• EU FT-ICR MS
End User School 2018
Department of Chemistry, University of Eastern Finland

Innovation with Integrity
Introduction

As a part of the activities of the first EU FT-ICR MS End User School, held at the Department of Chemistry, University of Eastern Finland during 20.-23.8.2018, a number of oral and poster presentations were given by younger scientists. This was a good chance for them to present and disseminate their research work to a larger audience as well as practice their skills for scientific presentations in general. Altogether, 18 poster or short talk presenters took part in a competition for a young scientist presentation prize sponsored by Bruker Daltonics. Overall, the level of talks and posters was very high, making it difficult to select a winner. The prize was awarded by the jury consisting of the principal investigators of the EU FT-ICR MS project, and was given to Anika Neumann from the University of Rostock. An abstract for her award-winning presentation as well as other contributions are made available in this electronic oral presentation and poster hall to the broader audience.

On behalf of the organizing committee for the first EU FT-ICR MS End User School,

Janne Jänis
Chair
Effect of Photochemical Aging on Water Soluble Organic Carbon from Small Scale Combustion

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Background

Small scale combustion (SSC) is an important source of ambient aerosol, having considerable environmental and health effects. Major part of organic combustion aerosol consists of water soluble organic carbon (WSOC). Hygroscopicity of WSOC makes it an important factor for e.g. cloud formation, but also for the biological impact of the aerosol.

The combustion emissions go through extensive atmospheric reactions, mainly with hydroxyl radical (OH). This continuous aging process creates significant amount of secondary organic aerosol and alters the chemical properties of aerosol.¹ In addition, majority of ambient WSOC stems from the oxidative aging.² However, the SSC-originating WSOC and its atmospheric behavior remain unassessed.

In this work, we combine different combustion and aging methods with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) in order to study the WSOC emitted from SSC. The first preliminary results of the effect of photochemical aging on the emissions are also reported.

Methods

Emissions from different combustion sources are studied, including a diesel engine, and a typical modern chimney stove with wood and lignite as fuels (Fig. 1). The emissions are aged using Photochemical Emission Aging Flow Tube Reactor (PEAR³) and a smog chamber in parallel in the UEF ILMARI laboratory, with OH exposures corresponding to one day in atmosphere.

Polar compounds in the WSOC of the particulate matter are prepared for negative electrospray ionisation with the method shown in Fig. 2. The contents of each sample are analyzed with FT-ICR MS in order to fully assess the chemical contents of the SSC WSOC.

Outlook

First comparison (Fig. 3) of fresh and aged wood combustion aerosol shows a slight increase in carbon oxidation state (-0.33→-0.25) and a decrease in double bond equivalent (8.3→7.4) during aging. Simultaneously, the nitrogen containing compounds are enhanced. Similar changes have previously been reported during atmospheric aging, indicating the method’s potential for WSOC characterization. Atmospheric organic aerosol is a diverse mixture of mainly unidentified compounds. This study of the molecular composition of real-life combustion sources, and their aging, is a notable step towards a full picture of the health- and environmentally relevant contents of small scale combustion emissions. Furthermore, it enables an intercomparison of different laboratory aging systems.

References:
³Ihalainen et al. submitted

Support by Academy of Finland (Grant: 304459), the European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers, the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE) is gratefully acknowledged.
2D FT-ICR MS/MS analysis of IgG1

Johanna Paris1, Tomos Morgan1, Christopher A. Wootton1, Bryan P. Marzullo3, Meng Li1, Frederik Lermyte1, Yuko P. Y. Lam1, Maria van Agthoven1, Mark P. Barrow1, John O’Hara2, and Peter B. O’Connor1
1. ICR lab, University of Warwick, UK 2. UCB, UK

Antibody (IgG1)

Recombinant monoclonal antibodies and derivatives are widely used as therapeutic drugs. They are susceptible to post-translational modifications that could occur during the manufacturing process and storage, resulting in product-related impurities. PTMs can change the efficacy, toxicity, or the clearance of the antibody, therefore they need to be well-monitored.

ECD and IRMPD fragmentations

ECD (Electron Capture Dissociation) fragmentation allows the analysis of labile modifications and, therefore, would be the technique of choice for the analysis of antibodies. However, the first attempts resulted in low fragmentation efficiency. The technique needs to be further optimised.

IRMPD (Infrared Multiphoton Dissociation) fragmentation (c and z).

IRMPD dissociation. Fragments follow the lowest energy pathway (b and y).

2DMS (Two-dimensional Mass Spectrometry) pulse sequence allows data independent acquisition while preserving the information of which fragment derives from which precursor.

Conclusion

The 2DMS technique offers an alternative to LC-MS with a different set of limitations. The processing of the data is challenging and needs further development.

References

Quantitating the Ratio of Isoaspartic Acid to Aspartic Acid in Proteins by Electron Capture Dissociation

Anisha Haris*, Yuko P. Y. Lam, Tomos E. Morgan, Cookson K. C. Chiu, Christopher A. Wootton, Mark P. Barrow, and Peter B. O'Connor

Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

Overview:

The ability to distinguish between the isomerization products of asparagine (Asn) deamidation and the quantitation of the ratio of these isomers help us to gain a deeper understanding of the effect that they have on protein structure, which is known to alter the activity and function of the protein. Furthermore, in mammalian antibodies, the isomerization of Asp to isoAsp can be challenging due to the slight structural differences between the isomers. Hence using ultra-Chromatography and tandem MS methods such as collisionally activated dissociation can be used to separate Asp from isoAsp.

Introduction:

- Deamidation of Asn is a common PTM resulting in the formation of the Asp and isoAsp product.
- Ratio of formation of isoAsp to Asp is generally 0.5:0.5, and the bound peptide.
- Reaction phase (P/FMS) can be used for separation and quantification of the ratio of the isomers can be affected by lyophilization in impact.
- Advantage of the HMMS peak in the peptide backbone by ECD-MS/MS generates diagnostic fragment ions for isoAsp residues, cl

MS Results:

- Deamidation peak (1st isotope)
- Deamidated peak (2nd isotope)
- Non-deamidated peak

Method:

BSA and HSA (purchased from Sigma Aldrich) were digested by trypsin for 16 hours at a temperature of 37 °C. Formic acid was added to the samples to a final concentration of 0.5%. After trypsin digestion, BSA and HSA (10 μM each) were incubated in ammonium bicarbonate at 60 °C for 5 days. 1 mL of each sample were taken out every 24 hours. Final samples were diluted with 75:25 water: acetonitrile with 0.1% formic acid.

ECD MS/MS Results:

- ECD MS/MS of the incubated HSA peptide LVNEVTEFAK.

Acknowledgements:

We thank EPSRC (J003022/1, N021630/1, N033191/1), BBSRC (P021875/1), Royal Society Translation Award, H2020 EU FT-ICR MS End User School 2018, Bruker Daltonics for support. We also thank Dr. Diana Palacio, Meng Li and Bryan Marzullo from the University of Warwick FT-ICR MS group for their assistance and helpful discussions relating to this project.

References:

2. Space charge effects present with increasing injection concentration and peak coalescence effect with increasing accumulation time.
3. Different i/z rejection conditions has no effects on the deamidation to non-deamidated peak ratio.
4. Different i/z rejection conditions has no effects on the deamidation to non-deamidated peak ratio.
How can FT-ICR-MS help me during my PhD Thesis?

Justine Hustin¹, L. Quinton¹, D. Debois², E. De Pauw¹

¹ Mass Spectrometry Laboratory, University of Liège, Belgium
² ZenTech S.A., Liège, Belgium
Contact: jhustin@uliege.be

The ultimate goal of my research project is to establish a correlation between the lipid composition present in stools and bacteria from the microbiota of children suffering from a certain disease in order to highlight a correlation between the lipid content (nature and/or concentration) and the targeted pathologies. Afterwards, the creation of a kit for the detection and quantification of these biomarkers will be considered to determine, through a non-invasive manner, whether a child is affected by the targeted disease.

Research project

How can I achieve this goal?

A) Extraction of lipids from mouse stools
   - Methyl tert-butyl ether/methanol
   - Methanol/chloroforme
   - Methanol/dichloromethane
   - Lyophilization and grinding

B) Development and optimization of a profiling method of the lipid content of these healthy mouse stools extracts
   - LC-ESI and LC-MALDI for qualitative and quantitative comparisons
   - Liquid chromatography with a HILIC phase to separate the lipids according to their polar heads and therefore according to their family

C) Upon optimization, determination of the lipid profile from mice with a specific disease
D) Establishment of the correlation between the lipid composition and the bacteria from the microbiota
E) Choice of biomarkers
F) Transition on human samples, method validation and creation of a kit

Use of FT-ICR-MS during my research

- Best way to compare LC-ESI and LC-MALDI because both sources are available on the same instrument
- Better resolution than the other mass spectrometers
- Possibility to have only one Matched Mass for each peak

Analyse with LIPIDMAPS

<table>
<thead>
<tr>
<th>Input Mass</th>
<th>Matched Mass</th>
<th>Name</th>
<th>Formula</th>
<th>Ion</th>
</tr>
</thead>
<tbody>
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<td>520.2921</td>
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<td>C₂₃H₄₄NO₃P</td>
<td>[M+H]⁺</td>
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<td>C₂₃H₄₄NO₃P</td>
<td>[M+K]⁺</td>
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</table>

Acknowledgments

This project is part of an Euregional project called EURlipids and financed by the Interreg Euregio Meuse-Rhine. It’s a collaboration between the Mass Spectrometry Laboratory from Liège and ZenTech S.A., a company specialized in newborn screening.
Findable: specific and codified name
Accessible: available to defined groups
Interoperable: same format for every metadata file
Re-usable: use repositories to store the data.

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MetadataFile3_010119.pdf

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- Name: Metadata 3
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User
- Name: Jon Snow
- Phone: 0601020304
- ...

Experiment
- m/z range: 100 - 1000
- ...

Sample
- ID: 123456789
- Solvent: H2O
- ...

Metadata File 1
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Jon Snow shared with The North:

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PROCESS YOUR DATA
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Display available for 1D and 2D spectrum
Show the previous processed samples

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F
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High Resolution Mass Spectrometry Characterization of Essential Oil and Extractives from Spruce Sprout

Omolara Mofikoya, Marko Mäkinen, and Janne Jänis
University of Eastern Finland, Department of Chemistry, P.O. Box 111, FI-80101 Joensuu, Finland

Introduction

- Spruces are coniferous evergreen trees that belong to the Pinaceae family. Their sprouts appear in the early spring.
- Essential oils and extracts from spruce sprout contain a variety of chemical compounds which contain considerable antioxidant and antimicrobial properties.
- In this study, we obtained essentials oil and other extractives from spruce sprout by hydrodistillation and solvent extraction and characterized their chemical composition using ESI/APPI-FT-ICR MS.

Materials & Methods

- Needle materials
  - Collected in May 2018 from the Ylä-Valtimo region located at the Eastern Finland.
  - Needles stored at 4 °C (prior to use) in order to avoid the loss of volatile constituents.
- Essentials oils production
  - Hydrodistillation with Clevenger apparatus (200 g needles per 400 mL of water; 3 h of distillation time).
  - Solvent extraction with continuous Soxhlet extractor (Buchi system B-811); 40 g of spruce sprout, 2.5 h of extraction time; solvents: hexane/methanol/water.
- Mass spectrometry
  - (-)ESI/(+)APPI FT-ICR MS.

Results

- Negative-ion ESI FT-ICR MS analysis of the essential oils samples revealed their complex nature.
  - The spectral fingerprints were unique for each of the sample (Figure 1).
  - The most abundant compounds for the non-polar solvent were resin acids while phenolic compounds were the most abundant in the polar solvent.
  - Other compounds detected were different acids, esters and terpenoids.
  - Some uniquely identified acids are presented in Figure 2.

- Positive-ion APPI FT-ICR MS analysis mostly targeted non-polar species (terpene hydrocarbons) present in essential oil samples (Figure 3).
  - More compounds were detected in the (+) APPI spectra compared to (+) ESI.
  - APPI provides complementary data to that of ESI.

Conclusions

- Extracts obtained from spruce sprouts are complex mixtures of terpene hydrocarbons and their derivatives, acids, esters and phenolic compounds.
- Lipophilic compounds (resin acids) were the most abundant from the hexane and the essential oil extract while hydrophilic (phenolic compounds) were the most abundant from the methanol and water extract.
- High-resolution ESI/APPI-FT-ICR provides a straightforward means to obtain comprehensive, semi-quantitative chemical fingerprints of the extracts.
Recovery of Valuable Chemical Compounds from Birch Outer Bark by Slow pyrolysis, Solvent extraction, and Alkaline hydrolysis

Qing Zhao,1,2 Ilja Miettinen,2 Marko Mäkinen,2 Antti Haapala,1 and Janne Jänis2
University of Eastern Finland, 1School of Forest Sciences & 2Department of Chemistry

1. Introduction
- In Nordic countries, Silver birch (Betula pendula) is a dominant tree species in forest industry.
- Bark is usually used as solid fuel burnt to produce energy, like heat, steam, and electricity. However, it is a potential material for value-added products including chemicals, materials and functional foods.
- Unlike wood, which mainly comprises cellulose, hemicellulose and lignin, tree bark has a high content of extractives (e.g. triterpenes), suberin, waxy cross-linked co-polymers made of different hydroxy/epoxy fatty acids or diacids, as well as phenolic substances.
- The aim is to isolate birch outer bark compounds and to characterize them with Fourier transform ion cyclotron resonance (FT-ICR) -mass spectrometer (Bruker Daltonics, Billerica, USA).
- Ionization method: electrospray ionization (ESI).

2. Materials and methods

3. Bulk chemistry results

<table>
<thead>
<tr>
<th>Samples</th>
<th>C (w-%)</th>
<th>H (w-%)</th>
<th>N (w-%)</th>
<th>O (w-%)</th>
<th>Water content (w-%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrolysis oil</td>
<td>86.0</td>
<td>10.1</td>
<td>0.57</td>
<td>3.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>60.7</td>
<td>11.6</td>
<td>0.0</td>
<td>27.7</td>
<td>12.9</td>
</tr>
<tr>
<td>Alkali hydrolysate</td>
<td>38.5</td>
<td>9.9</td>
<td>1.1</td>
<td>50.5</td>
<td>72.7</td>
</tr>
</tbody>
</table>

4. MS results

- FT-ICR MS: high mass resolution, high mass accuracy, no pre-separation needed to analyze complicated mixtures.
- The bulk analyses results were noticeable different between pyrolysis oil, ethanol extract, and alkali hydrolysates.
- Mass spectra of pyrolysis oil, ethanol extract, and alkali hydrolysate showed different chemical classes.
- The most common birch outer bark compounds were identified, such as betulin, betulinic acid, lupeol, linoleic acid, and oleic acid.

5. Conclusions
- FT-ICR MS: high mass resolution, high mass accuracy, no pre-separation needed to analyze complicated mixtures.
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References

Acknowledgments
The doctoral research has been funded by Fortumin säätiö, Teollisuusneuvos Heikki Väänäsen rahasto, and Suomen Kulttuurirahasto.

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Overview

- **Purpose**: Chemical fingerprinting of alcoholic beverages by using high-resolution mass spectrometry.
- **Methods**: FT-ICR MS coupled to (±) ESI was applied to the compositional analysis of whiskies and gins; Discrimination of the samples was performed by Principal Component Analysis.

Introduction

- Whisky contains a large variety of compounds, which are derived from different chemical families such as alcohols, fatty acids, phenols and esters.
- Monoterpenic, sesquiterpenic, and diterpenic compounds and their oxygenated derivatives are main compounds in gins.

Chemical Fingerprinting of Whisky and Gin by ESI FT-ICR MS
Yanning Dou, Marko Mäkinen & Janne Jänis

Results

- 124 compounds, mainly phenolics, esters, acids and carbohydrates, were identified from whiskies.
- In gins, 135 compounds, consisting of terpenoids, phenolics, esters, acids, and carbohydrates were found.
- PCA based on (±) ESI separated the studied alcohols into different groups according to their brands.

Conclusions

High-resolution FT-ICR MS proved to be a powerful technique for non-targeted chemical fingerprinting of whiskies and gins.
ESI-FT-ICR MS was successfully applied for the first time to the analyses of gins.
Abstract

As a type of renewable energy, one of the main concerns of biodiesel is its chemical instability under operation and storage conditions. Temperature-induced oxidation and decomposition of biodiesel have been widely studied in the past decades, using different experimental methods. While the modern diesel engine adopted the common-rail injector system to enhance the diesel energy efficiency and reduce pollution. The pressure in these key components are approaching greater than 0.25 GPa. This paper presents here the first approach of understanding the role of high pressure conditions in chemical reactions of biodiesel, using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR MS). Biodiesel samples after variable treatments: ambient conditions, 80 C/ambient pressure, room temperature/0.6 GPa, and 80 C/0.6 GPa were analysed by ultrahigh-resolution mass spectrometry. The assigned spectra were compared by several visualization techniques which clearly suggested that high pressure conditions efficiently preserved temperature-induced chemical reaction of biodiesel, while pressure is prone to inducing polymerisation.

Two-dimensional FT-ICR mass spectrometry as a tool to discriminate oil origin. Application to archaeological samples

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2 LNG: Laboratoty Nicolas Garnier, Vic Le Comte, France
E-mail : fabrice.bray@univ-lille.fr

Introduction. Two-dimensional Fourier transform ion cyclotron resonance mass spectrometry (2D FT-ICR MS) is an innovative tandem mass spectrometry method that correlates precursor and fragment ions without requiring prior ion isolation [1]. 2D FT-ICR MS is therefore very well adapted to the structural analysis of complex samples. Archaeological ceramics such as amphorae contained organics compounds which obtained information’s of human activities through years. We use two methodology lipidomic analysis and 2D FT-ICR MS methodology to discriminate moderns samples and identify archaeological samples.

Materials and methods. Triacylglycerols (TAGs) were extracted from 1 g of modern samples (vegetals oils, animals fats, dairy products) and archaeological sample (ceramic aryballos from Tenodo Necropolis, switzerland) [2]. TAGs fractions are extracted using two solid phase extractions on 50 mg DSC-NH2 columns. TAGs 50 microliter solution was then diluted 500 fold in methanol/chloroform 2:1 (v/v) with 0.1% of lithium chloride. Classical 1D MS analysis and bi-dimensional FT-ICR MS experiments were performed on an Apex 9.4 Tesla fitted with an Infinity cell using gas-free fragmentation method IRMPD as the lithiated TAG precursor ions are very cleanly fragmented by IMRPD allowing an easy determination of their fatty acids composition.

Results. Classical lipidomics analysis allowed to identify TAGs with a total carbon of 46 to 56 and unsaturation numbers from 1 to 5 may be identified and their individual fatty acid composition determined. Even and odd fatty acids are identified in animals fats and dairy products and identified specific markers of ruminants animals. This methodology allowed to identify specific markers of ruminants in archeological sample. From the low resolution 2D spectra, clear bidimensional patterns are obtained allowing to classify the moderns samples by 2D MSMS patterns. The 2D maps of moderns samples database allows to identify specific markers of ruminants animals in archelogical samples. The parent line (TAGs precursors) and 2D MSMS pattern (neutral loss of fatty acids) are specific of vegetals oils, animals fats, dairy products.

References:
Introduction. A novel experimental procedure is presented to measure binding energy for unimolecular (metastable) decay of multiply charged metal-ligand cluster ions in gas phase. The necessity to develop a new method arose from the limitations of the available experimental techniques in measuring binding energies of large cluster ions (such as the experimental methodologies developed by the groups of Peter B. Armentrout, A. Welford Castleman Jr., Paul Kebarle, and Evan R. Williams). Moreover, gas phase studies of the chemical and physical properties of metal ions have concentrated on singly charged species since they form easily. In contrast, the most frequent charge state of condensed phase metal ion chemistry is $2^+$. Technical difficulties with studying metal dication chemistry arise in the gas phase because of the very high probability of charge transfer, a condition easy to appreciate when the second ionization energy of a metal is typically $\geq 15$ eV, whilst the first ionization energy of an attached molecule is $\leq 10$ eV.

The determination of binding energy is a very important piece of information that an experiment can provide. The aim of measuring binding energies with experimental techniques is to study the process of solvation at a molecular level. In particular, the studies of small molecules aggregates and metal ion-molecules complexes in gas phase provide a mean to relate clusters properties with condensed phase behaviour, but it can also provide a reference against which quantum mechanical and molecular mechanics calculations can be judged.

Materials and methods. The in-house built experimental apparatus [1] is equipped with a:
(1) cluster chamber to generate mixed neutral clusters by the adiabatic expansion of a solvent/argon gas mix through a pulsed supersonic nozzle;
(2) “pick-up” [2] chamber containing a Knudsen effusion cell to produce a neutral vapor of metal atom, which collided with the molecular beam to produce various neutral clusters;
(3) no automated and high resolution double-focusing mass spectrometer having reversed sector geometry (VG Analytica LTD ZAB-E) to measure the kinetic energy release that accompanies fragmentation of the selected cluster ions with mass-analysed ion kinetic energy (MIKE) method;
(4) detection system: Daly scintillation, fast photomultiplier, and a lock-in amplifier;
(5) vacuum system; and
(6) in-house built console to control the components of the apparatus and electronic equipment.

For the purposes of analyzing MIKE spectra the measurement were only accepted when (i) the goodness of fit of the peak widths to a Gaussian profile was $>0.9$, where 1.0 is a perfect fit; (ii) the laboratory-frame FWHM peak widths of the precursor ions had to be all at least $<3$ eV. The background pressure in the 2nd ffr of the mass spectrometer was maintained at $<1 \times 10^{-7}$ mbar to ensured minimal interference from collision induced fragmentation. The data were analysed in order to determine binding energies for individual solvent molecule using heat bath theory [3]. Up to six laboratory-frame energy measurements were made for each fragmentation pathway to determine an error for the average peak width. The details of the calculation method, the choice of parameters and constants for these data analysis, omitted here, can be found in [4-7]. The method has been used to study $H^+(H_2O)_n$ and $H^+(NH_3)_n$ and $H^+(CH_3OH)_n$ for $3 \leq n \leq 30$; and how each of $Mg^{2+}$ and $Ca^{2+}$ and $Si^{4+}$ coordinates with each of $H_2O$ and $NH_3$ and $CH_3OH$.

Results. The study on $H^+(H_2O)_n$, and $H^+(NH_3)_n$, $H^+(CH_3OH)_n$, [4] was primarily used as calibration method to prove the reliability of the experimental procedure as being capable of yielding binding energies in large cluster ions containing up to 30 molecules.

(i) these experiments indicate that only a metastable peak, and hence a binding energy, is recorded for the mostly weak bound molecule present in a given cluster, which corresponds to the one bound with a single hydrogen bonding. The justification for this conclusion is given by considering that unimolecular (metastable) decay in these experiments is ruled by a competitive shift, so that the decay with the lowest activation energy is detected;

(ii) the only possible decay mechanism in these experiments is evaporative cooling, owing to the lifetime of the metastable ions $\sim 10^{-4}$ s and to the high vacuum pressure $1 \times 10^{-7}$ mbar at which the experiments are performed;

(iii) for the three system there is close agreement between the measured binding energies and the energy of a single hydrogen bond (evaporation enthalpy for the condensed phase equilibrium is ~42 kJmol$^{-1}$ for water, 19.86 kJmol$^{-1}$ for ammonia, and 37.43 kJmol$^{-1}$ for methanol).

The study on doubly charged metal ion complexes yielded:

(1) the first solvation shell (coordination number) is completed at
   (i) $[\text{Mg(NH}_3\text{)}_{4+5}]^{2+}$ and $[\text{Ca(NH}_3\text{)}_{6}^{2+}]$ [5]
   (ii) $[\text{Mg(CH}_3\text{OH)}_{6}^{2+}]$ and $[\text{Ca(CH}_3\text{OH)}_{6}^{2+}]$ and $[\text{Sr(CH}_3\text{OH)}_{6}^{2+}]$ [6]
   (iii) $[\text{Ca(H}_2\text{O)}_{6}^{2+}]$ [7]

(2) the species outside the first solvation shell interact through one hydrogen bond;

(3) the smaller clusters, which do not have a data on the figures, had charge separation via Coulomb explosion as the only metastable decay observable (see literature by Stace A.J.);

(4) the charge has an influence on the binding of the molecule in the second and quite possibly the third solvation shells to the central ion;

(5) the magnitude of +2 charge more than the charge density influences the strength of the interactions for $n > 6$: the charge on a metal cation is not contained and accommodated by a single shell of solvent molecules.

The comparison of these data with the available experimental and theoretical literature, which has been omitted, can be found in the references [4-7].

References.
GAS-PHASE KINETICS AND IRMPD SPECTROSCOPY TOWARDS A BETTER UNDERSTANDING OF CISPLATIN REACTIVITY

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Introduction. Cisplatin ([cis-PtCl₂(NH₃)₂]) is a widely used antineoplastic drug, particularly effective in the treatment of lung and prostate solid tumors. Its activity relies on the interaction with the nucleobases of DNA, in particular adenine and guanine, leading to inhibition of transcription and eventually cell death. However, the actual active species are recognized to be the products of cisplatin aquation (substitution of Cl with H₂O), making a matter of great interest the understanding of these aquated complexes reactivity. In this context, the use of a solvent-free environment permits to obtain an unambiguous characterization of these ions, while prototropic equilibria and the formation of hydroxo-bridged polinuclear complexes make hard to achieve the same result in water solution.

Materials and methods. Cisplatin from commercial sources was solubilized in a 1:1 water/methanol solution to a concentration of 10⁻⁵ M and eventually allowed to react with selected compounds in solution. The complexes of interest were ionized (ESI) and mass-analyzed using a Paul ion trap (Bruker Esquire 6000) and FT-ICR-MSs (Bruker APEX-Qe and 7T Solarix). Vibrational features of the mass-selected ions were assayed using IRMPD spectroscopy in the XH (X = C, N, O) stretching and fingerprint regions of the IR spectrum using respectively an OPO/OPA laser system and the FEL light of the Centre Laser Infrarouge d’Orsay facility. The assignment of the vibrational features in the experimental IRMPD spectra has been assisted by B3LYP computations. Moreover, cis- and transplatin aquated relatives were mass-selected, together with compounds which underwent subsequent hydrolysis, and allowed to interact with several neutral molecules in the cell of an FT-ICR mass spectrometer to obtain kinetic information about gas-phase reactivity. Finally theoretical calculations of the substitution reaction potential energy surfaces at the ωB97X-D level were used to interpret the experimental evidences.

Results. In this contribution we report the gas-phase kinetics for the substitution reaction of water from the primary and secondary aquation products of both cis- and transplatin ([PtCl(NH₃)₂(H₂O)]⁺ and ([Pt(OH)(NH₃)₂(H₂O)]⁺) with molecules representative of cisplatin binding motifs with biological targets -e.g. pyridine for nucleotides, thioanisole and dimethylsulfide for thiols-containing amino-acid residues and trimethylphosphate for ubiquitous inorganic phosphates as well as for phosphate groups present in the backbone of nucleic acids. The kinetic data (Table 1) showed a consistently higher reactivity for the primary aquation product compared to the complexes that have experienced a second hydrolysis process, [PtCl(NH₃)₂(H₂O)]⁺ and ([Pt(OH)(NH₃)₂(H₂O)]⁺ respectively.
Table 1. Reactivity of $[\text{PtX(NH}_3\text{)}_2(\text{H}_2\text{O})]^+$ ($X = \text{Cl, OH}$) with simple molecules in the gas-phase.

<table>
<thead>
<tr>
<th>Reagent Ion</th>
<th>Neutral</th>
<th>$k_{\text{exp}}$</th>
<th>Eff (%)</th>
</tr>
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$^a$ Second order rate constant in units of $10^{-11} \text{ cm}^3 \text{s}^{-1}$ at 298 K, estimated error ±30%. $^b$ Ref. $^4$ n. r. stands for non-reactive. Other tested and found to be unreactive compounds are $\text{H}_2\text{O}$ and $\text{H}_2\text{S}$.

In addition, ESI-MS have been employed to investigate the intermediates generated from the reaction of cisplatin with the selected nucleophiles in solution.$^4$ Intriguingly mass spectrometry enabled to reveal the addition complex generated by the interaction of the first cisplatin hydrolytic product ($\text{cis}[\text{PtCl(NH}_3\text{)}_2(\text{H}_2\text{O})]^+$) with the model ligands. IRMPD spectroscopy allowed us to unveil the nature of these species by comparing experimental spectra with theoretical ones of isomers which present the ligand either in the first coordination sphere of platinum or non-covalently bound to the water molecule of the aquo complex (Fig.1). Finally, we find direct proof for the substitution reaction to occur when the ions are activated by resonant IR photons. This evidence confirms we are sampling the encounter complex generated along the reaction path which, from the interaction of the cisplatin aquo complex with a certain nucleophile, leads to the substituted product.

Figure 1. IRMPD spectrum (lower panel) of $[\text{PtCl(NH}_3\text{)}_2(\text{Py}(\text{H}_2\text{O}))]^+$ and calculated IR spectra of $\text{Py}_1$ and $\text{Py}_3$.

INTERACTION OF TEAD1 TRANSCRIPTION FACTOR WITH ITS DNA RESPONSE ELEMENTS STUDIED BY STRUCTURAL MASS SPECTROMETRY

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Introduction. TEAD transcription factors mediate gene expression regulation through interactions with their DNA response M-CAT motif. They are active mainly during growth and development and induce gene expression of proteins involved in cell proliferation, differentiation or apoptosis prevention. TEADs, and many of their target proteins, are also known to be upregulated in several types of cancer cells. Thus, TEADs are considered as a possible target for anti-cancer therapy. Due to their aforementioned properties, strict regulation of TEAD proteins activity is required to prevent tumorigenesis or developmental disorders. To date, known ways of TEAD proteins regulation include mostly interaction with other regulatory proteins - coactivators such as YAP, TAZ or VglI. Nevertheless, the information on TEAD transcription factors activity regulation through interaction with DNA is still limited.

To study interaction of DNA binding domain of TEAD1 with M-CAT we focused on the effect of DNA binding motif surrounding sequence and orientation. Several M-CAT motifs originating from regulatory regions of human genes (namely CTGF, SRF, C-MYC and GLUT1) were selected and dissociation constant of each complex was determined using fluorescence anisotropy assay. Structural mass spectrometry techniques (H/D exchange and quantitative chemical cross-linking) and molecular docking were utilized for structural characterization.

Materials and methods. TEAD1-DBD was recombinantly expressed in E.coli. Six oligonucleotides containing M-CAT elements, occurring in human genome (namely CTGF, SRF, exon and enhancer of C-MYC and exon and enhancer of GLUT1 genes) and differing in M-CAT motif orientation or surrounding sequence were selected. TEAD1/M-CAT complex formation was checked by native nanospray coupled with FT-ICR MS and fluorescence anisotropy-based binding assay was used to determine Kd. H/D exchange was utilized for DNA-free TEAD1-DBD or in complex with each M-CAT using immobilized aspergillopepsin to obtain better spatial resolution. Regular and 13C-labelled forms of disuccinimidyl adipate (DSA) were used as reagents for quantitative protein-protein cross-linking. Lab-made software LinX and DeutEx were used for data processing. MS measurements were performed on 15T FT-ICR mass spectrometer coupled with reverse-phase chromatography. Molecular dynamics simulations were performed in Amber14 and Modeller 9.14 was utilized to build the initial model using the crystal structure of homologous TEAD4 protein-DNA complex1.

Results. Initially, complex formation of TEAD1-DBD with each M-CAT was confirmed by native nanospray coupled with FT-ICR MS and dissociation constants were determined utilizing fluorescence anisotropy-based binding assay. According to KD assay results, tested M-CATs could be divided into two groups one with approximately ten times higher affinity to TEAD1-DBD than the other (Fig. 1).

Figure 1. Comparison of binding properties of TEAD1-DBD complexes with dsDNA from different human genes. M-CATs on the left (originating from CTGF, SRF and exon of C-MYC genes) had significantly lower KD and thus higher affinity to TEAD1-DBD than those on the right (from enhancer of C-MYC, exon of GLUT1 and enhancer of GLUT1).

In H/D exchange results, differences in deuterium uptake were observed in helix H3, part of helix H2 and in the loop connecting them, identifying this as DNA-binding region (Fig. 2A). Minor protection from deuteration in complexed state, probably caused by stabilization of the helix, was observed in middle part of helix H1. The identified binding region was the same for all tested M-CATs but, in correlation with dissociation constants, protection from deuteration in higher-affinity complexes was more intense than in low-affinity complexes (Fig. 2B). Quantitative protein-protein chemical cross-linking resulted in 14 distance restraints. Three of them were not affected by presence of DNA, two were favored in presence of M-CAT and eight constrains were discriminated by DNA binding. Cross-link formation ability of lysines K57 and K88 significantly decreased in complexed state. Same as in H/D exchange, the effect of high-affinity duplexes was more significant than the effect of low-affinity ligands.

Figure 2. (A) Visualization of TEAD1-DBD protection induced by DNA binding after 10s of deuteration. The darker the blue colour, the more the region is protected. Black-coloured region wasn’t covered by the data. (B) Deuterium uptake plot of H3 helix illustrating the effect of M-CAT orientation on intensity of protection

Molecular docking revealed that TEAD1-DBD binds to 3’→5’ oriented M-CATs in 180° rotated orientation. Response motif recognition is led firstly by the overall shape of DNA major grove, which is similar for both motif orientations. Specific aminoacid-base interactions, which are more frequent and stronger in case of 5’→3’ oriented M-CATs, stabilize the complex once H3 helix is fitted inside the major groove (Fig. 3). In vitro results were correlated with the situation in living cells studied by the means of chromatin immunoprecipitation and qPCR. 3’→5’ oriented M-CAT motif reported lower occupancy by TEAD1 than 5’→3’ oriented M-CATs.

Figure 3. Structure superposition of DNA strands containing the 5’→3’ (red, green) and 3’→5’ (yellow, magenta) oriented M-CAT with TEAD1-DBD bound

References.

GRUBB'S REACTION APPLIED TO POLYOLEFIN MATERIALS DEPOLYMERIZATION AND ANALYSIS

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Polyolefin is a class of molecules formed by the polymerization of one simple olefin such as butadiene, isoprene or others. The characterization of this group of organic macromolecules by mass spectrometry continues to be very challenging due to their high hydrophobicity and non-ionizable character. The aim of this work is the depolymerization of a natural polymer, amber and the analysis of the obtained fragments by different techniques of mass spectrometry in order to elucidate its structure. However, due to the high complexity of the amber structure and its very low solubility in organic solvents, a preliminary study was performed. The depolymerization reaction was first optimized and tested on polyolefins, especially polybutadiene and polyisoprene polymers due to their structure resemblance to amber.

Thus, a controlled olefin metathesis between a polyolefin and an acrylate monomer in the presence of a ruthenium-based catalyst is used as an effective method for the cross-metathesis, depolymerization, functionalization and characterization of unsaturated polymers. Metathetic depolymerization of cis-polybutadiene (synthetic rubber) and cis-polyisoprene (natural rubber) was carried out under solvent free conditions using the second generation Hoveyda-Grubbs catalyst and butyl acrylate as chain transfer agent at 50°C under nitrogen. This catalytic reaction produced telechelic acrylate monomers, oligomers or polymers with different molecular weights. A trans-amidation reaction was performed between the produced telechelic molecules and amylamine to produce amide end-functionalized molecules. The functionalization of polybutadiene and polyisoprene allowed their analysis by Fourier-Transform ion cyclotron resonance (FT-ICR).

The adopted method contained two steps. It started with a cross-metathesis reaction between polymers and butyl-acrylate, followed by a trans-amidation reaction using amylamine to produce amide end-functionalized fragments. FT-ICR spectra confirmed that the double bonds were successfully broken leading to the depolymerization of the polymers and the formation of new acrylate functionalized fragments with different molecular weights. The advantage of FT-ICR technique is its ability to detect very low...
During this work, we presented a new method for the characterization, depolymerization and analysis of polymers using high-resolution mass spectrometry. This new method was based on a cross-metathesis reaction using butyl-acrylate (1) as chain transfer agent and the Hoveyda-Grubbs second-generation catalyst. The optimization of the different conditions of the cross-metathesis allowed the degradation of natural and synthesis rubber, and the production of new functionalized telechelic fragments with controlled molecular weights.
FT-ICR-MS METABOLIC APPROACH TO UNCOVER GRAPEVINE RESISTANCE TO DOWNY MILDEW

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Introduction. Grapevine (Vitis vinifera L.) is one of the most important and cultivated fruit plants in the world, mainly due to the wine industry. However, Vitis vinifera L. cultivars are highly susceptible to downy mildew, caused by Plasmopara viticola (Berk. et Curt.) Berl.et de Toni, which affects all the green parts of the vine, causing yield reduction and significant production losses. Thus, if not controlled, it presents serious negative effects in several countries’ economy1. To understand the innate susceptibility/resistance mechanism of Vitis, a metabolic characterization of grapevine leaves is of utmost importance, since plants contain a unique metabolome that change upon pathogen infections and could allow us to identify specific compounds associated to either resistance or susceptibility traits. The majority of grapevine metabolite studies were performed by Nuclear Magnetic Resonance (NMR) or Liquid Chromatography coupled to Mass Spectrometer (LC-MS). For NMR studies, the analysis was based on a single extract from leaves2 and the limit of detection was very low. Even using 1D/2D NMR techniques, the number of metabolites identified is usually less than 20. For LC-MS studies, although this methodology is more sensitive, permitting the identification and quantification of grapevine metabolites3, only 135 primary metabolites (sugars, amino acids, organic acids and amines) were identified and quantified in a 30-min hydrophilic interaction LC run coupled to a triple quadrupole mass spectrometer3. To achieve higher sensitivity and maximum metabolome coverage, we resort to mass spectrometry using high-resolution and high-mass accuracy instruments, based on Fourier transform technology. Our aim is to use an untargeted metabolomics based approach to understand the innate resistance mechanism of cultivars. To achieve that, we resort to Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS), which provides ultra-high-mass accuracy and the highest mass resolution4. Moreover, using direct infusion, metabolites are analysed in a high-throughput way, providing a rapid analysis of complex metabolite samples, and eliminating the time-consuming separation5. Once resistance associated metabolites are identified, these can be used as metabolic markers.

Materials and methods

1. Plant Material - The third to fifth leaves (from the shoot apex) of V. vinifera ‘Trincadeira’ (Susceptible to P. viticola) and ‘Regent’ (Resistant P. viticola) were collected at the Portuguese Ampelographic Grapevine Collection (CAN, international code PRT051), INIAV-Dois Portos. Leaves were harvested from 5 fully developed plants and combined in 1 biological replicate. Three biological replicates were considered for analysis.

2. Metabolite extraction and FT-ICR-MS analysis - Metabolite extraction was performed as previously described6. Methanol fraction was analysed by direct infusion on the Apex Qe 7-Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR-MS, Bruker Daltonics), in positive electrospray ionization (ESI) mode. Leucine enkephalin (YGGFL, Sigma Aldrich Portugal) was used as internal standard and added to all samples at a final concentration of 0.5 µg/mL, being considered a mass of [M+H]+ = 556.276575 Da for analysis. Spectra were recorded between 100 and 1000 m/z. Spectra analysis and alignment were performed according to Maia et al., 20166.

3. Multivariate analysis – For data normalization, intensity data were normalized by the internal standard (leucine enkephalin), generalized log transformed and Pareto scaled prior to multivariate methods. For missing values, these were substituted by half of the minimum positive value found within the data. Multivariate analysis considering peak intensities (Principal Component Analysis (PCA) and Partial Least Squares - Discriminant Analysis (PLS-DA)) was performed using MetaboAnalyst (http://www.metaboanalyst.ca/).

4. Metabolite identification – Mass lists were uploaded in the MassTRIX database (http://masstrix3.helmholtz-muenchen.de/). The adducts M+H, M+K and M+Na were considered and 2ppm was the maximum m/z deviation from theoretical mass. Vitis vinifera was the selected organism and the search was performed in the combined database of KEGG (Kyoto Encyclopedia of Genes and Genomes)/HMDB (Human Metabolome Data Base)/LIPID MAPS without isotopes.

Results. Comparing ‘Trincadeira’ and ‘Regent’ cultivars (without P. viticola infection), using an untargeted metabolomics approach with FT-ICR-MS, we were able to clearly discriminate and identify the most discriminatory compounds. We were also able to identify features that are exclusively from ‘Trincadeira’ and ‘Regent’. With this work, we were able to show the potential of metabolomics based on ultra-high resolution and ultra-high mass accuracy by FT-ICR-MS. This work will contribute, not only to grapevine variety discrimination, but also a deeper identification of compounds that participate in grapevine resistance mechanisms. This approach, will contribute for the identification of resistance associated metabolites that may contribute for the development of efficient biomarker assays, based on resistance-associated metabolites, to help future breeding programs and introgression line analysis7.

![Figure 1](image-url) - Metabolome difference and discrimination between ‘Trincadeira’ and ‘Regent’. (A) Common and exclusive peak counts. (B) PCA scores (‘Regent’ – Red; ‘Trincadeira’ – Green) with 95% confidence regions shown. (C) Hierarchical clustering and heatmap using the top 50 most significant (t-test p-values) MS peaks. (D) Variable Importance in Projection (VIP) scores of the first component in a PLS-DA classification model (adapted from Maia et al., 20187).

References.
High Resolution Mass Spectrometry Characterization of Essential Oils and Extractives from Conifer Needles

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Introduction. Plants serve as important raw materials in food and non-food industrial products. They are sources of bioactive compounds, which are useful in pharmaceutical, cosmetic, dyes and colorant, agricultural and other related industries.1-2 Bioactive compounds are mostly constituent of essential oils, which are complex compounds, mainly composed of terpenes and some non-terpene compound such as phenylpropanoids. Essential oils obtained from some conifer species needles and bark are widely used as bathing oils, ointments or inhaling drugs for treating a wide range of disorders of neuralgic, infectious and rheumatic origin. Several studies have also reported the antioxidant antimicrobial, antibacterial, larvicidal and repellency, antifungal, herbicidal, anti-inflammatory, and free radical scavenging properties of conifer essential oil.3 These properties are based on the chemical composition of the oils, which is dependent on the type of extraction method used. This study is aimed at analyzing and comparing the essential oils extracted from four different conifer species; scots pine (Pinus sylvestris), common juniper (Juniperus communis), Norway spruce (Picea abies), European larch (Larix decidua). Extraction were done by hydrodistillation and solvent extraction followed by chemical characterization by FT-ICR MS coupled with (-) ESI and (+) APPI.

Materials and methods. Plant materials. Needles were collected in June 2016 from Ylä-Valtimo region, Eastern Finland and stored at 4 °C (prior to use) in order to avoid the loss of volatile constituents.

Hydrodistillation. The hydrodistillation apparatus was assembled using a Clevenger apparatus and a condenser. (100 g needles per 150 mL of water; 3 h of distillation time)

Solvent extraction. The solvent extraction was carried out with Buchi extraction system B-811 (BÜCHI Labortechnik AG, Flawil, Switzerland). Solvent extraction was done only on pine needles. (20 g of pine needles, 2.5 h of extraction time; solvents: hexane/toluene).

Mass spectrometry. All essential oil samples were analysed with a 12.0 T solariX XR FT-ICR mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with Paracell ICR cell and Apollo-II ESI and APPI ion source. Both negative mode ESI and positive mode APPI ionization techniques were used to measure the samples. For the (-) ESI measurement, 10 µL of each of the samples were diluted with 190 µL of methanol (1:20 v/v). Injection of the dilute solution was done with a syringe pump at a flow rate of 2 µL/min. For the (+) APPI measurement 10 µL of the sample was diluted with 80 µL methanol and 10 µL toluene mix (8/1 v/v). The flow rate was 4 µL/min. Dry nitrogen was used as the drying and nebulizing gas and the drying temperature was 220°C. FT-ICR spectra was first calibrated externally using sodium trifluoroacetate for ESI and APCI-L tuning mix for APPI. Compass fmsControl software was used for the data acquisition and the spectra were analyzed with Data Analysis 4.0 software (Bruker Daltonik GmbH, Bremen, Germany).

Results. Negative-ion ESI FT-ICR MS analysis of the essential oils samples revealed their complex nature. The spectral fingerprints were unique for each tree species (Figure 1). The solvent extracts of pine were distinct from the hydrodistillation products. The main compounds detected were different acids, esters and terpenoids. Given the non-polarity of the solvent used, lipophilic compounds dominated the extracts. The extractive consisted of resin acid, free fatty acid, diterpenyl aldehyde, free sitosterol, and diterpenyl alcohol. The major component of both solvent extracts were resin acids and the most abundant compounds were labdane-type secondary metabolite. The extracts were dominated by pinifolic acid and its derivatives (Figure 2).

Positive-ion APPI FT-ICR MS analysis generated both the radical M+ and protonated [M+H]+ ions. It targeted non-polar species and it preferentially ionized the terpenoids and their derivatives. The hydrodistilled oils were mainly composed of terpenes (mono- and sesquiterpenes) while resin acid were the most abundant compounds in the solvent extracts. APPI provides complementary data to that of ESI.

Conclusions. Essential oils obtained from conifer needles are complex mixtures of terpene hydrocarbons and their derivatives, acids, esters and phenolic compounds. High-resolution ESI/APPI FT-ICR provides a straightforward means to obtain comprehensive, semi-quantitative chemical fingerprints of essential oil samples.

References:
2 Lubbe, A.; Verpoorte, R., Ind. Crops Prod. 2011, 34, 785-801
4 Motiejūnaitė, O.; Dalia Pečiulytė, D., Medicina (Kaunas) 2004, 8, 787-794.
Comparison of atmospheric pressure chemical and photo ionisation for evolved gas analysis high resolution FT-ICR MS for petroleum-derived material

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Introduction. For the last decades, atmospheric pressure ionisation (API) techniques raise in importance for state-of-the-art mass spectrometric analyses, and non-targeted analyses often need to cover a broad chemical space. The ionisation in API sources is most often based on a complex reaction cascade and ionisation can be promoted via certain substances like reactions with solvent molecules or dopants in spray-based techniques [1]. In contrast to liquid injection, we apply in this study solvent-free evolved gas analysis coupled to a 7 T Fourier transform ion cyclotron resonance mass spectrometry to investigate the gas-phase ionisation behaviour of complex petroleum-derived mixtures with different API techniques. [2,3]

Materials and methods. In this study, atmospheric pressure photo ionisation (APPI) utilizing a Xenon lamp with photon energies of 8.4/9.6 eV was deployed and compared to classical APPI utilizing a Krypton lamp with photon energies of 10/10.6 eV as well as atmospheric pressure chemical ionisation (APCI) utilizing a steel corona needle at low µA current. Samples were introduced as evolved gas from coupling of our gas phase API source to a thermo balance via a heated transfer line and interface (300 °C). Samples were evaporated/pyrolyzed using a temperature program from 20 to 600 °C with 10 K/min. The mass spectrometric analysis was conducted on a Bruker apex II ultra FT-MS equipped with a 7 T superconducting magnet. This allows for an elemental composition assignment and in-depth analysis of the occurring effects. Besides standard mixtures consisting of fatty acid methyl esters (FAME) and poly cyclic aromatic hydrocarbons (PAH), various petrochemical samples, such as defined distillation cuts, a light crude oil, marine gas oil and bitumen, were investigated.

Results. Analysis of the elemental compositions reveals that APCI produces protonated ions for each compound class and that it can also ionize low double bond equivalent (dbe) species more efficient than the APPI-techniques. APPI-Kr shows radical cations and protonated species, especially for low dbe species. APPI-Xe mostly shows radical cations and high dbe species are pronounced in ionisation. For APPI-Kr and APPI-Xe, a clear shift of the distribution between protonated and radical cations is observed for heavier petroleum samples. Although the lower energy flux Xe-APPI will result in a lower ionisation efficiency and higher limit-of-detection, it might be useful for investigations where sensitivity is not the most important aspect.
Because of producing mostly radical species, Xe-APPI leads to a further simplification of the spectra, which is of high interest for ultra-complex samples, such as in Petroleomics.

Stacked dba resolved compound classes for a light crude oil blend.

References

Using ECD to probe salt-bridges in phosphopeptides in conjugation with TWIMS and molecular modelling

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Introduction. Phosphoproteins are implicated in a wide range of diseases because many act as biomolecular switches in cascades that lead to disease when they become dysregulated. The analysis of phosphoproteins and phosphopeptides by electron capture dissociation (ECD) is complicated by the presence of salt-bridges reducing the number of fragments detected. We propose that this reduction in sequence coverage is due to salt-bridges in the molecule being maintained through the ECD process and preventing the cleaved molecule dissociating into two fragments. In order to investigate whether this effect can be used to propose candidate structures for biologically relevant peptides, we have derived an 18 residue model peptide from the sequence of GSK3β, a protein of mass 46,744 Da implicated in a number of neurological diseases.

Materials and methods. ECD experiments were performed on a solarix-XR mass spectrometer (Bruker, Bremen), and ion mobility was performed using a Synapt G2-S mass spectrometer (Waters, Wilmslow). Amino acid residues for molecular modelling experiments were built in Avogadro with an acetyl-glycine cap on the N-terminus and an amidated glycine cap at the C-terminus, then optimized using Gaussian 09 HF 6-31G (d,p). The charge distribution of the residue was calculated and the residue converted to a .off library, containing other amino acid residues with the same features, using the preppep, parmchk2 and tleap AMBER utility programs. Simulated annealing experiments were performed in the AMBER suite using the phosaa10 and ff99SB forcefields. Collision cross sections (CCS) for each molecular model were calculated using IMoS.3 The peptide Ac-QLVRGEPNVS(pY)I(acetamideC)SRYYR-Am was synthesized by GenicBio and reconstituted in 59.5:39.5:1 acetonitrile:water:formic acid, before being introduced to the mass spectrometer by nESI.

Results. A workflow was developed to combine molecular modelling, ion mobility and ECD data in order to propose candidate structures for phosphopeptides (Figure 1). The structure of the
phosphopeptide is modelled by simulated annealing (n=25,000), with each structure having its CCS (collision cross section) simulated. Simulated CCSs are compared to the experimental CCS derived from a TWIMS experiment, with those structures whose CCS does not match the experimental value being discarded. The remaining structures are clustered and the structure closest to the centroid of each cluster is proposed as a candidate structure for the peptide under the experimental conditions. The salt-bridge patterns of these candidate structures are compared to the proposed salt-bridges from the ECD fragmentation pattern.

The ECD fragmentation pattern of the peptide Ac-QLVRGEPNVS(pY)(acetamideC)SRYYR-Am (Figure 2.) shows no fragment coverage between the R4 and pY11 residues, and the R15 and pY11 residues, leading to the proposal of salt-bridges between these residues. Lower than expected fragment coverage at the C-terminal end of the peptide suggests that there may be some conformations in which the R18 residue participates in a salt-bridge with the pY11 residue.

The results of subjecting Ac-QLVRGEPNVS(pY)(acetamideC)SRYYR-Am to the workflow (Figure 3.) suggest 4 possible salt-bridging patterns: R4-pY11 and R15-pY11; R4-pY11 and R18-pY11; R15-pY11 and R18-pY11; and R4-pY11, R15-pY11 and R18-pY11.

Conclusions. We have developed a workflow for comparing ECD fragmentation patterns to TWIMS-derived CCSs and molecular modelling, the results of which broadly support the proposed salt-bridges from the ECD fragmentation pattern of Ac-QLVRGEPNVS(pY)(acetamideC)SRYYR-Am.

References.
1 M. J. Frisch et al., 2016, Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT.
2 D. A. Case et al., 2017, AMBER 2017, University of California, San Francisco.