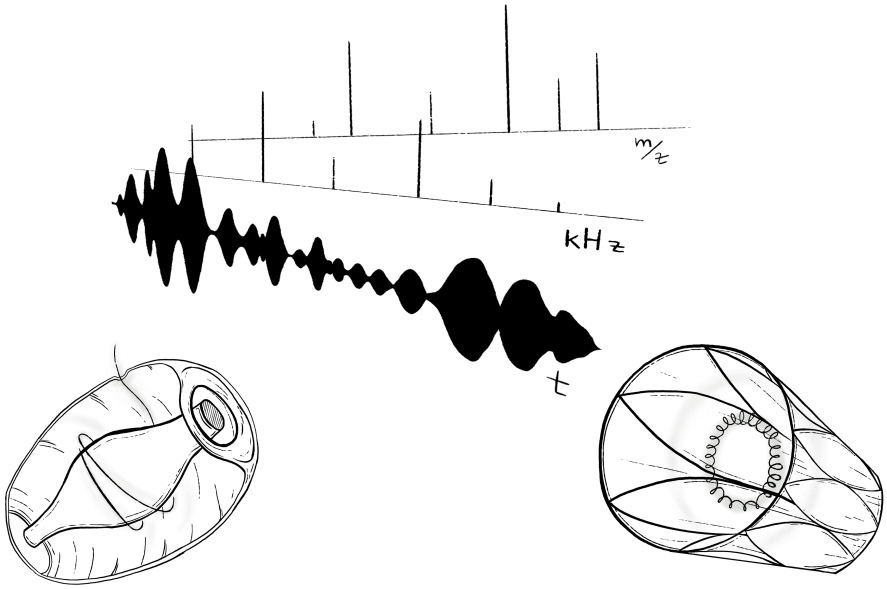

15th European Fourier Transform Mass Spectrometry Workshop
4th EFTMS School

Prague, April 17 – April 20, 2024

BOOK OF ABSTRACTS

Book of Abstracts from the Fifteenth European Fourier Transform Mass Spectrometry Workshop

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European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers

Institute of Microbiology of the CAS, v. v. i.

Faculty of Science, Charles University

Prague 2024

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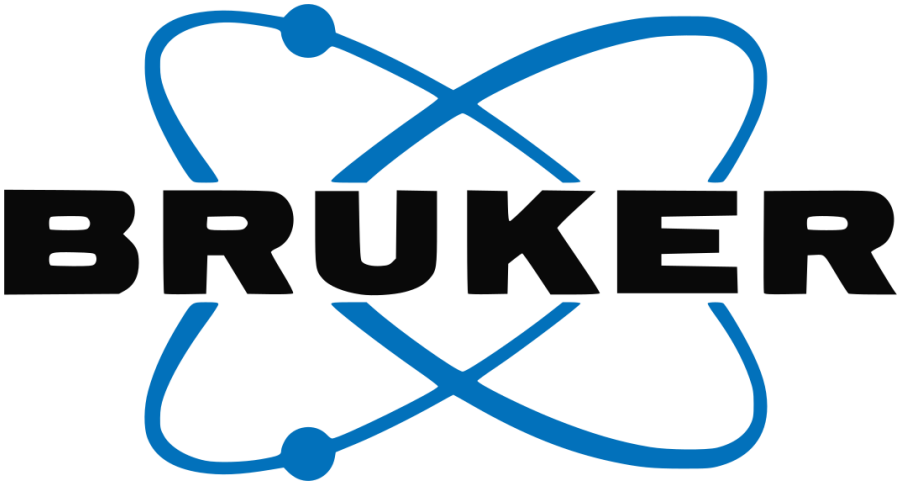

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Fifteenth European Fourier Transform Mass Spectrometry Workshop

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17th April – 20th April 2024

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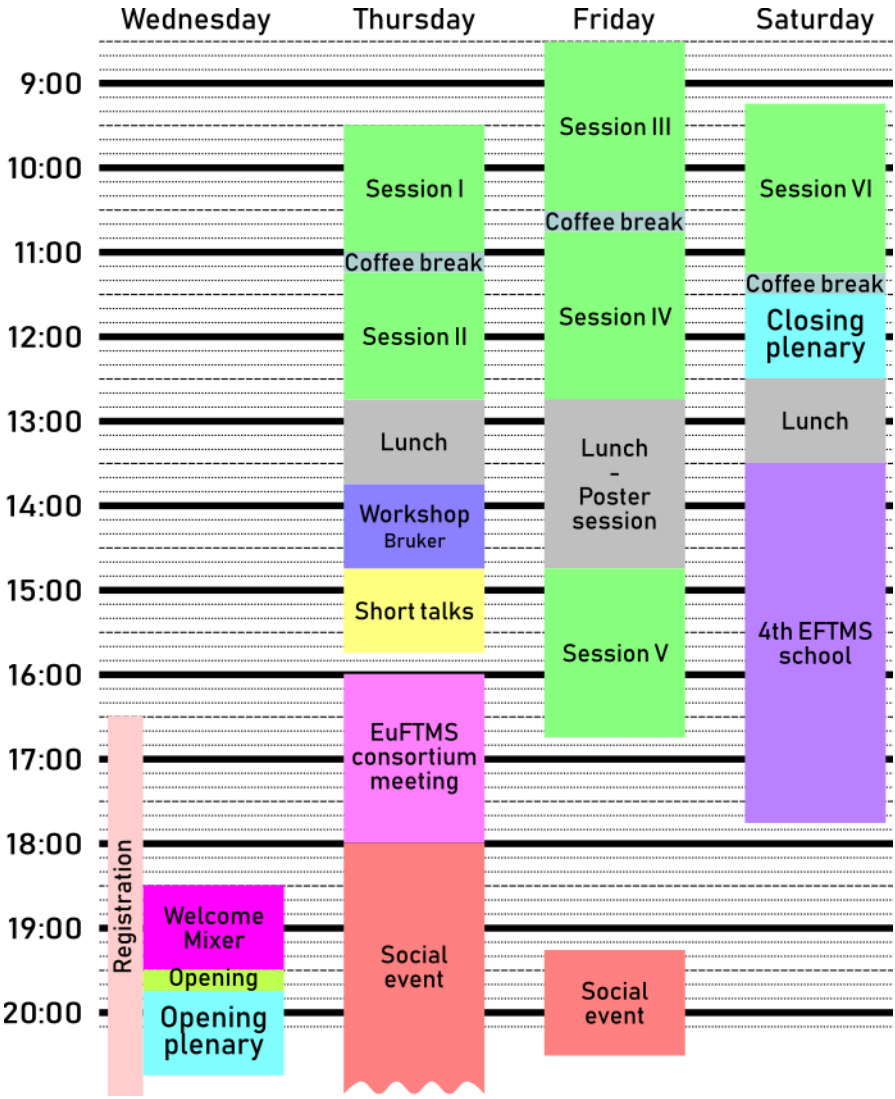
Wednesday
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9:00
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19:00
20:00
Registration
Welcome Mixer
Opening
Opening plenary
Session I
Coffee break
Session II
Lunch
Workshop
Bruker
Short talks
EuFTMS consortium meeting
Social event
Session III
Coffee break
Session IV
Lunch
-
Poster session
Session V
Social event
Session VI
Coffee break
Closing plenary
Lunch
4th EFTMS school

Wednesday 17th April 2024

16:30 – 21:00	Registration
18:30 – 19:30	Welcome mixer
19:30 – 19:45	Conference opening
19:45 – 20:45	**Opening plenary lecture: Michael L. Gross** (Chairperson: Petr Novák) *Mass Spectrometry Protein Footprinting: Principles and Application*

Thursday 18th April, 2024

9:30 – 11:00	**Session I** *(Chairperson: Petr Novák)*
9:30 – 10:00	Ryan P. Rodgers *Complex Mixture Analysis by Ultra-high Resolution FT-ICR Mass Spectrometry*
10:00 – 10:30	Bayan Almasri *Polyethylene terephthalates (PET) and microplastics identification by soft chemical depolymerization and FT mass spectrometry: New methodologies applied to environmental PET*
10:30 – 11:00	Marek Polák *Fast photochemical oxidation of nucleic acids coupled to high-resolution MS analysis*
11:00 – 11:15	Coffee break
11:15 – 12:45	**Session II** *(Chairperson: Yury Tsybin)*
11:15 – 11:45	Peter O'Connor *Advances in Two-Dimensional Mass Spectrometry*
11:45 – 12:15	Anika Neumann *Chemical Characterization of deposits formed during evaporation of fossil fuels and biofuels in stationary heating systems*
12:15 – 12:45	Pierre Pacholski *Thermodesorption/Pyrolysis (TDPy)-DART - FT-ICR MS for the characterization of synthetic fluoro-polymers*

12:45 – 13:45	Lunch
13:45 – 14:45	Company Workshop – Bruker Daltonics *Innovations in MRMS – from feasibility to ease-of-use*
14:45 – 15:45	**Short Talks** *(Chairperson: Carlos Cordeiro)*
14:45 – 14:55	Marta Cruz *FT-ICR-MS-based untargeted metabolomics: the path to precision medicine*
14:55 – 15:05	Helly Johanna Hansen *Humic-like Substances (HULIS) in Ship Engine Emissions Analyzed with ESI FT-ICR MS: Molecular Composition Effected by Fuel Type, Engine Mode, and Wet Scrubber Usage*
15:05 – 15:15	Callan Littlejohn *Tools to enhance MS/MS analysis of degraded polymers in FTICR MS*
15:15 – 15:25	Mariana Louro *Using MRMS based metabolomics to uncover the differential host response to SARS-CoV-2 variants infection*
15:25 – 15:35	Konstantin Nagornov *FTMS data analysis: software concepts and advanced workflows*
15:35 – 15:45	Inês Romão *Single shot top-down proteomics through direct infusion of complex protein mixtures, extreme resolution FT-ICR-MS and CapiDown*
16:00 – 18:00	EuFTMS Consortium Meeting – upon invitation – NLT meeting room *(Organizer: Christian Rolando)*
18:00 – 22:00	Social event and course dinner – Restaurant Větrník *(Group departure from NLT at 18:00)*

Friday 19th April, 2024

8:30 – 10:30	**Session III** *(Chair: Maria A. van Agthoven)*
8:30 – 9:00	Carlos Cordeiro *Single shot metabolomics: Challenges and solutions*
9:00 – 9:30	Pierre Giusti *Molecular Characterization of Electrodes Passivation Layers in Lithium-Ion Batteries*
9:30 – 10:00	Michael Volný *FTICR-MS expands the information about proteins detected on MALDI chips prepared by ion soft landing*
10:00 – 10:30	Christopher Wootton *A Gated-TIMS FT ICR-MS Instrument to decipher isomeric content of complex organic mixtures*
10:30 – 10:45	Coffee break
10:45 – 12:45	**Session IV** *(Chair: Christopher Rüger)*
10:45 – 11:15	Christian Rolando *Super-resolved spectra from FTMS signals by sinus_it, a non-Fourier Transform genetic evolution algorithm*
11:15 – 11:45	Théo Voellinger *Assessment of DIP FT-ICR MS for the study of polymer pyrolysis*
11:45 – 12:15	Vincent Carré *Metabolite mapping through MALDI FT-ICR MS: from plants to their microbiome*
12:15 – 12:45	Carlos Afonso *Advanced characterization of lignocellulosic biomass biooils by ultra-high resolution mass spectrometry using different separation and ionization methods*
12:45 – 14:45	Lunch & poster session

14:45 – 16:45	**Session V**
	(Chair: Alan Kádek)
14:45 – 15:15	Kristina Håkansson
	Electron Flux Dissociation: An Old New Twist on Ion-Electron Reactions in an FT-ICR Mass Spectrometer
15:15 – 15:45	Aleksandr Melikov
	Investigation of HSC70 oligomerization by structural mass spectrometry
15:45 – 16:15	Susanne Alfken
	MALDI MRMS for biomarker-based climate reconstruction from sediment archives with unmatched temporal resolution
16:15 – 16:45	Maria Elisa Crestoni
	Structure and reactivity of small carbon molecules probed by ion-molecule reactions and vibrational spectroscopy
19:15 – 20:30	Social event – *Guided ride through Prague's city centre on board a historical tramway car (1908-1942).*
	Group departure from NLT at 18:45.

Saturday 20th April 2024

9:15 – 11:15	**Session VI**
	(Chair: Michael Volný)
9:15 – 9:45	Yury Tsybin
	FTMS Booster: A high-performance data acquisition and processing system to advance FTMS applications
9:45 – 10:15	Alan Kádek
	Zapping ions – coupling an FTICR with UV and IR lasers for more informative top-down mass spectrometry
10:15 – 10:45	Maria A. van Agthoven
	From Rough Signal to Polished Spectrum: Absorption Mode Two-dimensional Mass Spectrometry
10:45 – 11:15	Konstantin Aizikov
	Ion Mobility Free High Throughput Peptide Collisional Cross-Section Measurements in Fourier Transform Mass Spectrometer

11:15 – 11:30	Coffee break
11:30 – 12:30	**Closing plenary lecture: Christopher L. Hendrickson** (Chair: Carlos Afonso) *21 Tesla FT-ICR Mass Spectrometer for Complex Mixture Analysis*
12:30 – 12:35	Closing remarks
12:35 – 13:30	Lunch
13:30 – 17:45	**4th EFTMS School**
13:30 – 14:30	FTMS Principles and spectra acquisition *Peter O'Connor, University of Warwick*
14:30 – 15:30	FTMS Data Processing *Yury Tsybin, Spectroswiss*
15:30 – 15:45	Coffee break
15:45 – 16:45	"How to mine a complex spectrum" - Dealing with Data from High-Resolution Mass Spectrometry of Complex Mixtures *Christopher Rüger, University of Rostock*
16:45 – 17:45	Beautiful friendship of high resolution mass spectrometry and structural biology *Petr Novák, Institute of Microbiology*

Mass Spectrometry Protein Footprinting: Principles and Application

Michael L. Gross[1,*]

1. *Department of Chemistry, Washington University in St Louis, St Louis, Missouri*

[*] *mgross@wustl.edu*

FPOP, first demonstrated in 2005, has proven to be a versatile tool for problem solving in protein chemistry and biophysics. Given the microsecond speed of the footprinting, FPOP can follow fast folding/unfolding of protein and map with FT mass spectrometers the regions of the protein that are undergoing the conformational changes. Its speed also permits location of regions with hidden conformational changes. In biopharmaceutical applications, it can identify protein conformational changes owing to thermal stress and can be productive in epitope mapping. It can also map conformational changes occurring upon binding to small ligands and metal ions, allowing metal-ion affinity and order of binding to be determined. A particularly relevant application in understanding Alzheimer's and other neurodegenerative diseases is mapping amyloid formation especially of soluble oligomers, the purported toxic substances in the disease. A current application where few biochemical tools are applicable is the emerging field of membrane proteins. Footprinting, both in membrane mimetics (e.g., nanodisks and liposomes) and in living cells, may be a significant option. A better-known footprinting method is HDX, and some examples of its complementary nature will be presented along with a description of FPOP and its applications.

21 Tesla FT-ICR Mass Spectrometer for Complex Mixture Analysis

Christopher L. Hendrickson[1,*]

1. Director, Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Distinguished University Scholar, Florida State University

[*] *hendrick@magnet.fsu.edu*

I will describe the design and current performance of the first 21 tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, which is the highest field ICR system to date. The instrument is a unique (and currently being upgraded!) combination of commercial and in-house hardware and software and features novel combinations of mass resolving power, mass measurement accuracy, dynamic range, and scan rate. We have coupled liquid chromatography, collisional, photo-, and electron transfer dissociation, proton transfer reactions, parallel ion parking, and high-resolution SWIFT ion isolation with the instrument for state-of-the-art top-down proteomics, mass spectrometry imaging, and characterization of complex mixtures of emerging environmental contaminants. The instrument is part of the National High Field FT-ICR User Facility at the National High Magnetic Field Laboratory, and is available to all qualified users.

Complex Mixture Analysis by Ultra-high Resolution FT-ICR Mass Spectrometry

Ryan Patrick Rodgers[1,2,3,*], Martha L. Chacon-Patino[1,2], Alvaro J. Tello Rodriguez[1], Christopher Holder Montenegro[1], Germain Salvato Vallverdu[2,3], Brice Bouyssiere[2,3], Pierre Giusti[2,4], Carlos Afonso[2,4], Christopher Ruger[5], Christopher L. Hendrickson[1]

1. *National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL USA*
2. *International Joint Laboratory (iC2MC), Harfleur, France*
3. *Université de Pau et des Pays de l'Adour, Pau, France*
4. *TotalEnergies OneTech, Harfleur, France*
5. *University of Rouen-Normandy, Mont-Saint-Aignan, France*

[*] *rodgers@magnet.fsu.edu*

Over the past 20 years, advances in modern high resolution / ultrahigh-resolution mass spectrometry have forever changed the expectations of complex mixture analysis. Aided by advances in ionization methodologies that provide course chemical selectivity in ionization that facilitates the molecular-level analysis of polar (acidic / basic) and aromatic species, ultrahigh-resolution mass spectrometry routinely resolves and identifies tens-of-thousands (at the level of elemental composition assignment) components in complex mixtures. Furthermore, the highest magnetic field instruments enable on-line coupling of separation methods with ultrahigh resolution mass spectrometry that provides unprecedented insight into even the most complex mixtures. Here, we present the latest efforts to address complex organic mixture analyses by high field Fourier Transform ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) and highlight on-line separation strategies used to alleviate / minimize the impact of selective ionization and aggregation. Applications highlighted include biomass pyrolysates, petroleum, dissolved organic matter, and complex emerging contaminants. Recent advances in data reduction / visualization strategies and updates to the PyC2MC software platform that facilitate rapid, confidence-based data processing are also discussed.

ACKNOWLEDGEMENT

This work was performed at the National High Magnetic Field Laboratory ICR User Facility, which is supported by the National Science Foundation Division of Chemistry through Cooperative Agreement No. DMR-1644779 and the State of Florida.

Polyethylene terephthalates (PET) and microplastics identification by soft chemical depolymerization and FT mass spectrometry: New methodologies applied to environmental PET

Bayan Almasri[1,2,*], Youssef Bakkour[2,3], Christian Rollando[1,4]

1. *Miniaturization for Synthesis, Analysis & Proteomics (MSAP), UAR 3290, CNRS, University of Lille, Faculty of Sciences & Technologies, 59655 Villeneuve d'Ascq, France*
2. *Laboratory of Applied Chemistry (LAC), Lebanese University, Faculty of Sciences, Tripoli, Lebanon*
3. *King Khalid University, Abha, Saudi Arabia*
4. *Shrieking Sixties, 1-3 Allée Lavoisier, Villenueve d'Ascq, France*

[*] *bayan.almasri1234@gmail.com*

Polyethylene terephthalates are one of the most abundant polymers in our daily life. These polyesters are formed of terephthalic acid and ethylene glycol units. In the environment, they degrade and release micro- and nano-PET, a major pollution source especially for marine ecosystems. Huge amounts of nano-PET were recently detected in water of PET bottles. Many analyses were performed on PET. Unfortunately, they were not satisfying for the study of PET structural modifications and their quantification. Our new methodology identifies and quantifies PET including their additives, allows the study of PET environmental modifications after ageing by UV, salinity, micro-organisms, waste-treatment, and permits the study of modifications' degree between micro-, nano- and macro-PET which affects their toxicity.

Our methodology starts by PET transamidation using N,N′-dimethyl-1,3-propanediamine (DMAPA) at room temperature after a solubilization step by 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). The resulting products were neutralized, extracted with ultrapure water and ethyl acetate, then analyzed using MALDI ionization on an ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) 9.4 Tesla and ESI ionization on an Orbitrap LC-MS. The step of PET pre-solubilization by HFIP allowed a transamidation at room temperature instead of 70°C and improved the extraction yield. To quantify PET, an internal standard was successfully synthesized. For better identifying polyols, we derivatized them with 4-N,N'-dimethylbenzoyl chloride prior to their analysis. This benzoylation allows the study of polyols modifications during PET manufacturing and their environmental modifications. Many PET containers and bottles from France and US of different brands and contents (oils, vinegar, water, wine, beer and carbonated liquids) were analyzed. PETs were also studied after accelerated ageing; and marine environmental PET were analyzed. For each, a specific signature was determined. Indeed, PET were differentiated by their chemical composition and by the difference in intensities of the common peaks; dimers, oligomers, crosslinked and oxidized products. For example, cyclohexanedimethanol of PET-G was detected in the carbonated-containing bottles. This methodology allows, in addition to the identification of PET and their additives (plasticizers and antioxidants), the detection of products embedded in PET. The micro-PET analysis and the study of the new similar biodegradable generation (PBAT) will be also presented.

Fast Photochemical Oxidation of Nucleic Acids coupled to High-Resolution MS analysis

Marek Polák[1,2], Jiří Černý[3], Vítězslav Brinsa[1,2], Daniel Kavan[1,2], Michael Volný[1], Alan Kádek[1], Petr Novák[1,2,*]

1. *BioCeV – Institute of Microbiology, The Czech Academy of Sciences, Prague, Czechia*
2. *Charles University, Faculty of Science, Prague, Czechia*
3. *BioCev – Institute of Biotechnology, The Czech Academy of Sciences, Prague, Czechia*

[*] *polakmare@natur.cuni.cz*

Recent years have seen significant growth in the methods of structural proteomics, which had a significant impact in the field of structural and molecular biology. These methods may unveil answers related to structure and dynamics of protein and protein complexes, making them favorable for studying protein-DNA interactions. One specific method, radical covalent labelling, has emerged as an effective analytical tool for characterization of biomolecules. Fast Photochemical Oxidation of Proteins (FPOP), the most common radical labelling method, is now exclusively employed only for mapping the structure and interaction of proteins. Nevertheless, during the early 80's, when radical chemistry was only involving, it was primarily used for assessing biding motif between DNA and transcription factors by hydroxyl radical damage of the DNA.

We show in this study an approach to analyze FPOP-induced damage of DNA coupled to high-resolution mass spectrometry analysis. Our model system 17bp double-stranded DNA, Insulin Response Element (IRE), was fragmented in FPOP apparatus in the absence and in the presence of its cognate binding partner, FOXO4. Residual protein was digested using Proteinase K, and resulting DNA fragments were separated in LC and analyzed using high-resolution 15T-FT-ICR mass spectrometry operated in negative ion mode. The study emphasizes the fragmentation mechanism of nucleic acid in solution, and outlines both benefits and drawbacks associated with analyzing DNA fragments throughout the experimental process, including FPOP oxidation, LC-MS, and data analysis.

Analysis of separated IRE fragments revealed that hydroxyl radicals cleave the DNA nonspecifically, creating a complementary set of 3'OH, 3'P, 5'OH and 5'P terminal fragment ions. Combining MS/MS analysis, mass accuracy precision and high resolution obtained by FT instrument and in-silico modeling of isotopic envelopes confirmed the presence of generated DNA fragments. Complementary fragments were found in the LC-MS trace and quantified. Comparison of IRE fragment ions revealed a significant protective effect around the binding sequence in the major groove of DNA, also lower protection in the minor groove. Expanding the scope, our study delved into a complex involving two transcription factors, FOXO4 and TEAD1, interacting with one oligonucleotide to form a ternary complex. This investigation illustrates how FPOP can effectively monitor minor structural changes in DNA upon the binding of transcription factors, underscoring its full potential for studying dynamic complexes in solution.

ACKNOWLEDGEMENT

This work was supported by the NPO-NEURO-EXCELLES – LX22NPO5107, Czech Science Foundation (grant numbers 19-16084S, 22-27695S), Grant Agency of Charles University (359521), European Commission Horizon H2020 (EU FT-ICR MS - grant agreement ID: 731077), and, in part, by the Czech Academy of Sciences (RVO61388971).

Advances in Two-Dimensional Mass Spectrometry

Peter O'Connor[1,*]

1. University of Warwick

[*] *p.oconnor@warwick.ac.uk*

Two-dimensional mass spectrometry (2DMS) allows acquisition of fragment information from all precursors simultaneously by modulation of a trapped ion packet position through a fragmentation zone. This fragmentation zone is best created using either an electron beam (ExD methods) or by using a laser (IRMPD or UVPD). The modulation is created using a classic Gaumann pulse sequence (a low amplitude frequency sweep pulse (P1), an iterated delay (T), and a second sweep pulse (P2=P1) or by using the SWIFT/SWIM pulse sequences from Ross and Marshall. In either case, all ions are modulated at their own cyclotron frequencies, through the fragmentation zone and fragments are born (or not) at the cyclotron frequency of the precursor ions, so a Fourier transform of the peak intensities of all peaks will extract the modulation frequencies of all ions, thereby correlating fragments with their precursor ions, even in complex mixtures.

The advantages of 2DMS are several. 2DMS does not require precursor ion isolation prior to fragmentation, so resolution in the precursor ion dimension is only limited by the number of scan lines chosen, and the ion beam stability. In 2DMS, accumulation of signal over a series of scans increases signal/noise as the square root of the number of scan lines (the Fellgett signal averaging advantage). Having all fragments from all precursors allows the user to avoid any ion selection biases (either by the user or by the DDA algorithm). Having all fragments from all precursors also allows interesting chemical correlations to be revealed in the spectra.

Over the past 10 years, our group has been working to implement this methodology and have done so using all the fragmentation methods above. We have also implemented an MS/2DMS methodology and a TIMS/2DMS methodology as well. Furthermore, we have shown the ability to differente fragments at greater than 4 sigma) from two different precursors which are only 19 mDa apart.

This presentation will discuss the current state of the art in 2DMS methodologies, and we will also attempt to project the longer-term utility of these techniques.

Chemical Characterization of deposits formed during evaporation of fossil fuels and biofuels in stationary heating systems

Anika Neumann[1,2,3,*], Benedikt Bender[4], Thorsten Streibel[1], Christopher Paul Rüger[1,3], Ralf Zimmermann[1,3]

1. *University of Rostock, Institute of Chemistry, Chair of Analytical Chemistry*
2. *University of Rostock, Faculty of Mechanical Engineering and Marine Technology, Chair of Piston Machines and Internal Combustion Engines*
3. *University of Rostock, Department Life, Light & Matter*
4. *OWI Science for Fuels gGmbH, RWTH Aachen University*

[*] *anika.neumann@uni-rostock.de*

Energy transition is a major topic facing climate change. Besides hydrogen, methanol, and ammonia, renewable carbon-based biofuels, such as fatty acid methyl esters (FAME), hydrated vegetable oils (HVO), or Fischer-Tropsch synthesis products raise in importance. [1] During combustion in heating systems, fossil fuels tend to form deposits over time. In this project, we investigated the influence of different fossil fuels, biofuels and fuel mixtures (fresh/aged) on the amount of formed deposit and their chemical composition. As the deposits' chemical composition was expected to be very complex, high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was applied for in-depth molecular characterization.

Fuels and fuel mixtures were aged with two different methods: 1) Storage at 40 °C for 6 month, 2) fast-aging with BigOxy (DGMK 763) at 105 °C, 4.8 bar and 32 h. Deposits were generated on a modified stationary heating with exchangeable evaporation unit.
Fresh/aged fuels were characterized by TAN, GC-MS and ESI/APPI-FT-ICR MS. Deposits were investigated by TGA, pyr-GC-MS and DIP-APPI-FT-ICR MS. [2,3]

Aging of fuels and fuels mixtures led to an increase of oxygen-containing compounds, especially when FAME was present. However, in the presence of octanol, no aging effects were observed.
The investigation of deposits revealed that they are composed of 70-85 % elemental carbon, 15-30 % pyrolyzable organic carbon, and less than 5% comprises remaining fuel. Pyr-GC-MS revealed the presence of alkyl-side chains with up to 20 carbons as well as the presence of alkylated benzenes and naphthalenes, which seem to be attached to higher molecular structures.
DIP-APPI-FT-ICR MS showed differences in the high complex molecular fingerprints of deposits from different fuels and fuel mixtures. Deposits are composed of high aromatic compounds, while alkylation differs between the investigated heating oil (higher alkylation) and diesel (lower alkylation). Main compound classes found for all types of deposits were the CH-class and O1-3-class. Furthermore, the addition of rapeseed oil methyl ester (RME) increased the amount of pyrolyzable organic carbon. Deposits of aged fuels showed compounds with slightly higher aromaticity compared to deposits from non-aged fuels.

LITERATURE:

[1] A. Krutof, Renewable Sustainable Energy Rev., 2016, 59
[2] C.P. Rüger, Anal. Chem., 2015, 87
[3] C.P. Rüger, Energy Fuels, 2018, 32

Thermodesorption/Pyrolysis (TDPy)-DART - FT-ICR MS for the characterization of synthetic fluoro-polymers

Pierre Pacholski[1,2,*], Sébastien Schramm[2], Umut Ugur Ozkose[3], Frank David-Quillot[4], Frédéric Progent[1], Bruno Améduri[3], Frédéric Aubriet[2]

1. CEA, DAM, DIF, F-91297 Arpajon, France
2. Université de Lorraine, LCP–A2MC (Laboratoire de Chimie et Physique-Approche Multi-échelles des Milieux Complexes), F-57000 Metz, France
3. Institut Charles Gerhardt, ICGM, Univ. Montpellier, CNRS, ENSCM, Montpellier, France
4. CEA, DAM, Le Ripault, F-37260 Monts, France

[*] *pierre.pacholski@gmail.com*

The analysis of polymer by mass spectrometry offers information on the repeating unit(s), average molar mass, end-groups and additives. Electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI) ion sources are commonly used. However, they can require sample preparation steps, as the solubilization of the sample in a sufficiently polar and volatile solvent for ESI, and homogeneous mixing with a matrix in MALDI.

In some cases, solubilization and preparation steps are not only time consuming but are real limitations, as exemplified by poly(vinylidene fluoride), PVDF, a polymer bearing a $C_2H_2F_2$ repeating unit. It is well-known that PVDFs are poorly (or non-soluble) in common organic solvents. Desorption/ionization techniques are useful in that case, but PVDF does not absorb UV and cannot be investigated by UV laser desorption/ionization. Direct analysis in real time (DART) is most suited and generates ions by shooting the sample with a hot metastable He or N_2 stream. DART-MS was successfully employed for the differentiation of PVDFs according to their end-groups. (Pacholski *et al.* 2023)

For in-depth PVDF characterization, a thermodesorption/pyrolysis (TD/Py) device was added to the DART ion source. A temperature gradient from 35 °C to 600 °C ensured to observe both thermal desorption and pyrolysis products. Classically, pyrolysis leads to the formation of a huge number of compounds. After ionization, the resulting mass spectrum may be very complex and bear thousands of features. For this reason, the TDPy-DART ion source was coupled to a 7T 2XR Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). Up to our knowledge, this study presents the first TD/Py-DART-FT-ICR MS coupling. The very high resolution and the high *m/z* measurement accuracy allowed the unambiguous ions formula attribution, leading to a deep understanding of the generated compounds. In one of the investigated PVDFs, an unexpected amount of fluorine (F/C greater than 1) revealed the presence of a perfluorinated comonomer, which was not evidenced by “simple”-DART analysis. This result was confirmed by ^{19}F NMR, which demonstrated the presence of 5% of hexafluoropropylene (HFP) comonomer. This sample was compared to a VDF homopolymer and a poly(VDF-*co*-HFP) copolymer with mol.%HFP > 5. A correlation between the HFP content in the polymer and the number of CF_2 units measured in pyrolysis product was observed.

The findings highlighted in this study showed the potential of TD/Py-DART-FT-ICR MS to quickly reveal (the time for an analysis is shorter than 10 minutes) insights on polymers and differentiate copolymers according to their monomer composition.

Single shot metabolomics: Challenges and solutions

Carlos Cordeiro[1,*]

1. *FT-ICR and Sctructural MS Lababoratory - ULisboa*

[*] *cacordeiro@fc.ul.pt*

Metabolomics offers the most direct readout of the phenotype, thus being of immense value to describe the current state of a living organism. In contrast to genomics or proteomics that rely on well established and virtually universal methods, the chemical diversity and dynamic range of the metabolome pose unique challenges. Thus, a plethora of analytical methods, straight from the analytical chemistry arsenal, has been applied to metabolomics, mainly CGMS and LCMS, that provide fractional and biased coverages of the metabolome. Moreover, all require extensive sample preparation and rely on the concept of sequential analysis through previous chromatographic or other separative techniques thus hindering throughput. Metabolomics calls for parallelization and simultaneous, unbiased high throughput analysis. Magnetic Resonance methods open that path, through nuclear magnetic resonance (NMR) or magnetic resonance mass spectrometry (MRMS). MRMS, correctly termed Fourier transform ion-cyclotron resonance mass spectrometry (FT-ICR-MS) offers a broad mass range coverage with extreme resolution and mass accuracy as well as a suitable dynamic range for the simultaneous analysis of all metabolites. Maximizing the information content in biological context of accurate mass measurements of whole metabolomes is the purpose of single-shot metabolomics. Here we show how mass spectra obtained from direct injection at extreme resolution and mass accuracy can be converted into biological information, addressing issues such as metabolite identification, quantification as well as future directions based on computational approaches.

ACKNOWLEDGEMENT

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Molecular Characterization of Electrodes Passivation Layers in Lithium-Ion Batteries

Pierre Giusti[1,2,3], Julien Maillard[1,2,3], Julien Demeaux[4], Charlotte Mase[1,2,3], Antonin Gajan[4], Cecile Tessier[4], Patrick Bernard[4], Carlos Afonso[1,3,*]

1. *TotalEnergies OneTech R&D, Total Research & Technology Gonfreville, BP 27, 76700 Harfleur, France.*
2. *Normandie Université, COBRA, UMR 6014 et FR 3038, Université de Rouen-Normandie, INSA de Rouen-Normandie, CNRS, IRCOF, Mont Saint Aignan Cedex*
3. *International Joint Laboratory - iC2MC: Complex Matrices Molecular Characterization, TRTG, BP 27, 76700 Harfleur, France.*
4. *Saft, Corporate Research, 33074 Bordeaux, France*

[*] *pierre.giusti@totalenergies.com*

The performances of lithium-ion batteries (LIBs) are closely related to the control of the electrolyte composition and solid-electrolytes interface (SEI) stability [1-2]. To decrease the capacity fading electrolyte formulation has been continuously optimized over years and much attention has been paid to identifying the SEI composition. However, deciphering the molecular diversity of both liquid electrolytes and solid interphase remains extremely challenging because few analytical techniques show the appropriate sensitivity and spatial resolution without modifying their composition. Here, Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) was used for the first time to characterize at the molecular level both parts of LIBs. The liquid electrolytes were characterized using atmospheric pressure chemical ionization to cover a broad polarity range. Surprisingly, obtained mass spectra revealed hundreds of signals even if the initial electrolytes cocktail was prepared using 10 species. Using mass defect molecular maps, it was possible to delimit regions of interest for each component and easily perform reverse engineering. Two anodes coming from LIBs with different performances were analyzed using laser desorption ionization operated in imaging mode. After the subtraction of species coming from the carbon reference electrode, the unique contribution of each distinct SEI was observed and known reaction products of electrolytes were researched and identified[3].

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FTICR-MS expands the information about proteins detected on MALDI chips prepared by ion soft landing

Michael Volny[1,2,3,*], Josef Dvorak[2,4], Jana Novakova[3], Jaroslav Hrabak[5], Zuzana Kalaninova[2,4], Petr Pompach[6], Petr Novak[2]

1. *Department of Analytical Chemistry, University of Chemistry and Technology, Prague, Czech Republic*
2. *Institute of Microbiology, The Czech Academy of Science, Prague, Czech Republic*
3. *AffiPro s.r.o., Vestec, Czech Republic*
4. *Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic*
5. *Department of Internal Medicine, Faculty of Medicine in Pilsen, Pilsen University Hospital, Charles University, Pilsen, Czech Republic*
6. *Institute of Biotechnology, The Czech Academy of Science, Vestec, Czech Republic*

[*] *volnyi@vscht.cz*

Deposition of ions from a gas phase, a process often referred to as ion soft landing, allows patterning of protein molecules onto surfaces in the form of different microarrays that can form protein chips with many different functionalities. Successful protein ion soft landing with retention of activity was first demonstrated by Cooks (1), while electrospray deposition of protein ions at atmospheric pressure was developed by Morozov (2). Ion soft landing can be used to prepare protein chips compatible with MALDI ionization, which gives the MALDI experiment new possibilities to perform in-situ affinity chemistry on the MALDI plate (3). Different workflows can be used to perform enrichment and clean-up prior MALDI ionization, a workflow previously named “lab-on-plate” (4). We have developed a method to use ambient ion soft landing, or controlled electrospray deposition, to prepare MALDI chips of large variety of protein functionalities that can be applied to perform different affinity assays detected by MALDI mass spectrometry (5). While MALDI has been coupled with time-of-flight mass analyzer from its inception, and this coupling has been extremely successful, sometime the MALDI performance is limited by the insufficient resolution of the time-of-flight. Here we show examples of biochemically and clinically relevant MALDI-MS assays, where ultrahigh resolution of FTICR significantly expanded information that can be obtained from MALDI protein chip applications.

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A Gated-TIMS FT ICR-MS Instrument to decipher isomeric content of complex organic mixtures

Christopher A. Wootton[1,*], Julien Maillard[2,3], Alina Theisen[1], Gregory F. Brabeck[1], Carlos L. Schat[1], Madeline E. Colley[4], Martin Dufresne[4], Jeffrey M. Spraggins[4], Christopher P. Rüger[3], Carlos Afonso[3,5], Pierre Giusti[2,3,5]

1. *Bruker Daltonics GmbH & Co. Kg, Bremen, Germany*
2. *TotalEnergies One Tech, R&D, Downstream Processes & Polymers, TotalEnergies Research & Technology Gonfreville, BP 27, 76700 Harfleur, France*
3. *International Joint Laboratory, iC2MC: Complex Matrices Molecular Characterization, TRTG, BP 27, 76700 Harfleur, France*
4. *Department of Biochemistry, MRBIII U9228, Vanderbilt University, USA*
5. *Univ Rouen Normandie, INSA Rouen Normandie, CNRS, Normandie Univ, COBRA UMR 6014, INC3M FR 3038, 76000 Rouen, France*

[*] *christopher.wootton@bruker.com*

Trapped ion mobility spectrometry (TIMS) has advanced greatly in recent years, yet FT-ICR MS has only had limited exposure to the IMS separation technique. A new FT-ICR MS instrument has been designed, constructed, and characterised which overhauls the entire front end of the SolariX MRMS platform to benefit from various ion optic advancements, including a contemporary, dual-accumulation and analysis TIMS cartridge as used on commercial TIMS Tof systems. The energy landscape of the system was retuned for ultra-low energy and low activation transmission of delicate analytes. Extensive ion current measurements were used to optimise each transfer and isolation region, with energy measurements informing optimum transfer and detection characteristics.

The TIMS MRMS system was modified to operate under gated TIMS (gTIMS) conditions using a dual-lens and/or fast switching quadrupole as a gating element. gTIMS allowed time-binned analysis of TIMS-separated ions in order to accommodate the FT-ICR MS's variable, and longer detection times required for ultra-high resolution MS analysis. This decoupling of the IMS separation (millisecond timescale) and MS (second timescale) allowed for ultra-high resolution analysis and the ability to accumulate large ion populations for subsequent analysis, enhancing dynamic range for complex mixture samples such as SRFA and alternative future fuels. Gated TIMS was optimised for a wide mobility range (e.g. 1/K0 0.01-2.3Vs/cm^2) and was operated in both sweeping mode to study mobility of species, or filtering mode to pass individual species/isomers on for MS^n and/or high resolution detection of gas phase fractionated ions.

Ultra-high resolution MS performance of FT-ICR and UHV was maintained; mass resolving power of several million in broadband mode, and over 14 million in narrowband mode was readily achieved. Front-end CID and in-cell ExD (ECD, EID, EDD) are compatible with up front gTIMS separation. Calibration against known compounds showed that gTIMS elution voltage to mobility relationship was preserved to an R^2 of 0.9999. The inherent mass accuracy of FT-ICR was achieved without compromise; 95ppb standard deviation for Tunemix ions 622-2722m/z on a 7T magnet system.

To demonstrate the capabilities of the prototype system a range of complex mixtures were analysed, such as Swannee river fulvic acids (SRFA) and alternative (green) future fuels. Combining ultra-high

MS resolution and gTIMS allowed m/z specific IMS analysis down to the milli-Dalton level, required for studying the isomeric complexity of such complex mixtures.

Initial MALDI imaging analysis of human brain and kidney tissue sections is also shown from the prototype instrument, showing 10um microprobe spatial resolution with high spatial fidelity and 500,000 to 1,000,000 MS resolving power (at 400m/z). Incorporation of a microgrid MALDI stage to achieve 5um spatial resolution is underway.

Super-resolved spectra from FTMS signals by sinus_it, a non-Fourier Transform genetic evolution algorithm

Marc Haegelin[1], Azad Kichibayov[1,2,3], Ulviyya Abdulkarimova[2,3], Pierre Collet[2,4], Christian Rolando[1,5,*]

1. *Université de Lille, Faculty of Sciences and Technologies, MSAP UAR 3290 CNRS, Villeneuve d'Ascq, France*
2. *Université de Strasbourg, ICube UMR 7357 CNRS, Strasbourg, France*
3. *French-Azerbaijani University (UFAZ), Baku, Azerbaijan*
4. *Universidad Andrés Bello, ITISB (Instituto Tecnológico para la Innovación en Salud y Bienestar), Viña del Mar, Valparaiso, Chile*
5. *Shrieking Sixties, 1-3 Allée Lavoisier, 59650 Villeneuve-d'Ascq, France*

[*] *christian.rolando@univ-lille.fr*

The raw signal from FT-ICR or Orbitrap MS is constituted by sum of damped sine in the time-domain called transient. From this transient a spectrum in the frequency domain is obtained Discrete Fourier Transform (DFT). Then the spectrum in frequency is transformed in the desired *m/z* scale. In mass spectrometry DFT has a major limitation: the peak with in the spectrum is proportional to the transient duration in the time domain. Obtaining peaks in the spectrum sharper than those obtained by DFT is called super-resolution. We will show that by searching a sum of damped sines in the time domain by a genetice algorithm we peak sharpening by a factor almost 4 which is close from the theoretically predicted limit.

The sinus_it algorithm runs on Nvidia RTX 2080 Ti Graphics Processing Units (GPU) hosted by a 2× 16 cores 2.1 Ghz Intel Xeon Dell Precision 7920 server. Nvidia RTX 2080 Ti GPU is a gaming CPU which is inexpensive (approximately 1500 USD). Sinus_it is written in C++ and CUDA the specific language for Nvidia GPU. We used the open source EASNA platform from ICube laboratory in Strasbourg (France) to implement it. Sinus_it was applied on simulated as well as experimental signals acquired on a Bruker SolariX 9.4 Tesla FTICR mass spectrometer.

Sinus_it searches a of sine sum that fits the transient using a genetic evolutionary algorithm. In a genetic algorithm, like in Nature, a population of solutions is evolved toward better solutions by mutation and mixing solutions. Sinus_it determines for each sine the intensity, frequency and damping factor. Preliminary results which showed that Sinus_it running time is proportional to data points in the transient and sines to be searched. So, Sinus_it was initially implemented with 2 search modes: coarse and fine. In coarse mode Sinus_it searches for the full set of isotopes of a substance whereas on fine mode Sinus_it searches for the fine structure of a given isotope. Transients with limited band width around relevant frequencies are obtained by classical Butterworth filtering. In all cases a super-resolution near the theoretical limit of 4 was obtained. In other words, we can use only the first quarter of the transient where the signal is more intense affording more accurate isotopic ratios. Genetic evolutionary algorithms are by nature stochastic, which mean that the results of a run depend on the random numbers drawn. By running sinus_it about thirty times we will show that the standard deviation on sine parameters is low and inversely proportional to the signal to noise. The high accuracy of sinus_it is proved by the perfect linear dependence of the phases with the starting point in the gradient.

Furthermore, we will present the possibility of performing information driven harmonic inversion when the expected *m/z* are known for example when performing isotope ratios measurements.

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Assessment of DIP FT-ICR MS for the study of polymer pyrolysis

Théo Voellinger[1,*], Sébastien Schramm[1], Frédéric Aubriet[1]

1. *Université de Lorraine, LCP-A2MC, 57070 Metz France*

[*] *theo.voellinger@univ-lorraine.fr*

The fast pyrolysis of synthetic and natural polymers is a promising source of renewable molecules and fuels. The parameters employed during the pyrolysis process (raw material, heating rate(1), gas flow) and the use of a catalytic treatment(2) have a significant impact on the physical properties of the resulting oil as well as its molecular composition. In addition, these oils contain tens of thousands of different compounds, whose chemical properties (weight, polarity, stability) can vary significantly. Optimizing pyrolysis parameters to obtain oils with the desired properties and yields for the targeted compounds is a critical step. This optimization process is complex and time-consuming and often require repeated production and characterization according to a petroleomic approach of the different oils in respect with the used pyrolysis parameters.

To shorten the time required to optimize the pyrolysis parameters, the on-line mass spectrometry analysis of the pyrolysis products is well suited. In this frame, a modification of the direct insertion probe (DIP) coupled to a FT-ICR Mass Spectrometer (FT-ICR MS) was developed. Indeed, the DIP device ensures to quickly heat a solid at temperatures reaching hundreds of Celsius. Adding a flow of inert gas inside the probe allowed to mimic the thermal degradation of raw materials occurring in pyrolysis reactors. To investigate the resulting species, an atmospheric pressure chemical ionization source (APCI) was used prior to the analysis of the resulting ions by FT-ICR MS. The proposed device allows the investigation in a few minutes of the thermal degradation products of natural and synthetic polymers.

The influence of the employed gas flow as well as the nature of the polymer's conversion products have been investigated. For cellulose, the pyrolysis produced well-known markers(3) such as cellobiosan and levoglucosan which have been unambiguously identified by tandem mass spectrometry. The dependence of the distribution and nature of the pyrolysis products of polystyrene with respect to its average molecular mass was also studied.

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DIP, FT-ICR MS, polymers, pyrolysis, analytical chemistry

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Metabolite mapping through MALDI FT-ICR MS: from plants to their microbiome

Farès Slimani[1], Marisa Maia[1,2,3], Patrick Chaimbault[1,4], Vincent Carré[1,4,*]

1. *LCP-A2MC, Université de Lorraine, Metz, France*
2. *GPS Lab, BioISI, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal*
3. *DBV, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal*
4. *MassLor Platform, Université de Lorraine, Metz, France*

[*] *vincent.carre@univ-lorraine.fr*

Plants and their environment, including microorganisms such as bacteria and fungi, are surprisingly complex at the molecular level. This is not only due to their nature, but also to their interactions, which can be symbiotic, neutral or pathogenic.

MALDI mass spectrometry is a powerful technique for probing and imaging this molecular diversity on the surface of a sample. In addition, the use of FT-ICR mass spectrometry with high mass resolution and mass measurement accuracy increases the confidence and selectivity of the spatial distribution of many compounds. However, application to plant and/or microbial samples remains an analytical challenge. Firstly, the topology and heterogeneity of the sample surface can vary greatly and influence the MALDI MSI response. Secondly, the molecular composition of these samples can be so extensive and complex that no relevant chemical variation from one region to another may be highlighted if no compounds are targeted first.

In this presentation we will discuss two examples of such MSI problems with plants and microorganisms and how they can be overcome. First, we will look at MALDI MSI of soil bacterial colonies on agar media, which introduces the problem of sample heterogeneity and topology. Secondly, we will look at the molecular complexity of a grape leaf and its response to biotic stress. In this last case, the large number of mass features led us to employ a new data analysis approach to treat non-targeted MSI data.

These two works highlight the ability of MSI to probe the dynamics and spatial variations of metabolic profiles of plants and their microbiome.

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Advanced characterization of lignocellulosic biomass biooils by ultra-high resolution mass spectrometry using different separation and ionization methods

Carlos Afonso[1,*], Charlotte Mase[1,2], Rémi Moulian[1,2], Marie Hubert-Roux[1], David C. Dayton[3], Ryan P. Rodgers[4], Christopher Wootton[5], Caroline Barrère-Mangote[2], Pierre Giusti[2]

1. *University of Rouen Normandy, Rouen, France*
2. *TotalEnergies, Harfleur, France*
3. *Research Triangle, North Carolina, USA*
4. *National High Magnetic Field Laboratory, Tallahassee, USA*
5. *Bruker Daltonics, Bremen, Germany*

[*] *carlos.afonso@univ-rouen.fr*

Bio-oils produced from the pyrolysis of lignocellulosic biomass have proven to be a promising renewable energy source. These bio-oils are highly complex organic mixtures consisting of thousands of compounds covering a wide range of mass and polarity. However, the liquid obtained after pyrolysis is rich in oxygen, which causes instability and corrosion problems. Therefore the use of processes such as hydrotreatment is required to increase the H/C ratio of the products to be used as fuels or chemicals. Advanced molecular level characterization of these samples is necessary to understand the efficiency of these processes. However, this requires the use of ultra-high resolution instruments such as the Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer. This instrument offers the best performance in terms of resolution, dynamic range, and mass accuracy. It allows unique molecular formulas to be unambiguously assigned to all compounds in the sample. To extend the range of molecules that can be characterized, the use of separation method on line or off line can be used to separate different families of molecules including isomers.

In this work high performance thin layer chromatography (HPTLC), flash chromatography (FC) an trapped ion mobility spectrometry (TIMS) coupled to FTICR MS were used to provide a more accurate chemical description of biooil composition [1]. In addition, the use of different ionization methods including ESI, APCI, APPI and MALDI was used to obtain different ionization selectivities [2, 3]. In particular, it was shown that APCI was particularly useful for the detection of aliphatic compounds, while MALDI was very effective for the detection of a wide range of molecules present in the biooils.

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Electron Flux Dissociation: An Old New Twist on Ion-Electron Reactions in an FT-ICR Mass Spectrometer

Steven A. DeFiglia[1], Neven N. Mikawy[1], Carson W. Szot[1], Teresa Lee[1], Kristina Håkansson[1,*]

1. *Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, United States*

[*] *kicki@umich.edu*

While the introduction of electron capture dissociation (ECD) in 1998 [1] was a catalyst for transformative radical-driven tandem mass spectrometry (MS/MS) of multiply-charged biomolecular cations, the idea of electron bombardment as an MS/MS activation method in FT-ICR MS had been previously introduced for singly-charged analytes under the names 'electron impact excitation of ions from organics' (EIEIO) [2] and 'electron induced dissociation' (EID) [3]. Furthermore, the term electron collision dissociation was used for related experiments in magnetic sector mass spectrometers [4]. Following the introduction of ECD, related techniques such as "hot" ECD [5] and electron ionization dissociation [6] (EIoD [7]) have been proposed to occur at higher electron energies than "true" ECD. Here, we show that phenomena previously attributed to "hot" ECD and EIoD can occur at low electron energy, similar to that in "true" ECD. These phenomena include secondary fragmentation and tandem ionization. We show that electron flux rather than energy is the main determinant for these processes, analogous to previously published findings from smaller cluster ions, demonstrating that multiple collisions with 5 eV electrons can raise analyte internal energy to ~15 eV [3].

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Investigation of HSC70 oligomerization by structural mass spectrometry

Aleksandr Melikov[1,2], Petr Novák[1,*], Matthias Mayer[3]

1. *Institute of Microbiology, CAS*
2. *The Department of Genetics and Microbiology, Faculty of Sciences of Charles University*
3. *Zentrum für Molekulare Biologie der Universität Heidelberg*

[*] *pnovak@biomed.cas.cz*

Heat shock cognate 71 kDa protein (Hsc70) is a representative of heat shock protein 70 family encoded by a HSPA8 gene in human genome. It fulfills several important tasks in cells, including proper folding of newly translated and misfolded proteins, as well as stabilize or degrade mutant proteins; it also participates in other biological processes including signal transduction, apoptosis, autophagy, protein homeostasis, cell growth and differentiation. HSC70 cellular pool regulation is thus tremendously important; one of the current hypotheses of HSC70 describes the inactivation through oligomerization, which was already proven by analytical size-exclusion chromatography. This work pursues the exploration of oligomerization properties of Hsc70 WT and its mutants by chemical cross-linking and mass spectrometry including stable isotope labeling of recombinant proteins by 15N. The last technique demands the production of 15N-versions of each studied protein.

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MALDI MRMS for biomarker-based climate reconstruction from sediment archives with unmatched temporal resolution

Susanne Alfken[1,*], Lars Wörmer[2], Kai-Uwe Hinrichs[2]

1. *Bruker Daltonics GmbH & Co. KG*
2. *MARUM – Center for marine environmental sciences, University of Bremen*

[*] *Susanne.Alfken@bruker.com*

The reconstruction of past environmental conditions from sedimentary archives via the extraction of organic biomarker molecules is a proven and well-established approach referred to as molecular stratigraphy (Brassel et al., 1986). Linked to biological precursor compounds, such as microbial membrane lipids, plant waxes, pigments, sterols, or hopanoids, these geochemical fossils are witnesses of past climate conditions and thus can provide key insights into understanding current and future effects of climate change.

Classical biomarker analysis requires the extraction of organic compounds from typically cubic-centimeter-sized samples. Considering that marine or lacustrine sediment deposits typically build up by a less than a few millimeters per year, a sample spanning one centimeter of depth averages the information deposited during decades, centuries, or more time into one signal.

Recently, the Organic Geochemistry working group at MARUM – Center for marine environmental sciences at the University of Bremen revolutionized the approach to molecular stratigraphy by using Matrix-Assisted Laser Desorption/Ionization coupled to a 7T solariX XR MRMS system to obtain molecular information directly from intact sediment samples, without previous sub-sampling or extraction. This approach offers unmatched spatial and thus temporal resolution for biomarker-based paleoclimate studies and further enables exploring the two-dimensional spatial distribution of the molecular information in a sample.

Here we will present some of the advances facilitated by this unique and dedicated facility for high-resolution paleoclimatology. For instance, on sediments deposited in the last century that can be compared with instrumental and historical climate data (Alfken et al., 2020; Alfken et al., 2021; Napier et al., 2022), to high-frequency fluctuations and abrupt environmental responses during the Younger Dryas (~12 ka) (Obreht et al., 2020, Wörmer et al., 2022) and the last warm climate period of the Last Interglacial (~129 - 116 ka) (Obreht et al., 2022), and even new findings into the drivers of the end-Permian mass extinction (~250 Ma) (Saito et a., 2023).

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Structure and reactivity of small carbon molecules probed by ion-molecule reactions and vibrational spectroscopy

Davide Corinti[1], Simonetta Fornarini[1], Paul Mayer[2], Maria Elisa Crestoni[1,*]

1. *Sapienza Università di Roma, Roma, Italy*
2. *University of Ottawa, Ottawa K1N 6N5, Canada*

[*] *mariaelisa.crestoni@uniroma1.it*

Methylenation reactions in the gas phase are of great significance in interstellar chemistry involved in carbon chain elongation of small molecules. Electron ionization of appropriate volatile precursors, like ketene, oxirane, and ethers, has been employed to simulate these processes. Preliminary experiments in the cell of an FT-ICR mass spectrometer were carried out, where ionized ethylene oxide was allowed to react with CH_3CHO. Interestingly, a net $CH_2^{+\cdot}$ transfer occurred forming $[C_3H_6O]^{+\cdot}$ (m/z 58) ions, whose structure was probed by ion-molecule reactions (IMR), collision induced dissociation (CID) and mid-infrared vibrational spectroscopy. In particular, candidate isomers obtained by ionization of suitable neutral precursors, including 4-methyl-1,3-dioxolane and acetone, were assayed and compared to characterize different isomers possibly participating in the sampled ion population. [1, 2]
The same multimethodological approach was applied to the study of a bare prototypical perfluoroalkyl anion, $C_2F_5^-$, to reveal the possible existence of isomeric structures, either a covalently bound species or a loosely bound complex, and the operation of negative hyperconjugation effects. This species is relevant in fluorocarbon plasmas broadly used for the deposition and etching in semiconductor industry. [3] The structure and bonding features of $C_2F_5^-$ conforms to the behavior of a covalently bound ion, according to the evidence gained by the reactivity pattern, the IRMPD spectrum and the molecular geometries optimized at MP2/cc-pVTZ level. [4]

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FTMS booster: a high-performance data acquisition and processing system to advance ftms applications

Yury Tsybin[1,*], Konstantin Nagornov[1], Anton Kozhinov[1]

1. *Spectroswiss, 1015 Lausanne, Switzerland*

[*] *tsybin@spectroswiss.ch*

Hardware architectures of contemporary built-in data acquisition and processing (DAQ/P) systems in the FTMS instruments often remain sub-optimal in performance, including phase distortions requiring post-acquisition phase correction, reduced resolution and duty cycle, and compromised S/N and sensitivity. A direct consequence of these limitations is restricted access to the absorption mode FT (aFT) mass spectra. Furthermore, the current generation of built-in DAQ/P systems, accompanied by rigid data acquisition control software, may inhibit experimental flexibility, for instance, by prohibiting the stable acquisition of ultra-short or ultra-long time-domain transients.

Previously, we demonstrated that the state-of-the-art field-programmable-gate-array (FPGA) technology enables a new-generation DAQ/P architecture with powerful in-line digital signal processing helping to overcome the said disadvantages. The new-generation high-performance DAQ/P systems, e.g., the FTMS Boosters, can directly yield the in-hardware phased transients, which can be readily converted into equally informative aFT mass spectra. The flexibility of the external and in-parallel interfaced high-performance DAQ/P systems to commercial and custom FTMS instruments provides additional benefits.

We will overview recent advances in high-performance external phased transient acquisition and processing technology and its use in selected applications. The recent examples include advancing Orbitrap-, ICR-, and custom FTMS-based charge detection mass spectrometry (CDMS), mass spectrometry imaging and bottom-up/top-down proteomics applications [1-3].

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Zapping ions – coupling an FTICR with UV and IR lasers for more informative top-down mass spectrometry

Alan Kádek[1,*], Petr Novák[1,2]

1. *BIOCEV – Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*
2. *Faculty of Science, Charles University in Prague, Czech Republic*

[*] *alan.kadek@biomed.cas.cz*

Efficient and informative fragmentation is key to the success of many MS-based experiments studying both small molecules as well as large multi-protein assemblies. Especially so, in the case of proteins, since the realization of both structural biologists and mass spectrometrists that there exists yet another level of complexity involved in the regulation and controlling of protein structure and functions beyond pure single gene = single protein equation. Through RNA splicing, mutations as well as post- and co-translational modifications and involvement in larger non-covalent assemblies, individual proteins generate complex landscapes of proteoforms with huge implications on their functioning in organisms. [1]

Therefore, both proteomics as well as structural MS have been often dependent on various means of fragmentation for which diverse MS dissociation techniques and their combinations are often needed. This contribution will report on the successful implementation of both 10.6 μm CO2 and 193 nm ArF lasers for infrared multi-photon dissociation (IRMPD) and ultraviolet photodissociation (UVPD), respectively, for in-cell dissociation inside the 15T SolariX Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer at BIOCEV in Prague. It will also highlight a particular success story, where the molecular structure of nostatin A – a novel polypeptidic highly bioactive compound synthesized and heavily co-translationally modified in algae has been solved by top-down MS/MS after years of resisting efforts to crystallize it or determine it using nuclear magnetic resonance alone.

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From Rough Signal to Polished Spectrum: Absorption Mode Two-dimensional Mass Spectrometry

Maria A. van Agthoven[1,*], Sarah V. Heel[2], Marek Polák[3], Alan Kádek[3], Petr Novák[3], Kathrin Breuker[2], Carlos Afonso[1], Marc-André Delsuc[4]

1. *Laboratoire COBRA UMR CNRS 6014, University of Rouen-Normandy, France*
2. *Center for Chemistry and Biochemistry, University of Innsbruck, Austria*
3. *BIOCEV, Czech Academy of Sciences, Czech Republic*
4. *IGBMC, University of Strasbourg, France*

[*] *maria.van-agthoven@univ-rouen.fr*

Two-dimensional mass spectrometry (2D MS) is a tandem mass spectrometry method where precursor and fragment ions are correlated without isolation. A multifaceted data-independent acquisition technique, 2D MS has found applications from agrochemicals to top-down proteomics with both laser-based and electron-based fragmentation [1]. Developments in signal processing have shaped 2D MS into a fully-fledged analytical method. For narrowband 2D mass spectra with limited precursor m/z ranges, phase-corrected absorption mode, which improves resolving power and signal-to-noise ratios, has enabled the distinction between fragments from histone peptides with m/z 0.006 difference [2]. This study seeks to extract analytical information from all 2D mass spectra with maximum signal-to-noise ratio and resolving power by expanding phase-corrected absorption mode to broadband 2D MS.

2D mass spectra of diverse samples (intact proteins, peptide isoform mixtures, protein digests, plant extracts) were recorded on FT-ICR mass spectrometers with magnetic fields between 7-15 T (Bruker Daltonics, Germany) with Gäumann's pulse sequence and both laser-based and electron-based fragmentation [1]. The frequency range for precursor ions in the vertical dimension ranged from 10 to 1000 kHz (i.e. precursor m/z 5-2000 ranges). All data processing and visualization was performed with the open-source Spectrometry Processing Innovative Kernel (SPIKE) software, developed in 64-bit python language [3]. For all 2D mass spectra, parameter optimization was performed in python notebooks before data processing in batch processing to eliminate limitations in dataset size due to RAM.

Phase progression in 2D mass spectra is linear with frequency in the vertical precursor ion dimension and quadratic in the horizontal fragment dimension [4]. For accurate phase correction, different parameters need to be optimized: 3 coefficients for horizontal phase correction, 2 coefficients for vertical phase correction, and the modulation frequency. All parameters are dependent on the experimental conditions determined in the pulse sequence. Phase-corrected absorption mode narrowband 2D mass spectra show that both signal-to-noise ratio and resolving power can be doubled compared to magnitude mode. In addition, phase correction acts as a filter for artefacts from harmonic peaks in 2D mass spectra. Data processing for absorption mode is performed in batch processing to eliminate caps on dataset size imposed by available RAM. We discuss the stability of empirically optimized parameters (modulation frequency, all phase correction coefficients) for automated processing of multiple 2D mass spectra acquired with identical experimental conditions. We establish routines in SPIKE for front-end data processing to produce internally calibrated peaklists for input in

data analysis software (e.g. MASCOT for bottom-up proteomics, FAST-MS for top-down proteomics, PyC2MC for petroleomics). We examine disturbances introduced by factors like frequency range and speed of phase accretion as a function of cyclotron frequency (for fragment ion m/z) or of modulation frequency (for precursor ion m/z). We discuss solutions to these issues: asymmetrical apodisation, reconstruction of initial datapoints through autoregressive models, non-uniform sampling, and one-dimensional phase correction.

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Ion Mobility Free High Throughput Peptide Collisional Cross-Section Measurements in Fourier Transform Mass Spectrometer

Konstantin Aizikov[1,*], Ulises H. Guzman[2], Kyle Fort[1], Pedro Navarro[1], Martin Rykaer[2], Jeppe Madsen[2], Ana Martínez-Val[2], Alexander Makarov[1], Jesper V. Olsen[2]

1. Thermo Fisher Scientific, Bremen, Germany
2. Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Denmark

[*] *konstantin.ayzikov@thermofisher.com*

The integration of Ion Mobility (IM) devices with mass spectrometers has facilitated the precise determination of peptide collisional cross sections (CCS), introducing an additional dimension to Mass Spectrometry (MS)-based analysis. Utilization of transient decay rate in Fourier Transform Mass Spectrometry (FTMS) for assessing CCS values has been reported for Orbitrap and Ion Cyclotron Resonance (ICR) MS systems, eliminating the need for a dedicated IM device. This study presents a new method that enables accurate and consistent measurement of CCS using FTMS in high-throughput LC/MS analyses on the proteome scale.

All the experiments were conducted on a modified Thermo Scientific™ Orbitrap Exploris™ 480 MS. HeLa cells were harvested in boiling SDS lysis buffer. Protein aggregation capture (PAC) was induced by the addition of organic solvent and magnetic microbeads before overnight on-bead digestion with Lys-C and Trypsin. Offline High pH Reversed-Phase HPLC Fractionation was performed using a Waters XBridge BEH130 C18 3.5 µm 4.6 × 250 mm column on an Ultimate 3000 high-pressure liquid chromatography (HPLC) system. Briefly, phosphopeptide enrichment was performed using TilMAC-HP beads on a KingFisher™ Flex robot. The ionic CCS values of peptides were evaluated based on the reported resolution values in MS1 scans. The processing was done using an in-house developed computational pipeline.

The inferred CCS values showed strong correlation with the IM-MS values from the literature and demonstrated comparable separation of different peptide charge states in the m/z vs CCS space. The reproducibility of the FTMS CCS measurements in the shotgun proteomics experiments is on the same scale as the reported CCS values obtained with the dedicated IM devices. Moreover, our findings illustrate the utility of the CCS in the analysis of the peptide secondary structures and suggest that the conformation of peptides is likely determined by the balance between hydrophobic interactions driving more compressed forms and charge repulsion promoting extended conformations. Additionally, in the analysis of Post Translational Modifications (PTM), the CCS showed that phosphopeptides generally exhibit smaller CCS values compared to their unmodified counterparts; this trend particularly affects peptides with higher charge states. Conversely, methylated and acetylated peptides have larger CCS compared to their unmodified versions, likely due to increased hydrophobicity and loss of charge. Finally, we envision that this strategy can benefit the understanding and utility of CCS in proteomics workflows, contributing to increased confidence in peptide, phosphorylation site, and protein identification.

FT-ICR-MS-based untargeted metabolomics: the path to precision medicine

Marta Cruz[1], Petr Novák[2], Marta Sousa Silva[1], Carlos Cordeiro[1,*]

1. *FT-ICR and Structural Mass Spectrometry Laboratory- ULisboa, BioISI, FCUL*
2. *Institute of Microbiology - BioCeV, Academy of Sciences of the Czech Republic, Prague, Czech Republic.*

[*] *fc54343@alunos.fc.ul.pt*

The metabolome, a highly dynamic entity, as a product of the genome, transcriptome, proteome, and environment, provides us with an extremely detailed real-time picture of what is happening inside our bodies. Thus, metabolomics can be the great propeller of a new era of precision medicine. As firm believers in that, we propose a non-invasive method based on untargeted fingermarks metabolomics profiling to diagnose and monitor cancer patients. To get high metabolome coverage while keeping sample preparation to a minimum, we selected FT-ICR mass spectrometry, whose resolving power and mass accuracy are unmatched. Method performance was evaluated with one stage IV Hodgkin lymphoma patient, monitored from diagnosis until seven months after complete remission, and four healthy sex-matched volunteers. Very promising results were obtained as cancer and treatment-response-specific metabolic signatures were captured.

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Humic-like Substances (HULIS) in Ship Engine Emissions Analyzed with ESI FT-ICR MS: Molecular Composition Effected by Fuel Type, Engine Mode, and Wet Scrubber Usage

Helly Johanna Hansen[1,*], Eric Schneider[1], Hendryk Czech[1], Martin Sklorz[2], Uwe Etzien[1], Bert Buchholz[1], Thorsten Streibel[1], Thomas Adam[2,3], Christopher Paul Rüger[1], Ralf Zimmermann[1,2]

1. *University of Rostock*
2. *Helmholtz Centre Munich*
3. *University of the Bundeswehr Munich*

[*] *helly.hansen@uni-rostock.de*

Humic-like substances (HULIS) denote a class of high molecular weight organic compounds prevalent in the water-soluble organic carbon (WSOC) fraction of particulate matter (PM), influencing various properties of atmospheric aerosols. The chemical characterization of HULIS emitted from various sources is becoming an increasingly relevant topic, as their impact on the environment and health depends on their source-related molecular composition.

Detailed chemical characterization of HULIS emitted by ship engines is essential for estimating the regional and global relevance of ship emissions with respect to environmental and health implications. As the HULIS fraction consist of high polarity compounds, electrospray ionization (ESI) is used for investigating their chemical composition. The combination with the ultrahigh mass resolving power and accuracy of Fourier-Transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) allows accurate mass assignments, enabling the derivation of sum formulae, as well as the calculation of sum parameters. Thus, the in-depth molecular characterization of HULIS is facilitated, revealing detailed insights into their high molecular composition.

Marine diesel engine exhaust PM samples of five different ship fuels were collected from a single-cylinder four-stroke research and development engine. The five marine fuels include two high-sulfur heavy fuel oils (HFO) of differing sulphur content, which are non-compliant with the sulphur content regulations established by the International Maritime Organization (IMO), as well as three IMO compliant marine fuels: commercial marine gas oil (MGO), a low-sulfur HFO and a noncommercial aromatic-rich ultralow-sulfur heavy fuel oil. Optionally, the exhaust gas was passed through a wet sulfur scrubber unit operated with Baltic Sea water under open-loop or closed-loop conditions. For HULIS isolation, filter samples were extracted with 10 mL water in an ultrasonic bath for 30 min. The extracts were filtered through a 0.2 µm PTFE membrane and subjected to a preconditioned solid-phase extraction (SPE) cartridge for desalting and enrichment. The retained HULIS fraction was eluted with 1.5 mL of methanol. All samples were analyzed by direct-infusion ESI in negative and positive ionization mode.

The chemical composition of ship HULIS shows a dependency on the properties of the marine fuel. Each fuel yields several unique compounds, which are detected only for the respective fuel. The highest number of unique elemental compositions in negative ESI is observed for the HFO with the highest sulphur content. Evident differences are also found when comparing the two engine modes for each fuel in regard of the number of detected compounds and the molecular weights. The elemental composition of ship HULIS is affected by wet scrubber usage. In positive ESI a decrease in the number and intensity of CHOS compounds after the scrubber is observed, whereas the CHNO compounds are intensified after the closed-loop scrubber but unchanged for the open-loop scrubber.

Tools to enhance MS/MS analysis of degraded polymers in FTICR MS

Callan Littlejohn[1], Meng Li[1], Sam Weekes[1], Anna Cordiner[1], Mark P. Barrow[1], Peter B. O'Connor[1,*]

1. *University of Warwick, Coventry, UK*

[*] *callan.littlejohn@warwick.ac.uk*

Environmental degradation of polymers can result in the buildup of plastic particulates, in the form of micro and nano plastics; within the ecosystem. These particles are then accumulated in the food chain until potentially dangerous levels of plastic occur in larger predatory animals. The current methods of analysis of polymer degradation are primarily based on changes in the functional groups of the polymer such as infra-red spectroscopy which yields very little insight into the method of degradation. Tandem mass spectrometry has already shown promise in the study of polymer degradation and could be used to accurately describe the mechanisms of degradation of polymers by environmental means.

Tandem Mass spectrometry was performed using, a Bruker 15T solariX and a Bruker 12T solariX, using a multimodal approach. The high complexity of polymer degradation products lends itself to analysis using FTICR-MS due to the high peak capacity. The high mass accuracy offered by FTICR allows for assignments to be made of complex polymer mixtures and allows for enhanced mechanisms of analysis such as the Kendrick mass defect.

The Kendrick mass defect is a function of the difference between a molecule to a reference medium. Typically, the reference medium is an infinitely long alkyl chain but in the case of the modified Kendrick mass defect (mKMD) this medium can be altered to fit the polymer of analysis, which means that any heteromonomer content will be evidenced by a shift in the mKMD. The mKMD analysis can be useful in the study of polymer degradation as it allows the user to quickly identify peak series of interest.

Once these peak series of interest are identified it is possible to further apply the mKMD analysis to any MS/MS type analysis, because each fragmentation type introduces a new heteromonomer unit into the chain and therefore the different fragmentation types are clearly and visibly separated in mKMD space. Modified Kedrick Mass Defect analysis allows for rapid analysis of these spectrum as well as increasing the possibility of automation.

mKMD analysis has also been applied to 2DMS analysis for 4D DIA analysis of polymers. Using mKMD it was possible to simplify the complex task of analysing the dense spectrum produced by 2DMS. By scanning the reference mass through common monomer residues it was possible to identify several common polymers within a mixture, which could be used in further environmental studies to identify plastics of interest.

The combination of mKMD with tandem mass spectrometry methods will make analysis of chain like molecules simpler and increase the speed of analysis for the complex datasets that result from the degradation products of these molecules.

Using MRMS based metabolomics to uncover the differential host response to SARS-CoV-2 variants infection

Mariana Louro[1], Carlos Flores[2], Conceição Godinho[2], Fernando Maltez[2], Marta Sousa Silva[1], Carlos Cordeiro[1,*]

1. *FT-ICR and Structural Mass Spectrometry Laboratory, Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Portugal*
2. *Hospital Curry Cabral, Centro Hospitalar Lisboa Central, Lisboa, Portugal*

[*] *cacordeiro@fc.ul.pt*

SARS-CoV-2 suffered many mutations causing the emergence of several variants. Each of the new predominant variants was usually characterized by higher transmissibility, increased immune evasion, and unique host responses. MRMS is a powerful metabolomics enabling technique due to its high resolving power and dynamic range allowing the identification of metabolites with an extreme mass accuracy, in the nanomolar concentration range while covering a wide range of metabolite classes. By not relying on chromatography, MRMS-single shot metabolomics allows an enormous amount of information to be collected in a matter of minutes. In a clinical setting, this is an important advantage. Each of the predominant SARS-CoV-2 variants was characterized by unique host responses, which had a corresponding unique impact on the host metabolome. Here we compare the effects of the original Wuhan strain with the ones caused by omicron. Analysis of these samples by untargeted metabolomics using an MRMS 7T SolariX demonstrated the capability of the instrument to distinguish between variants and elucidate the most impacted pathways for each. Moreover, we show that MRMS can be used to quantify metabolites of interest in complex samples, in a high-throughput fashion with the same accuracy as the traditional quantification methods, without the need to optimize a new method for every metabolite, metabolite class, or sample type. Extreme-resolution mass spectrometry promises to be much more than one analytical tool, having proved to be a versatile technique that combined with its high throughput has unlimited potential.

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FTMS data analysis: software concepts and advanced workflows

Konstantin Nagornov[1,*], Anton Kozhinov[1], Yury Tsybin[1]

1. *Spectroswiss, 1015 Lausanne, Switzerland*

[*] *nagornov@spectroswiss.ch*

FTMS instruments produce highly complex, large mass spectral datasets. Computationally efficient and automatable modern FTMS software is crucial for processing various FTMS data types, including time-domain transients and full or reduced profile FT mass spectra in different modes (absorption, magnitude, enhanced). Here, we will discuss concepts and considerations that we employ in developing advanced FTMS data processing and analysis software tools.

The unique challenges of FTMS data, such as resolution variation with m/z and peak interferences, necessitate specific data analysis approaches. Our FTMS Simulator software addresses this by accurately simulating FTMS data, for small and large molecules, e.g., monoclonal antibodies [1,2].

We utilize the FTMS Simulator capabilities to create advanced workflows to analyze small molecules, including metabolites, and characterize biotherapeutics, including glycoproteins. These workflows benefit from feature extraction through isotopic envelope matching, enhancing analysis in metabolomics, imaging, and protein-ligand interactions (affinity-selection FTMS) applications.

Our approach speeds up data analysis and increases accuracy, supported by the *.H5 file structure for efficient data cataloging and the Python-based Peak-by-Peak framework for simplified data processing and visualization for experts and non-experts. This comprehensive framework supports large-scale data processing and analysis, including statistical analyses and enhanced sensitivity for specific analyses like glycoprotein site-specific investigation.

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Single shot top-down proteomics through direct infusion of complex protein mixtures, extreme resolution FT-ICR-MS and CapiDown

Inês Romão[1], Francisco Traquete[1], João Luz[1], António Ferreira[1], Petr Novák[2], Marta Sousa Silva[1], Carlos Cordeiro[1,*]

1. *Structural Mass Spectrometry Laboratory, Biosystems and Integrative Sciences Institute, Faculty of Sciences, Lisbon University, Lisbon*
2. *Institute of Microbiology of the Czech Academy of Sciences, 14220 Prague, Czech Republic*

[*] *fc54357@alunos.fc.ul.pt*

Top-down proteomics aims to identify and characterize all proteoforms through the direct analysis of intact proteins. Only by avoiding enzymatic digestion, the heterogeneity of proteoforms can be revealed and connected to function or disfunction in pathologies or be used for the molecular based classification of living organisms. Top-down proteomics is mainly limited by low throughput protein separation methods and sequential analysis of proteoforms through classic LC-MSMS methods. Here we show that the extreme resolution of FT-ICR-MS can be harnessed to resolve multiple proteoforms by direct injection of a whole cell lysate. For protein identification, CapiDown (Computational Annotation of Proteins in Top-Down Proteomics) was developed. This software processes MSMS protein spectra after deconvolution to single charge states, matching and ranking high quality MSMS spectra to in silico generated fragments from the target taxonome. Protein matches are sorted according to their z-scores, using a MOWSE inspired algorithm. CapiDown will evolve to incorporate MSMS data generated by multiple fragmentation methods (CID, ECD, UVPD) and will allow the PTM analysis. By improving and automating ion-precursor selection we will achieve a fast and accurate way for proteoform identification in proteomes, without separation methods, thus ushering in a new era of top-down proteomics.

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Two-dimensional Fourier transform ion cyclotron mass spectrometry applied to bio-oil: a step beyond molecular formulae for complex mixture characterization.

Frédéric Aubriet[1,*], Anthony Abou-Dib[1], Bryan Marzukki[2], Jasmine Hertzog[1], Diana Catina Palacio Lozano[2], Peter O'Connor[2], Mark Barrow[2], Vincent Carré[1]

1. *LCP-A2MC, University of Lorraine, F-57070 Metz (France)*
2. *Department of Chemistry, University of Warwick, Coventry, CV4 7AL (United Kingdom)*

[*] *frederic.aubriet@univ-lorraine.fr*

Bio-oils are complex mixtures with tens of thousands of components with different chemical functions covering a wide range of mass and polarity. Before being used as a petroleum alternative, bio-oils require to be upgraded. To define the most appropriate up-grading treatments, as well as to evaluate their efficiency, a fine description of the raw and up-graded bio-oil composition is required. A preferred method for bio-oil characterization is the non-targeted analysis using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). It enables the detection and assignment of up to hundreds of thousands of molecular formulae. Still, it fails to give structural and chemical function information, which is paramount for a better understating of the upgrading pathways. Such information can be notably obtained by tandem mass spectrometry. Alternatively, to the classical tandem mass spectrometry approach (1DMS), two-dimensional mass spectrometry (2DMS) can provide structural insights on a broad range of bio-oil compounds without requiring their individual isolation. In this work, 2DMS using UVPD as an activation method, was applied for the first time to gain an insight into the structure and functional groups of bio-oil lignin compounds. The extraction of specific lines from the 2DMS contour plot allowed us to highlight specific functional groups and identify the nature of the lignin monomer part of the bio-oil components. For example, the loss of as phenol and guaiacol revealed the presence of paracoumaryl and coniferyl units in phenolic 8-end position of some lignin pyrolysis derivates.

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FT-ICR Tandem Two-Dimensional Mass Spectrometry (MS/2DMS) with 3D Peak Picking to Analyse the Complex Product Ion Spectrum of Ticagrelor.

Anna Cordiner[1], Caitlin Chapman[1,2], Andrew D. Ray[3], Stephen W. Holman[4], Callan Littlejohn[5], Peter B. O'Connor[5], Jackie Mosely[1,*]

1. *Department of Chemistry, University of York, York, YO10 5DD, United Kingdom*
2. *Department of Chemistry, University of Teesside, Middlesbrough, Tees Valley, TS1 3BX, United Kingdom*
3. *New Modalities & Parenteral Development, Product Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Macclesfield, UK*
4. *Chemical Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Macclesfield, UK*
5. *The Department of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom*

[*] *jackie.mosely@york.ac.uk*

Ticagrelor is a small pharmaceutical molecule, belonging to the triazolopyrimidine class. It is currently used to treat Acute Coronary Syndrome (ACS) by inhibiting the platelet $P2Y_{12}$ receptor, and was first approved for use by the FDA in 2011.

When protonated ticagrelor undergoes collision induced dissociation (CID), it gives a dense product ion spectrum, including multiple areas containing peaks of the same nominal mass, for example, one 30 mDa window contains 3 clearly resolved product ion peaks. While these peaks can be mass resolved in a high-resolution tandem MS experiment, quadrupole isolation limits further MS^n analysis due to their close proximity. In order to overcome this MS/2DMS was used as it allowed for those proximal MS^2 ions to be fragmented while keeping the resulting MS^3 fragments correlated with their MS^2 precursor ion.

A sample of ticagrelor was analysed using a 9.4T SolariX FT-ICR MS (Bruker, GmBH) using electrospray ionisation (ESI). The protonated molecule was isolated in the quadrupole using a typical width window of 10 Da, and CID to give the complex product ion MS^2 spectrum. All MS^2 product ions were transmitted into the FT-ICR analyser cell simultaneously where they were all further fragmented, without additional isolation, using InfraRed MultiPhoton Dissociation (IRMPD), resulting in an MS^3 spectrum. This is repeated over many scans and by tracking the change in peak intensity in the MS^3 spectrum relative to the change in MS^2 precursor peak intensity, the MS^3 product ion can be correlated to the MS^2 precursor. This experiment describes MS/2DMS.

In MS/2DMS a 3 dimensional spectrum can be generated, wherein the X-axis shows all MS^3 ions and the Y-axis indicates their corresponding MS^2 ion, with the Z-axis showing the intensity of the signal. For peaks in close proximity, like those seen in ticagrelor, their MS^2 peaks were seen to overlap due to being significantly broader in the Y-dimension. To resolve these peaks, and thus be able to correctly align MS^3 product ions with MS^2 precursor, 3D peak picking was used whereby the centre of the peak was calculated in the X, Y and Z dimension. These datapoints were then sorted by their Y-axis values to give an MS/2DMS peak list for each MS^2 precursor. These peak lists gave sufficient separation to

identify separate close proximity peaks, and assign their corresponding fragment ions. For ticagrelor, this enabled confident structure determination in key parts of the molecule that were not possible with only MS^2 data.

Ticagrelor represents an example where MS^2 may not be sufficient for structure determination, and MS^3 is not possible with most instrumentation. This work shows MS/2DMS together with the developments in peak picking, is capable of extracting this information for multiple ions within a 0.03 Da window and when some ions are less than 0.012 Da apart. This work is unique in combining MS/2DMS and 3D peak picking to analyse and characterise a single molecule with a complex MS^2 product ion spectrum as this has yet to be seen in the literature.

This also allowed peak lists to be generated for every precursor in the MS/2DMS dataset and consolidated into a single simple dataset for easy navigation and visualisation, and has the potential to be incorporated into existing data independent computational workflows.

Metabolomic Characterization of three Ecotypes of Burdock (Arctium lappa L.) roots by the means of FT-ICR Mass Spectrometry.

Francesca Del Cioppo[1,*], Alba Lasalvia[1], Enrico Romano[1], Maria Elisa Crestoni[1], Cinzia Ingallina[1]

1. *Dep. Chemistry and Technology of Drugs, Sapienza University of Rome*

[*] *francesca.delcioppo@uniroma1.it*

Burdock (Arctium lappa L.) is a medicinal plant used in Chinese traditional medicine and cuisine and is known for its health benefits attributed to the high content of secondary metabolites. [1] In this study, an untargeted metabolomic analysis was performed to characterize the phytochemical composition of the root's extracts from an organic burdock ecotype (OE), a land spontaneous ecotype (LSE) and a mountain spontaneous ecotype (MSE), by the means of electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS). With its high resolution, sensitivity, and accuracy, FT-ICR MS is a valuable tool for studying complex mixtures and understanding cellular regulatory processes. [2] Plant materials were subjected to the Bligh-Dyer protocol and the diluted solutions were directly infused for untargeted ESI FT-ICR MS analyses in both positive and negative ionization modes. Mass spectra were processed using DataAnalysis 3.4 software for peak picking and mass calibration. Accurate m/z values were annotated using the free tool MassTrix, associating each value with a molecular formula and then to one or more candidate metabolite isomers. ESI FT-ICR MS allowed to detect more than 200 metabolites in OE and LSE, while MSE exhibited a lower compounds density. The molecular formulas obtained from each sample were transposed in two-dimensional van Krevelen diagrams, to visualize the predominant metabolite classes. [3] The diagrams of OE, LSE and MSE show similarities, with the highest metabolite density in the area of lipids and polyketides, followed by polyalcohols and amino acids. Relative frequency histograms classified metabolites into seven classes based on elemental composition: CHO class, mainly characterized by lipids, carbohydrates, alcohols, and polyphenols, results the most abundant set of species (about 60%). ESI FT-ICR MS analyses furnished a fingerprinting of Burdock roots, confirming the presence of amino acids, fatty acids, organic acids, carbohydrates, vitamins; secondary metabolites included monoterpenes, plant hormones, lignans (e.g., lappaol A/B, arctignan) and flavonoids, mainly detected in O-glycosylated form. Moreover, the study provides insights into how environmental factors may influence the phytochemical profile, highlighting the importance of understanding optimal growth conditions to enhance the production of secondary metabolites. These results will be complemented by high-resolution NMR-based targeted analysis, to provide a more comprehensive characterization of the samples.

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Spray-dried Acheta domesticus powder, a recent novel food characterized by ESI FT-ICR MS

Alba Lasalvia[1,*], Mattia Spano[1], Giacomo Di Matteo[1], Marta Sousa Silva[2], Carlos Cordeiro[2], Maria Elisa Crestoni[1], Luisa Mannina[1]

1. *Dipartimento di Chimica e Tecnologie del Farmaco, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185,Roma, Italy*
2. *Laboratório de FT-ICR e Espectrometria de Massa Estrutural, Universidade de Lisboa, Campo-Grande, 1749-016 Lisboa, Portugal*

[*] *alba.lasalvia@uniroma1.it*

The expected large population increase in the very next future and the necessity of reducing the ecological problems related to the intensive food production result in a growing request for alternative protein sources. In this context, edible insects, with their environmental and nutritional advantages, represent a new and innovative food source able to satisfy both sustainability and nutritional demands [1]. Entomophagy, the practice of eating insects, represents a common use in several cultures, such as Latin America, Asia, and Africa. Conversely, in Europe, the use of insects as edible food is limited since the idea of their consumption often suggest feelings of disgust. However, entomophagy deserves to be promoted for three main reasons: i) health, since insects are nutritious alternative to traditional food sources, being rich in proteins, fatty acids and minerals; ii) environmental, since they require lower water and feed consumption, and their farms generate fewer greenhouse gases and ammonia; iii) economic factors, since insect rearing needs smaller spaces for farming and low-tech and low-capital investment. In the present study, for the first time, a spray-dried A. domesticus (house cricket) powder produced in Italy has been investigated through a multimethodological approach. The improved knowledge of the chemical profile of this Novel Food opens up new horizons both for the use of the cricket products and for the use of specific components that could be isolated for the production of new formulations. For these reasons, an untargeted characterization was achieved thanks to the high resolution of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) allowing, by a fast single measurement, the identification of several classes of metabolites present in the matrix [2]. Furthermore, complementary methodologies such as untargeted Nuclear Magnetic Resonance (NMR) spectroscopy and targeted Gas-Chromatography Mass Spectrometry (GC-MS) were employed to maximize the metabolomic coverage. Samples were extracted with Bligh-Dyer procedure obtaining two different phases (hydroalcoholic and organic); after appropriate dilution, the solution were directly submitted to electrospray ionization (ESI). Preliminary ESI-MS analyses were carried out with a Bruker BioApex FT-ICR both in positive and negative ionization mode. Ultrahigh-resolution mass analyses were carried out on a Bruker SolariX FT-ICR equipped with a 7 T superconducting magnet, a ParaCell and an Apollo II ESI source. The unsurpassed accuracy of high resolution gives a univocal molecular formula which has been assigned to several metabolites with an uncertainty of less than 1 ppm. Complementary information for the determination of fatty acids was gathered by using ESI (-) coupled to a linear ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific). Analyses revealed the presence of more than 500 molecular formulas, with almost 40% common to both extracts. Visualization of the main molecular classes was gathered by van Krevelen diagrams (vKd) and

histograms of relative frequencies. VKd allows to get an immediate overview of several molecular family densities based on their individual H/C versus O/C ratios. Herein, the diagrams showed that both the extracts came out rich in lipids, terpenoids and polyketides, followed by amino acids with less hits. The averaged relative frequency distribution histogram revealed that both extracts were largely populated by CHO and CHNO with more entries in organic extract, followed by CHNOS and CHOP species with more entries in hydroalcoholic extract. Semi-quantitative analysis was carried out on lipids molecular formula extrapolated from the mass list gained by ESI (-) MS analyses of the organic extract. Notably, among saturated fatty acids, the most abundant is palmitic acid, whereas in the C18 series the highest percentage was found for linoleic acid, an essential fatty acid. The application of a multimethodological approach allowed to define a very rich chemical profile of this Novel Food confirming the presence of relevant compounds from a nutritional point of view, such as essential amino acids and polyunsaturated fatty acids. At the same time, this chemical characterization allowed to highlight how edible insects represent a very innovative food source with a low environmental impact.

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Comprehensive honey metabolome profiling using ultrahigh resolution mass spectrometry

Michael Liss[1,*], Stefan Pieczonka[1], Leopold Weidner[1], Philippe Schmitt-Kopplin[1,2]

1. *Technical University Munich*
2. *Helmholtz Munich*

[*] *michael.liss@tum.de*

In the present study, honey samples of different botanical and geographical origin were investigated with DI-FTICR-MS revealing the chemodiversity of honey. For sample preparation, a SPE method was developed to remove interfering matrix compounds. The final SPE method was performed with Bond Elut PPL cartridges (100 mg, 1 mL) at pH 5. For the washing step, 2 mL H2O/FA (pH 5, v/v) was applied, and elution was performed with 1 mL MeOH. Applying multivariate statistics by means of PCA and HCA, it was shown that honey samples predominantly clustered based on botanical origin. Heatmap clustering allowed the assignment of distinct features to specific honey types which were displayed in Van Krevelen plots. Distinct clusters were found for fir, spruce, rapeseed, eucalyptus, chestnut, manuka, lavender, erica and basswood honeys, respectively. FTICR MS is a powerful tool that quickly characterizes the molecular fingerprints of each cluster by assigning molecular formulae to exact masses. Thereby, it was possible to compare annotated molecular formulae with specific honey markers described in the literature. Kynurenic acid, 3-pyrrolidinyl-kynurenic acid and its γ-lactam derivate were shown to be distinctive for chestnut honeys. UPLC-DAD-ESI-MS confirmed the presence of these compounds in chestnut honeys. Basswood honeys revealed a metabolite with an absorbance maximum at 300 nm which was reported as 4-(1-hydroxy-1-methylethenyl)cyclohexa-1,3-diene-carboxylic acid. Applying supervised OPLS DA for basswood and chestnut honeys confirmed the correlation between absorbance maximum and the features found in the heatmap clustering. Confirmation of single substances with targeted techniques is an important step in order to provide evidence about the presence of marker compounds. Consecutive studies should therefore determine the potential markers in fir, spruce, rapeseed, manuka, lavender and erica honeys in order to verify the clustering of unsupervised multivariate statistics. Altogether, analysis with DI-FTICR-MS revealed promising results for the distinction of honeys of different botanical and geographical origin. SPE enabled high repeatability and reproducibility in the DI-FTICR-MS measurements and is therefore suggested as a sample preparation technique in further studies.

Mass Spectrometry for Protein Structure Analysis

Petr Pompach[1,*], Pavla Vankova[1]

1. *Institute of Biotechnology*

[*] *petr.pompach@ibt.cas.cz*

Structural mass spectrometry (MS3D) is a fast growing field on analytical chemistry representing a new approach for protein structural studies. In CMS, the tools of structural mass spectrometry, including native mass spectrometry, hydrogen-deuterium exchange, chemical cross-linking and other labelling method, are well established and allow to look beyond the edge of traditional structural techniques. The structural mass spectrometry core facility is equipped with state-of-the-art instrumentation such 15T FT-ICR, timsToF Pro, timsToF CSP, automation system for HDX, UPLC and nanoUPLC systems. Besides the MS3D, the core facility offers other services including identification and quantification of proteins, precise determination of protein molecular mass, characterization of various posttranslational modifications (phosphorylation, glycosylation, acetylation …).

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Thermal Analysis High-Resolution Mass Spectrometry for Molecular Insights into Complex Soil Organic Matter

Christopher Paul Rüger[1,2,*], Eric Schneider[1,2], Anika Neumann[1,2], Lukas Friederici[1,2], Vera Samburova[3], Hendryk Czech[1], Ralf Zimmermann[1,2]

1. *University of Rostock, Chair of Analytical Chemistry, Joint Mass Spectrometry Centre (JMSC), 18059 Rostock, Germany*
2. *University of Rostock, Department Life, Light & Matter (LL&M), 18059 Rostock, Germany*
3. *Desert Research Institute (DRI), Division of Atmospheric Sciences, Reno, NV 89512, USA*

[*] *christopher.rueger@uni-rostock.de*

It is well known that wildfires affect flora and fauna, burn various fuels, and emit large quantities of gases (*e.g.*, CO_2 and CO) and particles (*e.g.*, black, brown, and organic carbon) that are further modified during atmospheric transport. Wildfires significantly affect air quality, human health, cloud formation and properties, and atmospheric light absorption and radiative forcing in the atmosphere and after deposition onto snow. Moreover, wildfires also alter soil properties, including soil wettability (or water repellency). Fire-induced soil water repellency (SWR) decreases water infiltration, leading to increased runoff, soil erosion, flooding, and debris flows.

Despite a significant effort and several comprehensive studies on the chemistry of post-fire organic constituents in soils, a large gap remains in the current knowledge and understanding of which organic compounds are responsible for post-fire SWR. This study attempts to shed more light on the chemical nature of fire-induced SWR, using thermogravimetry (TG) atmospheric pressure photoionization (APPI) in combination with Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) (or TG APPI FT-ICR MS). The TG APPI FT-ICR MS method is a comprehensive state-of-the-art method to characterize complex mixtures of organic molecules, including organic constituents in soils. The positive correlation between water repellency measures and aromaticity, derived from the APPI FT-ICR MS spectra for the desorption (or pre-pyrolysis) phase (~20–270 °C temperature range), suggests that burned soils may become water repellent because of the formation and/or deposition of PAH-like organic species on the soil surface. Moreover, we found that organic molecules with a higher amount of oxygen in their structure are more common in unburned than in burned soil samples, which may also have contributed to the more hydrophobic (or more water-repellent) behavior of the burned compared to the unburned soils.

DIP-APCI potential for real-time analysis of thermal conversion products of lignin

Nathan Traullé[1,*], Théo Voellinger[1], Jasmine Hertzog[1], Vincent Carré[1], Frédéric Aubriet[1]

1. *Université de Lorraine, LCP A2MC, 57070 Metz, France*

[*] *nathan.traulle@univ-lorraine.fr*

The utilization of fossil fuels such as gas and oil is unsustainable. Among the available renewable pathways, lignocellulosic biomass emerges as a promising resource. Lignin represents about 15% to 30% of the total mass of lignocellulosic biomass depending on the considered feedstock. It is depicted as a compound of great interest due to its rich aromatic content, that used to be wasted in inefficient combustion processes. Today, industrial processes such as pyrolysis or catalytic fast pyrolysis can be used to efficiently valorise lignin for energy and production of added-value chemicals (1).
Evaluating the efficiency of various conversion conditions (including temperature, catalyst, and biomass) requires a high-throughput approach integrating high performance analytical system in real time.

To address this need, a high-resolution mass spectrometer was used for the analysis of pyrolysis products after their post-ionisation in the ion source. A modification of a direct injection probe (DIP) has been implemented to replicate the conditions of fast pyrolysis (FP) within an atmospheric pressure chemical ionization (APCI) source, coupled with a Fourier-transform ion cyclotron resonance mass spectrometer (FT ICR-MS). This solvent-free method involves the introduction of an inert gas flow into the DIP capillary to shorten the residence time of pyrolysis products and effectively simulating FP reactors.

The ability to scan the whole pyrolysis process and to investigate pyrolytic products by MS in real time allows for a time-dependant analysis, and a more thorough study of the conversion processes. Additionally, the analysis of samples in DIP-APCI does not exceed 3 minutes allowing a high-throughput screening of different feedstock and catalysts. Using this method, the influence of temperature on thermal conversion of lignin samples was studied and post-analysis data processing using statistical tools allowed the identification of the original lignin feedstock.

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