



Application of Mass Spectrometry to Speciation Analysis in the Life Sciences

Arranged by the Automation and Analytical Management Group
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Thursday 20th September 2007

**At the Society of Chemical Industry (S C I)
Belgrave Square, London**

Email: conference@aamg-rsc.org
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**Application of Mass Spectrometry to Speciation Analysis in the Life Sciences
Conference with Exhibition**

**Thursday, 20th September 2007
At The Society of Chemical Industry (S C I)
London**

- 10:00 **Registration and Coffee**
- 10:30 *Introduction and Welcome*
Mike Sargent
L G C Limited, Teddington
- 10:40 *The Role of Selenium in Cancer Prevention and Treatment*
Simon Joel
Institute of Cancer and the CR-UK Clinical Centre, London, UK
- 11:10 *Qualitative and Quantitative Approaches via ICP-MS for Problem-Solving in Heteroatom-Tagged Genomics and Proteomics*
Maria Montes-Bayón,
University of Oviedo, Oviedo, Spain
- 11:40 *Elemental Speciation – A Two Pronged Attack*
Chris Harrington
University of Leicester, Leicester, UK
- 12:10 *Mass Spectrometry for Selenium Speciation Analysis in the Life Sciences*
Heidi Goenaga-Infante
L G C Limited, Teddington, UK
- 12:45 Lunch and Exhibition
- 14:00 *Selenium Species in Foods and Supplements*
Margaret Rayman
University of Surrey, Guildford, UK
- 14:30 *Importance of Speciation in Plant Physiology – Key Analytical Applications*
Jórg Feldman
University of Aberdeen, Aberdeen, UK
- 15:00 *Advances in ICP-MS Speciation Analysis*
Ed McCurdy
Agilent Technologies, Santa Clara, CA, USA
- 15:30 *Elemental and Organic Mass Spectrometry as Complementary Techniques in Proteomics and Life Science*
Shona McSheehy
Thermo Fisher Scientific, Bremen, Germany
- 16:00 Close

ABSTRACTS

THE ROLE OF SELENIUM IN CANCER PREVENTION AND TREATMENT.

Simon Joel

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ABSTRACT

Selenium is a trace element that is vital to human health. In the cancer setting its role in chemoprevention has received much attention, but recent data from our own group, and others, suggests that selenium may also be important in patients with established cancers. Ongoing laboratory studies have shown that organic selenium compounds can sensitise tumour cells to the effects of cytotoxic drugs, most likely by altering stress responses. Additionally, selenium may also protect normal cells from the toxic side effects of chemotherapy. An important part of our own work, in collaboration with LGC, has been the development of methods that allow the quantitation of intracellular selenium species. These methods are not only important in elucidating which selenium species may be mediating the effects observed in the lab, but will also be useful in planned clinical trials investigating the use of selenium supplementation at the time of chemotherapy.

QUALITATIVE AND QUANTITATIVE APPROACHES VIA ICP-MS FOR PROBLEM-SOLVING IN HETEROATOM-TAGGED GENOMICS AND PROTEOMICS.

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ABSTRACT

In recent years, genomics and proteomics have received great attention from the viewpoints of gene and protein sciences. Genomics and proteomics are indeed the fundamentally important scientific fields, because genes (DNAs and RNAs) contain the genetic information codes to synthesize various proteins. However, genes and proteins cannot be synthesized without the assistance of metalloenzymes containing, for example, zinc or other metals. In these sense, “metallomics” may stand in the same position in scientific significance as genomics and proteomics.

Inductively coupled plasma mass spectrometry (ICP-MS) being recognized as the detector of choice for elemental determinations at trace and ultratrace levels, offers analytical signals directly proportional to the mass of the selected element (of a given biocompound) present in the sample. A remarkable advantage over soft-ionisation methods is that the signal sensitivity is mainly species- or compound-independent. Consequently, elemental ion sources as the ICP may open the door for real absolute quantification of protein traces in complex samples.

Therefore, the status and potential of metal elemental speciation in biocompounds using ICP-MS detection will be illustrated regarding these two different aspects (qualitative and quantitative) in genomic and proteomic studies. In the first case, the interaction between cis-Pt and DNA *in-vivo* and *in-vitro* will be described by HPLC-ICP-MS. Additionally the coupling of Gel Electrophoresis to ICP-MS with on-line isotope dilution will show the capabilities of this system to conduct kinetic studies of such interaction. This is an important pharmacokinetic parameter to optimize in cancer therapy with Pt drugs.

In the second part, the use of iron stable isotope labelling and HPLC-ICP-MS measurements allow accurate determination of several iron status parameters of clinical relevance. These studies, conducted by combination of Fe isotope dilution and isotope pattern deconvolution methods, permit the accurate determination of individual iron-transferrin isoforms as well as serum iron, unsaturated iron binding capacity and transferrin saturation simultaneously.

ELEMENTAL SPECIATION – A TWO PRONGED ATTACK

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ABSTRACT

The determination of the different individual chemical species that comprise the total concentration of a given metal(loid) in a sample has collectively been referred to as: elemental; chemical; or metal speciation. To carry out speciation analysis, a chromatographic separation step such as gas chromatography (GC), high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) is coupled to a suitable detector. The most effective and up-to-date detectors are either based on element-specific detection or mass spectrometry. Recent enhancements in instrumental design have made the coupling or hyphenation of the chromatographic separation, be it HPLC, GC or CE, to the ICP-MS detector much more practicable. Developments in data acquisition and analysis software have improved the automation of sample injection, species quantitation and data reporting. Detection based on molecular mass spectrometry using atmospheric pressure ionisation (API) via either electrospray (ESI) or chemical ionisation (APCI) has also been used for chemical speciation analysis. Unlike with ICP-MS detection, where identification of the analyte is based on retention time matching with an authentic standard using a number of different separation conditions, with API-MS/MS species identification is based on structural elucidation of each species. The advantages and disadvantages of both techniques for speciation analysis will be discussed and a number of applications using both elemental and molecular mass spectrometry with a chromatographic separation common to both detectors, will be highlighted. Another significant advantage of using mass spectrometry is the ability to carry out accurate and precise quantitation, which for the highest accuracy applications involves the use of isotope dilution analysis (IDA). This combined approach to speciation analysis, using elemental and molecular mass spectrometry, will be discussed in the context of the structural analysis and high accuracy quantitation of organometallic environmental pollutants, metalloprotein species and DNA biomarkers, of interest from a toxicological, clinical and nutritional perspective.

MASS SPECTROMETRY FOR SELENIUM SPECIATION ANALYSIS IN THE LIFE SCIENCES

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ABSTRACT

Selenium (Se) is covalently bound in selenocysteine (known as the 21st amino acid in the genetic code), which is incorporated into selenoproteins with functions of relevance to human health. While the nutritionally essential functions of Se are understood to be fulfilled by the selenoproteins, dietary Se can be metabolised to low molecular weight species (e.g. methyl-Se compounds) that have more recently generated interest because of putative anti-cancer effects.¹

Biochemical and clinical evidence indicates that monomethylated selenium metabolites are involved in the anti-cancer role of selenium, and methylselenol (CH_3SeH) is a key metabolite. Precursors of methylselenol, typically methylseleninic acid ($\text{CH}_3\text{SeO}_2\text{H}$) in experimental *in vitro* systems have been shown to induce apoptosis of cancer cells. Moreover, methyl-Se compounds are known to improve the activity of cytotoxic drugs against cancer in pre-clinical models.²

The mechanisms by which Se benefits human health remain unclear. Therefore, speciation (profiling) methods for Se metabolites in food, supplements, body fluids and tissues and cancer-type models are much needed since they will help to determine Se requirements and to study the relationships between Se status and health and disease.³ Such methods will also assist in hypothesis testing and the development of mechanism-based Se status markers for cancer prevention.

This presentation gives an overview of the most relevant advances of mass spectrometry (ICP, ESI, MALDI) in combination with chromatography which enable acquisition of selenium speciation information relevant to nutrition, cancer research and, in general, to life sciences. Important topics such as complexity of Se speciation in biofortified food, pharmaceutical supplements and clinical samples will be critically discussed. Quality control aspects such as stability of the species and quality of the measurements and how they are addressed in the different applications will also be discussed in this section.

[1] M. P. Rayman, *Proc. Nutr. Soc.*, 2005, **64**, 527-542.

[2] K. Last, L. Maharaj, J. Perry, S. Strauss, J. Fitzgibbon, T. A. Lister and S. P. Joel, *Ann. Oncol.*, 2006, **17**, 773-779.

[3] H. Goenaga Infante, R. Hearn and T. Catterick, *Anal. Bioanal. Chem.*, 2005, **382**, 957-967.

SELENIUM SPECIES IN FOODS AND SUPPLEMENTS

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ABSTRACT

Selenium (Se) is an essential trace element of considerable interest owing to its health-related functions, many of which are ascribed to the antioxidant and anti-inflammatory effects of the selenoproteins. It is required for a healthy immune system, for successful reproduction, it has powerful anti-viral effects, and there is considerable evidence for its having a role in cancer prevention. There is a growing appreciation that it is not just the total intake of dietary selenium (Se) that is important to health but that the species of Se ingested may also be important. Se enters the food chain through plants. The biosynthetic pathways involved in Se assimilation by plants and the way in which Se species are metabolized in animals give an insight into the species likely to be present in plant and animal foods. Known data on the species of Se in the food chain and in food supplements will be presented along with their concentrations and the analytical methodology used. The latter is important since identification that is only based on retention time matching with authentic standards must be considered as tentative: for evidence of structural confirmation, fragmentation of the molecular ion in addition to MS data is required. Bioavailability, as normally defined, is higher for organic Se species. Health effects, both beneficial and toxic, have been associated with particular Se species. Potent anti-tumour effects have been attributed to the low-molecular-weight species, *Se*-methyl-selenocysteine and its γ -glutamyl-derivative, found in a number of edible plants of the *Allium* and *Brassica* families which can be metabolised to methyl selenol. The latter can cause cell-cycle arrest, apoptosis induction and angiogenesis inhibition. There remain considerable gaps in our knowledge of the forms of Se that naturally occur in foods. Without adequate knowledge of Se speciation, false conclusions may be drawn when assessing Se requirements for optimal health. Further speciation studies carried out in parallel with human interventions using some of these novel Se compounds are likely to inform the development of functional foods for the benefit of human health.

IMPORTANCE OF SPECIATION IN PLANT PHYSIOLOGY – SOME KEY APPLICATIONS

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ABSTRACT

The exposure to arsenic is correlated with an increased risk to contract skin, bladder and other forms of internal cancer. More than 10 years ago it has been surfaced that millions of people in Asia have been and are exposed to elevated levels of arsenic in their drinking water and suffer increasingly the detrimental consequences. However, the arsenic exposure through food has been neglected in the past decade. It has only recently been revealed that a major contribution of arsenic exposure can come from rice intake – and not only in the arsenic affected areas.

It will be shown in this lecture how speciation can explain how arsenic is taken up from the paddy fields into the rice plant and then translocated into the shoots and grains and how it accumulates in the different parts of the crop. Here state-of-the-art mass spectrometry is coupled simultaneously to liquid chromatography (HPLC-ICP-MS/ES-MS) in order to determine the different arsenic species in the soil porewater as well as in the plant and their interactions with peptides such as phytochelatins. Additionally X-ray absorption spectroscopy (XANES) have been used to complimentary supports the identification of the molecular form of accumulating arsenic in the plant.

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- A. Raab, P.N. Williams, A.A. Meharg, J. Feldmann, Uptake and translocation of arsenic species by plants, **Env. Chem.** (2007) 4, 197-203.
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- J. Feldmann, What can the different current detection methods offer for element speciation ?, **Trends in Analytical Chemistry**, (2005) 24, 228-242
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APPLICATION OF LASER ABLATION ICP-MS TO MULTI-DIMENSIONAL IMAGING OF BIOLOGICAL TISSUES

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ABSTRACT

In order to understand the biology of a living organism, one must understand not only the complete complement of cellular components in the given biological system and how they interact, but also their spatial distribution. In typical proteomics and/or metabolomics studies today, tissue samples are lysed followed by analysis of their components. Hence, the important information about their exact localization within the biological system is lost. That's why imaging mass spectrometry is such an important technology. In this work, laser ablation ICP-MS is used in raster scanning mode to scan thin sections of biological tissues and organs for both metallic and non-metallic elements of interest. The resulting data is used to generate 2 or 3 dimensional images mapping the relative elemental composition of the sample sections. This technique has been termed Metal Imaging Mass Spectrometry (MIMS) by its developers. These elemental maps can be compared with comparable maps generated by electron microscopy or other MS imaging techniques to gain a more complete picture of the role of metals and certain non-metals in biological function. Since approximately 30% of all proteins contain metals, MIMS promises to be a powerful tool in the understanding of the role of metals in the metabolism of both normal and diseased cells and tissues. Preliminary results showing MIMS images of normal and diseased tissues in rats and humans will be presented.

ELEMENTAL AND ORGANIC MASS SPECTROMETRY AS COMPLEMENTARY TECHNIQUES IN PROTEOMICS AND LIFE SCIENCE

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ABSTRACT

Modern proteomics and life science studies increasingly try to incorporate a degree of quantitative information as well as qualitative to offer the best understanding of a system under investigation. Organic mass spectrometers (MS) provide the necessary molecular information for identification but often, the quantitative abilities of organic MS are limited, whether due to interfering matrix or the low levels of analyte. Where organic MS fails, elemental MS can often offer an alternative. As well as quantitative information, elemental analysis can provide elemental and stoichiometric information. Metal tagging is a recently developed technique which clearly demonstrates this ability and allows analysts to determine proteins at levels which would otherwise be undetectable by organic MS. Bio-molecules which naturally contain or accumulate elements also benefit from the complementarity of organic and elemental MS. For example, the ability of yeast to incorporate and accumulate metals (e.g. Cr, Zn, Se) from enriched media is a well known phenomenon. Moreover, yeast has the capacity to metabolize or biotransform metals into bioavailable chemical forms, readily used by the human organism. This has led to many investigations on incorporation mechanisms and metabolic pathways with the ultimate goal of identifying the metal containing species active in human health. In addition to bioinorganic and metalloproteomic fields; diagnostic, forensic and molecular biological studies also benefit from MS for quantification of nucleic acids. These investigations ultimately include both protocols to achieve the maximum of information in molecular biology or medicine. In this presentation some of the aspects of organic and elemental MS will be shown exemplified by some recent research in various life science themes.