Speciation of metals in biomolecules by use of inductively coupled plasma mass spectrometry with low and high mass resolution

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Speciation and metabolization of platinum and selenium in biological systems was investigated by HPLC coupled to low and high resolution ICP-MS. Using size exclusion chromatography more than 90% of the Pt found in a grass sample was present in an inorganic fraction while the remaining 10% occurred in 4 different organic fractions. Selenium was studied in sea-gull eggs by use of reversed-phase HPLC. Six separated Se-organic compounds were observed, the major compounds being selenocystine and selenocystamine.

For trace analysis of toxic elements it is not only the requirement to determine the total content of the element in question at lower and lower levels, but additionally to elucidate the binding state in which it is appearing with the aim to evaluate the ecotoxicity and to follow the pathways of these elements in the environment and their metabolism in biological systems. Speciation of toxic as well as of essential elements is therefore of increasing interest.

For analysis of metals bound to biomolecules usually organic mass spectrometry is applied convincing by high sensitivity and abilities for identification. On the other hand atomic spectrometry has often been applied to determine toxic and essential metals in many different compounds and is more and more used for speciation in most cases as a single element detector with high sensitivity.

Speciation of metals and semi-metals has a long analytical tradition, as has been reviewed recently by Lobinski [1]. For separation of the different inorganic and organic species, chromatographic techniques, especially liquid chromatography (LC) [2], gas chromatography (GC) [3–5], size-exclusion chromatography (SEC) [6] and high performance LC (HPLC) [7–10] have been applied in combination with different detectors. While for gas chromatographic detection a number of different powerful detectors exist, there is a certain lack of sensitive and selective detectors for liquid chromatography. UV-Vis detection, for example, is often applied, but its selectivity as well as sensitivity often are not sufficient, whereas electrochemical detection shows both high sensitivity and selectivity but is applicable only for charged compounds. This is the reason why for LC techniques new detectors are still required. Recently, new techniques based on emission spectrometry are introduced, among which excitation sources as microwave induced plasmas (MIP) or inductively coupled plasmas (ICP) were favoured, partially in combination with hydride techniques. However, in most cases the detection limits were not low enough for determination of all species of interest at trace levels in complex matrices. Therefore improvement of detection limits was in particular expected from application of mass spectrometry.

Inductively coupled plasma mass spectrometry (ICP-MS) was shown to be a powerful detection technique for speciation by LC, HPLC and GC [11–13]. ICP-MS is not only a very selective but also a very sensitive detection technique and it can be coupled to many different chromatography systems in on-line operation. The speciation of metals and of organometallic compounds has been investigated for coupling LC and HPLC to ICP-MS utilizing different sample introduction techniques as to guarantee highest sensitivity. Beside pneumatic nebulization, mainly high efficiency sample introduction techniques such as direct injection nebulization (DIN) [14–16], ultrasonic nebulization (USN) [17] and hydride generation [18] are applied. In contrary to other techniques of atomic spectrometry ICP-MS offers true multielement capability, which can be a versatile tool if metals have to be identified in biomolecules. This shall be discussed here in more detail at hand of two examples, Se and Pt, for which speciation in the environment and metabolization in biological systems is a particular challenge due to several reasons.

The introduction of automobile exhaust catalysts and the use of platinum containing drugs (e.g. “Cis-platinum”) in cancer chemotherapy have considerably stimulated the interest in the analytical determination of this element. It is estimated that nowadays about 2.6 t of Pt per year are emitted by exhaust catalysts in Germany [19]. Furthermore, an average consumption of 25 kg Pt anticancer drugs is estimated to be used per year by each hospital in Germany [20]. As a consequence Pt will appear in the environment by aerosol deposition of Pt-containing particles and by discharge of waste waters. Thus Pt can enter the food chain, either through deposition or uptake from water or waste water sludges used as fertilizers for plants. Concerning the toxicity of Pt, it is well known that salts of the hexa-chloro platinum acid are among the strongest anorganic allergenes, even at ng-levels. Furthermore Pt(II)- compounds, especially Cis-platinum and its analogues, have shown genotoxicity and mutagenic properties in biological tests. To assess health risks for animals and men, speciation of Pt metabolites in the food chain is a new challenge. Single element determination of Pt was therefore investigated in different compartments by use of atomic absorption spectrometry and inverse voltammetry [21]. It was now the aim of a recent study to investigate the metabolization of Pt by simultaneous analysis of further selected elements as bio-indicators. For this

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purpose we have applied size-exclusion-chromatography (SEC) coupled to ICP-MS to get more detailed insight in the metabolization of Pt in grass cultures.

The behaviour of selenium, as the second example, in biological systems is ambivalent. It is known to be essential for plants, animals and men. Deficiency can produce disease as it is well-known from areas with low geogenic selenium levels [22]. On the other hand it may become toxic at higher levels. The fact that there is only a small concentration gap between deficiency and excess, additionally complicated by dependence on the species form [23], stresses the need for an accurate determination of Se with respect to its species. On the other hand Se is of interest because it can reduce the toxicity of heavy metals like Hg by formation of SeHg. This however depends also strongly on the species form and thus on the source from where it is taken [24]. Recently a new impact for application of Se arose from medical science owing to experiences in the therapy for cancer and heart disease [25,26]. Therefore the analysis of inorganic Se and especially of its species is actually of considerable interest for drinking water, food and other systems of biological relevance [27]. For reason of completeness, it should also be mentioned that the metabolization of inorganic selenium species and the transformation to organometallic compounds and their detection in biological systems is the topic of actual research work as reviewed by several authors [28–32]. In a recent study we have investigated LC for separation of Se-organic compounds. By use of Reversed Phase Chromatography (RP) in combination with ICP-MS we have obtained detection limits at 0.1 μg L^{-1} levels for selenocystine, selenocystamine, selenomethionine and selenoethionine [33]. We have now applied this technique for speciation of Se-organic compounds in sea-gull eggs from a German aquatic eco-system, which is contaminated by heavy metals like As, as an example of a real life sample.

For both examples considered, the metals of interest have to be determined in biomolecules with the consequence that for most of the substances involved standard reference materials are still missing. Therefore the mulitelement capabilities of ICP-MS have been used to draw some initial conclusions, because certain elements can be used as indicators for prominent binding partners if biomolecules are investigated. Additionally to Pt other heavy metals like Pb can be set free by combustion engines, and their distribution in plants is important too. Although UV-Vis detection is often applied for the identification of metalorganic compounds a sensitive detector for total carbon is still missing and the same holds true also for S and Ca. Sulphur is an indicator element for many organic compounds like aminoacids and peptides. It is present in many vitamines and encymes. Ca plays an important role as structural component for the plant stroma and bones of animals and men. For all the elements mentioned, except As, Pt and Pb, ICP-MS based on quadrupole mass analyzers is not the best choice, because they are spectrally interferred to a certain extent. Here application of double focusing sector field ICP-MS (ICP-SFMS) equipment operated in high mass resolution - a description of these instruments is given elsewhere [34] - is the method of choice with the additional advantage of improved sensitivity. It is the aim of this paper to demonstrate the capabilities of ICP-MS for speciation analysis of metals bound to biomolecules by use of its inherent multielement capabilities and to demonstrate in particular the usefulness of high mass resolution for this purpose.

Instrumentation

A schematic diagram of the whole experimental arrangement for speciation with ICP-MS is presented in figure 1. The instruments, components and operational conditions are compiled in table I. The chromatographic equipment consists of an HPLC pump (Type 64, Knauer, Berlin, Germany) with a metal free pumping head, a sample loop with a volume of 100 μL (Pt) or 200 μL (Se) and a separation column. Samples were injected with an inert syringe made of plastic (Terumo, Leuwen, Belgium).

Two ICP-MS instruments were used; the operational paramters are compiled in table I. A “VG PlasmaQuad 2+” (VG Elemental, Winsford, UK) with low mass resolution was applied for speciation of Pt and Pb. A prototype of the “ELEMENT” (Finnigan MAT, Bremen, Germany) capable of high mass resolution (HMR) was applied to trace C, S, Ca, As and Se in the chromatograms. As can be seen from table II, these elements are spectrally interferred and therefore cannot be investigated by a quadrupole instrument, except As. Table II shows additionally the resolution setting required to separate the isotope of interest from the interfering molecule. Although high mass resolution is the most straightforward way to overcome problems caused by spectral interferences, it should be mentioned that each increase of resolution results in a reduction of sensitivity. Therefore, as a rule for selection, isotopes with highest natural abundances and with lowest resolution required for separation are always preferred. For this investigation the isotopes compiled in table II have been selected due to the following reasons. For determinatin of C the main isotope at mass 12 leads to over-load of the detector so that ^{13}C was chosen. However, due to interference from the highly abundant signal at mass 14 (N) HMR had to be applied. For Ca the minor isotope at mass 44 was the best choice in terms of signal to noise ratio. Although As can be interferred if Cl is present in the solution, no interference of this kind was observed and an interference by organic compounds contributed only to a

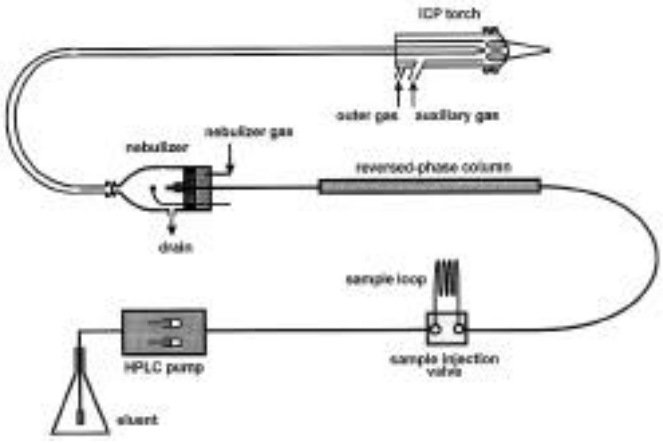

Figure 1. Experimental setup.

Table I. Working conditions of the HPLC-ICP-MS system.

a) Working conditions of the HPLC system for Pt speciation	
Analytical column	Bio-gel SEC 20XL, 300 × 7.8 mm (BioRad, München, Germany)
Flow-rate	0.7 mL min^{-1}
UV-detector	WellChrom K2000 (Knauer)
HPLC pump	HPLC Pump 64 (Knauer, Berlin, Germany)
b) Working conditions of the HPLC system for Se speciation	
Analytical column	Nucleosil 120 Å, C18, 5 µm (Knauer, Berlin, Germany)
Flow rate	1.20 mL min^{-1}
Mobile phase	30 mM ammonium formiate buffer, methanol/water 5:95 (v/v)
pH	3.0
Sample injection volume	200 µL
Sample introduction system	
– nebulizer	Hydraulic high pressure nebulization, (Knauer, Berlin, Germany)
– sample uptake rate	1.20 mL min^{-1}
– pump	HPLC-pump
Desolvation system	
– heating temperature	140 °C
– cooling temperature	first stage −5 °C, second stage −10 °C
c) Working conditions of the ICP-MS system	
PQ2 turbo + (VG Elemental, Winsford, UK)	
– incident power	1400 W
– reflected power	< 10 W
– cooling gas flow	14 L min^{-1}
– auxiliary gas flow	1.7 L min^{-1}
– nebulizer gas flow	0.90 L min^{-1}
– analyser pressure	1.5×10^{-6} mbar
– intermediate pressure	$< 10^{-4}$ mbar
– expansion pressure	2.4 mbar
ELEMENT (Finnigan MAT, Bremen, Germany)	
– incident power	1400 W
– reflected power	≈ 2 W
– cooling gas flow	14 L min^{-1}
– auxiliary gas flow	1.6 L min^{-1}
– nebulizer gas flow	1.0 L min^{-1}
– fore vacuum	4.5×10^{-3} mbar
– high vacuum	1.0×10^{-6} mbar

Table II. Selected isotopes for Pt and Se speciation.

Isotope	*Interfering molecule*	*Required resolution to separate from isotope of interest*
^{13}C	–	
^{32}S	$^{12}C\ ^{1}H_4\ ^{16}O^+$	590
	$^{16}O\ ^{16}O^+$	1800
^{33}S	$^{13}C\ ^{1}H_4\ ^{16}O^+$	570
	$^{1}H\ ^{16}O_2^+$	1260
	$^{16}O\ ^{17}O^+$	1460
^{34}S	$^{18}O\ ^{16}O^+$	1300
^{75}As	$^{12}C_6\ ^{1}H_3^+$	740
	$^{12}C_2\ ^{1}H_3\ ^{16}O_3^+$	870
	$^{40}Ar\ ^{35}Cl^+$	7770
^{76}Se	$^{12}C_6\ ^{1}H_4^+$	680
	$^{12}C_2{}^{1}H_4{}^{16}O_3^+$	780
	$^{40}Ar\ ^{36}Ar^+$	7080
^{77}Se	$^{12}C_6\ ^{1}H_5^+$	650
	$^{12}C_5\ ^{1}H\ ^{16}O^+$	930
	$^{40}Ar\ ^{37}Cl^+$	9180
^{78}Se	$^{12}C_6\ ^{1}H_6^+$	600
	$^{12}C_5\ ^{1}H_2\ ^{16}O^+$	840
	$^{40}Ar\ ^{38}Ar^+$	9970
^{80}Se	$^{40}Ar\ ^{40}Ar^+$	9690
^{82}Se	$^{12}C_6{}^{1}H_{10}{}^+$	510
	$^{12}C_5{}^{1}H_6{}^{16}O^+$	660
	$^{12}C_4{}^{1}H_2{}^{16}O_2^+$	920
	$^{82}Kr^+$	25400
^{195}Pt	–	
^{208}Pb	–	

small blank and therefore the double focusing instrument could be operated in a low mass resolution mode. For determination of Se the isotopes at mass 77 and 82 could be used even for application by a low resolution instrument. The dynamic range, however, is limited due to blank values from interfering Ar dimers and hydrocarbons from the organic eluent. Due to a very low contribution from Kr, which is present in the discharge gas Ar, the best signal to noise ratio has been realized for ^{82}Se if a mass resolution of about 1700 was applied.

For identification purposes of the metal organic compounds an organic mass spectrometer instrument, the LCQ (Finnigan MAT, Bremen, Germany), was used additionally. The chosen working conditions are described elsewhere [35].

Speciation of Pt

For Pt speciation grass cultures have been chosen as a bioindicator as well as a typical entry to the food chain [36]. The grass samples (Welsches Weidelgras) were grown in commercial garden mould. One part of the cultivation grew up as reference without any special treatment; the other part was treated by watering with $[Pt(NH_3)_4](NO_3)_2$. A total amount of 150 mg was applied over a period of 6 weeks and the platinum was taken up only by the roots. The grass was cut when it had grown to a length of 5 − 7 cm, and five cuts were harvested in a sequence. From each grass cut 5 g were triturated with a quartz pestle in a quartz vessel together with 25 mL of an extraction buffer (20 mmol L^{-1} of ammonium acetate adjusted to pH 8.0 with TRIS). The extracts were separated by ultrafiltration in a low and high molecular

weight fraction, and for this investigation only the low molecular fraction < 10 kDa were investigated. Finally the filtrate was lyophilized at −20 °C. For speciation analysis this samples are freshly re-conditioned using 10 mL of the extraction buffer and 100 µL of this solution were injected by use of the sample loop.

For speciation of Pt a large variety of different compounds were expected, which could consist of charged and un-charged species, polar and nonpolar ones and molecules of different hydrophobicity. Therefore size-exclusion chromatography was chosen as separation technique, which separates molecules on the basis of their size only. Additionally it is well-known that there is nearly no interaction of the bed material with the species involved, which is important to conserve the species properties as much as possible. A SEC column (Biogel SEC 20XL, 300 × 7.8 mm, BioRad, München, Germany) with a cut-off < 10 kDa was used for this investigation. For this application it was difficult to find an eluent which fulfills two requirements, best chromatographic separation as well as lowest influence of spectroscopic and non-spectroscopic interferences. Just the latter, the problem of interferences of ICP-MS, was the most stringent requirement, because most eluents caused spectral interferences for a number of elements of interest or non-spectral interferences, which resulted in a loss of sensitivity or in drift effects [37]. This was the reason why NaCl was chosen as a compromise but the maximal concentration was limited to about 25 mmol only due to non-spectroscopic interferences. Aerosol generation for sample introduction to the ICP-MS was performed by a conventional pneumatic nebulizer fitted to a water cooled spray chamber, which was sufficient to detect Pt in all investigated fractions. Before entering the nebulizer of the ICP-MS system, the analyte liquid passed through a conventional HPLC-filter photometer.

Performing the speciation experiment for the treated plant material with the developed procedure, we finally obtained the ^{195}Pt chromatogram shown in figure 2a with the quadrupole based instrument. In this figure a group of 4 minor fractions and a fifth dominating fraction can be discovered. It was known from previous work, that for native untreated grass, Pt is completely bound to a protein of high molecular weight (> 100 kDa) [21], whereas for treated cultivations most Pt occurs in the five fractions shown in this figure. In figure 2b the UV-Vis registration of the chromatogram is shown additionally, from which the conclusion can be drawn that the major fraction, indicated as fraction 5, does not show any absorption in the UV wavelength range chosen, whereas the minor fractions consist of UV active substances. This is a hint for organic molecules, but no proof at all. Only 10% of the Pt is found in these 4 fractions. From the calibration of the retention time it can be concluded that the molecules concerned have molecular weights above 400 but below 800 Da.

In figure 2c the signal of ^{13}C measured in HMR shows, that most carbon is present in the first two fractions. This is a proof for organic molecules. The major fraction (No. 5 in Fig. 2a) however, which represents about 90% of the Pt found in the grass leaves, does not contain any carbon, so that the conclusion is obvious that this fraction consists of inorganic molecules. Comparing the retention time of the original Pt containing watering solution (not shown here), which gives a sharp peak in the chromatogram, confirms that this fraction consists of molecules from the original water-

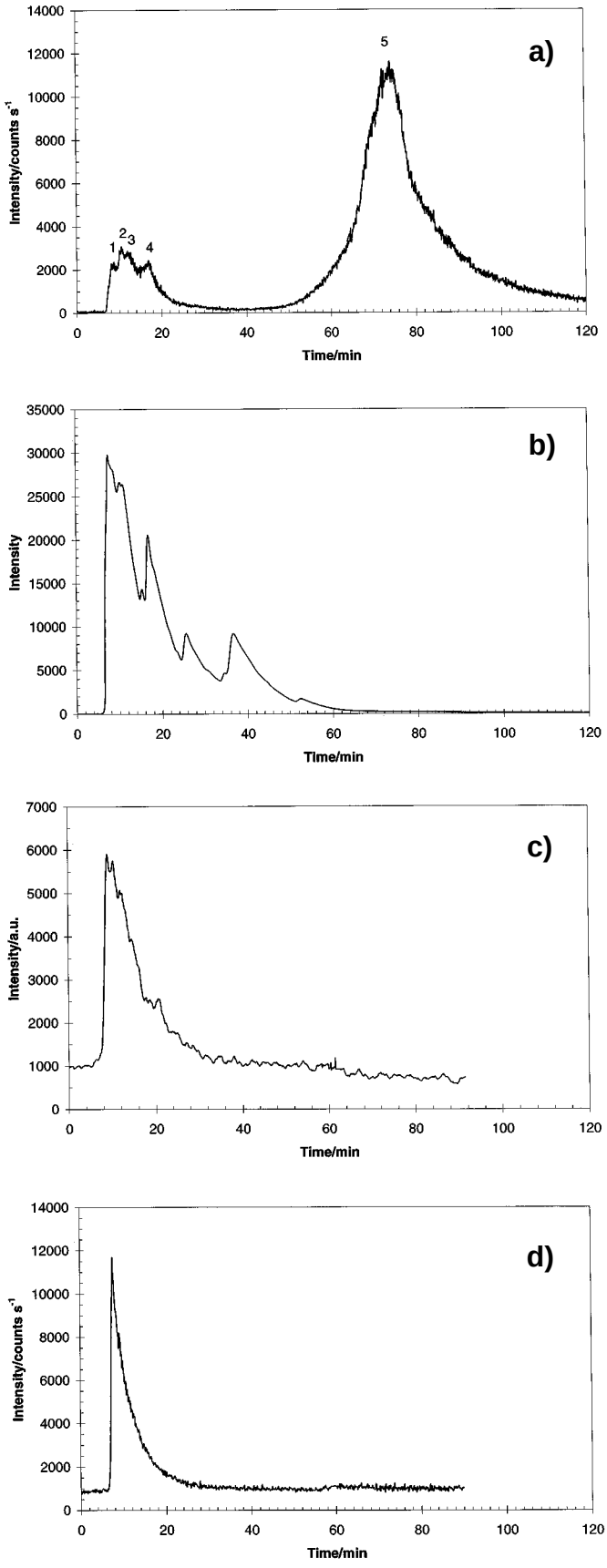

Figure 2. Comparison of different chromatograms from grass extracts obtained by a) ICP-MS for ^{195}Pt; instrument: PlasmaQuad 2+,b) UV absorption, c) ICP-MS for ^{13}C, instrument: ELEMENT; *R* = 3000, d) ICP-MS for ^{34}S; instrument: ELEMENT; *R* = 3000.

ing solution. This is surprising, because an extremely high concentration of 10 μg g^{-1} Pt has been determined in the grass, a level which for heavy metals can cause significant stress reactions. To see, if the reaction mechanism is different from heavy metals, Pb was investigated too. This heavy metal was present in the garden mould at elevated levels and a concentration of about 3 μg g^{-1} has been determined in the grass. Pb was identified in fraction 1 only, so that it can be concluded that Pt chemically reacts in this fraction to a certain extent like a heavy metal. The occurence of three other organic compounds demonstrate that the reaction mechanism of Pt is more complex than that of heavy metals.

Another important feature of the C-signal should be pointed out here. In comparison with the Pt signal a fifth signal is observed in the UV chromatogram. This may lead to the interpretation of an organic molecule, however would be wrong, because the C channel does not detect any organic compound. This shows that an additional detector for total C is a powerful tool for speciation analysis.

For further conclusions high mass resolution had to be applied, as in particular for S. A chromatogram for ^{34}S is shown in figure 2d. A correlation of S exists only in the first fraction with Pt and Pb. For heavy metal stress it is well-known that phytochelatines are produced by the organism for detoxification [38-40]. They consist of a series of γ-glutamylcysteine-dipeptides and the binding with the heavy metal is realized by active sulphydryl groups in the cysteinyle molecule. Although the existance of a S signal in the chromatogram is a hint for phytochelatines, it is of course not a definitve identification.

Another element which has been investigated by application of the double focusing instrument was Ca (not shown here). This element is only correlated to Pt in the second fraction. It should be mentioned that the concentration of Ca in the untreated culture was even higher than that in the treated one. Therefore, it can be concluded that Ca was exchanged by Pt. Owing to Ca deficits in plants as a reaction to metal stress, an exchange mechanism has already been discussed in literature on the basis of poly-galacturonic acids as complexing agents [41]. In poly-galacturonic acids, which are known to be important poly-saccharide components of the plant stroma, the Ca^{2+} ion acts as counter-ion of the carboxylic function and can be exchanged in the presence of an excess of other doubly charged cations. Considering that the retention time for this fraction indicates molecules of low molecular weight, it can be supposed that fraction 2 represents pre-cursors or fragment of macro-molecules on the basis of polygalacturonic acids. Again, the occurence of Ca is not a proof at all, and an independent method is required for verification of this statement. Nevertheless, it is an important hint for further investigations.

Speciation of selenium

For speciation of Se, eggs of sea-gull were chosen because they represent the end of a food chain of the marine ecosystem under actual investigation. After collecting, the eggs of sea-gull were deep-frozen in liquid nitrogen to avoid any chemical alteration. After grinding and homogenization of the deep-frozen samples the resulting powders are divided into sub-samples of about 10 g fresh weight. Half of the sub-samples were extracted by 20 mL of a methanol/water mixture (9:1) over night. After ultrafiltration of the extract and decantation of the above solution the residue was washed 3 times with a methanol/water mixture (9:1). After washing, the remaining solutions were centrifuged for 30 min. The methanol/water mixture was carefully removed by evaporation under reduced pressure and the residue was diluted in 10 mL bidistilled water and stored at +4 °C in the dark. The overall extraction efficiency was about 30%. 200 μL of this extract was injected by use of the sample loop.

Different chromatography techniques such as ion exchange and reversed phase chromatography have been investigated using the above mentioned standards (selenocystine, selenocystamine, selenomethionine and selenoethionine and Se(IV), Se(VI)) as test objects. It was not possible to separate all the species with one column only. Best results for the organic compounds have been realized by reversed phase chromatography and a RP-HPLC column (Nucleosil, 120 Å, C_{18}, 5 μm) was therefore selected for further investigations. As before, the eluent has to be chosen carefully to avoid spectroscopic and non-spectroscopic interferences which are otherwise caused by the organic eluents (see Tab. II). Methanol was chosen as eluent because by keeping its concentration < 10%, the plasma could be operated stable and any clogging of the sampler and skimmer orifice in the interface could be avoided even without addition of oxygen. The Se organic standards served as test compounds, for which the separation conditions were optimized, and for identification. A pH value of 3.0 gave best separations by using an ammonium formiate buffer and finally a mixture of methanol and water with a ratio of 5:95 was chosen as eluent.

For this application highest sensitivity was required and therefore hydraulic high pressure nebulization (HHPN) was applied as a high efficiency sample introduction technique instead of pneumatic nebulization. This helps to improve the sensitivity for most elements by about one order of magnitude in comparison to a Meinhard nebulizer [42,43].

The extract of the sea-gull eggs was diluted in bidistilled water (1:5) to avoid overload of the analytical column. Figure 3a shows a chromatogram of ^{82}Se of the original sample. For comparison of the retention time figure 3b shows a chromatogram of the four Se organic compounds with a Se concentration of 10 ng mL^{-1}. About 6 different peaks in the chromatogram of figure 3a can be observed for Se, but only 2 of them can be identified by reference to the standards. The first Se organic compound which can be identified via its retention time is selenocystine, which is present in the first peak. Standard addition shows that in this fraction a second un-identified Se compound is contributing. It should be mentioned, that this first peak is not resolved from the void volume and therefore the inorganic Se species (IV and VI) could be present in this fraction, too. By application of cation- as well as anion-exchange chromatography no one of them could be detected, so that the conclusion becomes obvious that one or more Se organic compounds are involved. The second compound, selenocystamine, which can be identified without any doubt, is identical with the second peak in the chromatogram. By standard addition a concentration of 7.5 ng mL^{-1} in the solution or 14.1 ng g^{-1} in the sample has been determined. Here one important

advantage of ICP-MS should be pointed out. If standards are available quantification is straightforward realized by standard addition only. Although two of the six Se organic compounds have been identified, it is not clear yet, if these are products of a detoxification process or if they are produced and enriched in the food chain.

In figure 3c the chromatogram for As is shown. Although the main As signal coincidences with the main Se signal in fraction 2, it cannot be concluded that both are bound to the same molecule. By comparison of the retention with As standards it could be shown that several As organic compounds are not resolved in this peak. This is not surprising, because the separation conditions have been optimized for Se speciation and therefore are not optimal for As speciation too. Nevertheless, more than 50% of the signal intensity of this peak can be attributed to arseno-betaine, which is a well-known metabolization product of As found in many organisms.

In figure 3d the chromatogram of S is shown too. As can be seen from this figure most of the S is correlated to the first fraction and therefore is eluted in the void volume, together with selenocystine. Nevertheless a small peak in the tailing can be identified which correlates to the second fraction. It is only small in comparison to the S signal, but it is high in comparison to the As- and Se-signal. It is not surprising that Se is found in correlation to S, because this element behaves very similar in terms of chemical reactions. Further investigations are necessary to clearify if S- or Se-containing glutathione is involved in a detoxification process and to identify the remaining species.

In a preliminary study we have tried to use this HPLC procedure for organic mass spectrometry also for determination of the 4 Se organic standards. The detection limits in this case were about two orders of magnitude worse than required for the real samples without change of the chromatographic parameters. Furthermore different sensitivities resulted from the standards so that it was concluded that the ionization mechanisms depend on the specific substance and may be influenced if a matrix is present.

With respect to this experience, ICP-MS looks promising for speciation of metals in biomolecules if standards are available for identification. In this case also quantification is still straightforward and matrix effects although well-known for ICP-MS are only moderate for both examples.

Conclusion

ICP-MS with low and high mass resolution has been used here as a screening technique to discover metals bound to biomolecules. The examples presented, show that ICP-MS is a powerful tool for speciation of metals even in biomolecules and two main advantages can be seen for ICP-MS. First its extraordinary high sensitivity is convincing which even can compete with organic mass spectrometry. Second the full multielement capabilities are a versatile means to draw conclusions on prominent binding partners of the metals. In many biological systems, elements like C, P, S, Ca and Fe play an important role, however they can only be made accessible for measurement by high mass resolution. Further research is required to improve the extraction

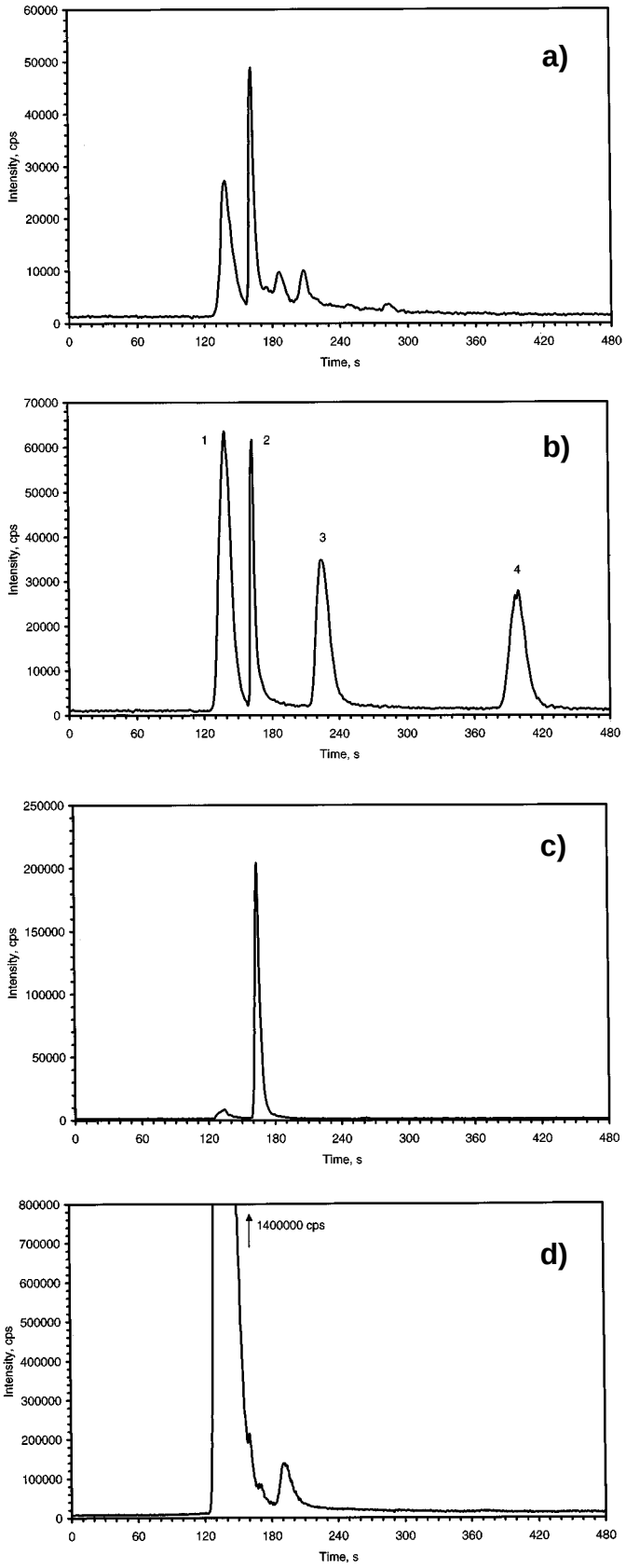

Figure 3. Comparison of different chromatograms from extracts of sea-gull eggs obtained by a) ICP-MS for ^{82}Se; original extract; instrument: ELEMENT; *R* = 1400, b) ICP-MS for ^{82}Se; chromatogram of standards; instrument: ELEMENT; *R* = 1400, c) ICP-MS for ^{75}As; original extract; instrument: ELEMENT; *R* = 300, d) ICP-MS for ^{34}S; original extract; instrument: ELEMENT; *R* = 1700.

process and to check the stability of the species investigated during the whole analytical procedure.

The only disadvantage of ICP-MS for this kind of application is that this technique is only an atomic detector and does not give by itself any molecular information. Therefore standard reference materials are a pre-requisite whenever atomic spectrometry is applied for speciation of metals in biomolecules. As long as such materials are generally not available, both mass spectrometry techniques - organic and inorganic - are complementing techniques, in which the inorganic techniques is a valuable tool to give assistance for both identification and easy quantification.

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