutes with a limit of detection of low nanograms on column, making this approach ideal for the analysis of amino acids standards and starting materials in quality control. The work was expanded with a comparison of charged aerosol detection with inverse gradient and UV detection for monitoring digestion of bovine serum albumin by Trypsin. This technique offers a complementary approach to the traditional UV @ 214 nm with potential for more uniform response across both large and small peptide chains and the ability to measure free amino acids which may be missed because of insufficient chromophores. These fast UHPLC methods provide a simple alternative to the more time consuming or less sensitive amino acid methods typically used to measure analytes in a simple matrix.

72 Electrospray Ionization – High Performance Ion Mobility Spectrometry: A Precise and Sensitive Tool with Pharmaceutical Applications

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Electrospray ionization – high performance ion mobility spectrometry (ESI-HPIMS) provides a high resolution separation for many general analytical and pharmaceutical applications. HPIMS is a gas phase technique that separates molecules based on their mobility in a gas medium. The measured drift time in milliseconds can be directly related to an analyte's molecular weight and structure. The recent development of superior resolution in our lab has enabled the ESI-HPIMS as an orthogonal separation method to HPLC and MS. This method reaches sub-microM sensitivity with a resolving power of 60-90. Its major advantage is that it can separate compounds in seconds with minimal method development. In addition, ESI-HPIMS can successfully separate isomers and molecules that are chromatographically sensitive, nonvolatile, thermally labile, or that lack a UV chromophore. Precision, sensitivity, resolution, linear response range and LOD/LOQ of the ESI-HPIMS method were carried out in order to determine the instrument's feasibility for use in many pharmaceutical applications. These applications include dissolution testing, content uniformity studies, reaction monitoring, and cleaning validation/verification.

73 NMR Spectroscopy in the Study of Natural Product Curcumin

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Curcumin is a natural product of interest to many researchers due to the diversity of medicinal applications and properties. This natural product is derived from the rhizome of the Curcuma longa plant and comes from turmeric that has been used for nearly 5000 years. Tumeric has been used as a condiment, as a yellow dye, as a food preservative, as an antioxidant, and in traditional medicine [1-6].

Structurally, curcumin is a 1,3-dicarbonyl compound that we have investigated by NMR in terms of keto/enol equilibria in comparison to other ß-dicarbonyl systems [7,8]. For curcumin, only the enol proton resonance was observed. The diacetyl analogue of curcumin gave a similar result.

The results obtained for curcumin and diacetylcurcumin were compared to other small ß-dicarbonyl compounds including: 2,4-pentanedion, ethyl acetoacetate, dimedone, 1,3-cyclohexandione, and 1,3-pentanedione. The latter group of compounds had keto and enol tautomers.

All of the compounds were subjected to deuterium exchange by dissolving them in mono-deutero methanol. Following deuterium exchange, ^{1}H and ^{13}C NMR spectra were recorded to observe ^{2}H-^{13}C coupling and the decrease of the keto and enol signals.

Curcumin and diacetylcurcumin both exhibited a decrease in their enol proton resonances but ^{2}H-^{13}C couplings were not observed. In contrast, all of the other small molecules studied exhibited a decrease in both signals and all exhibited ^{2}H-^{13}C couplings. With this results we can see in a direct way the presence of the keto/enol equilibria be it by the decrease of the ^{1}H NMR signals and/or the presence of the ^{2}H-^{13}C coupling.

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74 New Strategy for Assisted Diastereotopic Protons Assignment Using a Combination of *J* Scaled BIRD HSQC and *J* Scaled BIRD HMQC/HSQC

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A new strategy to unambiguously assign diastereotopic protons was developed on the basis of Residual Dipolar Couplings collected in compressed poly(methylmethacrylate) PMMA based gels [1]. A combination of 2D *J* scaled BIRD HSQC [2] and *J* scaled BIRD HMQC-HSQC [3] NMR experiments was used to collect the RDC data. In the proposed strategy, the first experiment is used to measure $^1D_{CH}$ for methine groups, the sum $^1D_{CHa}$ + $^1D_{CHb}$ for methylene groups and the average $^1D_{CH3}$ value for methyl groups. In turns, the small molecule's alignment tensor is calculated using these *D* values without the *priory* assignment of CH_2 diastereotopic protons. The *D* value of each individual CH bond (CHa and CHb) of each methylene group in the molecule is predicted using the calculated alignment tensor and these values compared with the results from the HMQC-HSQC experiment, leading to their unambiguous assignment. This strategy is demonstrated with the alkaloid strychnine that contains five methylene groups with diastereotopic protons and our results fully agree with previously reported assignment using permutation [4].

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75 The Through Space Transmission of ^{19}F-^{15}N Coupling Constant

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Indirect spin–spin coupling constants (SSCCs) has an important role in the structural studies of molecules, independently of the nature (organic or organ metallic) or length (small molecules, peptides or proteins). Until 1970's decade were believed that the transmission of the scalar coupling could occur just through electrons of the covalent bonds.[1] However, a great number of experimental and theoretical studies have been showed that the transmission of the nuclear information can occur "*Through Space* (^{TS}J)". These couplings cover an extensive range of values (4 Hz until 800 Hz), depending of the nucleus involved.[2,3]

Fluorine nucleus (^{19}F) is a spin-½ species, which exits in 100% natural abundance and possesses the second greatest magnetogyric ratio, excluding tritium, this high sensitivity makes ^{19}F a probe in a large number of studies involving protein structure, dynamics, and function. While traditional ^{1}H, ^{13}C, and ^{15}N solution state NMR protocols provide a wealth of data on protein structure and dynamics, ^{19}F provides a unique perspective of conformation and topology.[4] With this in mind, we focus our attention in rationalizing the mechanisms involved in the transmission of the coupling between ^{19}F-^{15}N using like model compounds fluoroximes rigid. Mallory et al [3] studied these couplings however any detailed rationalization was assessed. In this way, we obtained theoretically the most stable geometries and also analyzing the molecular electronic structure through, Natural Bond Orbitals (NBO) and the Quantum Theory of Atoms in Molecules (QTAIM) methodologies. Several compounds was taking into account in the present studied, such as 2-fluorobenzaldehyde oxime, (E)-8-fluoro-5-methyl-3,4-dihydronaphthalen-1(2H)-one oxime, (E)-7-fluoro-4-methyl-2,3-dihydroinden-1-one oxime and (E)-9-fluoro-4-methyl-2,3-dihydrocyclopenta[a]naphthalen-1-one oxime. It has been observed in the present study that J_{FN} coupling constant is dependent not only for the distance between coupled nuclei as suggested by Mallory [3], but the most important effect involved in the transmission of J_{FN} coupling is related to the overlap between LP(F) and LP(N), which is dependent of the orientation of fluorine and nitrogen. In the case where FC term is the main mechanism involving into transmission the % s character of LP(F) is also important.

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