POSSIBILITIES OF THE APPLICATION OF HIGH EFFICIENCY SAMPLE INTRODUCTION METHODS IN TRACE ELEMENT SPECIATION

Summary of the PhD thesis

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INTRODUCTION

Definition and importance of trace element speciation

The effects of trace elements depend not only on the concentration but also on their chemical forms in which they enter into the living organism. In the aspect of the biological effect the given form of a trace element (e.g. oxidation state, organic and inorganic complexes and interactions with organic molecules) is very important. The new field of analytical chemistry, which is suitable for the determination of the different chemical forms of elements, is called speciation analysis. Speciation analysis is one of the most innovative fields of analytical chemistry; this fact is proved by the number of publications on this field that increased ten times in the last decade.

The importance of this scientific area incited the international scientific society to define the concepts of this new field of analytical chemistry (TEMPLETON 2000):

"Chemical compounds that differ in isotopic composition, conformation, oxidation or electronic state, or in the nature of their complexed or covalently bound substituents, can be regarded as distinct chemical species.

i. Chemical species. Chemical elements: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure
ii. Speciation analysis. Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample

Speciation of an element; speciation. Distribution of an element amongst definedchemical species in a system"

The investigated field of speciation and the problems of sample introduction

In the first part of my PhD work I focused on the determination of different organic and inorganic selenium species employing a hyphenated system based on high performance liquid chromatography (HPLC) separation and atomic fluorescence (AFS) detection. The simultaneous determination of the different isotopes of elements was studied in the second part of the present work as an interesting field of speciation. For this latter purpose, an inductively coupled plasma time-of-flight mass spectrometer (ICP-TOFMS) was used.

One of the main difficulties encountered in atomic spectrometry is sample introduction, as the transport efficiency of conventionally used pneumatic nebulizers is in the range of only 2-3%. An

additional problem arising when pneumatic nebulizers are in use is the effects of sample matrices entering into the detector and increasing the possibility of interferences.

The target of the work

The main target of the presented work is to overcome the above mentioned problems of sample introduction. The method development focuses on high efficiency sample introduction techniques, which are suitable not only for the chosen separation method but also for the detector applied in the study.

The requirements of sample introduction methods are as follows:

- High transport efficiency that improves the detection limit and the sensitivity of the detector;
- Selectivity for the analytes of interest along with the elimination of possible interferences;
- Easily hyphenation to the selected separation technique and detector;
- The hyphenation techniques using the developed sample introduction methods should be applicable to the speciation analysis of real samples;
- The developed method should be applicable to the simultaneous determination of elements/analytes of interest reducing running costs and increasing the information gained per analytical runs.

MATERIALS AND METHODS

During my experiments two large parts of speciation analysis were deeply studied. The first one was the determination of inorganic selenium species and selenoamino acids based on HPLC separation and AFS detection. In this part of the work hydraulic high pressure nebulizer (HHPN), hydride generation (HG) and hydride generation combined with thermal digestion (TD) and ultraviolet irradiation (UV) were used as sample introduction methods.

The second part comprised the isotopic composition of elements. In this part of the study an ICP-TOFMS detector was employed combined with HHPN in flow-injection mode and tungsten filament based electrothermal vaporisation (WETV) as sample introduction techniques.

RESULTS

HPLC-HHPN-AFS

- I was the first to publish in analytical communications an HPLC-HHPN-AFS system for the speciation analysis of three selenoamino acids (SeMet, $SeCys_2$ and SeEt), and two inorganic Se-species, selenite and selenate.
- I determined the analytical performance of the system and provided a critical overview of the limits of application of the system developed for speciation analysis.
- The background problem – that I described first – encountered in case of complex sample matrices when using HHPN sample introduction and AFS detection requires background correction.
- Through the lack of certified reference materials and independent analytical methods the method was validated by a spiking procedure. The results confirm that the method is suitable for selenium speciation of food samples.

HPLC-HG-AFS

- I developed a hydride generation method that does not require any pre-treatment steps for the determination of selenoamino acids. The method had not been published before in scientific papers. However the formation of volatile selenium species does not reach 100 %, the transport efficiency of the method exceeds those of the commonly used pneumatic nebulizers.
- I described a method in the literature first time for the quantification of the rate of generated volatile selenium species. By applying this method I determined the volatilisation rate of the species first time as well.
- The method was found applicable to the analysis of real samples, which was demonstrated by the speciation analysis of a selenium-containing food supplement.

HPLC-TD-UV-HG-AFS

- As a final step of the method development based on HPLC separation and AFS detection, a process including thermal digestion and ultraviolet irradiation was combined with hydride generation in order to improve the volatilisation of selenium species. Using this

sample introduction method a considerable improvement of LODs was observed compared to the methods described before.

- I showed through the example of Brazil nuts that the method is suitable for the analysis of seleno-cystine, seleno-methionine, selenite and selenate in food samples.

WETV-ICP-TOFMS

- I described a novel combination of tungsten based electrothermal evaporation (WETV) and ICP-TOFMS. The system combines the high transport efficiency of WETV and the suitability of ICP-TOFMS for the simultaneous detection of large number of analytes during a fast transient signal.
- The developed method is suitable for the simultaneous determination of 17 elements. The LODs are in the ng-pg ml^{-1} range.
- I confirmed that isotope ratio determination could be completed with high precision using the WETV-ICP-TOFMS system.
- I confirmed that some interferences can be eliminated with the developed system, e.g. $^{56}ArO^{+}$ on $^{56}Fe^{+}$ and $^{52}ArC^{+}$ on $^{52}Cr^{+}$.

FI-HHPN-ICP-TOFMS

- The combination of HHPN and ICP-TOFMS in flow-injection mode resulted an analytical system suitable for the determination of large number of analytes (19 elements) with good detection limits.
- I showed that the accurate optimisation of the desolvation unit could reduce the interferences caused by oxides in plasma.
- The method was validated against certified reference materials (CRMs) of biological origin. The results showed good agreement with the certified values of the CRMs applied.

Scientific results

1. A speciation system based on HPLC separation and AFS detection was developed that is capable to be coupled to different sample introduction methods depending on the quality and quantity of the species of interest. I showed that the method development had improved the analytical performance of the system and had resulted in the theoretically best LODs.
2. In connection with the different sample introduction methods used in the HPLC-AFS system I established that:
    a) The HHPN can be easily connected to the AFS detector, but the hyphenation is not sufficient from analytical point of view. The system suffers several limitation e.g. pure limits of detection, unsuitable robustness against sample matrix. The signals of analytes depend on the sample matrix, therefore a background correction is required, which is not usual in the case of AFS detection.
    b) Volatile selenium species can be generated from selenoamino acids without any pre-treatment procedure. I determined – first time in the scientific literature – the volatilisation efficiency of different selenium species.
    c) The ultraviolet irradiation improves the volatilisation efficiency of selenoamino acids and slightly influences that of the inorganic selenium species.
    d) The atomic fluorescence signal of different selenium species depends on the quality of the species. This fact can be explained with the different volatilisation efficiency and atomisation properties of the species.
3. The HPLC-AFS system using the different sample introduction methods is applicable for the analysis of the five frequently investigated selenium species (SeMet, $SeCys_2$, SeEt, selenite and selenate). Failing certified reference materials the methods used for the analysis of food samples (e.g. mushroom, brazil nut, food supplement) were validated by applying standard addition and spiking for the determination of the accuracy of the methods.
4. In connection with the different sample introduction methods connected to ICP-TOFMS I established that:
    a) The tungsten based electrothermal vaporizer is an ideal sample introduction method for ICP-TOFMS; high transport efficiency, low sample volume demand and fast analysis are the characteristic features of the developed system.

b) Using the HHPN a considerable improvement of LODs was observed compared to other sample introduction techniques (e.g. conventional nebulization, hydride generation). Through the high efficiency of the HHPN the system can be used in flow-injection mode, which improves the sample throughput.

c) I confirmed that some interferences can be eliminated with the help of WETV and HHPN systems, e.g. the $^{56}ArO^{+}$ on $^{56}Fe^{+}$ and the $^{52}ArC^{+}$ on $^{52}Cr^{+}$ in case of WETV sample introduction; furthermore, the interferences caused by oxides in plasma can be reduced through the accurate optimisation of the desolvation unit of the HHPN.

5. I confirmed that ICP-TOFMS is an ideal detector for simultaneous determination of large number of analytes during fast transient signals, where the number of analytes does not influences the precision of the measurement.

List of publications

Hungarian publications

Scientific papers

1. **Stefánka Zs**., Fodor P., Szelén specieszek meghatározása csiperkegombából. Anyagvizsgálók Lapja, 12, 117, 1999
2. Ipolyi Ildikó, **Stefánka Zsolt** és Fodor Péter, Módszerfejlesztés szeleno-aminosavak és szelén(IV) közvetlen hidridfejlesztéssel történő meghatározásához, Magyar Kémiai Folyóirat, 107, 31-35, 2001
3. **Stefánka Zsolt,** Fodor Péter, Nyomelem meghatározás vízmintákból – Egy jártassági vizsgálat eredményei, Anyagvizsgálók Lapja, 21-23, 2002
4. **Stefánka Zsolt**, Dernovics Mihály, Ipolyi Ildikó, Mátyás András, Abrankó László és Fodor Péter, HPLC-UV-HG-AFS módszer szeleno-aminosavak és szervetlen szelén specieszek meghatározására, Anyagvizsgálók Lapja, 13, 23-27, 2003

Conference contributions

1. Száraz L., **Stefánka Zs**., Divinyi Zs., Vitányi Gy., Lelik L., Maráz A., Fodor P., Bartha E., Pais I., Titán kötődésének vizsgálata saccharomices cerevisiae sejtek RNS bázisaihoz, poster pres., Vas Károly Tudományos Ülésszak, 1996
2. Ipolyi I., **Stefánka Zs**., Fodor P., Multielemes speciációs rendszerek fejlesztése arzén és szelén módosulatok szimultán meghatározására, 43. MSV Kiadványa (Zalaegerszeg), oral pres., 51, 2000
3. **Stefánka Zs**., Ipolyi I., Fodor P., Módszerfejlesztés szeleno-aminosavak és SeIV meghatározásához direkt hidridfejlesztéssel HPLC-HG-AFS rendszeren, 43. MSV Kiadványa (Zalaegerszeg) oral pres., 96, 2000
4. Dernovics M, **Stefánka Zs**, Ipolyi I és Fodor P, Mintaelőkészítési módszerek összehasonlítás szelénnel dúsított csiperkegomba módosulat-analitikai vizsgálatához, 44. MSV (Baja), oral pres., 29, 2001
5. **Stefánka Zs**, Dernovics M, Ipolyi I és Fodor P, Mintabeviteli technikák összehasonlítása biológia minták szelén speciációjánál, 44. MSV (Baja), oral pres., 106, 2001
6. Abrankó L, Ipolyi I. **Stefánka Zs** és Fodor P., Az induktív csatolású plazma repülési idő tömegspektrometria (ICP-TOFMS) analitikai alkalmazhatósága, 45. MSV (Siófok), oral pres., 2002
7. **Stefánka Zs**, Körelemzések kiértékelése, Magyar Kémiai Egyesület Analitikai Napok, 23, oral pres., 2003

International publications

Scientific papers

1. Ipolyi I., **Stefánka Zs**., Fodor P., Speciation of Se(IV) and the Selenoamino Acids by HPLC-direct HG-AFS System, Analytica Chimica Acta 2001, 435, 367-375
2. **Zs. Stefánka**, I. Ipolyi, M. Dernovics and P. Fodor ,Comparison of sample preparation methods based on proteolytic enzymatic processes for Se-speciation of edible mushroom **(***Agaricus bisporus)* samples, Talanta 55, (2001), 437-447
3. M. Dernovics, **Zs. Stefánka** and P. Fodor, Improving selenium extraction by sequential enzymatic processes for Se-speciation of selenium-enriched Agaricus bisporus, Anal. Bioanal. Chem. 372, (2002), 473–480
4. N. H. Bings, **Zs. Stefánka**, S. R. Mallada Flow injection electrochemical hydride generation inductively coupled plasma time-of-flight mass spectrometry for the simultaneous determination of elements forming hydride, Analytica Chimica Acta, 479, (2003), 203-214
5. E. T. Bodó, **Zs. Stefánka**, I. Ipolyi, Cs. Sörös, M. Dernovics and P. Fodor, Preparation of Laboratory Reference Material (LRM) for selenium speciation, Anal. Bioanal. Chem. (accepted for publication)

Conference contributions

1. Ipolyi I., **Stefánka Zs**., Fodor P., Simultaneous Se and As Speciation Using an HPLC-HG-AfS System 10th Biennial National Atomic Spectroscopy Symposium (Sheffield), poster pres., **222**, 2000
2. Ipolyi I., **Stefánka Zs**., Fodor P., Possibilities of simultaneous Se and As speciation using HPLC-AFS system, 4th Euroconference on Environmental Analytical Chemistry (Visegrád), oral pres., 82, 2000
3. Ildikó Ipolyi, Mihály Dernovics, **Zsolt Stefánka**, Péter Fodor, Investigation of matrix effects occuring during the Se speciation of *Agaricus bisporus* with HPLC-HHPN-AFS system., European Winter Conference on Plasma Spectrochemistry (Lillehammer), poster pres., 44**,** 2001
4. **Zs. Stefánka**, I. Ipolyi, M. Dernovics, P. Fodor, Comparison of sequential sample preparation methods for Se-enriched *Agaricus bisporus,* Second International Conference on Trace Element Speciation in Biomedical, Nutritional and Environmental Sciences (Munich-Neuherberg), oral pres., 48, 2001
5. P. Fodor, I. Ipolyi**, Zs. Stefánka**, M. Dernovics, New directions and results on speciation analysis at the Department of Applied Chemistry of the Szent István University, X. Hungarian-Italian Symposium on spectrochemistry – Trace substances in the Biosphere, (Eger), oral pres., 2001
6. N. H. Bings, **Zs. Stefánka,** S. R. Mallada, Flow injection electrochemical hydride generation inductively coupled plasma time-of-flight mass spectrometry for the simultaneous determination

of elements forming hydride, 2002 Conference on Plasma Spectrochemistry, (Scottsdale, USA), poster pres., 2002

7. M. Dernovics, A. Mátyás, I. Ipolyi, **Zs. Stefánka** and P. Fodor, A complex speciation study of a sample with high SeMet-content: brazil nuts (Bertholletia excelsa), 11th Biennial National Atomic Spectroscopy Symposium (Loughborough), oral pres., 2002
8. N. H. Bings, **Zs. Stefánka**, ICP-TOFMS in combination with electrothermal vaporization from a W-coil for the analysis of minute amount of samples, 12th Euroanalysis, (Dortmund), poster pres.,,363, 2002
9. **Zs. Stefánka**, L. Abrankó and P. Fodor, Flow injection ICP time-of-flight spectrometry coupled to hydraulic high pressure nebulizer for the analysis of low volume biolgical samples, European Winter Conference on Plasma Spectrochemistry (Garmisch-Partenkirchen) poster pres., 195, 2003
10. N. H. Bings, **Zs. Stefánka,** Analysis of whole blood and serum samples by tungsten coil electrothermal vaporization in combination with inductively coupled plasma time-of-flight spectrometry, European Winter Conference on Plasma Spectrochemistry (Garmisch-Partenkirchen) poster pres., 380, 2003
11. E. T. Bodó, M. Dernovics, **Zs. Stefánka** and P. Fodor, Preparation of Laboratory Reference Material (LRM) for selenium speciation, European Winter Conference on Plasma Spectrochemistry (Garmisch-Partenkirchen) poster pres., 401, 2003