

Development of hyphenated analytical technique for selenium speciation in environmental samples

Research Team

Maria Ochsenkühn-Petropoulou (P.I.), *Professor, N.T.U.A., Greece*

F. Tsopelas, (*Ph.D. Student*), *N.T.U.A., Greece*

L. Tsakanika, (*Ph.D. Student*), *N.T.U.A., Greece*

K. Ochsenkühn, (*Senior Researcher*), *N.C.S.R. "Demokritos", Greece*

Introduction

The aim of the present project is the development of a hyphenated technique for selenium (Se) speciation and its application to environmental samples. The term "Se speciation" refers to the separation and determination of different Se species, such as selenites (Se(IV)), selenates (Se(VI)), selenomethionine (Se-Met), selenocystine (Se-Cyst), selenocystamine (Se-CM), selenourea (Se-U), dimethylselenide ((CH₃)₂Se₂) and dimethyldiselenide ((CH₃)₂Se₂), which occur in environmental matrices and food. Each Se species exhibits different physicochemical properties, toxicity, bioavailability and tendency to bioaccumulate in organisms and, therefore, the information of the total selenium content of a sample is not sufficient and it can even be misleading in understanding the assessment of the environmental risk as it is suggested by Cornelis et al. [1]. Selenium speciation can be achieved, according to Zheng et al. [2], Uden [3], Zheng et al. [4], Chatterjee et al. [5], Ruiz Encinar et al. [6] and Ogra et al. [7], using mainly chromatographic techniques, such as ion-pair, ion-exchange, and size-exclusion chromatography coupled usually with inductively coupled plasma mass spectrometry (ICP-MS), while capillary electrophoresis coupled with ICP-MS or electrospray ionization mass spectrometry (ESI-MS) has also been used by Schramel et al. [8] and Michalke et al. [9]. However, the use of the described methods has some problems, such as the strong interferences of Ar compounds with ⁷⁷Se and ⁸²Se isotopes, when the sample contains high chloride and bromide matrices. The blocking of the ICP-MS detector by high salt content as it was reported by Zheng et al. [4], and the moderate ionization efficiency which has also been reported by Ruiz Encinar et al. [6], while the drawbacks of ESI-MS include poor sensitivity in case of complex matrices and the formation of doubly charged ions, which make the recognition of Se isotopic pattern in a mass spectrum difficult as it was discussed by Chatterjee et al. [5]. Most importantly, the use of ICP-MS requires very expensive instrumentation, which in most cases is not accessible. Therefore, the development of alternative analytical approaches based on lower cost instrumentation is of high interest for Se speciation. Especially, the use of UV spectrometry as detector in liquid chromatographic (LC) systems for Se speciation is of high interest because it is the simplest, the most inexpensive detector for LC systems and general accessible, suited to routine applications. Furthermore, voltammetric techniques appear also great interest for the determination of Se species because they are inexpensive, se-

lective and very sensitive. However, very few electrochemical techniques have been developed for Se speciation, although they are a powerful tool due to their low detection limits, especially if they are used in combination with simple procedures, such as extraction or ion-exchange procedure and almost of all refer to inorganic Se species as it is reported by Susnjevic et al. [10] and Lange et al. [11] or the determination of the total Se content of the sample after appropriate digestion procedure, as it is presented by Inam et al. [12] and Stoica et al. [13].

Electrochemical speciation of Se species

In this project, the electrochemical behavior of 8 selenium species, namely Se(IV), Se(VI), Se-Met, Se-Cyst, Se-CM, $(\text{CH}_3)_2\text{Se}_2$ and $(\text{CH}_3)_2\text{Se}$ was investigated and the direct voltammetric determination of the 5 Se species, Se(IV), Se-Cyst, Se-CM, Se-U and $(\text{CH}_3)_2\text{Se}_2$ was carried out, while Se(VI), Se-Met and $(\text{CH}_3)_2\text{Se}_2$ are electrochemically inactive. The voltammetric parameters of their determination, such as the kind and the concentration of the supporting electrolyte, the deposition voltage and time were investigated and optimized. The half-wave potentials, sensitivities, detection limits and the RSD values for the developed voltammetric techniques are presented in Table 1.

Table 1. Half-wave potentials, sensitivity, repeatability and detection limits of selenium species by the developed voltammetric technique

Se species	Half-wave potential (-V)	Sensitivity ($\text{nA}\cdot\text{ng}^{-1}\text{ Se}\cdot\text{ml}$)	Detection limit ($\text{ng Se}\cdot\text{ml}^{-1}$)	RSD
Aqueous phase, Electrolyte: HCl 0.1-2 M Deposition: -200 mV/180 s (for Se(IV)) or -50 mV/180 s (for Se-Cyst and Se-CM)				
Se(IV)	-0.46 ± 0.05	7 ± 2	0.12	4%
Se-Cyst	-0.33 ± 0.05	0.7 ± 0.3	3	7%
Se-CM	-0.26 ± 0.03	5 ± 0.7	0.3	4%
Aqueous phase, Electrolyte Na_2CO_3 0.5M Deposition: -600 mV/180 s				
Se-U	-0.85 ± 0.03	1 ± 0.15	2	3%
Organic Phase, CH_2Cl_2 : 0.2 M $\text{LiClO}_4/\text{EtOH}$ 1:1 + 0.03 M HCl Deposition: -50 mV/180 s				
$(\text{CH}_3)_2\text{Se}_2$	-0.22 ± 0.03	2.2 ± 0.3	0.23	3%

The obtained detection limits for the investigated Se species are extremely low, comparable with hyphenated techniques, such as HPLC-ICP-MS. In Table 2 the detection limits of Se-U and Se-CM achieved by using voltammetric techniques are compared with the corresponding values of liquid chromatography coupled with ICP-MS and capillary electrophoresis coupled with ICP-MS detector.

Table 2. Comparison of detection limits of Se-U and Se-CM by using hyphenated techniques and the developed voltammetric technique.

<i>Method</i>	<i>Detection limit (ng Se/ml)</i>	
	Se-CM	Se-U
IPRPC ¹ -ICP/MS [2]	2	2
IPRPC ¹ -ICP/MS [4]	1	1
CE ² -ICP/MS [14]	35	-
SQWCSV (This work)	0.3	2

¹: Ion-pair reversed-phase liquid chromatography

²: Capillary electrophoresis

Furthermore, the developed voltammetric techniques are not affected by the presence of common compounds with similar structure, which occur in environmental samples even in a much higher concentration. For example, urea and S-urea do not affect the voltammetric determination of Se-U even in a 100-fold greater concentration or more.

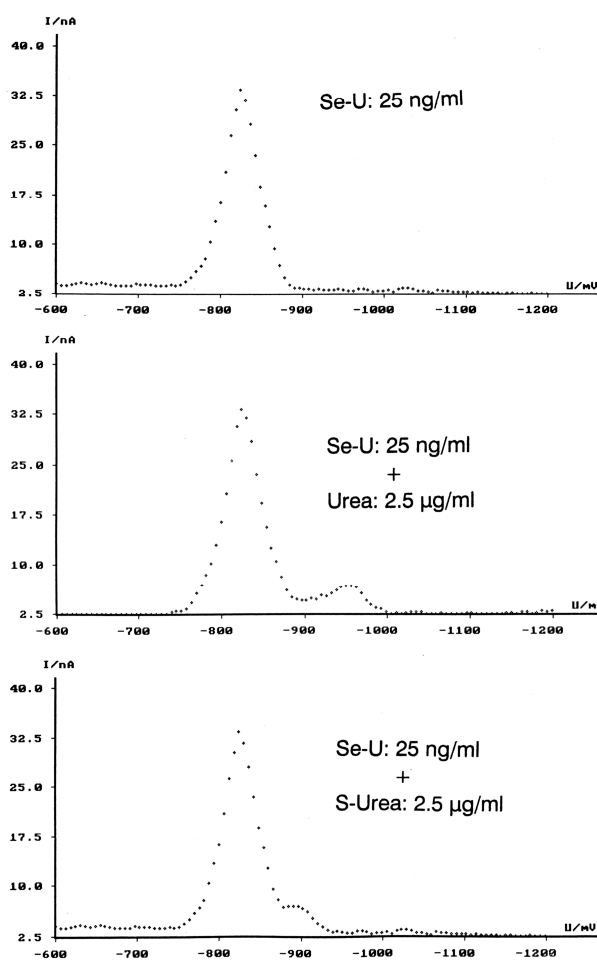


Figure 1. Effect of the presence of urea and S-urea in 100-times greater concentration on the voltammetric determination of Se-U using 0.5 M Na₂CO₃ as electrolyte.

Chromatographic separation of Se species

A chromatographic separation of Se(IV), Se(VI), Se-Met, Se-Cyst, Se-CM and $(\text{CH}_3)_2\text{Se}_2$ using reversed-phase liquid chromatography was also developed in this project. For this purpose, a wide range of chromatographic parameters, such as the kind, the concentration and the pH value of the eluent, the kind and the concentration of the ion-pair reagent were investigated and optimized. The best results were obtained using the BIO Wide Pore C18 column (150 mm x 4.6 mm) Discovery (Supelco) and as eluent 0.01 M H_2SO_4 after adjusting the pH to 7 with 1 M of NaOH and using 0.007 M of sodium salt of n-octanesulfonic acid as ion-pair reagent. The on-line quantification of the separated Se species was achieved using inductively coupled plasma atomic emission spectrometry (ICP-AES) as well as UV spectrometry at the optimum wavelength of 192 nm. The obtained separation using the above described conditions and UV detection, is presented in Figure 2.

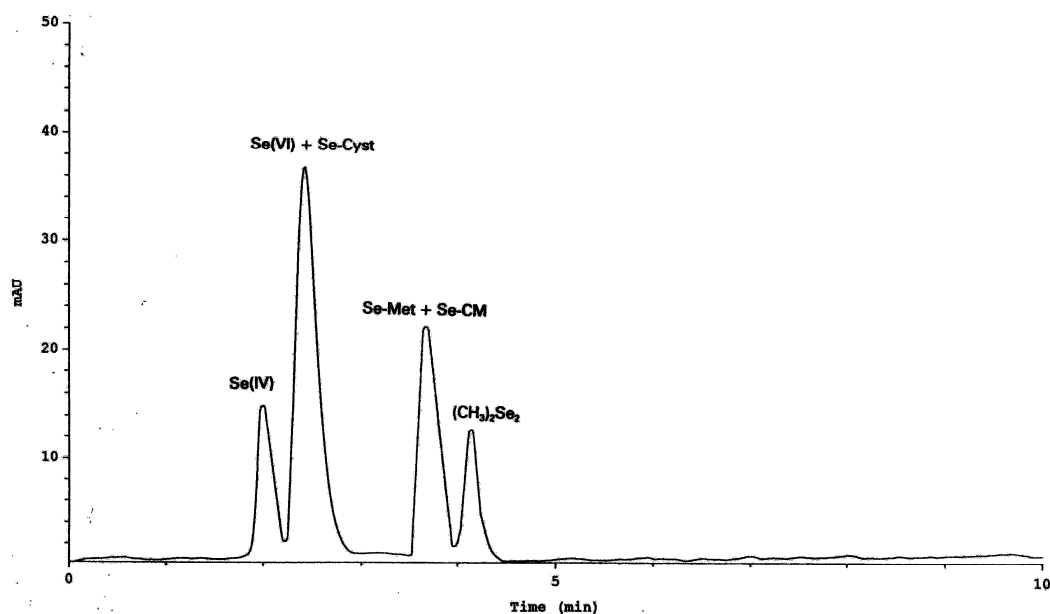


Figure 2. Separation of Se(IV), Se(VI), Se-Met, Se-Cyst, Se-CM and $(\text{CH}_3)_2\text{Se}_2$ (synthetic solution, 2 μg Se/ml each compound) using 0.01 M $\text{H}_2\text{SO}_4/\text{NaOH}$, pH=7 and 0.007 M of sodium salt of n-octanesulfonic acid and UV detection at 192 nm.

The sensitivities, detection limits and RSD values of the ICP-AES and UV spectrometry as detectors for the investigated Se species after their chromatographic separation are presented in Table 3. ICP-AES has higher sensitivity and lower detection limits in comparison with UV spectrometry and as element specific detector is likely not to have substantial interferences caused by the matrix of the sample.

Application to environmental samples

The developed techniques were successfully applied to environmental samples, such as soil samples from Thermopiles Spa, seawater samples, marine organisms, airborne particulates and to a coal fly ash reference material. In the case of soil samples from Thermopiles Spa Se speciation was performed using voltammetric techniques after leaching

procedure with 0.1 M HCl. The determination of Se(IV) in these samples using voltammetric techniques and the standard addition procedure is illustrated in Figure 3.

Table 3. Sensitivity, detection limit and RSD values of Se species by using the LC-ICP-AES and LC-UV systems

Method/ Se species	ICP-AES (196.090 nm)			UV Spectrometry (192 nm)		
	Sensitivity (counts/ ($\mu\text{g Se/ml}$))	Detection limit (ng Se/ml)	RSD (%)	Sensitivity (mAU/ ($\mu\text{g Se/ml}$))	Detection limit (ng Se/ml)	RSD (%)
Se(IV)	36.0	30	3.0	7.4	300	3.2
Se(VI)	35.5	32	3.1	7.0	300	3.3
Se-Met	34.7	34	3.3	7.2	300	3.4
Se-Cyst	39.8	27	3.0	10.9	200	3.0
Se-CM	34.2	39	3.7	4.2	500	3.5
(CH ₃) ₂ Se ₂	29.3	49	4.0	3.1	700	5.0

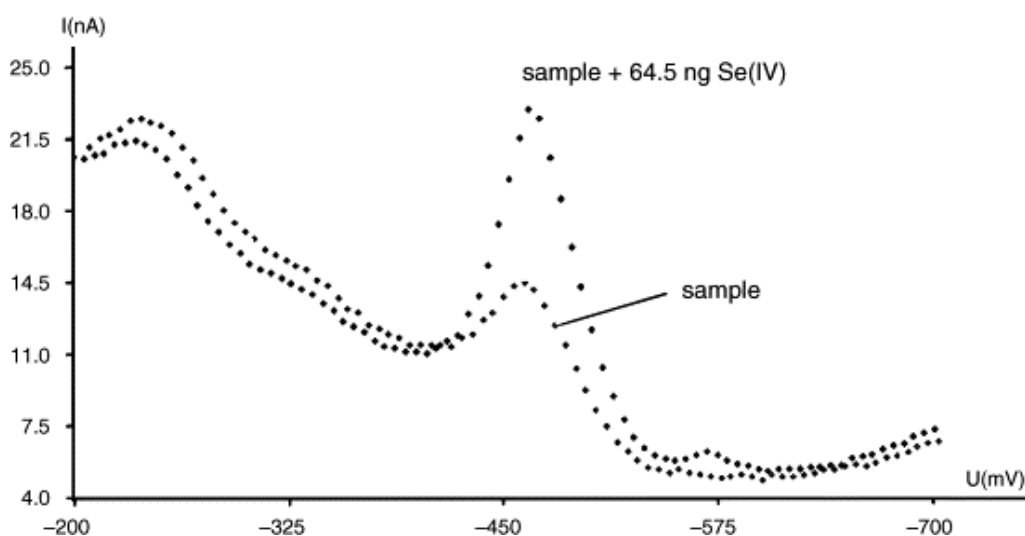


Figure 3. Determination of Se(IV) in the sediments of Thermopyles Spa by DPCSV using the standard addition method

Finally, the results obtained by the voltammetric techniques for the soil samples of Thermopyles Spa was similar with the corresponding ones using HPLC-ICP-MS in Institute of Ecological Chemistry, GSF, Germany as it is shown in Table 4.

Table 4. Comparison of the results obtained by developed technique to sediment samples from Thermopyles Spa (Greece) with the corresponding obtained by HPLC-ICP-MS in Institute of Ecological Chemistry, GSF, Germany.

Se species [results in $\mu\text{g Se/g sample}$]	This work	Institute of Ecological Chemistry GSF (HPLC-ICP-MS)
Total Se	1.90 ± 0.05	1.82 ± 0.05
Se(IV)	0.050 ± 0.008	0.030 ± 0.005
Se-U	0.15 ± 0.02	0.12 ± 0.02
Se-Cyst	< 0.1	-
$(\text{CH}_3)_2\text{Se}_2$	< 0.003	-

Conclusions

It is concluded that the developed analytical techniques are sensitive, selective and they can both successfully be applied to environmental samples.

References

1. Cornelis, R., Caruso, J., Crews, H. and Heumann, K., "Handbook of Elemental Speciation- Techniques and Methodology", Wiley Publications, 2003
2. Zheng, J., Ohata, M., Furuta, N., Kosmus, W., *J. Chromatogr. A*, **874**, 55, 2000
3. Uden, P.C., *Anal. Bioanal. Chem.*, **373**, 422, 2002
4. Zheng, J., Shibita, Y., Furuta, N., *Talanta*, **59**, 27, 2003
5. Chatterjee, A., Tao, H., Shibata, Y., Morita, M., *J. Chromatogr. A*, **997**, 249, 2003
6. Ruiz Encinar, J., Sliwka-Kaszynska, M., Polatajko, A., Vacchina, V., Szpunar, J., *Anal. Chim. Acta*, **500**, 171, 2003
7. Ogra, Y., Ishiwata, K., Ruiz Encinar, J., Lobinski, R., Suzuki, K., *Anal. Bioanal. Chem.*, **379**, 861, 2004
8. Schramel, P., Michalke, B., Kettrup, A., *Fresenius J. Anal. Chem.*, **363**, 452, 1999
9. Michalke, B., Schramel, P., *Electrophoresis*, **19**, 270, 1998
10. Susnjevic, D., Blagojevic, S., Vidic, J., Erceg, M., Vuculic, D., *Microchem. Journal*, **57**, 255, 1997
11. Lange, B., Van den Berg, C.M.G., Khan, S., *Anal. Chim. Acta*, **418**, 33, 2000
12. Inam, R., Somer, G., *Food Chemistry*, **69**, 345, 2000
13. Stoica, A.I., Babaua, G.R., Iorgulescu, E.E., Marinescu, D., Baiulescu, G.E., *J. Pharm. Biomed. Anal.*, **30**, 1425, 2002
14. Michalke, B., *Fresenius J. Anal. Chem.*, **351**, 670, 1995