ICP MS

Why use an ICPMS in radioecology ?

In the early 1980's the coupling between plasma and mass spectrometry (coupling ICP MS) was initiated for elemental analysis and the inductively coupled plasma mass spectrometer (ICP MS) instrument was introduced in mid-1980 for the analysis of trace metals. Fast, multi-elemental, combining both low detection limits (see Table 1 for examples) and wide linear range, ICP MS is used both in laboratories for research developments and in routine laboratories.

	Elt	DL (ng/L) in ultrapure HNO3		Elt	DL (ng/L) in ultrapure HNO3)		Elt	DL (ng/L) in ultrapure HNO3
7	Li	18	78	Se	35	153	Eu	0,19
9	Be	8,8	79	Br	130	157	Gd	0,23
11	Be	88	82	Se	26	159	Tb	0,07
23	Na	490	85	Rb	0,87	163	Dy	0,2
24	Mg	1,6	88	Sr	0,35	165	Ho	0,06
27	Al	26	89	Y	0,09	166	Er	0,13
28	Si	360	90	Zr	0,17	169	Tm	0,04
31	P	560	93	Nb	0,25	172	Yb	0,33
34	Si	19600	95	Mo	1,1	175	Lu	0,11
35	Cl	4040	103	Rh	0,1	178	Hf	0
39	K	400	105	Pd	3,3	181	Ta	83
44	Ca	21	107	Ag	0,72	182	W	0,09
45	Sc	1,3	114	Cd	0,27	185	Re	1,1
47	Ti	3,7	115	In	0,35	189	Os	2,7
51	V	0,28	118	Sn	0,87	193	Ir	0,53
52	Cr	0,53	121	Sb	1	195	Pt	1,1
55	Mn	0,79	126	Te	5,2	197	Au	0,97
56	Fe	9,4	127	In	20	202	Hg	0,56
59	Co	0,5	133	Cs	0,5	205	Tl	0,71
60	Ni	1,7	137	Ba	0,85	208	Pb	0,29
63	Cu	2	139	La	0,13	209	Bi	0,33
68	Zn	3,1	140	Ce	0,1	232	Th	0,77
69	Ga	0,47	141	Pr	0,07	238	U	0,16
72	Ge	1,3	146	Nd	0,35			
75	As	1,4	147	Sm	0,43			

Table 1: Elements which can be measured and example of detection limits

In the field of radioecology, ICPMS can be used i) to analyze trace metals, analogous to radionuclides or radioisotopes, but also essential metals of which homeostasis can be modified by the presence of radionuclides/radioisotopes, ii) to analyze long life radionuclides (such as uranium, thorium, etc) with a high sensitivity, to determine their isotopic composition or even to speciate them in different media.

What can it be used for?

Many examples of ICP MS measurements are described in the literature. We have chosen in this document to illustrate i) the isotopic measurement of U and ii) the analysis of transient signals in a context of speciation studies of U in a biological medium.

i) Isotope Dilution Mass Spectrometry (IDMS) for $^{238}U/^{235}U$ and $^{234}U/^{235}U$ ratios determination

The IDMS technique involves addition of a known amount (spike) of enriched isotope of the element of interest (U) to the sample. This addition is made prior to sample preparation during which the spiked addition of the enhanced isotope is 'equilibrated' with the sample. By measuring the isotope ratio of the mixture (sample + spike) and knowing the isotopic ratio of the spike, the sample concentration can be calculated. The entire measurement is based upon ratio measurements of one isotope of the element to another. Mass bias was corrected applying an exponential law. The protocol description is:

- Mass scan of the sample to verify the presence of ^{232}Th, ^{236}U and ^{239}Pu; in case of presence of ^{236}U and ^{239}Pu, the analysis is impossible without a further procedure of chemical separation.
- Spike addition for which the ratio $^{233}U/^{236}U$ is known as well as its ^{236}U concentration.
- Measures of ^{233}U, ^{234}U, ^{235}U, ^{236}U, ^{238}U and ^{239}U isotopes.
- Corrections: uranium hydrides through ^{239}U and mass bias by $^{233}U/^{236}U$ ratio of the spike; in case of presence ^{232}Th, it is necessary to add correction of thorium hydrides.
- Calculation of ^{234}U, ^{235}U and ^{238}U concentrations as well as $^{234}U/^{235}U$ and $^{238}U/^{235}U$ ratios.

The quantification limit is estimated to 0.08 $ng.L^{-1}$ for ^{234}U and 2 $ng.L^{-1}$ for ^{235}U.

ii) Transient signal Analysis: elemental distribution over a biological sample

To better understand intake, incorporation, storage and elimination mechanisms and then potentially elucidating associated toxicity of metals (here uranium), the study of U compartmentalization and more specifically the identification of U target biomolecules is a key step. Several methodologies were then set-up to enable the assessment of U speciation in target organs of aquatic species. Due to the sensitivity of the ICP mass spectrometry and the ability of measuring transient signals, coupling with separation methods (LC, GC) is especially suitable.

In the developments presented in the following parts, ICP MS is a powerful tool that enables to map U distribution in proteins from organs of waterborne exposed fish or crayfish, after their chromatographic or electrophoretic separation.

*LC-ICP MS coupling (Results from **Bucher et al Chemosphere 2014**)*

This technique was used to perform a screening of the uranium distribution within a pool of gills cytosolic proteins in a fish model after different waterborne exposure.

The cytosol of gills cells was analysed by Size Exclusion Chromatography (SEC) coupled with UV (280 nm) and Inductively Coupled Plasma - Sector Field Mass Spectrometry (ICP-SFMS; Element XR, ThermoFisher, Bremen, Germany, monitoring ^{235}U, ^{238}U, ^{54}Fe, ^{63}Cu and ^{64}Zn) detectors (**Figure 1**).

SEC column was a Superdex 200 HR (10–600 kDa linear mass separation range, GE Healthcare, Uppsala, Sweden) and mobile phase was 100 mM NH4Ac, pH 7.4 delivered at 0.7 mL min^{-1}. Proteins were quantitatively recovered from the column with a mean recovery of 110 ± 12% (n = 4). However, U recovery ranged only between 10% and 50%.

This recovery was assessed by comparing U amount in the whole eluate (ca. 42 mL) and the corresponding amount in the 100 µL of cytosol injected (ICP MS measurement after acidic digestion). A large amount of U was trapped on the column and may correspond to either unbound cytosolic U or U-biomolecule complexes with a weaker stability constant than the one of U-stationary phase.

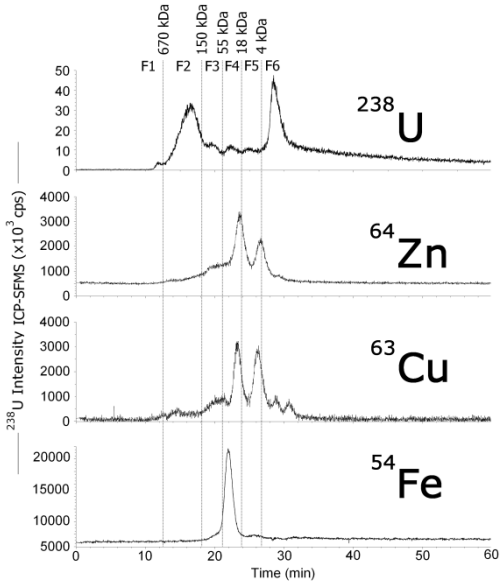

Figure 1: Typical U, Fe, Cu, Zn and UV chromatograms of gill epithelium cytosols (c250-3d sample)

This showed that the recovery is required to be calculated in order to get if the fraction analyzed is representative from the whole sample.

Even that, the results obtained provided new information to identify the distribution of U in the cytosol of cells and gave information on potential interaction and competition with endogenous essential metals (fe, Cu, Zn).

*LA-ICP MS coupling - Uranium quantitative analysis in proteins by LA-ICP MS (Results from **Xu et al ABC 2014**)*

The ND-IEF gel electrophoresis has been investigated for quantitative analysis of *in vivo* U-protein complexes by LA-ICP MS in cytosol of digestive gland of crayfish waterborne exposed to U. These developments were performed in a context of U speciation studies in biological media after waterborne exposure of crayfish.

As the limitations of the LA may be the heterogeneity in the sample and the lack of availability of solid standards, particularly at trace levels, the first step of this work was thus to make standards to assess the concentration of U.

Before quantitative analysis, sampling parameters by laser ablation, *i.e.* laser beam diameter (150, 200 and 250 µm) and scan speed (40, 60, 80, 100, 120 and 140 µm sec^{-1}) of laser, were then optimized for U maximum sensitivity using an *in vitro* U-BSA complex focused on the IPG strips. Then U-protein complexes in digestive gland cytosols of crayfish were quantified by LA-ICP MS utilizing a U-BSA complex standard curve with U concentrations ranging from 0.8 to 5.0 nmol L^{-1} (**Figure 2**). Utilizing the U-BSA standard, the limit of detection (U concentration producing the signal superior to 3 times standard deviation of the number of counts of ^{238}U in the blank electropherogram) was assessed to 19.3 pmol L^{-1} or 4.6 ng L^{-1} (4.8 fmol).

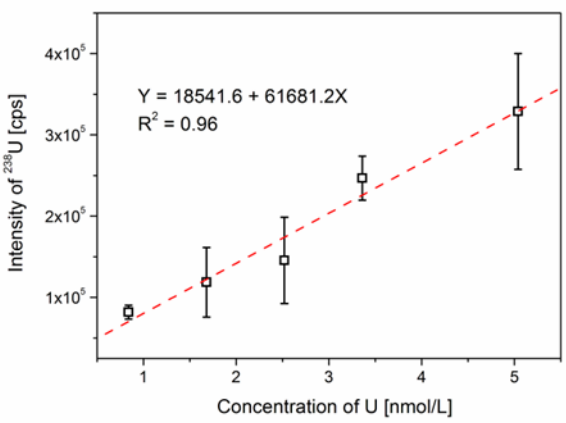

Figure 2: ^{238}U Standard curve for quantitative analysis of U-protein complexes by ND-IEF-LA-ICP MS. For each prepared IPG strip, three parallel scans were carried out by LA-ICP MS.

The cytosol samples were diluted with H_2O to adjust the U concentrations in the concentration range of standard curve. Then the cytosol samples (200 µL) were focused by ND-IEF gel electrophoresis and resulting strips were scanned in triplicate by LA-ICP MS. Finally, the U-protein complexes in cytosol samples were quantified by means of the standard curve. This methodology enabled the quantification of U-protein complexes at 9.03 ± 0.23, 15.27 ± 0.36 and 177.31 ± 25.51 nmol U L^{-1} in digestive gland cytosols of the crayfish, *Procambarus clarkii*, exposed respectively to 0, 0.12 and 2.5 µmol of waterborne depleted U L^{-1} during 10 days.

These results are closed to (<15%) the one found in the same samples after acidic digestion and ICP MS measurement. That validated the methodology and enables to know the recovery of this technique and the amount of uranium at each peak level.

Advantages and disadvantages of using ICPMS, problems associated.

Advantages

ICP MS measurement is extremely sensitive for a large range of elements.

Easy to handle and to make work, an accurate and appropriate optimization enables a concentration assessment in 1h and measurement at 7-8% in 2h depending on the number of elements measured.

The optimization of the signal is mainly related to plasma operating parameters *e.g.* the RF generator power, speed and amount of spray, distance and turn-interface, and to ion lenses settings to focus the signal on isotopes to be analyzed.

For quantitative analysis, most laboratories work by external calibration and then measurement of unknown solutions or by standard addition in unknown solutions (case of complex matrices). These solutions are of various origins *e.g.* some are samples obtained after acidic digestion (case of solid samples), other are liquid samples (water, biological samples); they can be directly analyzed or previously purified on a column. In addition, according to the elements to be analyzed, solutions can be previously acidified. Moreover, the presence of an internal standard is highly recommended in order to follow the stability of the introduction and ionization parameters and to check the signal reduction due to a complex matrix. This internal standard, if different than an isotope from the element of interest, has to be chemically closed to these elements to be analyzed (m/z, ionization potential, pH sensitivity, …) and without any interference.

Disadvantages

Price of equipment

Necessary to count twice to three times the price of ICPAES apparatus for quadrupolar ICPMS that are the most widespread (cost / wide opportunity of analyses / easiness). Other systems are commercially available, mainly magnetic sector systems with one or more detectors (multicollection) but cost again 2-3 times more.

Interferences

For elemental analysis, the spectra obtained by mass spectrometry would be very simple since they come from monoatomic ions and their isotopes. However, the situation is more complex as some spectroscopic interferences exist. They result in an overlap of signals for ions of the same ratio mass/charge; they are divided into 4 groups: isobaric (mainly corrected by equations when the ratios of isotopes is known), due to a double charge (minimized during the optimization of sample introduction parameters), due to oxide formation (also minimized during optimization of sample introduction parameters) and due to polyatomic ion formation (molecule).

Quadrupolar ICP MS have a mass resolution (defined as the ratio M / ΔM) between 0.3-1 amu, preventing the solution of most mass interference previously presented, generated by ionization or the matrix. Sector field systems, can reach a resolution up to 10 000, but with loss of sensitivity. In the early 2000s, to remove the interference due to the formation of molecular species, quadrupolar ICP MS were equipped with collision/ reaction cell. Multipole placed before quadrupole and after the cones and ion lenses, the device can be filled with a single gas or a mixture of gas which removes polyatomic interference by collision (rare gas acting through a physical process) or reaction (reagent gas).

Other interferences, physical or due to matrix can also occur. They are related to the viscosity of the matrix, the charge space effects (light ions are more defocused than heavy ions), changes in the degree of ionization of elements (cancellation of analyte ionization by major elements that ionize more easily).

Drawback of its sensitivity: analysis would always give a result… but…

ICP MS sensitivity requires the analyst to be extremely vigilant with regard to matrices, interference, sample preparations and blanks. Indeed, the problems of signal loss or contamination are really critical at these concentration levels, especially when a large number of elements is investigated.

Work on basal levels and blanks

Some elements (B, I, Th in a less extent) are difficult to rinse; memory effects can be minimized by flushing the system with a rinse blank and regular analysis of reactant blanks.

Depending on the levels needed to be studied, reactants are also very important to check as they contain traces of elements.

Work on matrices

A first assay in a new matrix should start by tests dedicated to i) the determination of interferences, ii) of signal absorption or enhancement, due to the matrix and presence of major cations taking the most part of the plasma energy. The use of internal standard can partly solve the problem.

Salt content is critical and should be less than 0.5 $g.L^{-1}$ for ICPMS analysis. Some increasing spikes of the elements to be analyzed should be analyzed in several dilution of the matrix of interest to characterize the best the signal. In addition, this first characterization of the matrix could be also done by ICP emission spectrometry.

Web links for this technique

http://en.wikipedia.org/wiki/Inductively_coupled_plasma_mass_spectrometry

http://icp-ms.wikispaces.com/ (includes 30 minutes video guide).