# Integrated mass spectrometry in (semi-)metal speciation and its potential in phytochemistry

Juris Meija, Maria Montes-Bayón, Joseph A. Caruso, Alfredo Sanz-Medel

**Despite the advances in modern molecular biology, dissecting the molecular mechanisms of metal and semi-metal transformations in plants is still among the greatest challenges in phytochemistry. The use of complementary atomic (elemental) and molecular techniques is often required for complete characterization of the interactions of (semi-)metals with plant biomolecules. Thus, hyphenated techniques that use inductively coupled plasma mass spectrometry (ICP-MS) for an element-specific detector coupled to liquid or gas chromatographic separations have been applied for studying (semi-)metal–biomolecule interactions in plant tissues. We give numerous examples to demonstrate the role of integrated molecular and atomic MS in advancing basic chemical knowledge in phytochemistry.**

*Keywords:* Element-specific detector; Gas chromatography; Inductively coupled plasma; Liquid chromatography; MS; Metal; Phytochemistry; Semi-metal

**Juris Meija, Joseph A. Caruso**
Department of Chemistry,
University of Cincinnati,
Cincinnati, OH 45221-0172,
USA

**Maria Montes-Bayón,**
**Alfredo Sanz-Medel***
Department of Chemistry,
University of Oviedo, c/Julian
Claveria 8, 33006 Oviedo,
Spain

## 1. Introduction

Although advances in modern molecular biology allow numerous manipulations with plant genes, we still lack the most fundamental knowledge of ionomics and metallomics in plant tissues. Thus, the dissection of the molecular mechanisms involved in (semi-)metal hyperaccumulation in land plants is one of the biggest challenges in molecular biology. Understanding the molecular mechanisms of (semi-)metal uptake by plants has the potential to transform societies and phytoremediation of polluted soils is a prime example. For example, arsenic uptake by rice has great implications for East Asia, but understanding these processes at the molecular level is only beginning [1].

A biochemical approach to solving such problems involves identifying genes involved in the accumulation process that can also provide valuable genetic resources for the future development of plants ideally suited to phytoremediation. In this regard, molecular approaches are increasingly used to provide new insights into the role of metal transporters and assimilation enzymes in the uptake and the metabolism of (semi-)metals. An alternative approach to the metabolism of an element in plant tissues is to study the metabolic pathway of (semi-)metals through elucidation of their complexes with bioligands and their compartmentalization within the plant cells. For this purpose, the joint research effort of analytical chemistry and plant physiology provides fruitful results in molecular characterization of metallocomplexes in plant cells (so-called "plant metallomics").

In this regard, most scientific contributions so far have dealt with evaluating the phytotoxicity of a certain element in plant development. As is well known, for non-essential elements, plants have evolved homeostasis mechanisms to maintain low cellular concentrations of free ions. Frequently, such mechanisms involve the induction of bioligands that generally bind to the toxic species, diminishing its harm to plant growth. Consequently, monitoring the physiological changes associated with toxic-element uptake and metabolism is difficult. Also, acquiring direct evidence of their complexation and intracellular compartmentalization is a challenge to modern bioanalytical chemistry. Although an increasing number of publications address the capabilities of analytical tools in studying the effect of certain genetic modifications in the metabolism of metals and metalloids in plants, they are still rare. This is probably due to the limitations of conventional biochemical approaches in terms of specificity and sensitivity. However, the

---

*Corresponding author.
E-mail: asm@uniovi.es

0165-9936/$ - see front matter © 2005 Elsevier Ltd. All rights reserved. doi:10.1016/j.trac.2005.04.003

instrumental developments in organic and inorganic mass spectrometry (MS) techniques over the last decade have enhanced our ability to do such complex studies. Thus, elemental speciation studies, which combine a powerful separation with atomic (elemental) detectors, provide selective and sensitive detection of the (semi-)metals present in the complexes formed in plant tissues.

Trace-element speciation has now become an important facet in current analytical chemistry, resulting in a gain of more biochemical knowledge through integrating atomic and molecular MS techniques [2]. Inductively coupled plasma MS (ICP-MS) could play an enormous role in determining genomic-scale metal profiling in exploring plant ionomics and metallomics [3]; however, many achievements clearly demonstrate the pivotal role of ICP-MS in elemental speciation studies when coupled to various chromatographic techniques. With respect to this, ICP-MS currently is the ultimate technique for selective detection of (semi-)metal-tagged biomolecules [4]. Among the emerging plasma-based MS techniques, laser-ablation (LA) ICP-MS is a rapid, accurate and spatially resolved analytical technique that can be used directly for investigating mass loading of (semi-)metals onto plant surfaces. The spatial resolution capabilities of this technique offer significant advantages over conventional ICP-MS. Moreover, LA-ICP-MS is also able to distinguish between metals located inside biological tissues and those just adhering to the plant surface [5].

For detecting molecules, electrospray ionization MS (ESI-MS), introduced in 1985, combines a soft ionization source with MS detection. The capability of ESI-MS for studying weak, non-covalent interactions between metal cations and organic ligands was recognized almost immediately after the introduction of this technique and a significant number of papers on this subject have already been published. However, this advantage was not commonly acknowledged among analytical chemists until recently, as discussed in the analysis of intact molecules produced during metal accumulation in plant tissues [6]. The new developments in nanospray sources are advantageous since sample consumption is very low. The balance between the ionization efficiency and species stability is a ubiquitous problem in metallobiomolecule analysis.

Matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF-MS) is the most used alternative to ESI. While the advantages of this technique are greater sensitivity and less formation of solvent adducts, it cannot be easily coupled to a chromatographic separation system. Also, the ability to detect non-covalent complex ions in MALDI-MS depends greatly on the matrix chosen. MALDI-MS has had little use in studying coordination complexes of (semi-)metals in plant tissues, although some examples can be found in the literature of (semi-)metals covalently bound to an organic moiety. The structural features of organic compounds are most often elucidated from tandem mass spectra (e.g., ESI-tandem MS (MS$^2$) and ESI-quadrupole (Q)-TOF); however, MALDI/post-source decay is a less expensive alternative to this and its use in peptide structure analysis has been demonstrated [7].

We next briefly summarize how synergic studies in analytical chemistry and plant physiology can address problems on (semi-)metal–biomolecules interactions in phytochemistry.

## 2. (Semi-)metallobiomolecule characterization

Multi-element specificity and accurate quantification are the key features of ICP-MS, whereas molecular weight determination and structural information are those contributed by electron impact (EI), ESI and MALDI techniques. Both elemental and molecular MS, supported by on-line or off-line coupling to chromatographic or electrophoretic separation methods, have created the basis for new structural and/or quantitative insights, demonstrating the analytical excellence of this approach [8].

Molecular biology and analytical chemistry methods do not overlap much in research laboratories. Developments in (semi-)metal metabolism have largely been made through separate accumulation of knowledge. However, it is a becoming fashionable to combine the best of both worlds. For example, in a recent study by Vacchina et al. [9], hyphenated analytical chemistry techniques [high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) coupled to ICP-MS and ESI-MS] are assisted by molecular biology techniques (functional yeast complementation) to offer a new, attractive way of investigating the processes of metal accumulation and metal tolerance in hyperaccumulating plants. 2D-PAGE electrophoresis combined with laser-ablation ICP-MS is another prime example of the advantageous combination of molecular biology and analytical chemistry techniques, allowing for selective visualization of a particular (semi-)metal-containing protein among numerous proteins resolved by a gel.

After the metal–complex species have been detected and identified using chromatography and MS techniques, it is common practice among analytical chemists to simulate the chelation chemistry in isolated ligand–(semi-)metal systems. This approach allows careful exploration of the complexation phenomena in easily controlled conditions. Almost as a rule, all (semi-)metal and biomolecule interactions have been studied using this approach. Among the most common examples are studies of complexes of glutathione and arsenic (using ICP and ESI) [10] or selenium (ICP and ESI) [11], phyto- chelatin (PC) complexes with arsenic (ICP and ESI) [12], mercury [13], cadmium (ESI) [14], mercury complexes with biological thiols (MS) [15] and S/Se chalcogenide-exchange-reaction simulations