TRANSACTIONS OF THE UNIVERSITIES OF KOŠICE

ISSN 1335-2334 **2-3/2006**

Editorial Board „Transactions of the Universities of Košice“

http://library.upjs.sk

Estimating Uncertainty and Lead Quality Determination in Blood in the Occupation Exposure

D. Borošová[1)], Z. Klöslová[2)]
Regional Institute of Public Health, Cesta k nemocnici 1, 975 56 Banská Bystrica
[1)] Department of chemical analyses, daniela.borosova@vzbb.sk
[2)] Department of risk assessment and genetic toxicology, zuzana.kloslova@vzbb.sk

Abstract

The determination of lead in blood of workers, occupationally exposed in the accumulator production, was assessed due to the toxicity of lead. The method for the direct determination of lead by AAS in whole human blood is presented. The previous experience and validation data are suggested as sources of performance information. The method recovery, sample recovery, homogeneity, precision and calibration were included to estimate measurement uncertainty compliant with ISO/IEC 17025 : 2005. The results of measurements using this method have uncertainty (52-23) % in the working range (70 – 700) μg/L. Two groups of workers were examined. The significantly higher lead concentration in blood as occupation exposure impact was found in workers who were in a direct or physical contact with lead accumulators and lead waste in the battery factory.

Key words: *blood, lead, occupational exposure, uncertainty*

Introduction

Occupational exposure to lead presents a significant hazard in the onset of serious intoxication. Potentially high levels of lead may still occur in the lead smelting and refining industries, battery manufacturing plants, steel welding or cutting operations. Lead is a potent, systemic poison that causes unknown harm once absorbed by body [9].

Significant portion of the lead that is inhaled or ingested, gets into blood stream [3]. Once in blood stream, lead circulates throughout body and stored in various organs and body tissues. Some of this lead is quickly filtered out of body and excreted, but some remains in the blood and other tissues. When exposure to lead continues, the amount stored in body increases if absorption of lead is higher than excretion [3]. Chronic overexposure to lead may result in severe damage to blood-forming, nervous, urinary and reproductive systems [4], kidney disease.

The ideal biomarker of lead exposure is a measurement of total lead body burden. Biomarkers of exposure in practical use are measurements of total lead levels in tissues or body fluids, such as blood, bone, urine, or hair.

In compliance with Slovak regulation limit, the concentration of lead in blood referred to as harmful in occupation exposure is 700 μg/L. Medical examination is recommended if concentration of lead in blood exceeds the level of 400 μg/L.

The result of measurement is unacceptable and may even be misleading if the quality of the method is not declared. Laboratories that are authorised with the respect to analytical methods shall continuously document the quality of this method. Moreover, all results are supposed to be estimated in the range, within which the true value lies. Under STN EN ISO/IEC 17025 [10], testing laboratory shall have and shall apply procedures for estimating uncertainty of measurement. In severe cases a reasonable estimation shall be based on knowledge of method performance and on the measurement scope and shall make use of previous experience and validation data.

Analytical method

The method for the direct determination of lead by AAS in whole human blood is presented [8]. A mixed matrix modifier solution containing nitric acid, ammonium dihydrogen phosphate and Triton-X-100 was used for preparation venous blood samples.

After venipuncture, blood samples were collected in plastic 2.7 mL Li-Heparin sample tubes (SARSTEDT, Monovette), which contained EDTA as anticoagulant [5]. 200 μL portions of blood sample were mixed with 1200 μL of the mixed matrix modifier solution. Mixed matrix modifier solution (0,2 % HNO_3, p.a. and 0,5% $NH_4H_2PO_4$, Suprapure (Merck) in 0,4% TRITON - 100 p.a. SERVA (FENBIOCHEMICA)) was prepared by mixing of 10 ml of 0,5 % HNO_3 + 0,125g $NH_4H_2PO_4$ + 5 ml of 2% TRITON-X, filled with deionized water in 25 ml volumetric flask. Blood test samples were left at rest for 5 minutes and were centrifuged at 3000 rpm within 6 min. Samples were poured into measuring vial. The blank sample was prepared from the matrix modifier solution.

The Perkin Elmer 4100 ZL atomic absorption spectrometer with transversely heated graphite furnace atomiser with Zeeman background correction and lead hollow cathode lamp at 283.3 nm were used for all analyses. The peak area was applied for evaluation of lead response. Temperature set for the Pb determination in whole blood is given in Table 1.

Table 1. The furnace AAS programme

Step	Temp, °C	Ramp Time, s	Hold Time, s	Internal Flow, mL/min	Read Step
1	110	1	60	250	
2	140	10	40	250	
3	1000	10	10	250	
4	1800	0	5	0	X
5	2400	1	2	250	

The blood sample with low lead was used for the method of standard additions for calibration. Lead intermediate standard of 100 mg/L and 1 mg/L were prepared by adjusting of stock standard solution of 1.000 g/l (the Slovak Institute of Metrology, Bratislava). Then there were 10, 20, 50, 75, 100 μL of 1 mg/L standard solution diluted with 0.5% HNO_3 in 10 mL volumetric flask. This corresponded to 70, 140, 350, 525, 700 μg Pb/L.

Uncertainty estimate

The method shall have uncertainty estimated especially in the case if the observed concentration is compared with reference value. Under regulation limit, the concentration of lead in blood referred to as harmful in occupation exposure is 700 μg/L. Medical examination is recommended if the concentration of lead in blood exceeds the level 400 μg/L.

In principle the development of a comprehensive mathematical model describing the test procedure can be impractical. Factors such as diffusion between matrix modifier solution and sample solution, temperature, the use of volumetric flasks, centrifuge, operation on AAS and process of calibration contribute to the uncertainty. Rigorous identification and statistical quantification can be long lasting and non-effective. Therefore, the sources of uncertainty were identified in accordance with Armishaw's estimating measurement uncertainty in the practical application of measurement uncertainty [1] of toluene measurement in water. Armishaw identified method recovery, sample recovery, precision, homogeneity and calibration as sources of uncertainty in GC-MSD measurement. All these components were calculated using the AAS method of lead determination in blood sample.

Quantitative measurement in atomic absorption, used in the method of addition calibration [2], are based on an equation (1):

$$C = -K_1 \cdot A \tag{1}$$

C is a concentration measured in an aliquot of sample, *A* is a difference between the absorbance for the aliquot with added standard and the absorbance measured for the sample. The final sample concentration is calculated by multiplying the slope $(-K_1)$ times the absorbance of the sample. The least square technique is used to determine the K_1 coefficient when two or more standards are used for calibration. Method of standard addition is used on the first sample and then group of samples, having a similar matrix is analysed. The concentrations of the remaining samples are determined from the calibration curve, generated with the first sample.

The effect of uncertainty components can modify [7] the equation (1)

$$C = -K_1 \cdot A \cdot f_{rm} \cdot f_{rs} \cdot f_{\text{hom}} \cdot f_{std} \tag{2}$$

f_{rm} – recovery method, f_{rs} – recovery sample, f_{hom} – homogeneity, f_{st} – preparation of the standards.

There is a condition when the method is under statistical control in the definition range and the uncertainty of the method is the standard deviation of the normal distribution (σ_y) at a given true value and must be constant in the definition range of the method.

Combined standard uncertainty for the model [1] above is given by the equation (3):

$$\frac{u_{(c)}}{C} = \sqrt{\left(\frac{u_{rm}}{rm}\right)^2 + \left(\frac{u_{rs}}{rs}\right)^2 + \left(\frac{u_{dup}}{dup}\right)^2 + \left(\frac{u_{std}}{std}\right)^2} \tag{3}$$

Method recovery uncertainty (u_{rm}) - a series of seven spiked blood samples at concentration (100 – 700 μg/L) of lead equidistantly covered definition range was selected to estimate method recovery uncertainty. The spiked blood samples went through the whole analytical procedure and thus represent many particular contributions in course of sample preparation. Least square regression analysis was used to estimate the standard deviation of predicted values, standard deviation - s_x obtained 16.17 μg/L. This value is considered to be uncertainty of the method recovery (u_{rm}) calculated at the concentration 400 μg/L in the centre of linear regression.

Sample recovery uncertainty (u_{rs}) – fresh-prepared matrix blood control samples spiked with 100 μg/L of lead were analysed with each series of measured sample. The obtained average recovery was 97.6% and standard deviation 4.2 % ($n = 13$ control sample)

Sample homogeneity uncertainty (u_{dup}) - six blood samples selected at random were analysed in duplicates. Variability between duplicates was normalised to the mean ratio of duplicates according to equations (4):

$$\frac{A}{(A+B)/2} \qquad \frac{B}{(A+B)/2} \tag{4}$$

A, B are concentrations of each duplicate. The average ratio of duplicate series is 1.0 and the standard deviation of ratio series is 0.106.

Calibration standard uncertainty (u_{std}) - the lead standard of purity 100.02±0.19% was used for the calibration. The rectangular distribution modifies the uncertainty to the value of 0.19/√3 = 0.11%.

The obtained results of uncertainty calculated are summarized in Table 2.

Table 2. Summary of measured uncertainty contributions

Uncertainty contributions:	Value	Standard deviation
method recovery (u_{rm}), μg/L	400	16.17
sample recovery (u_{rs}), %	97.6	4.2
sample homogeneity (u_{dup})	1.0	0.106
calibration standard (u_{std}), %	100.02	0.11

The combined standard uncertainty calculated is 97 μg/L, (cca 24%) when using equation 3 for the concentration 400 μg/L and the coverage factor *k=2*.

$$u_{(400)} = 400 \cdot \sqrt{\left(\frac{16.17}{400}\right)^2 + \left(\frac{4.2}{97.6}\right)^2 + \left(\frac{0.106}{1.0}\right)^2 + \left(\frac{0.11}{100.02}\right)^2} \tag{5}$$

$$U = u \cdot k = u_{(400)} \cdot 2 = 97\ \mu g / L$$

The combined standard uncertainties at other concentrations were calculated and the results are given in the Figure 1 where the relative combined uncertainty is a function of concentration. The relative combined uncertainty decreases from 52% to approximately 23% in the concentration range 70 – 700 μg/L. These results are comparable with the Armishaw's conclusion [1]. High level of relative standard uncertainty at low concentration is connected with high variability at low concentrations.

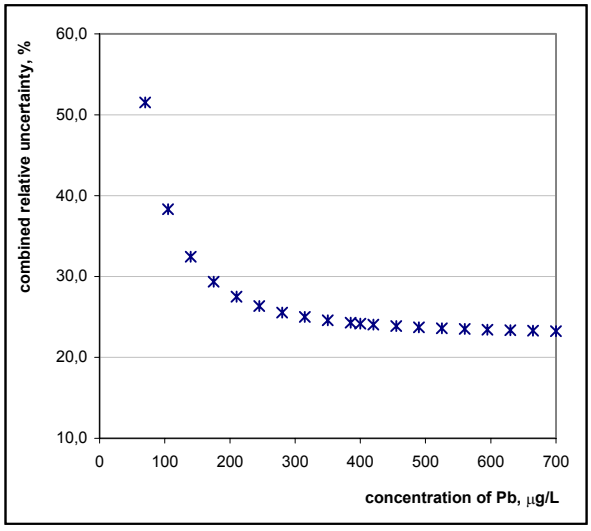


Figure 1. Relation of combined relative uncertainty and concentration in Pb blood

This fact reflects the ULA [6] computation limit of detection, based on one-sided upper confidence limit of the blank signal, critical value of *t*-distribution, residual standard deviation, s_{yx}, and degrees of freedom ν, ν = n-2. LOD and LOQ computed by this way were 13 μg/L and 38 μg/L respectively.

Occupational exposure

The lead exposure of 18 employees in 2 enterprises in Slovakia was studied. The determination of blood lead (PbB) levels was performed in a lead battery factory (*n=7*) and at the balancing weights for cars and trucks operation (*n=11*).

The results of PbB determination indicate that exposure to lead continues to be a serious problem in the Slovak industry. The Slovak binding biological exposure limit (BEL) value of 700 μg/L for workers was not exceeded. However, PbB concentrations were higher than the indicative BEL value of 400 μg/L in about 28 % of employees. The indicative BEL value of 100μg/L for female workers under 45 years was not exceeded.

The arithmetic mean PbB level in the lead battery factory was much higher (409 μg/L) than at the of balancing weights operation (161μg/L). PbB levels were significantly increased in workers who directly manipulated with lead accumulator and lead waste in the battery factory. The observed results are given in Figure 2 and 3.

No significant association between exposure time and PbB levels was observed. The highest PbB levels (390 – 551μg/L) were found in workers with the average duration of exposure of 4.3 months.

Lead is a component of tobacco and tobacco smoke, and smokers often have higher lead blood levels than non-smokers. PbB levels in smokers and non-smokers were analyzed and correlation between tobacco smoke and exposure levels was observed. The arithmetic mean PbB level in smokers was higher (324 μg/L) than in non-smokers (198 μg/L). The size of the group as well as ignorance of the exact exposure dose, do not enable to postulate explicit conclusions.

It could be considered that the lead hazard is particularly acute in small enterprises and some employees in Slovakia are still at risk to health due to adverse effects from Pb exposure. However, occupational exposure to lead is dependent not only upon the concentrations of lead in workplace air but also upon the personal hygiene and personal habits of the worker.

The necessity of PbB determinations, the improvement of working conditions and the implementation of the health education for workers are the measures to be promptly taken. In order to achieve these goals, a close cooperation between the Authorities of Public Health and the Labour inspectorates as well as the employers are required.

Conclusion

The AAS method was performed to determine the lead in blood (PbB) of workers occupationally exposed in the lead battery factory and at the balancing weights operation. The validation data were used to estimate the measurement uncertainty. Significantly higher PbB levels were found in workers who were in a direct or physical contact with lead accumulators and lead waste in the battery factory.

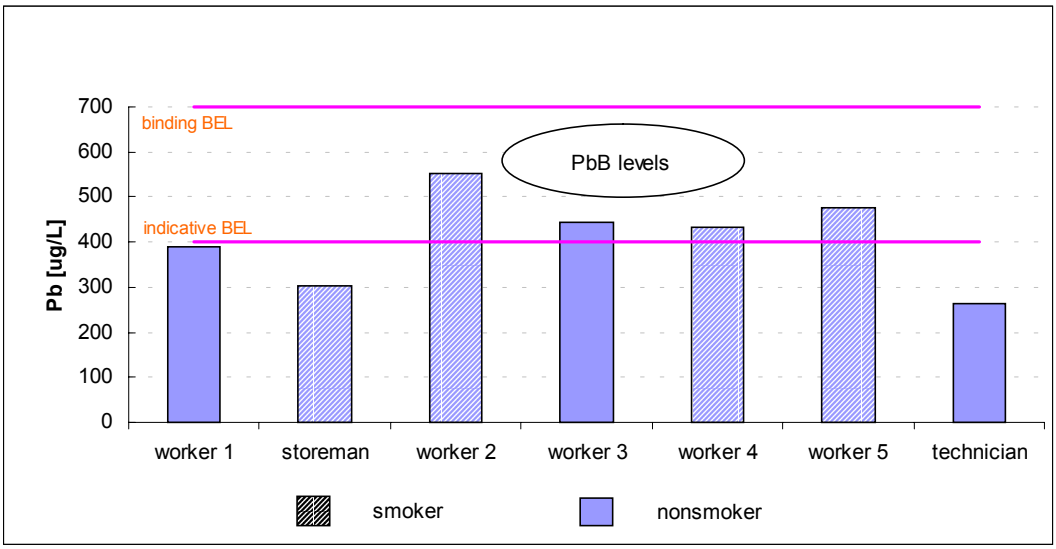


Figure 2. Manufacture of accumulator's batteries, purchasing of lead waste

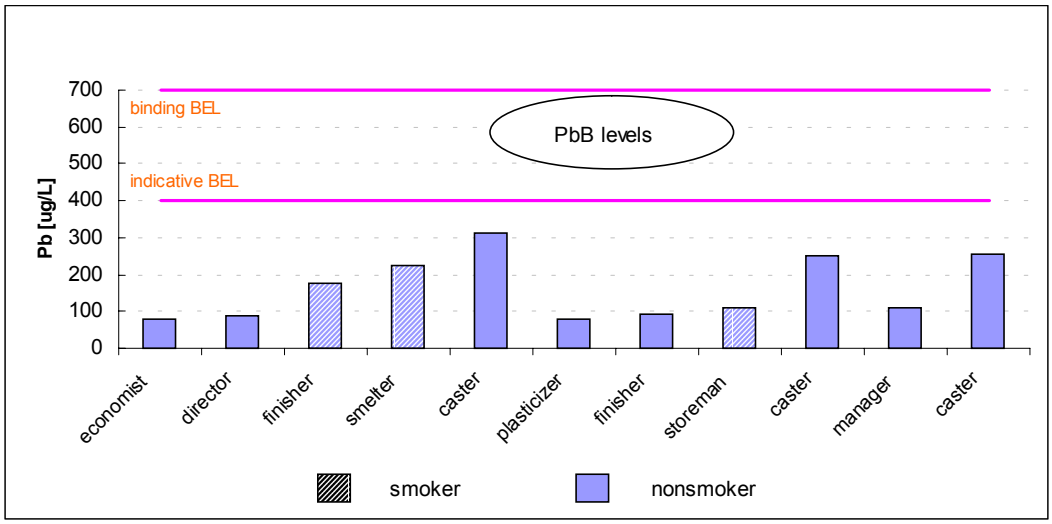


Figure 3. Balancing weights for cars and trucks, lead casting

References

[1] Armishaw, P.: Estimating measurement uncertainty in the afternoon. A case study in the practical application of measurement uncertainty. *Accred. Qual. Assur., 2003, 8, 218-224*

[2] *Atomic Absorption Laboratory Benchtop*, *User's Guide*. Perkin Elmer, Ueberlingen, 1992

[3] *ATSDR toxicological profile for lead. Draft.* Agency for Toxic Substances and Disease Registry, Atlanta, 200.

[4] *Environmental health criteria 165: Inorganic lead.* World Health Organization, Geneva, 1995

[5] Liang, L.: *The Use of Graphite Furnace AAS for the Determination of Al, Fe, Pb, Cd, and Cd in Biological Materials*. *Dissertation to the degree of Doctor in Sciences*. University of Antwerp, Belgium, 1991

[6] Mocak, J., Bond, A.M., Mitchell, S., Scollary, G.: A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: application to voltammetric and stripping techniques. *Pure & Appl.Chem. 1997, 69, 297-328*
[7] Mocák, J., Kordík, D., Máriássy, M., Vyskočil, L.: Vyjadrovanie neistôt pri meraní. In: *Súčasné trendy pri vyhodnocovaní analytických metód a postupov. CHEMIS, Bratislava, 1996, 26-38*
[8] Morton, S.: Lead in whole blood. *Atom. Spectr., 2000, 12, 5, 24-28*
[9] *NIOSH pocket guide to chemical hazards.* Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, 2005
[10] *STN EN ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories*. SÚTN, Bratislava, 2005

Vapor Generation of Cadmium for its Determination using Atomic Absorption Spectrometry – Recent Developments

Marek Bujdoš
Comenius University in Bratislava, Faculty of Natural Sciences, Geological Institute, Mlynská dolina G, 842 15 Bratislava, Slovakia, e-mail: bujdos@fns.uniba.sk

Abstract

The determination of trace elements at very low concentration levels is an actual analytical problem. Atomic absorption spectrometry (AAS) with different methods of atomization is frequently used for the determination of metals at ppb and sub-ppb concentration levels. Powerful sample introduction method to AAS is vapor generation (VGAAS). Among various chemical VG systems, volatile hydride generation is the widely used in analytical atomic spectrometry because of its ease of implementation, low reagent cost, high yield and corresponding analytical benefits that accrue when hydride generation (HG) sample introduction is coupled with atomic spectrometric detection. In this brief review a development of new vapor generation techniques for cadmium determination using photochemical vapor generation and preconcentration on quartz surface are described.

Key words: *cadmium – photochemical vapor generation – hydride – atomic spectrometry – quartz trap*

Introduction

Cadmium is a well-known toxic and persistant pollutant; therefore, it is important to be able to accurately determine its content in all components of the environment. Concentrations of cadmium are often detemined using atomic absorption spectrometry techniques, especially, electrothermal AAS (ETAAS) and recently VGAAS. This technique has low detection limits, rapid analysis times and the ability to utilize simple and inexpensive instrumentation. The most frequently determined elements are hydride-forming elements such as As, Bi, Ge, Hg, In, Pb, Sb, Se, Sn, Te and Tl. Fortunately, in recent years, determination of other elements, e.g., Cd [1], Ni, Co, Cu, Fe, [2,3], Ag [4,5,21] become feasible.

Chemical vapor generation of volatile species of Cd

Hydride generation AAS (HGAAS) is still the most utilised vapor generation approach, using chemical reduction employing sodium (or potassium) tetrahydroborate from strongly acidic media of strong mineral acid [6-9,34-36]. In case of typical hydride-forming elements this method is well

evolved and it is used as routine analytical technique. More recently VGAAS has been used also for the determination of cadmium in a variety of matrices [10-14]. Sanz-Medel et al. [10] revealed that cold vapor generation of monoatomic Cd^0 can be accomplished by reduction of Cd^{2+} solutions with $NaBH_4$ from an organized (vesicular) medium. The detection limits obtained using VGAAS are usually lower than using ETAAS. However, some of the properties of Cd vapor generation, particularly very narrow optimal concentration range of strong acids in sample solution, prevent the routine use of this technique in analytical laboratories [15].

Other problem common to all chemical vapor generation approaches is interferences that usually decrease sensitivity and reproducibility. Typically, these occur during the generation step, due to the coproduction of the active metals that catalytically decompose $NaBH_4$. In addition, the efficiency of vapor generation may depend strongly on the chemical form of the analyte in the sample. Furthermore, $NaBH_4$, as well as other derivatization reagents, is relatively expensive and a potential source of contamination [3]. The effort to develop new vapor generation systems that may replace or reduce the use of chemical reagents remains an important research area in VGAAS.

Photochemical generation of volatile species

It is well known that reactive free radicals can be generated by the application of ultraviolet light. These include oxidizing species (electron acceptors or holes, e.g., the hydroxyl radical). In contrast to oxidation, free radicals were used for the purpose of reduction. Kikuchi and Sakamoto [16] reported the formation of volatile species of selenium (presumably SeH_2) when photolyzing aqueous solutions fortified with formic acid in the presence of TiO_2 photocatalyst. Guo et al. [17-19] used for the determination of selenium using VGAAS photochemical formation of its volatile species from inorganic Se(IV) in the presence of low molecular weight (LMW) organic acids. They constructed a flow-through photoreactor consisting of PTFE tubing wrapped around a low-pressure Hg vapor UV lamp. The volatile alkyl Se species were detected using AAS or ICP-MS or after cryogenic trapping they were analyzed by GC-MS. In the presence of formic, acetic, propionic and malonic acids, inorganic selenium (IV) is converted by UV irradiation to volatile selenium hydride and carbonyl, dimethylselenide and diethylselenide, respectively. In acetic acid solution, the efficiency of generation was estimated to be 50 ± 10%. A detection limit of 2.5 $\mu g.l^{-1}$ was obtained.

Guo et al. [29] and McSheehy et al. [30] generated volatile arsenic species by UV irradiation of aqueous solutions of arsenite in LMW carboxylic acid media (formic, acetic, propionic and butyric). They revealed that inorganic arsenite can be alkylated to yield AsH_3 in the presence of formic acid, trimethylarsine in acetic acid, triethylarsine in propionic acid and tripropylarsine in butyric acid solutions. Mixed ligand arsenic species such as methylarsine, dimethylarsine, dimethylethylarsine and diethylmethylarsine are also formed when a mixture of acids is used. Using 0.05 mg l^{-1} As(III) as the test solution in 3.1 mol l^{-1} acetic acid, a methylation efficiency of approximately 75% was achieved. Guo et al. [3] generated volatile species of the conventional hydride-forming elements, some transition metals (among others Cd), noble metals and nonmetals following UV irradiation of their aqueous solutions to which LMW carboxylic acids (formic, acetic, propionic) had been added. They used batch reactor with UVC pen lamp. The most effective reaction of cadmium species occurred in an acetic acid medium with signal-to background ratio 20 for 10 $\mu g\ l^{-1}$ of Cd.

Coupling of vapor generation with ETAAS

Coupling vapor generation with the graphite furnace permits significant enhancement in relative detection power over conventional batch and continuous generation approaches. The pre-heated graphite furnace (200-600 ºC) is used to decompose the volatile species and trap the analytes on tube surface, thereby effecting a clean, rapid separation from the matrix as well as the collection and preconcentration. Then a conventional atomization step is performed. Graphite tubes with permanently

modified surfaces (usually by Ir or Pd) are used. A detailed review of AAS detection of hydride forming elements following in-situ trapping within a graphite furnace was given by Matusiewicz a Sturgeon [20]. In recent years graphite tube preconcentration technique was used for cadmium, too. Moreda-Piñeiro et al. [22] optimised hydride generation procedures for As, Cd, Sb and Se. Ir-coated graphite tubes were used for preconcentration and atomisation of the hydrides generated. For Cd they obtained LOD 200 ng l^{-1} during 30 s of trapping time. The within-batch precision was lower then 10%. Luna et al. [23] generated volatile species of Ag, Cu, Cd and Zn at room temperature by the addition of $NaBH_4$ to an acidified solution of the analytes in a micro batch reactor. The vapor-phase species were transported to a pre-heated graphite tube, the surface of which was previously treated with Ir as a permanent modifier. The limits of detection were 0.6 μg l^{-1}. Lampugnanie et al [24] overviewed literature data on vapour generation techniques for Cd and compared them with their own experiments. They researched several different types of HG-ETAAS systems (batch, semi-batch, continuous-flow and flow-injection as well as different gas-liquid separators. The best overall efficiency of HG, transportation and trapping was 41% and LOD was 2 ng l^{-1}.

The common drawback of graphite tube trapping approach is the need of expensive graphite furnace and its high operational cost.

Trapping of volatile species on quartz surfaces

Until recently the only widely used approach to volatile species preconcentration was in situ trapping in graphite furnaces. Now there is another, new approach to in-atomizer trapping – to collect analytes in a bare quartz tube. External quartz traps or commonly used quartz atomizers are employed for this purpose. One of the first papers on this novel approach was published by Korkmaz et al. [25] for determination of lead. They generated lead hydride vapor by a conventional hydride-generation flow system. A trap from silica tubing was made and it was placed between the gas-liquid separator and silica T-tube. Trapping was performed at 500 ºC and revolatilisation of analyte at 750 ºC at the presence of H_2 and O_2 gases. The overall efficiency was 49 %. For 60-s trapping, the detection limit was 19 ng l^{-1}. The very brief overview of quartz trapping techniques was given by Cankur et al. [26]. The method was used for trapping of stibine [27,28], arsine [31], bismuthine [32]. In works of Kratzer and Dědina [28,32] in situ trapping of analytes in the quartz T-tube atomizer, without any interfaced trapping device was employed. Cadmium cold vapor generation followed by on-line preconcentration of analyte in a quartz trap was performed by Korkmaz et al. [33]. The trapping device was formed by external heating of the inlet arm of a quartz T-tube. The collection temperature was 350 ºC. The collected species were released when the trap was heated further to revolatilization temperature, 1000 ºC, and hydrogen gas was introduced in the trapping medium. For a collection period of 3.0 min, i.e., 6.0 ml sample volume the detection limit was 1.8 ng l^{-1}. The enhancement factor with trapping for LOD was found to be 90 as compared with conventional FI-HGAAS.

Acknowledgements

The work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-042002.

References

[1] Matusiewicz, H., Kopras, M., Sturgeon, R.E.: Determination of cadmium in environmental samples by hydride generation with in situ concentration and atomic absorption detection, Analyst 122 (1997) 331-336

[2] Sturgeon, R.E., Liu, J., Boyko, V.J., Luong, V.T.: Determination of copper in environmental matrices following vapor generation, Anal. Chem. 68 (1996) 1883-1887
[3] Guo, X., Sturgeon, R.E., Mester, Z., Gardner, G.J.: Vapor generation by UV irradiation for sample introduction with atomic spectrometry, Anal. Chem. 76 (2004) 2401-2405
[4] Matoušek, T., Sturgeon, R.E.: Chemical vapor generation of silver: reduced palladium as permanent reaction modifier for enhanced performance, J. Anal. At. Spectrom. 19 (2004) 1014-1016
[5] Duan, X., McLaughlin, R.L., Brindle, I.D., Conn, A.: Investigations into the generation of Ag, Au, Cd, Co, Cu, Ni, Sn and Zn by vapor generation and their determination by ICP-AES, together with a mass spectrometric study of volatile species. Determination of Ag, Au, Co, Cu, Ni and Zn in iron, J. Anal. At. Spectrom. 17 (2002) 227-231
[6] D'Ulivo, A., Onor, M., Pitzalis, E.: Role of hydroboron intermediates in the mechanism of chemical vapor generation in strongly acidic media, Anal. Chem. 76 (2004) 6342-6352
[7] D'Ulivo, A., Baiocchi, C., Pitzalis, E., Onor, M., Zamboni, R.: Chemical vapour generation for atomic spectrometry. A contribution to the comprehension of reaction mechanisms in the generation of volatile hydrides using borane complexes, Spectrochim. Acta Part B 59 (2004) 471-486
[8] D'Ulivo, A.: Chemical vapor generation by tetrahydroborate(III) and borane-complexes in aqueous media. A critical discussion of fundamental processes and mechanisms involved in reagent decomposition and hydride formation, Spectrochim. Acta Part B 59 (2004) 793-825
[9] Sturgeon, R.E., Mester, Z.: Analytical applications of volatile metal derivatives, Appl. Spectrosc. 56 (2002) 202-213
[10] Sanz-Medel, A., Valdés-Hevia y Temprano, M.C., Bordel Garcia, N., Fernández de la Campa, M.R.: Generation of cadmium atoms at room temperature using vesicles and its application to cadmium determination by cold vapor atomic spectrometry, Anal. Chem. 67 (1995) 2216-2223
[11] Vargas-Razo, C., Tyson, J.F.: Determination of cadmium by flow injection-chemical vapor generation-atomic absorption spectrometry, Fresenius J. Anal. Chem. 366 (2000) 182-190
[12] Feng, Y.L., Sturgeon, R.E., Lam, J.W.: Generation of atomic and molecular cadmium species from aqueous media, Anal. Chem. 75 (2003) 635-640
[13] Bujdoš, M., Streško, V., Medveď, J., Kubová, J.: Evaluation of cadmium determination in soil extracts by hydride generation atomic absorption spectrometry (HG AAS), Fresenius Environ. Bull. 11 (2002) 686-690
[14] Bujdoš, M., Streško, V., Medveď, J., Kubová, J.: Optimization of cadmium determination by vapour generation AAS, Chem. Pap. 57 (2003) 201-203
[15] Lampugnani, L., Tsalev, D.L., D'Ulivo, A.D., Salvetti, C., Zamboni, R., Mascherpa, M.C.: Vapour generation–electrothermal AAS for Cd–mixed feelings, Proceedings of the 4th European Furnace Symposium and 15th Slovak Spectroscopic Conference, Podbanské, 2000, 31
[16] Kikuchi, E., Sakamoto, H.: Kinetics of the reduction reaction of selenate ions by TiO_2 photocatalyst, J. Electrochem. Soc. 147 (2000) 4589-4593
[17] Guo, X., Sturgeon, R.E., Mester, Z., Gardner, G.J.: UV vapor generation for determination of selenium by heated quartz tube atomic absorption spectrometry, Anal. Chem. 75 (2003) 2092-2099
[18] Guo, X., Sturgeon, R.E., Mester, Z., Gardner, G.J.: UV light-mediated alkylation of inorganic selenium, Appl.Organomet. Chem. 17 (2003) 575-579
[19] Guo, X., Sturgeon, R.E., Mester, Z., Gardner, G.J.: Photochemical alkylation of inorganic selenium in the presence of low molecular weight organic acids, Environ. Sci. Technol. 34 (2003) 5645-5650
[20] Matusiewicz, H., Sturgeon, R.E.: Atomic spectrometric detection of hydride forming elements following in situ trapping within a graphite furnace, Spectrochim. Acta Part B 51 (1996) 377-397
[21] Feng, Y.-L., Sturgeon, R.E., Lam, J.W.: Chemical vapor generation characteristics of transition and noble metals reacting with tetrahydroborate(III), J. Anal. At. Spectrom. 18 (2003) 1435-1442
[22] Moreda-Piñeiro, J., Moscoso-Pérez, C., López-Mahía, P., Muniategui-Lorenzo, S., Fernández-Fernández, E., Prada-Rodríguez, D.: Multivariate optimisation of hydride generation procedures

for single element determinations of As, Cd, Sb and Se in natural waters by electrothermalatomic absorption spectrometry, Talanta 53 (2001) 871-883
[23] Luna, A.S., Borges Pereira, H., Takase, I., Araújo Gonçalves, R., Sturgeon, R.E., de Campos, R.C: Chemical vapor generation–electrothermal atomic absorption spectromery: new perspectives, Spectrochim. Acta Part B 57 (2002) 2047-2056
[24] Lampugnani, L., Salvetti, C., Tsalev, D.L.: Hydride generation atomic absorption spectrometry with different flow systems and in-atomizer trapping for determination of cadmium in water and urine–Overview of existing data on cadmium vapour generation and evaluation of critical parameters, Talanta 61 (2003) 683-698
[25] Karadeniz Korkmaz, D., Ertaş, N., Ataman, O.Y.: A novel silica trap for lead determination by hydride generation atomic absorption spectrometry, Spectrochim. Acta Part B 57 (2002) 571-580
[26] Cankur, O., Korkmaz, D., Ertaş, N., Menemenlioğlu, İ., Öztürk, Ç.P., Dinçer, S., Ataman, O.Y.: Atom trapping in atomic spectrometry, 4th AACD Congress, 29 Sept.-3 Oct. 2004, Kuşadası-Aydin, Turkey, Proceedings Book, 27
[27] Korkmaz, D., Dědina, J., Ataman, O.Y.: Stibine preconcentration in a quartz trap with subsequent atomization in the quartz multiatomizer for atomic absorption spectrometry, J. Anal. At. Spectrom. 19 (2004) 255-259
[28] Kratzer, J., Dědina, J.: In situ trapping of stibine in externally heated quartz tube atomizers for atomic absorption spectrometry, Spectrochim. Acta Part B 60 (2005) 859-864
[29] Guo, X.M., Sturgeon, R.E., Mester, Z., Gardner, G.J.: Photochemical alkylation of inorganic arsenic, Part 1. Identification of volatile arsenic species, J. Anal. At. Spectrom. 20 (2005) 702-708
[30] McSheehy, S., Guo, X.M., Sturgeon, R.E., Mester, Z.: Photochemical alkylation of inorganic arsenic, Part 2. Identification of aqueous phase organoarsenic species using multidimensional liquid chromatography and electrospray mass spectrometry, J. Anal. At. Spectrom. 20 (2005) 709-716
[31] Kratzer, J., Dědina, J.: Arsine trapping at the quartz surface: a powerful tool for AAS, XXXIV. CSI, 4. - 9. Sept. 2005, Antwerp, Belgium, Book of Abstracts, 297
[32] Kratzer, J., Dědina, J.: In situ trapping of bismuthine in externally heated quartz tube atomizers for atomic absorption spectrometry, J. Anal. At. Spectrom. 21 (2006) 208-210
[33] Korkmaz, D., Demir, C., Aydın, F., Ataman, O.Y.: Cold Vapour Generation and on-line Trapping of Cadmium Species on Quartz Surface Prior to Detection by Atomic Absorption Spectrometry, J. Anal. At. Spectrom. 20 (2005) 46-52
[34] Farkašovská, I., Závadská, M., Žemberyová, M.: Determination and speciation of antimony in environmental samples by AAS techniques, Chem. Listy 93 (1999) 173-180
[35] Farkašovská, I., Žemberyová, M.: Determination and speciation by AAS techniques of selenium in environmental and biological samples, Chem. Listy 93 (1999) 633-638
[36] Hagarová, I., Žemberyová, M.: Determination of arsenic in biological and environmental samples by AAS techniques, Chem. Listy 99 (2005) 578-584

Determination of Arsenic by On-Line Electrochemical Preconcentration/GFAAS

František Čacho, Milan Střelec, Ernest Beinrohr, Alena Manová
*Institute of Analytical Chemistry, Slovak University of Technology, **Radlinského 9,** 812 37 Bratislava, Slovakia*
e-mail: cacho@atlas.sk

Abstract

The aim of this work was to develop an on-line coupling of electrochemical preconcentration to AAS method and the utilization of the developed system for the determination of ultra-trace amounts of As in water samples. A flow-through electrochemical analyzer EcaFlow (Istran, Bratislava) was used as the preconcentration unit with a two-electrode cell. The working electrode was an RVC electrode coated with gold. An AAS spectrometer SP9 with graphite furnace atomizer (Pye Unicam) was used to measure the preconcentrated As species. The preconcentration parameters for the electrochemical process were optimized. The deposition runs at a constant current of -2500 μA and the optimum stripping current was found to be 100 μA. Determination of Sb by FTC-GFAAS we develop in this time. Moreover, the determination of Sb by GFAAS was optimised enabling a direct measurement also from HCl media. The preconcentration step was optimised and characterised.

Keywords: *Arsenic – GFAAS – electrochemical -- preconcentration*

Introduction

The increasing of concentration of the toxic element (As, Hg, Cd, etc.) and their compound in the water, soil and air present serious problem for humans. Toxic properties of the elements could be transferred from the soil to the foodstuff and mammals by erosion cycles. Consequences of medical research indicate, that the many compounds exhibits like injurants at inconsiderable amounts as was suppose years ago. Determination of the toxic elements represent typical problem of ultra-trace analysis.

In this work we have focused for determination of As (III). Toxic level of the arsenic starts at 30-50 mg, smallest lethal dose is 60 – 80 mg for adult [1,2].

Atomic absorption spectrometry (AAS)

Atomic absorption spectrometry (AAS) is one of the most widespread analytical methods. The principal is hidden in absorption of radiation by free atoms in gaseous state (produced in atomizer). This work is aimed to coupling of atomic absorption spectrometry with electrochemical preconcentration of the analyte, in our case – arsenic.

The sub-goal of this work was to develop the suitable method for the determination of the arsenic by GFAAS without matrix modification. Determination of arsenic without modification is still problematic [3]. Detection limits for determination of the arsenic spread between 1 to 10 $\mu g.dm^{-3}$ [4–14]. Interference emerges as a big problem in the determination too [13,14]. Standard technique for the determination of the arsenic is HGAAS. Detection limits for this technique could be round 0.1 $\mu g.dm^{-3}$ [15-18].

Flow through chronopotentiometry (FTC)

Determination of As was done by GFAAS, but with preconcentration electrochemical unit. We inspected considerable decreasing limit of detection and influence of interferences could be removed too. On the surface of working electrode will be deposited quantitatively only As (III). The deposit is that stripped in on-line system directly to the graphite cuvette of the electrothermal atomizer. This system was the same as we used for electrochemical determination of the As (III). This system works on FTC principle, which is full validated for the determination of As (III) [19-21].

On-line coupling FTC and GFAAS

On-line take advance of coupling of two steps to one's. At first step, analyte is deposited on working electrode from flowing sample. At second step, the analyte is then quantitatively determined by suitable analytic method.

The main topic of preconcentration step is maximize the concentration of the analyte with the simultaneously suppress the effect of interference.

There were published a lot of articles about the As preconcentration among last decade. Basic principles utilized for on-line preconcentration and published detection limits are:

- on-line preconcentration on columns with utilization of different sorbents. Published detection limits differs from: LOD = 0.02 $\mu g.dm^{-3}$ at sampling volume 10 mL [6,10,11,22–30]
- on-line "cloud-point" extraction with LOD = 0.01 $\mu g.dm^{-3}$ at sampling volume 10 mL [8]
- "in situ" preconcentration in atomizer (both GF-AAS and HG-AAS atomizers were utilized). LOD = 0.05 $\mu g.dm^{-3}$ at sampling volume 10 mL [11,15,17,31,32]
- preconcentration with cryogenic trapping (only HG-AAS). LOD = 0.02 $\mu g.dm^{-3}$ at sampling volume 10 mL [16,33]
- preconcentration by liquid extraction: LOD = 0.05 $\mu g.dm^{-3}$ at sampling volume 10 mL [5]

Electrochemical processes were also used the on-line flow-through systems which utilized electrochemical cells. Applications for this systems were performed for preconcentration of following elements Pd(II), Pb(II), Cd(II), Cr(III), Cr(VI). Godlewska [34] and Bulska [35] used electrochemical measured cell for on-line preconcentration Pd (II), Pb (II) a Cd (II). Their construction wasn't suitable for measurement and preconcentration of the As (III). Even FAAS was utilized to the on-line electrochemical preconcentration. In this work, authors apply sorption cell for separation and consequently determination of the Cr species by FAAS [36].

Material and methods

GFAAS

An AAS spectrometer SP9 with graphite furnace atomizer (Pye Unicam) was used to measure the preconcentrated As species.

FTC

A flow-through electrochemical analyzer EcaFlow (model GLP 150, Istran, Bratislava) was used as the preconcentration unit with a two-electrode cell. The working electrode was a RVC electrode coated with gold (Fig. 1).

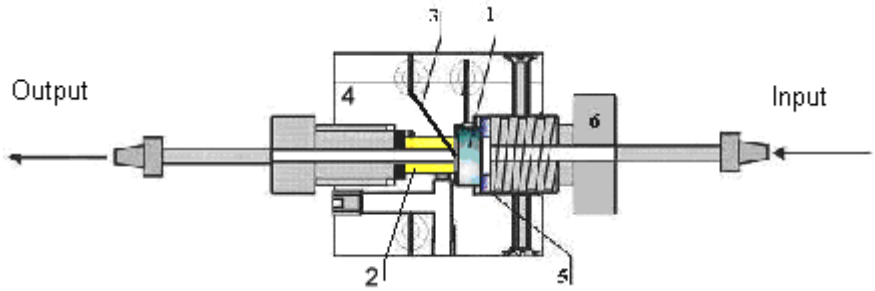


Fig. 1. Scheme of the flow-through cell with dual electrode connection
Legend: 1 - working electrode E-53 Au, 2 - inert inset, 3 -auxilliary Pt electrode, 4 - plexi glass body, 5 - packing, 6 - screw

Scheme of the on-line coupling FTC-GFAAS

The 6-way valve was connected with sampling loop with sampling volume 57.2 µl. Thereby on-line connection of electrochemical analyzer EcaFlow could by make by coupling with GFAAS over the 6-way valve (Fig. 2).

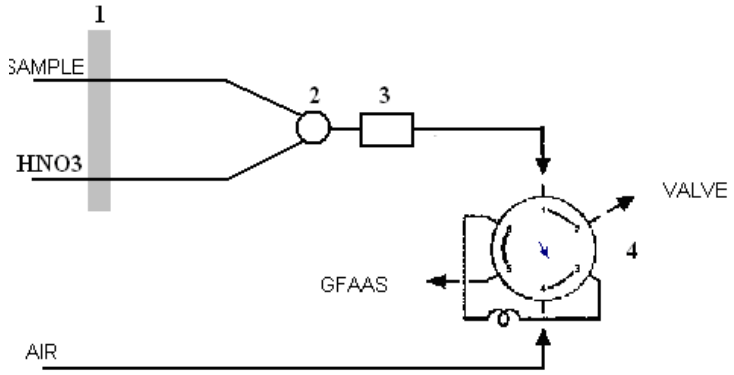


Fig. 2. Scheme of the flow-through system FTC-GFAAS
Legend: 1 - pump, 2 - valve, 3 - preconcentration cell, 4 - 6-way valve with sampling loop calibrated to the sampling volume 57.2 µl

Results and discussion

For validation process of the new developed coupling FTC-GFAAS were chosen three different feeding volumes of the sample and complete validation set were survey for all feeding volumes. The linear range, limit of detection and limit of quantification were appointed, respectively. Finally were assessed repeatability, reproducibility and accuracy by analyzing the standard reference material.

Linear ranges

Linear ranges are summarized in Table 1. Listed results show broad ranges, parameters of the calibration curves, coefficients of determination and regression coefficients for different feeding volumes.

Table 1. Linear ranges, equations of the linear regressions and their coefficients for different feeding volumes As (III)

Feeding volume [ml]	Linear Range [μg·dm^{-3}]	Equation of regression	R^2
1	0.20 – 5.0	A = 0.00154+0.13932ρ	0.99986
5	0.06 – 2.0	A = -0.01273+0.36449ρ	0.99968
10	0.01 – 0.5	A = 3.01584E-5+1.08665ρ	0.99854

Limit of detection (LOD) and limit of quantification (LOQ)

Assessed values for limit of detection and limit of quantification for determination of As (III) are listed in Table 2.

Table 2. Summarized values of slopes and increments for assessment of LOD and LOQ over ULA-2 approach [37]

volume [ml]	δ_0	b_1	LOD [μg.dm^{-3}]	LOQ [μg.dm^{-3}]
1	0.0027568	0.3829	0.06	0.2
5	0.0022268	0.38214	0.02	0.07
10	0.0015779	1.0625	0.004	0.01

Repeatability

Fig. 3 represent the dependence of measured concentration of the As(III) against measurement order (significance level α = 0,05, selected concentration was 1 μg.dm^{-3}). Repeatability characterized by RSD was 3.5 % (n=11).

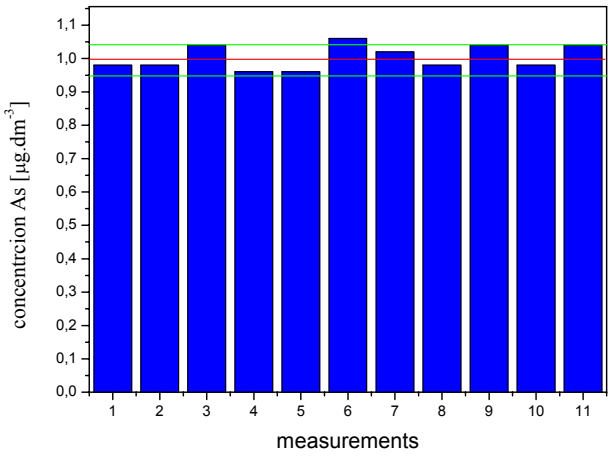


Fig. 3. Repeatability of the determination of the As (III) (ρ = 1.00 μg.dm^{-3}, feeding volume 1 ml)

Trueness

Trueness was determined in standard reference material CRM 12-3-10 by on-line FTC-GFAAS of the total As.

The FTC-GFAAS technique developed at these conditions has some limitations. It is suitable only for determination of As (III) species and total As must be converted to the As(III) form. Many publications were pointed to the selective reduction of total As to As (III) form. As appropriate procedure for selective reduction step seems to be utilizing L-cysteine [38].

Table 3 report the results of the determination of the total As in certified reference material after selective reduction step by L-cysteine. Analyse was done with 1 ml of the sample volume.

Table 3. Results of the total As in CRM 12-3-10

FTC-GFAAS	ρ As [μg.dm^{-3}] found	ρ As [μg.dm^{-3}] certified
	21.0 ± 0.1	**21 ± 5**

Preconcentration factor and speed of the analysis

Preconcentration factor (PF) was calculated by equation:

$$PF = \frac{concentration\ As\ after\ preconcentration\ step}{concentration\ As\ before\ preconcentration\ step} \qquad (1)$$

It is a value, which determines how many times have been increased the concentration of the analyte by preconcentration step. The speed of the analysis expresses how many of the analysis could be finished per hour.

Table 4 summarized determined preconcentration factors and predicted speed of the analysis, respectively at different feeding volumes.

Table 4. Summarized preconcentration factors and speed of the analysis

feeding volume [ml]	**ρ As(III) before preconcentration step [μg.dm^{-3}]**	**ρ As after preconcentration step [μg.dm^{-3}]**	**PF**	**Speed of the analysis [number/hour]**
1	1	8	8	27
5	1	42	42	23
10	1	84	84	11

Conclusions

The aim of this work was to develop an on-line coupling of electrochemical preconcentration to AAS method and the utilization of the developed system for the determination of ultra-trace amounts of As in water samples. A flow-through electrochemical analyzer EcaFlow (model GLP 150, Istran, Bratislava) was used as the preconcentration unit with a two-electrode cell. The working electrode was a RVC electrode coated with gold. An AAS spectrometer SP9 with graphite furnace atomizer (Pye Unicam) was used to measure the preconcentrated As species. The preconcentration parameters for the electrochemical process were optimized. The deposition runs at a constant current of -2500 μA and the optimum stripping current was found to be 100 μA.

The deposition was made from a hydrochloric acid solution, which was then on-line replaced by a diluted nitric acid facilitating the AAS measurement of As. The advantage of the used preconcentration system was in an automatic and reliable matrix exchange facility. Optimum electrolyte concentrations were found to be 0.1 mol.dm^{-3} HCl and 2 mol.dm^{-3} HNO_3 for the deposition and stripping/AAS measurement, respectively.

The trueness was determined by means of a certified reference material CRM 12-3-10 (SMÚ) with As content of 21.0 ± 5.0 μg.dm^{-3}. The analysis was performed after a fast and simple selective reduction of As (V) to As (III) by L-cysteine.

We are developing suitable method for determination of Sb by FTC-GFAAS at this time. Moreover, the determination of Sb by graphite furnace atomic absorption spectrometry was optimised enabling a direct measurement also from hydrochloric acid media. The preconcentration step was optimised and characterised. We are planning to elaborate condition for on-line determination of Sb by FTC-GFAAS.

References

[1] Prousek. J.: Rizikové vlastnosti látok. STU, Bratislava 2001
[2] Pradosh Roy, Anupama Saha: Current Science, 1997, 82, 1
[3] STN EN ISO 15586: Kvalita vody: Stanovenie stopových prvkov atómovou absorpčnou spektrometriou s grafitovou pieckou, 2003
[4] Burguera Marcela, Burguera, Josh Luis: Talanta, 1997, 44, I58I – 1604
[5] Torgov V.G., Vall G.A., Demidova M.G..: Chemical Geology, 1995, 124, 101-107
[6] Iglesias M., Antico E., Salvado V.: Talanta, 2003, 59, 651-657
[7] Latva S., Hurtta M., Peräniemi S., Ahlgrén M.: Anal. Chim. Acta, 2000, 418, 11–17
[8] Shemirani F., Baghdadi M., Ramezani M.: Talanta, 2005, 65, 882–887
[9] Gonzalez M.M., Gallego M., Valcarcel M.: Talanta, 2001, 55, 135–142
[10] Hung D.O., Nekrassova O., Compton Richard G.: Talanta, 2004, 64, 269-277
[11] Alonso E.V., de Torres A.G., Cano Pavón J.M.: Talanta, 2001, 55, 219-232
[12] Man-Ching Hsiang, Yu-Hsiang Sung, Shang-Da Huang: Talanta, 2004, 62, 791–799
[13] Cacho F.: Bakalárska práca, STU, Bratislava, 2004
[14] Střelec M.: Diplomová práca, STU, Bratislava 2003
[15] Cacho F., Střelec M., Hlubikova S., Beinrohr E., Manova A.: Proceedings of 25th industrial toxicology, Bratislava, 2005, 170-174
[16] Burguera J.L., Burguera M., Rival C., Carrero P.: Talanta, 1998, 45, 531–542
[17] Kalihne R., Henrion G., Hulanicki A., Garbo S., Walcerz M.: Spectrochimica Acta Part B, 1997, 52, 1509-1516
[18] Nielsen S., Sloth Jens J., Hansen Elo H.: Talanta, 1996, 43, 867–880
[19] Profumo A., Merli D., Pesavento M.: Analytica Chim. Acta, 2005, 539, 245–250
[20] Švancara I., Vytoas K., Bobrowski A., Kalcher K.: Talanta, 2002, 58, 45
[21] He Yi, Zheng Y., Ramnaraine M., Locke D.C.: Anal. Chim. Acta, 2004, 511, 55–61
[22] Dressler V.L, Pozebon D., Curtius A.J.: Spectroch. Acta Part B, 1998, 1527–1539
[23] Karthikeyan S., Prasada Rao T., Iyer C.S.P.: Talanta, 1999, 49, 523–530
[24] Gomes Neto J. A., Montes R., Cardovo A.A.: Talanta, 1999, 50, 959–966
[25] Narcise C.I.S., Coo L., del Mundo F.R.: Talanta, 2005, 68, 298–304
[26] Jitmanee K., Oshima Mitsuko, Motomizu Shoji: Talanta, 2005, 66, 529–533
[27] Leal L.O., Semanova N.V., Forteza R., Cerdà V.: Talanta, 2004, 64, 1335–1342
[28] Aristidis N. Anthemidis, Evdoxia K. Martavaltzoglou: Anal.Chim.Acta, 2006
[29] Jitmanee K., Oshima Mitsuko, Motomizu Shoji: Talanta, 2005, 66, 529–533
[30] Leal L.O., Semenova N.V., Forteza R., Cerdà V.: Talanta, 2004, 64, 1335–1342
[31] Niedzielski P., Siepak M., Siepak J.: Microchemical Journal, 2002, 72, 137–145
[32] Yang Li-Li, Zhang De-Qiang: Analytica Chimica Acta, 2003, 491, 91–97
[33] Pinillos S.C., Adensko J.S., Bernal J.G.: Anal. Chimica Acta, 1995, 300, 321-327
[34] Godlewska-Zyłkiewicz B., Zaleska M.: Anal. Chim. Acta, 2002, 462, 305–312
[35] Bulska E., Walcerz M., Jedral W., Hulanicki A.: Anal. Chim. Acta, 1997, 357,133 – 140
[36] Beinrohr E., Manov A., Dzurov J.: Fresenius J Anal Chem, 1996, 355,528-531
[37] Mocák J, Bond A.M., Mitchell S., Scollary G.: Pure and Appl Chem, 1997, 69, 297 – 328
[38] Hengwu Chen, Ian D., Brindle, Xiao-Chun Le: Anal. Chemistry, 1992, 64, 667-672

The Control Charts in Quality Assurance

Ludmila Dempírová
*Czech Geological Survey, Klárov 3, 118 21 Prague **1,** Czech Republic*

Abstract

Construction of control charts according to the Czech standard Cz ISO 8258 is dealt with. Being in accordance with quality assurance requirements instructs us to monitor permanently two quality signs – precision and the central value. Therefore, each individual analytical method is covered with two up to five control samples to monitor them. The standard persists on repeated measuring on each sample. Precision is calculated as standard deviation from the mean range of the results reached within subgroups of 20-25 data sets. Central value is arithmetic mean of all results. Statistically ruled status ensures that variations both in precision and in mean value, are random only. The assessment of charts with calculation of method capability index is shown on the Al determination in groundwater sample CRM BCR 609. The instrumental method used was electrothermic atomic absorption spectrometry.

Key words: a*nalytical method - control samples - R, $\overline{X}$ -diagrams - statistically ruled status – precision – tolerances - method capability index*

Introduction

Analytical method resembles by sample treatment procedures industrial processes. In industry, the machined products are checked; the products not corresponding to industrial standards are discharged.

The industrial practice is not applicable onto the field of analytical chemistry. The easy tools for discovering and excluding faults are missing. The prevention of faulty results suits best onto this field. From two up to five control samples, covering the method concentration range, are recommended to analyze with the routine samples. If the repeated results of control samples vary within control limits, the routine samples are presumed to give accurate values then. Matrix and analyte content of an unknown and control sample should be similar.

As follows from the Gauss equation

$$y = \frac{\exp\left\{-\left(\bar{x}-\mu\right)^2 / 2\sigma^2\right\}}{\sigma\sqrt{2\pi}} \tag{1}$$

two method criteria are being collected. Precision (σ) and mean value ($\bar{x}$). In the Cz ISO 8258 the precision is calculated from the repeated measurements as standard deviation from the mean range measured within subgroups of 20-25 data sets. For the control charts construction, formulae for their control lines and numerical coefficients are needed. Formulae and coefficients can be found in the

appendices of the standard mentioned above. In the Shewhart diagrams, 3σ probability concept is employed rather than 2σ probability concept which is largely accepted in the field of analytical chemistry.

For the precision, $R-diagram$ investigating ranges within subgroup of repeated measurements is made use of. In $\overline{X}$ - diagram the variations in the means of repeated measurements are in detail observed.

Table 1: Formulae for the control limits of Shewhart diagrams. Diagram type: the values acquired by measuring

Statistics	**Basic values σ, $\overline{x}$ of a control sample are collected**	
	Central line	UCL and LCL
$\overline{X}$	$\overline{\overline{X}}$	$\overline{\overline{X}} \pm A_2\overline{R}$
R	$\overline{R}$	$D_4\overline{R}$

Explanatory notes: $\overline{\overline{X}}$ - mean of the means, $\overline{R}$ - average range, A_2, D_4 - numerical coefficients, UCL – upper control limit, LCL-lower control limit

Statistics	**Basic values σ, $\overline{x}$ have been determined yet**	
	Central line	UCL and LCL
$\overline{X}$	X_0	$X_0 \pm A\sigma_0$
R	$d_2\sigma_0$	$D_2\sigma_0$

Explanatory notes: X_0, σ_0 - average values determined in the previous step, A, d_2, D_2 - numerical coefficients

Table 2: Coefficients for calculating the lines of Shewhart diagrams. Extracted from the standard

Number of repeated measurements n	**Coefficients**				
	A	A_2	D_2	D_4	d_2
2	2.121	1.880	3.686	3.267	1.128
3	1.732	1.023	4.358	2.574	1.693
4	1.500	0.729	4.698	2.282	2.059
5	1.342	0.577	4.918	2.114	2.326

At first, 20-25 repeated measurements of the control sample data are collected. From them, basic values X_0, σ_0 of the process are calculated. In the further measurements, the once established parameters of the method are inspected to lie within the control lines. As follows from 99% probability, none must lie outside the limits.

Experimental part

As an example for discussing Shewhart diagrams, Al determination in waters by atomic absorption spectrometry with electrothermic atomization was chosen. The working range of a method is 3-100 μg/L, the lower value is the limit of determination. Two control samples CRM BCR 609 and TM 23.2 with central values 47.7 μg/L and 96 μg/L respectively are analysed with routine samples. The choice depends on Al content in routine samples.

Further details about Al determination are following: wavelength 309.3 nm, slitwidth 0.7 nm, background corrector used, volume of a sample 20 μl, graphite cuvette, current 10-25 mA, drying temperature 120°/10s, pyrolysis 1500°/20s, atomization 2600°/4s. Atomic absorption spectrometer Perkin Elmer type 4100+HGA 700 was used.

Control samples are analysed with routine samples twice: firstly at the beginning and secondly at the end of a workshift under the same instrument calibration. The analysis is performed approximately once a week. As emerges, the data for CRM BCR 609 discussed here were collected within a period of a year. The results in table 3 are arranged under numbers 1-50.

Table 3: Source data for Al [μg/L] in CRM BCR 609. Figures in brackets mean 1st value, 2nd value, range R, mean $\overline{X}$

a) The first step - basic values $\overline{R}, \overline{\overline{X}}$ of CRM are collected

No.	1	2	3	4	5
Results	[47,47,0,47]	[49,53,4,51]	[46,52,6,49]	[49,53,4,51]	[46,41,5,43.5]
No.	6	7	8	9	10
Results	[40,42,2,41]	[42,37,5,39.5]	[44,43,1,43.5]	[44,39,5,41.5]	[43,47,4,45]
No.	11	12	13	14	15
Results	[47,38,9,42.5]	[42,37,5,39.5]	[41,40,1,40.5]	[43,45,2,44]	[47,41,6,44]
No.	16	17	18	19	20
Results	[49,50,1,49.5]	[45,49,4,47]	[49,45,4,47]	[46,47,1,46.5]	[45,46,1,45.5]
No.	21	22	23	24	25
Results	[45,43,2,44]	[50,49,1,49.5]	[47,47,0,47]	[40,42,2,41]	[50,44,6,47]

b) The second step - basic values $X_{0,}\ \sigma_0$ of CRM are checked

No.	26	27	28	29	30
Results	[48,48,0,48]	[49,42,7,45.5]	[48,42,6,45]	[44,51,7,47.5]	[45,43,2,44]
No.	31	32	33	34	35
Results	[45,41,4,43]	[40,42,2,41]	[41,50,9,45.5]	[46,40,6,43]	[47,54,7,50.5]
No.	36	37	38	39	40
Results	[45,50,5,47.5]	[47,45,2,46]	[45,42,3,43.5]	[43,51,8,47]	[46,46,0,46]
No.	41	42	43	44	45
Results	[40,43,3,41.5]	[42,47,5,44.5]	[41,45,4,43]	[44,50,6,47]	[40,46,6,43]
No.	46	47	48	49	50
Results	[49,46,3,47.5]	[46,51,5,48.5]	[47,43,4,45]	[45,44,1,44.5]	[49,41,8,45]

Results and discussion

The procedure of charts construction is described step by step now. From the data given in the part a) of the table 3, the lines for the R-diagram were computed. Mean range $\overline{R}$ is 3.24 μg/L, upper control line is 10.6 μg/L ($D_4\overline{R} = 3.267 * 3.24$). The inspection of the each range from 25 groups indicates that there are no data above the upper control line. The mean is then calculated. Central line in $\overline{X} - diagram$ is in the position of 45.1 μg/L. Upper control limit is 51.2 μg/L $(45.1 + 1.880 * 3.24)$, lower control limit is 39.0 μg/L $(45.1 - 1.880 * 3.24)$. Again, there is no point above and under these lines. Variations of the ranges and the means are of random occurrence. The analytical method is considered to be in the statistically ruled status. The precision is computed according to the relationship $\sigma_0 = \overline{R}/d_2$. σ_0 is 2.9 μg/L (3.24/1.128).

The Al determination in CRM BCR 609 is now monitored for permanent consistenty of the values X_0, σ_0 [45.1; 2.9]. The shifts from established values are permitted in the frame of random occurrence, only. The lines in the Shewhart diagrams are computed for the part b) of the table 3. The $\overline{X} - diagram$ is considered first. Central line lies at the value 45.1 μg/L. Upper control limit is 51.3 μg/L (45.1 + 2.121*2.9), lower control limit is 38.9 μg/L (45.1 – 2.121*2.9). None of the 25 means lies outside the limits. In the R-diagram the central line for the mean range lies at the value 3.3 μg/L (1.128*2.9), upper control limit is 10.7 μg/L (3.686*2.9). None of the 25 ranges from the part b) of the Table 3 lies above this limit. From the viewpoint of variability the determination of Al reached stable and predictable performance. The situation is demonstrated in the Figure 1.

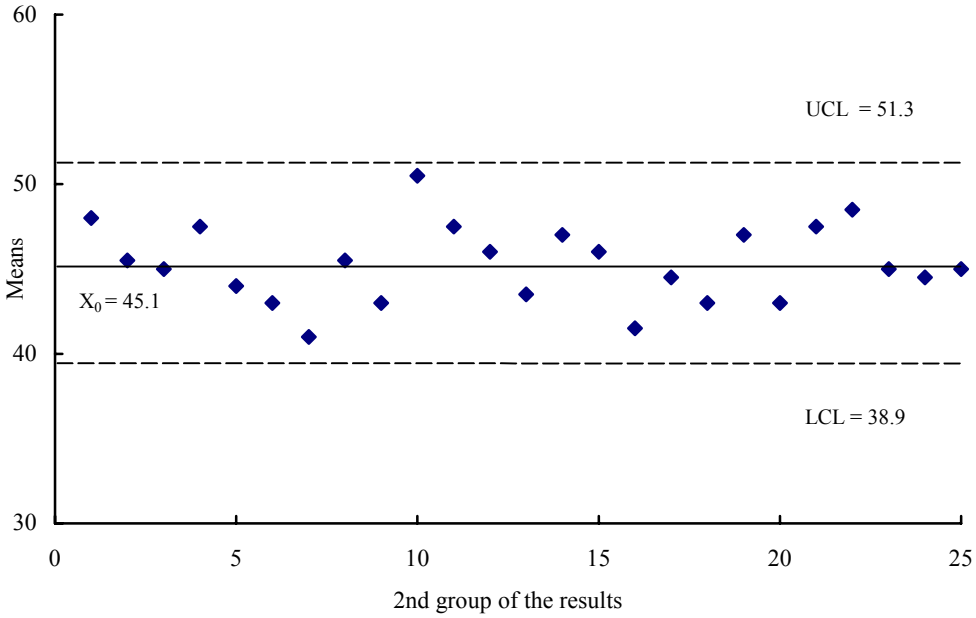


Fig. 1: $\overline{X} - diagram$ of Al in BCR 609 (Result numbers 26-50)

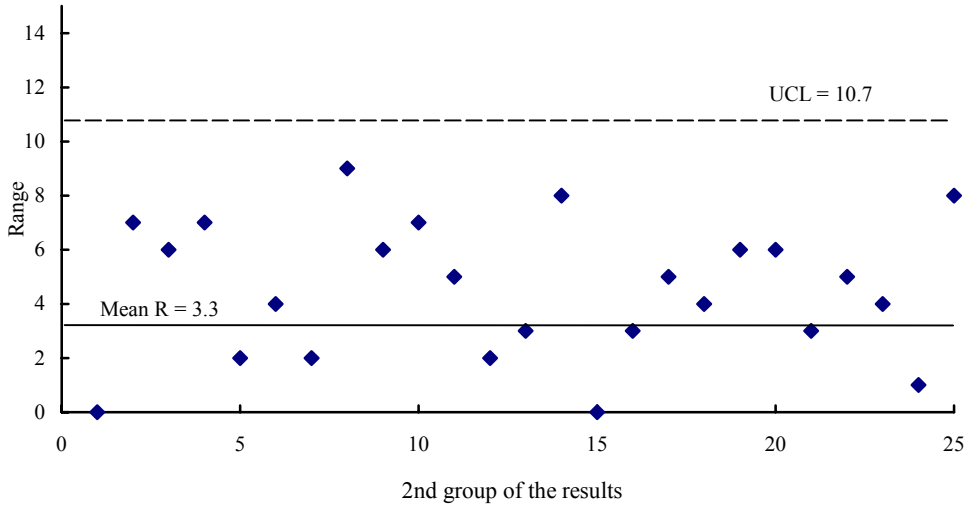


Fig. 1: $R-diagram$ of Al in BCR 609 (Result numbers 26-50)

The control sample analyzed is certified reference material with the certified value 47.7 μg/L. It is possible to construct $\overline{X}$-diagram with the central line 47.7 μg/L. Upper control line is 53.9 μg/L (47.7 + 2.121*2.9), lower control line is 41.5 mg/L (47.7 - 2.121*2.9). The difference in the central values 2.6 μg/L creates long branches that lie beyond the certified value. In the practice of Central laboratory of CGS, the difference is included in measurement uncertainty. Laboratory standard uncertainty is 0.125% of the Al concentration and is constant within the method working range.

In the 1st group of 25 results, small inconsistency in means towards higher values was proved. In the 2nd group of the results, the effect was not observed. The trial was performed according to Neumann [4].

The Al determination by electrothermic AAS has in every laboratory different parameters. Approximate demands on analysis precision can be found in the standards relating to waters [2]. Further source of information about the Al determination were the reports from interlaboratory proficiency tests. Permitted deviation from the target value was expressed as percentages of a central value here. Interlaboratory tolerances are adjusted more loosely so that the majority of laboratories fulfill the comparison exercise.

Method capability index takes into account prescribed tolerance and method precision. In this relationship the strict requirement on accurate central value is omitted.

$$MCI = \frac{method\ tolerance\ required}{method\ \mathrm{var}iability} = \frac{UTL - LTL}{6\sigma} \qquad (2)$$

If the MCI ≥ 1.33 [1], the determination is considered to be capable for routine performance. The values of capability index for two tolerance zones are given in the table 4.

Table 4: Capability index for variant tolerances

Tolerances	Method variability	MCI
± 37.5% (laboratory uncertainty)	2.9 μg/L	1.94
± 30% (proficiency test)	2.9 μg/L	1.56

The standard uncertainty was calculated as a combination of possible uncertainty sources by „step by step“ procedure [3]. Method variability was not explicitly included in the budget. In comparison trials good results for the Al determination were achieved. Some evidence is given here. Figures in brackets are arranged in sequence: target value [μg/L], laboratory value [μg/L] and z-score: [353; 370; +0.48], [105; 101; -0.40]. The determination of Al meets laboratory quality demands on routine performance.

Conclusion

In this article the Central Laboratory experience in quality assurance is dealt with. In quality assurance control samples (CRMs, home made standards) are largely used. The control samples are analysed at the beginning and at the end of laboratory work under the same calibration. From the pair of the results, range is computed and compared with upper control limit. The results of routine samples fulfill the determination when the results of the control samples meet control limits of the Shewhart diagrams.

References

[1] Czech national standard Cz ISO 8258. Shewhart control charts. CNI, Prague, 1993
[2] Czech national standard Cz EN ISO 15586. The determination of trace elements by atomic absorption spectrometry with graphite cuvette. CNI, Prague, 2004
[3] Eurachem-Cz: Qualimetry VI. Prague 1996
[4] Meloun, Militký: The statistical evaluation of experimental data. Plus, Prague 1994, p. 95

DC-ARC-OES *versus* ETV-ICP-OES in Solid Sampling Atomic Spectrometry: Competitive or Complementary Methods?

[1]Karol Flórián , [2]Jürgen Hassler, [1]Vladislava Boková
[1]Department of Chemistry, Faculty of Metallurgy, Technical University in Košice, Letná 9, SK-042 00 Košice, Slovakia
[2]ESK Ceramics GmbH & Co. KG Max Schaidhauf Str. 25, D-87437 Kempten, Germany

Abstract

At the beginning of the quantitative atomic spectroscopic methods, which could be placed in 30thies of last century, the methods based on the use of various electric discharges played an important role in the direct analysis of the solid samples. During the following years of the development, the direct current arc (DC-ARC) has achieved the leading position and also a lot of new constructions and ideas (e.g. double-arc, magnetic field-stabilized arc etc.) were based also on this one. The above mentioned leading position was lost in moment, when the inductively coupled plasma source (ICP) was successfully combined with electrothermal atomisation/ evaporation device implemented from the atomic absorption spectrometric (AAS) methods. In this time, various instrumentations were proposed, tested and used, but only the development in the last decade has also a commercial output in the ETV-ICP-OES technique. When the mentioned technique was widely implemented in solid sampling spectrometry, the carrier of DC-ARC technique seemed to be finished. This statement was true only for a very short period of spectroscopic history and nowadays exists in this field a commercially modernized instrumentation, successfully tested and also used in the round-robin test of solid samples.
This work is dedicated to short historical overview of the above mentioned development, as well as to comparison of both leading methods of the solid-sampling-spectrometry, i.e. ETV-ICP-OES and DC-ARC-OES. In this comparison the most important validation characteristics as precision, accuracy, range, linearity were estimated and compared.

Key words: *solid sample spectrometry – comparison DC-ARC-OES vs. ETV-ICP-OES - validation procedure*

Introduction

About 20 years ago a comprehensive article [1] about free-burning DC-arc and its use in the spectrographic analysis of the solid samples was published. The work was devoted to evaporation and excitation capability of the DC arc discharge in the analysis of the powdered samples as well as to some methods validation criteria. This technique seems to be out of the spectrochemists' interest nowadays. This fact has been strongly influenced by the declaration " the DC-arc is dead" declared [2] by the author of excellent book [3] and plenty of articles about this technique in the past. In the time of mentioned 20 years other spectrometric techniques were preferred, among them first of all the excitation in various ICP-sources, later connected also with the solid-sampling instrumentation as e.g.

ETV-ICP-OES, LA-ICP-OES, etc. Since the middle of the 90thies of the last century some reports about newly designed and instrumentalised DC-arcs appeared from time to time, especially in the field of the analysis of various types of advanced ceramic materials. As the most frequently used solid-sampling technique, the ETV-ICP-OES is limited by the maximum achievable temperature about 2900°C, the evaporation of the carbide-forming elements could not be complete. Therefore the use of the special working atmospheres or additives is needed. On the other hand, the temperature of DC-arc excitation about 4000°C fulfils the requirements of the total evaporation of the refractory elements.

The new technologies, namely the use of a modern, programmable and computer-controlled DC-arc has therefore turned back the interest of the spectrochemist's to this old instrumentation. This fact could be documented by very nice presentation "The awakening of the sleeping beauty" [4] in this year, but also by the acceptance of this old technique in more laboratories over the world (see e.g. [5-7]). Since 1999 when the modern design and new possibilities of the DC-arc technique were described [8], more presentations were given about special applications in analysis of non-oxidic ceramic powders [9-11], one of them also in this journal [12]. The round-robin-test of the SiC standard, reported in [13] was based on the modernized DC-ARC-OES, too. As an interesting aspect of the DC-arc-applications is – and it should be not forgotten – that some tandem techniques were applied in solid sampling of the discussed materials. Preuss [14] has described the first combination of "ETV" with DC-arc. Besides this also the combination of the DC-arc - Marinković plasma source should be mentioned, where the arc serves as the evaporation source and the Marinković plasma as an excitation source [15]. Reviews [16,17] about arc and spark optical emission spectroscopy include also other recent applications.

The short overview of development in DC-ARC-OES and ETV-ICP-OES

Two different constructions should be distinguished in solid sampling atomic emission spectrometry:

- the so called contact technique, where the evaporation of the solid sample and its excitation is running on the same place, a typical example is the DC-ARC-OES,
- the tandem technique, where the both above described processes are separated on different places, mainly the vapour of the sample is transported using a carrier gas into excitation source; typical examples are various ETV-ICP-OES constructions.

The first tandem technique was neither the first, nor the second of the above defined instrumentations. Preuss [14] has described a construction, where the volatile elements of the powdered sample were evaporated in an electric (resistive) heated carbon tube furnace and the vapours were transported into DC-ARC discharge (Fig. 1).

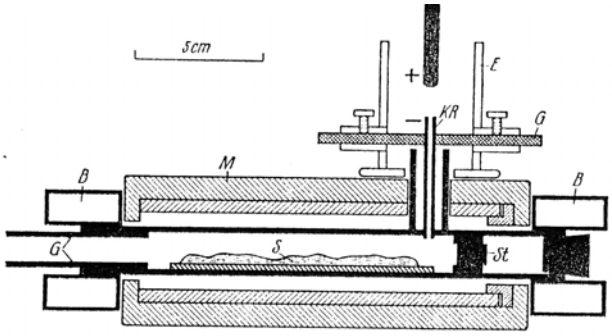


Fig. 1. The tandem construction described by Preuss [14]

This idea of ETV + DC-ARC combination was rediscovered 50 years later [18,19], but in this time the benefits in comparison to ETV-ICP-OES technique were not found. Therefore the later

revitalization of the DC-ARC as excitation source, described in [8,9,20] was concentrated only on the contact technique. This modernization lies in two moments:

i) the computer controlling of the whole evaporation and excitation procedure realized on basis of the burning programme, defining the change of the arc current (and from this depending temperature of discharge) in time, as is shown in Fig. 2.

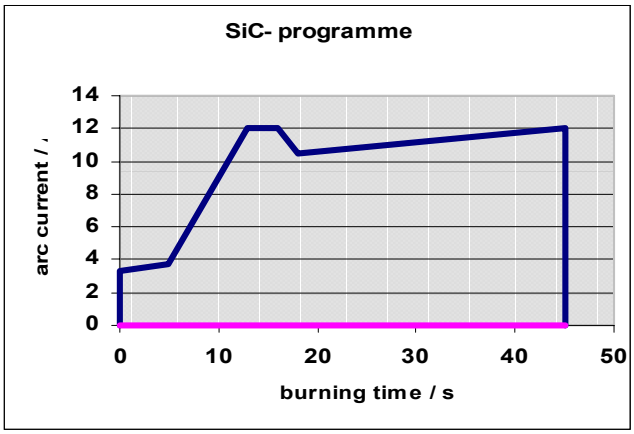


Fig.2. The burning program of the DCA-301 (Spectral-Systems) DC-ARC-source

ii) the projection of the radiated light from excitation source on the entrance slit of the spectrometer using an double-optics projection system connected to spectrometer with quarz-fibre. The double-optic projection system should avoid the un-certainty due to so called wondering of the arc. The mentioned system as well as its optimisation was described in detail in [8].

Another, also programmable but not computer-controlled DC-ARC discharge is integrated into *ATOMCOMP 2000* commercial spectrometer. The burning of the arc is programmable in three steps, defined by arc current and burning time, as it is shown in Fig. 3.

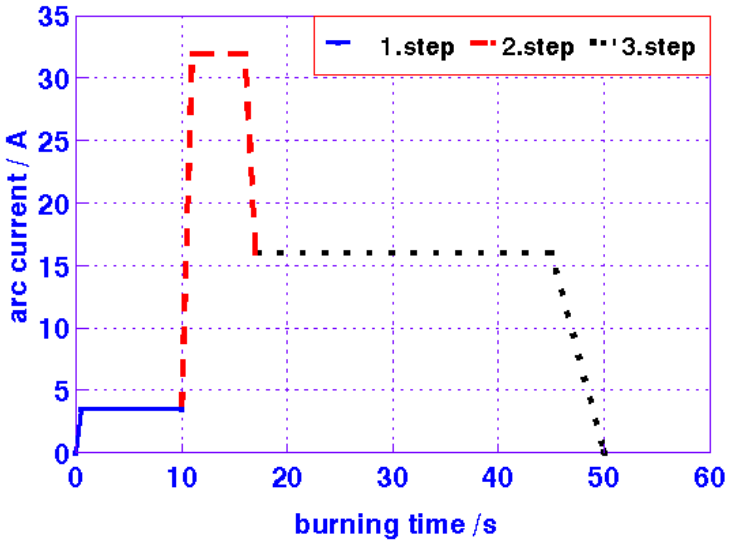


Fig. 3. The 3-step burning program of DC-ARC-source, integrated into spectrometer ATOMCOMP 2000

The commercial ETV-ICP-OES instrumentation represents nowadays the automated ETV unit ETV-4000, described in [9,20]. The way to this construction, used in connection to various ICP-

spectrometers in daily practice in a lot of spectrochemical laboratories was long. Since a huge amount of various constructions was developed and tested and also a plenty of reviews were presented in last decades, only the milestones are going to be shortly mentioned.

At the beginning of the history of the ETV-ICP-OES methods, some electrically heated cups or electrodes were used for the evaporation of the solid sample [21-24]. Later a new construction was developed, useful for both DC-ARC and ICP source [25], which have achieved more variations [26]. In the same time, other group have combined [27] the ICP and later the ICP-MS with the commercial

ETV-furnace developed for solid-sampling AAS by Grün-Systems Wetzlar. As a next step the complete optimization of the graphite tube and the connection to ICP was applied [9] and at the end the first model of half-automatic ETV device was introduced. A very good detailed overview of the development of the ETV-ICP-OES techniques is given in reviews [28,29], as well as in monograph [30].

Experimental

In comparison studies of both DC-ARC-OES and ETV-ICP-OES methods the instrumentation, described in the Table I. was used.

Table I.: Experimental conditions

spectrometer	adapted LECO-750 vacuum-spectrometer (Paschen-Runge-construction), holographic grating (2400 groves/mm) entrance slit = 20μm; secondary slit = 40μm spectral range = 150-456 nm 39 photomultiplier
ICP (ETV)	radial plasma; HF-power = 1150 W ETV-4000 automatized furnace (boot in tube system) computer-controlled furnace and measure programme
DC-ARC	DCA-301 computer-controlled programmable discharge water-cooled electrode holders double-optics projection system, quarz-fibre optics high-resistance carbon electrodes (Elektrokarbon-Topolčany)
samples	laboratory SiC standards (ESK, Kempten)

As a typical material for solid-sampling spectrometry the silicon carbide was chosen, in the calibration procedure a set of laboratory SiC standards (ESK, Kempten) was used. The calibration was done using a linear model

$$y = a + b \times c \tag{1}$$

(integrated intensity vs. concentration).

In order to check the stability of the modernized DC-ARC-source, 12 series of the same sets of 4 laboratory standards, each of them with 6 repetitions were analyzed. The following validation criteria were calculated and evaluated:

- precision, calculated as average relative standard deviation (RSD_a) [30], the average of RSD_i values, characterizing each of individual calibration samples
- correlation coefficient (**r**), this is commonly used for the assessing of linearity in validation procedure and graphs with r > 0,995 are considered to be linear
- residual variance - one of the most important comparative characteristics [7], calculated as:

$$s_{res} = \sqrt{\frac{1}{N-2}\sum_{i=1}^{N}(\bar{y}_i - \hat{y}_i)^2} \quad (2)$$

(y_i are the mean of signal values = integrated intensities, $\hat{y}_i$ = calculated values).

- the, in the German norm [31] defined precision of the method:

$$RSD_{METHOD} = \frac{1}{b} \times \frac{s_{res}}{\bar{c}} \times 100/\% \quad (3)$$

This figure of merit should also serve as comparative characteristics.

- linearity - besides of correlation coefficient **r**, which was declared by several authors not to be a useful indicator of linearity [32], the quality coefficient ***QC*** calculated as :

$$QC = 100 \times \sqrt{\frac{(\sum_{i=1}^{n} \frac{y_i - \hat{y}_i}{\bar{y}})^2}{n-1}} \quad (4)$$

where the value of $QC < 5$ % should characterise the suitable calibration.

All results are presented on example of analytes V and Ti, as these are the most complicated. Both chosen analytes are typical carbide forming elements, their carbides are typical heavy volatile ones with boiling temperatures over 3000°C.

Results and discussion

The above described validation criteria obtained for both analytes using the DC-ARC-OES technique are summarized in the Tables II. and III.

Table II. The validation characteristics obtained for the analyte **V** in 12 series of the measurements using the DC-ARC-OES technique

series	1	2	3	4	5	6	7	8	9	10	11	12
RSD_a / %	6.6	8.4	7.9	9.6	7.1	8.9	7.9	9.8	9.1	9.2	6.2	7.7
r	0.973	0.999	0.999	0.994	0.991	0.996	0.999	0.988	0.998	0.999	0.997	0.995
s_{res}	6.1	1.1	0.67	2.7	3.4	2.3	0.87	4.7	1.8	1.4	2.1	2.5
RSD_{method} / %	23	3.5	2.1	9.8	13	8.4	2.7	14	6.2	4.4	7.7	8.9
QC / %	15	2.8	1.7	7.1	9.4	6.1	2.1	12	4.5	3.5	5.8	6.6

Table III. The validation characteristics obtained for the analyte **Ti** in 12 series of the measurements using the DC-ARC-OES technique

series	1	2	3	4	5	6	7	8	9	10	11	12
RSD_a / %	5.3	7.4	6.9	7.9	6.3	8.4	7.5	9.2	6.8	8.2	7.0	5.9
r	0.999	0.999	0.999	0.998	0.999	0.994	0.998	0.999	0.993	0.996	0.999	0.990
s_{res}	2.04	0.53	4.33	1.61	2.10	1.90	2.69	1.27	4.29	3.29	1.18	3.18
RSD_{method} / %	3.1	0.74	6.6	3.0	3.5	2.8	3.8	1.9	7.0	5.1	1.8	6.1

In order to evaluate the linearity of the calibration functions also in another way, in case of the analyte V also the values of the QC were calculated. Comparing these values with the values of

residual variances, a very good correlation was found, as it is shown on Fig. 4. Therefore the decision was made, to evaluate the quality of calibration only on the basis of residual variance, the methods precision (3) as suitable comparative characteristics without the necessity to calculate other values

In the Table IV are compared the best values from 12 series of measurements obtained by using the DCA-301 instrumentation with values achieved for the same samples using similar experimental conditions and the ATOMCOMP-2000 spectrometer.

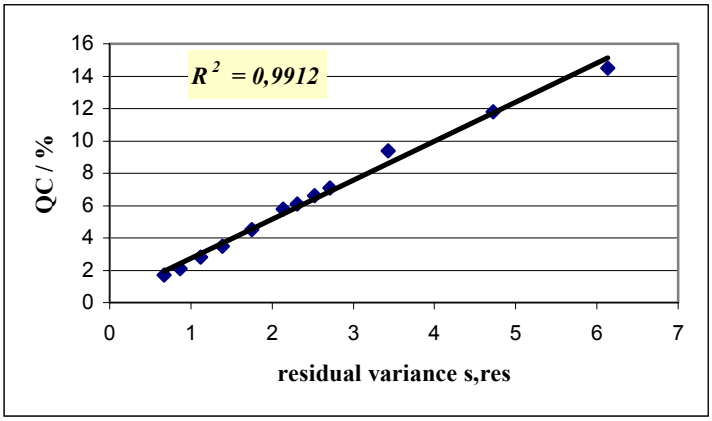


Fig. 4: The dependence of the parameter QC on the value of residual variance

Table IV: Comparison of the optimum validation criteria for both DC-ARC techniques

element	V		Ti	
method	DCA-301	Atomcomp 2000	DCA-301	Atomcomp 2000
RSD_a / %	7.9	6.8	7.0	4.0
r	0.999	0.990	0.999	0.999
s_{res}	0.67	13.2	1.18	1.08
RSD_{method} / %	2.1	13	1.8	2.6
QC / %	1.7	12		1.7

The best results obtained using the DC-ARC-OES technique were compared with similar results, published earlier [9,12,20] obtained for the ETV-ICP-OES technique. The most important characteristics are summarized in the Table V.

Table V. Comparison of most important validation criteria for both DC-ARC as well as ETV-ICP-OES techniques

element	V		Ti	
method	DC-ARC*	ETV-IC-OES ♣	DC-ARC*	ETV-IC-OES ♣
RSD_a / %	**7,9** *(9,8)*	7,1	**7,0** *(6,8)*	8,8
r	**0,999** *(0,988)*	0,993	**0,999** *(0,993)*	0,997
s_{res}	**0,67** *(4,7)*	9,1	**1,18** *(4,29)*	7,0
RSD_{method} / %	**2,1** *(14)*	11	**1,8** *(7.0)*	7,2
QC / %	**1,7** *(12)*	11		4,2

* The **best values**, obtained in case of DCA-301 technique were used; in italics are given the ***worst data*** of the serie

♣ variable sample amounts were used, the signal data were normalized on sample amount 3 mg

Conclusions

The presented results allow the following conclusions on the DC-ARC-OES methods:

a) the stability, as well as the calculated validation criteria of the modernized DC-ARC source are acceptable for a solid-sampling method

b) the both instrumentations produces similar validation characteristics, which are acceptable for the direct analysis of the solid samples

c) both instrumentations do not need any additives or special working atmospheres, which is also an important economic factor.

The comparison of DC-ARC-OES method on the one hand, and the ETV-ICP-OES method on the other hand shows, that there are no significant differences between both different instrumentations. The advantage of ETV-ICP-OES lies in the automation possibility. As a disadvantage the necessity of special working atmosphere should be mentioned, as well as the higher influence of the samples non-homogeneity, given by small analysed sample amounts. The advantage of DC-ARC-OES instrumentation lies in the simplicity of the system and in possibility of the direct analysis of the powdered samples after their weighing in into carrier electrodes. The higher sample amounts could partially decrease the influence of non-homogeneity of samples.

Acknowledgements

This work was supported by Slovak Grant Projects VEGA No. 1/3149/06 and APVT No.20-0094-04 as well as by the Slovak-German DAAD Project 11/2005. The authors are obliged to express their gratitude for this support.

References

[1] Flórián K., Zimmer K.: *Spectrochim. Acta* 1986, **41 B**, 1025–1041
[2] Round-table discussion, 1986, Analytiktreffen ’86, Neubrandenburg
[3] Boumans P.W.J.M.: *Theory of Spectrochemical Excitation*. Hilger, London, 1966
[4] Brenner I.B., Hassler J., Perzl P.R.: 2006, http://ca.pittcon.oro/technical+Program/TPabstra06.nst
[5] Brenner I.B., Zander A.: *Fres. J. Anal. Chem*., 1996, **355**, 559-570
[6] Atomic Minerals Directorate for Explorations and Research, Department of Atomic Energy, Goverment of India: http://www.dae.gov.in/labs/spec.htm17
[7] Methods of Sampling and Inorganic Analysis of Coal, 2003, *U.S. Geological Survey Bulletin,* 1823
[8] Flórián K., Haβler J., Surová E.: *J. Analyt. Atom. Spectrosc*., 1999, 14, 559 - 564
[9] Haβler J., Perzl, P.R.: *GIT Lab. Fachz*.,1996, **40**, 989
[10] Flórián K., Haßler J., Förster O., Boková V.: *Microchim. Acta*, 2006, published online, June 6
[11] Brenner I.B., Haβler J., Barth P., Perzl P.: *ICAS-2006 Moskow, Book of abstracts*, 2006, abstr.32-P3A
[12] Flórián K., Haβler J., Förster O.: *Transactions of Košice Universities* **1/2006**, 27-35
[13] Matchat R., Haβler J., Traub H., Dette A.: *Anal. Bioanal.Chem*., 2005, **383**, 1060-1074
[14] Preuss E.: *Z. Angew. Mineralogie* **1941**, 8-20
[15] Ružičková S., Koller L., Matherny M.: *Chem. Papers, 2006*, **60**, 116-121
[16] Zhou Z., Zhou K., Hou X., Hong L.: *Appl. Spectroscopy Rev*., 2005, **40**, 165-185
[17] Zhou K.Z., Hou X.D, Kou X.M., Feng Y.J.: *Appl. Spectroscopy Rev.,* 2003, **38**, 295-305
[18] Haßler J., Förster O., Perzl P.R.: Book of Abstracts of 40th Hungarian Spectrochemical Conference and 8th Hungarian-Italian Symposium on Spectrochemistry, Debrecen 1997, HI33

[19] Flórián K., Haβler J., Marquardt D.: GIT Lab. Fachz., 2002, **46**, 1144-1148
[20] Haβler J., Detcheva A., Forster O., Perzl P.R., Flórián K.: Annali di Chimica (Rome), 1999, **89**, 827-836
[21] Matusiewicz H., Barnes R.M.: Spectrochim. Acta, 1985, 40 B, 41-47
[22] Nickel H., Zadgorska Z.: ICP Information Newsletter, 1993, **19**, 73-75
[23] Ohls K., Dewies J., Loepp R.: ICP Information Newsletter, 1986, **12**, 177-179
[24] Golloch A., Haveresch-Kock M., Platnikow-Vossgätter, F.: Spectrochim. Acta, 1995, **50 B,** 501-516
[25] Kántor T., Záray Gy.: Fresenius J Anal Chem., 1992, **342**, 927 -935
[26] Záray Gy., Kántor T., Wolf G., Zadgorska Z., Nickel H.: Mikrochim. Acta, 1992, **107**,345-358
[27] Verrept, P., Galbács, G., Moens, L., Dams, R.: Spectrochim. Acta, 1993, **48 B,** 671-689
[28] Kántor T.: Spectrochim. Acta, 2001, 56 B, 1523-1563
[29] Matusiewicz, H.: J. Anal. Atom. Spectrom., 1986, **1,** 171-184
[30] Kurfürst, U. (ed.): Solid Sample Analysis**.** Springer Verlag, Berlin-Heidelberg, 1998
[31] Damman V., Donnevert G., Funk W.: Quality Assurance in Analytical Chemistry, VCH, Weinheim, 1995
[32] Van Luco L., Elskens M., Croux Ch., Beernaert H.: Accred. Qual. Assur., 2002, **7**, 281- 285

Comparison of Various Reaction Media for the Speciation Reliability of Arsenic by Hydride Generation Atomic Absorption Spectrometry

Ingrid Hagarová, Marek Bujdoš
Comenius University, Faculty of Natural Sciences, Geological Institute, Mlynská dolina G, 842 15 Bratislava, Slovakia

Abstract

In waters, arsenic is generally found in the inorganic forms of arsenite (As(III)) and arsenate (As(V)). Because these inorganic species show different toxicity, mobility, and behavior in biological systems, their differentiation has generated considerable interest. Many methodologies exist for the speciation of arsenic in waters. Selective reduction procedures based on the highly pH-dependent reduction reaction between arsenic species and $NaBH_4$ to generate arsine in HGAAS systems are relatively commonly used. In this case, for As(V) strongly acidic solutions are required (pH ≤ 1), while for As(III) hydride formation occurs in mildly acidic solutions. The aim of this study was to critically evaluate the most frequently used reaction media for the speciation of arsenic by HGAAS. The main attention was paid to the determination of arsenite in the presence of arsenate at different ratios. Four various reaction media has been used to achieve a selective volatilization of arsenite: 0.1 mol/l acetic acid (pH 2.9), acetate buffer (pH 5.0), phosphate buffer (pH 7.2) and citrate buffer (pH 3.1).

Key words: *arsenite -- speciation -- hydride generation atomic absorption spectrometry*

1. Introduction

Hydride generation (HG) is a well-known technique for the determination of arsenic at trace levels, which consists of the reaction of some arsenic compounds with sodium tetrahydroborate in acidic medium to produce various arsines [1,2]. Arsenite (As(III)) and arsenate (As(V)), the most commonly found arsenic species in waters, create AsH_3. However, the formation of the arsine from these species is pH dependent. The pH requirements of the reduction reaction indicate that the arsenic species must be fully protonated before they can be reduced to the arsine [3]. The pK_1 for arsenic acid is 2.3, the reaction must therefore be carried out at very low pH (1-2 mol/l HCl is commonly employed). On the other hand, arsenite is protonated under most conditions ($pK_1 = 9.2$) and will react with $NaBH_4$ under conditions, which are only mildly acidic. In the absence of other arsenic species, differentiation of arsenate and arsenite can be achieved simply by exploiting the pH dependency of the $NaBH_4$ reaction.

The aim of this study was to critically evaluate the most frequently used reaction media [4-7] for the speciation reliability of arsenic by HGAAS. The main attention was paid to the determination of arsenite in the presence of arsenate at different ratios.

In this work, four various reaction media has been used for study of selective volatilization of arsenite: 0.1 mol/l acetic acid (pH 2.9), acetate buffer (pH 5.0), phosphate buffer (pH 7.2), and citrate buffer (pH 3.1).

2. Experimental

2.1. Apparatus

Measurements were performed with a Perkin Elmer 1100 atomic absorption spectrometer equipped with continuous-flow hydride system Labtech HG-2. The model solutions (containing As(III) or As(III)+As(V)) prepared in the various studied reaction media and sodium tetrahydroborate stabilized in 1 % sodium hydroxide were introduced into the system trough the peristaltic pumps. The produced gaseous hydrides of arsenic were separated from the solution (in the gas-liquid separator) and swept by a stream of argon (flow rate 300 ml/min) to an electrically heated quartz tube mounted in the light path of an electrodelless discharge lamp for arsenic (power supply 10 W). A spectral band pass of 0.7 nm was selected to isolate the 193.7 nm line. Stabilization time was 60 s.

2.2. Reagents and solutions

The reagents used were of p.a. grade. Arsenic stock solution for As(V) (1000 mg/l) – H_3AsO_4 in 0.5 mol/l HNO_3, As_2O_3, concentrated HCl, $NaBH_4$, NaOH, concentrated acetic acid, sodium acetate, citric acid, sodium citrate, K_2HPO_4, KH_2PO_4, were from Merck (Darmstadt, SRN). Doubly distilled water was used for all dilution procedures.

Arsenic stock solution for As(III) (1000 mg/l) was prepared dissolving 0.1320 g of As_2O_3 in 2 ml of 1 mol/l NaOH, followed by neutralization with 5 ml of 0.5 mol/l HCl and diluting to 100 ml with doubly distilled water.

Working standard solutions of As(III) were prepared by appropriate stepwise dilution of the standard stock solution in the studied reaction media just before use.

The $NaBH_4$ solutions were freshly prepared by dissolving the reagent in 1 % sodium hydroxide.

Acetate buffer (pH 5.0) was prepared mixing 200 ml of 2 mol/l sodium acetate and 200 ml of 1 mol/l acetic acid. Phosphate buffer (pH 7.2) was prepared mixing 200 ml of 1 mol/l KH_2PO_4 and 200 ml of 1 mol/l K_2HPO_4. Citrate buffer (pH 3.1) was prepared mixing 200 ml of 1 mol/l citric acid and 200 ml of 1 mol/l sodium citrate. Then 10 ml of the studied buffer was added to the calibration or model solutions. The prepared solutions were immediately used for measurement.

3. Results and discussion

3.1. Optimization of hydride generation conditions for As(III)

Several variables such as $NaBH_4$ concentration, $NaBH_4$ flow rate and sample flow rate were studied in detail. Arsine from As(III) was generated in various reaction media such as acetic acid, acetate buffer (pH 5.0), phosphate buffer (pH 7.2) and citrate buffer (pH 3.1). Solutions containing 10 µg/l of As(III) (in acetic acid, acetate buffer or citrate buffer) and 20 µg/l of As(III) (in phosphate buffer) were used in this optimization.

3.1.1. Reducing solution

The effect of the variation of the $NaBH_4$ concentration on the absorbance of As(III) was studied in the four reaction media. The first studied reaction medium was acetic acid. Different concentrations of acetic acid were used and compared (0.10 mol/l; 0.25 mol/l; 0.50 mol/l; 0.75 mol/l; 1.00 mol/l; 1.25

mol/l; and 1.50 mol/l). The best sensitivity was achieved in 0.1 mol/l acetic acid (Fig. 1); therefore, this concentration of acetic acid was selected for further experiments.

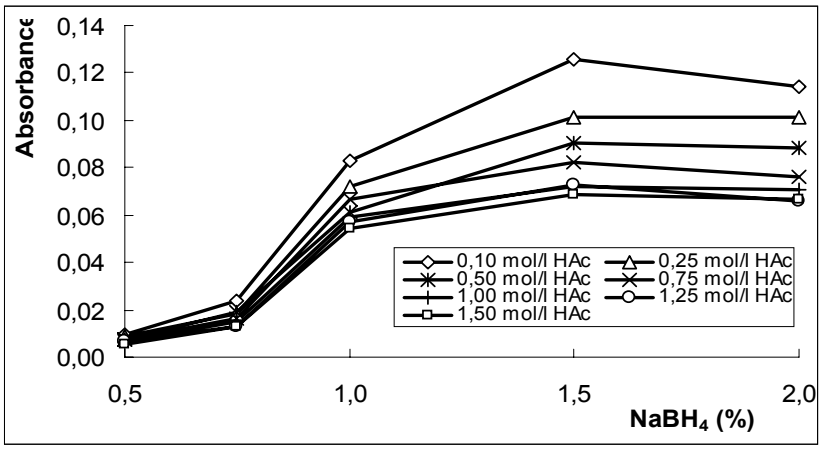


Fig. 1. Effect of $NaBH_4$ concentration on the response of As(III) in acetic acid medium

The effect of the variation of the $NaBH_4$ concentration on the absorbance of As(III) in the all studied reaction media is compared on Fig. 2. As it can be seen, the absorbance of As(III) increases with increase of $NaBH_4$ concentration up to 1.5 % in acetic acid and acetate buffer, up to 3 % in phosphate buffer, and up to 2 % in citrate buffer. Optimal $NaBH_4$ concentrations for As(III) determination in the studied reaction media are shown in Table 1.

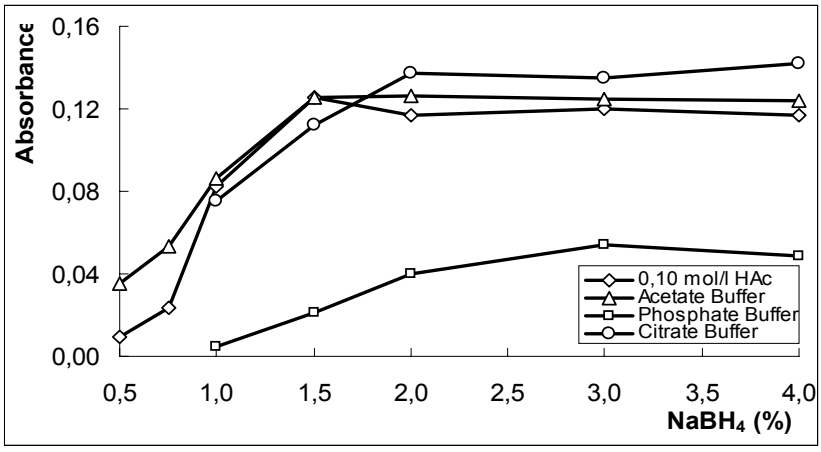


Fig. 2. Effect of $NaBH_4$ concentration on the response of As(III) in acetic acid medium, acetate buffer medium (pH 5.0), phosphate buffer medium (pH 7.2) and citrate buffer medium (pH 3.1)

3.1.2. Flow rate parameters for reducing solution and sample

The efficiency of the arsenic hydride generation was studied for different pump speeds (different flows of the reducing solutions and sample). Effect of the $NaBH_4$ flow rate on the response of As(III) in the various studied reaction media is shown on Fig. 3. Effect of the sample flow rate on the response of As(III) in the various studied reaction media is shown on Fig. 4.

Results illustrated on Fig. 3 show an increase of the As(III) absorbance for all the studied reaction media with the increasing pumping speed until a point where plateau was achieved (in citrate buffer), slightly decreasing absorbances were observed (in phosphate buffer) or dramatically decreasing absorbances were observed (in 0.1 mol/l acetic acid and acetate buffer). Optimal flow rates for $NaBH_4$ solutions in the studied reaction media are shown in Table 1.

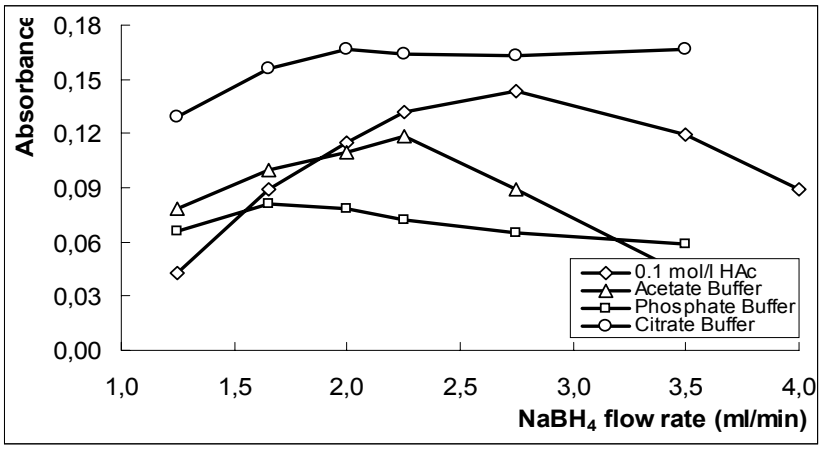


Fig. 3. Effect of the $NaBH_4$ flow rate on the response of As(III) in 0.1 mol/l acetic acid medium, acetate buffer medium (pH 5.0), phosphate buffer medium (pH 7.2) and citrate buffer medium (pH 3.1)

The effect of the sample flow rate on the As(III) absorbance in the studied reaction media is demonstrated on Fig. 4. There, curves with different shapes can be seen. Slightly increasing absorbances with increasing sample flow rate until a plateau was achieved (in phosphate buffer and citrate buffer), dramatically (almost linearly) increasing absorbances with increasing sample flow rate until a plateau was achieved (in acetate buffer) or "S" shaped curve (in 0.1 mol/l acetic acid). Optimal flow rates for sample solutions in the studied reaction media are shown in Table 1.

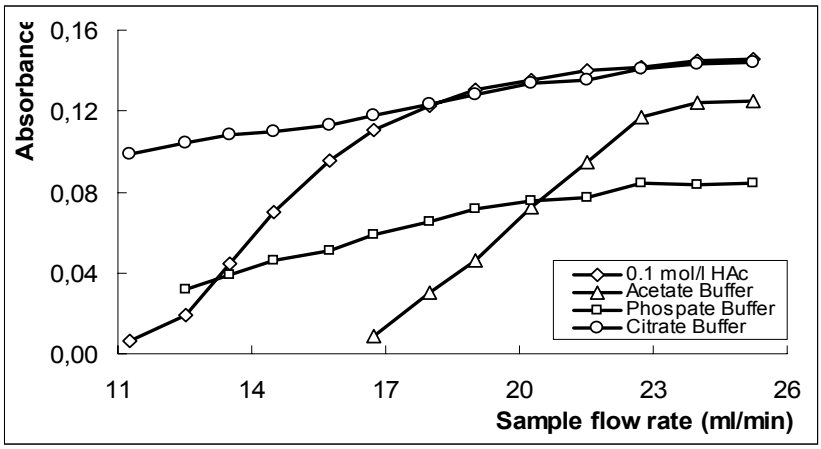


Fig. 4 Effect of the sample flow rate on the response of As(III) in 0.1 mol/l acetic acid medium, acetate buffer medium (pH 5.0), phosphate buffer medium (pH 7.2) and citrate buffer medium (pH 3.1)

Table 1 Optimal conditions for As(III) determination by continuous-flow HGAAS in the various studied reaction media

Reaction medium	$NaBH_4$ concentration (%)	$NaBH_4$ flow rate (ml/min)	Sample flow rate (ml/min)
0.1 mol/l CH_3COOH (pH 2.9)	1.5	2.7	21.5
Acetate buffer (pH 5.0)	1.5	2.2	23.0
Phosphate buffer (pH 7.2)	3.0	1.6	23.0
Citrate buffer (pH 3.1)	2.0	2.0	23.0

Table 2 Comparison of sensitivity for As(III) determination by continuous-flow HGAAS in the various studied reaction media

Reaction medium	Slope of the calibration curve (l/µg)	LOD* (µg/l)	Linear range (µg/l)
1.5 mol/l HCl	0.0222	0.198	up to 12
0.1 mol/l CH_3COOH (pH 2.9)	0.0185	0.238	up to 12
Acetate buffer (pH 5.0)	0.0140	0.314	up to 30
Phosphate buffer (pH 7.2)	0.0043	1.02	up to 60
Citrate buffer (pH 3.1)	0.0169	0.260	up to 12

*LOD calculated as: 3SD of the blank / slope of the calibration graph

Limits of detection (LOD) for As(III) determination in the studied reaction media were compared with LOD achieved in 1.5 mol/l HCl (medium the most often used for the total inorganic arsenic determination). The worse sensitivity was observed in all the studied reaction media (1.2 times in acetic acid, 1.6 times in acetate buffer and 1.3 times in citrate buffer). However, there is no such a big difference between sensitivity in these studied media. On the other hand, sensitivity in phosphate buffer is more than 5 times worse than sensitivity in 1.5 mol/l HCl.

3.2. Interference effect of As(V)

In order to evaluate the As(V) interference effect, the mixtures containing 60 µg/l of total inorganic arsenic with corresponding relative contents of 1 %, 2 %, 5 %, 10 %, 20 %, 35 % and 50 % of As(III) were prepared in the studied media. Then, the interferences due to the different amounts of As(V) on the absorbance of As(III) were expressed as the percent analytical error. This error was calculated for each case as follows:

$$e = \frac{A_2 - A_1}{A_1}.100 \qquad (1)$$

Where, e – analytical error (%); A_2 – absorbance of As(III)+As(V); A_1 – absorbance of As(III).

Mean percent analytical errors ± SD are shown on Fig. 5.

Using 0.1 mol/l acetic acid medium, acetate buffer medium (pH 5.0) or citrate buffer medium (pH 3.1) for selective volatilization of As(III) in the presence of different amounts of As(V) [different ratios of As(III):As(V); 1:1; 1:1.86; 1:4; 1:9; 1:19; and 1:99], similar errors in As(III) determination caused by As(V) interferences were observed. In the optimized conditions, As(V) interferences were less than 10 % in the case of model solution with 6 µg/l of As(III) in the presence of 54 µg/l of As(V) [i.e. 10 % of As(III); ratio 1:9]. The percent analytical errors due to the As(V) interferences were dramatically increased if relative contents of As(III) were less than 10 % [As(III):As(V) ratios more than 1:9].

Using phosphate buffer medium (pH 7.2) for selective volatilization of As(III) in the presence of different amounts of As(V), the percent analytical errors due to the As(V) interferences were less than 10 % in all the studied cases. However, sensitivity for As(III) determination in this medium was lower, so the speciation can be done only if relatively high contents of As(III) in the samples are present.

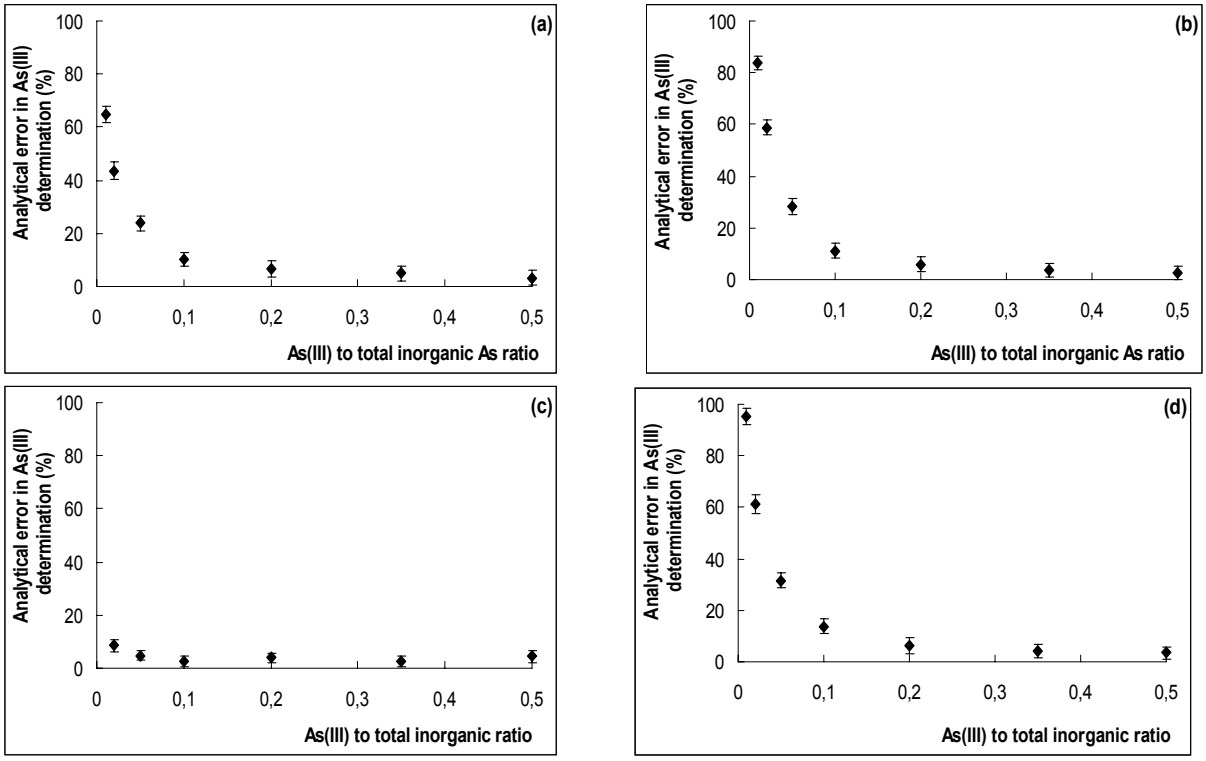


Fig. 5. Effect of As(V) on the response of As(III) in: (**a**) 0.1 mol/l acetic acid medium, (**b**) acetate buffer medium (pH 5.0), (**c**) phosphate buffer medium (pH 7.2) and (**d**) citrate buffer medium (pH 3.1)

4. Conclusion

All the studied reaction media can be used for selective volatilization of As(III) but the serious problem caused by interferences of As(V) was observed (in 0.1 mol/l acetic acid, acetate buffer and citrate buffer) when the ratio of As(III):As(V) was more than 1:9. Over this ratio, analytical error in As(III) determination dramatically increases. However, natural waters usually contain higher ratios of As(III):As(V), so the speciation in the real samples could be accompanied with another speciation procedure to confirm accuracy of the measured data. This problem was not observed in phosphate buffer but sensitivity, in this case, is significantly lower and speciation in this medium can be done only if relatively high content of As(III) is present.

Speciation of As(III) by HGAAS using pH controlled conditions, is really simply, fast and inexpensive but interferences caused by As(V) cannot be ignored.

Acknowledgement

The work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-042002 and Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences under the contract No. VEGA 1/3561/06.

References

[1] Dedina J., Tsalev D.L.: Hydride Generation Atomic Absorption Spectrometry. J. Wiley, Chichester, 1995
[2] Burguera M., Burguera J.L.: Analytical Metrology for Speciation of Arsenic in Environmental and Biological Samples. Talanta, 1997, 44, 1581-1604
[3] Howard A.G.: (Boro)Hydride Techniques in Trace Element Speciation. J. Anal. At. Spectrom., 1997, 12 , 267-272
[4] Torralba R., Bonilla M., Palacios A., Camara C.: Flow-Injection Analysis and Continuous-Flow Systems to Determine As(III) and As(V) by Hydride Generation Atomic Absorption Spectrometry. Analusis, 1994, 22, 478-482
[5] Molenat N., Astruc A., Holeman M., Maury G., Pinel R.: Arsenic Speciation by Hydride Generation-Quartz Furnace Atomic Absorption Spectrometry. Optimization of Analytical Parameters and Application to Environmental Samples. Analusis, 1999, 27, 795-803
[6] Quinaia S.P., Rollemberg M.C.E.: Selective Reduction of Arsenic Species by Hydride Generation Atomic Absorption Spectrometry. Part 1 – Reduction Media. J. Brazil. Chem. Soc., 1997, 8, 349-356
[7] Quinaia S.P., Rollemberg M.C.E.: Selective Reduction of Arsenic Species by Hydride Generation Atomic Absorption Spectrometry. Part 2 – Sample Storage and Determination in Natural Waters. J. Brazil. Chem. Soc., 2001, 12, 37-41

Laboratory Instruments and Equipment as Supplements or Substitutions to Spectrometric Methods

Václav Helán, [2]Edward Reszke, [3]Ernest Beinrohr, [4]Ján Dzurov
2 THETA ASE, s.r.o., Jasná 307, 735 62 Český Těšín, Czech Republic
[2] ERTEC Poland, ul. Rogowska 146/5, 54 440 Wrocław, Poland
[3] Department of Analytical Chemistry, Slovak Technical University, Radlinského 11, 812 37 Bratislava, Slovak Republic
[4] ISTRAN, s.r.o., Radlinského 27, 811 07 Bratislava, Slovak Republic

Abstract

Systems for high pressure mineralization in focused microwave field are useful tools for preparation of samples prior to spectrometric analysis. In many cases, however, the conventional spectrometric analysis can be replaced with striping chronopotentiometry, which in fact has advantages not only in low costs of instrumentation and low operating expenses.

Key words: *microwaves - mineralization – decomposition - sample preparation – coulometry – chronopotenciometry – FIA - trace element.*

Microwave decomposition

Microwave digestion unit ERTEC MAGNUM is a laboratory dedicated equipment devoted for wet digestion of analytical samples with the help of microwave excitation of the reagents in a closed high pressure vessel. The decomposition processes is accelerated by microwave field. Microwaves are effectively absorbed by solutions, which contain polar liquids such as mixtures of concentrated acids, which after reaching the boiling point cause the vapour pressure to increase and ultimately accelerate the desired sample digestion. The sample obtained this way can be analysed quantitatively or qualitatively applying one of the known methods of chemical or physical/chemical analysis.

Standard version of the instrument is manufactured as one-stand high pressure module with built-in control of pressure in the reaction vessel, with continuous monitoring of reaction temperature and with an option to open the closed vessel system and to run it as a digestion system at atmospheric pressure.

Microwave digestion unit consists of metal enclosure, inside which there is a power supply and microwave power generator with wave guide output. One per module water cooled stainless steel digestion vessel with a bayonet mount head is fixed on the wave guide. The vessel head is covered with a plastic shield, which is devoted for catching the fumes of the solvents (Fig. 1).

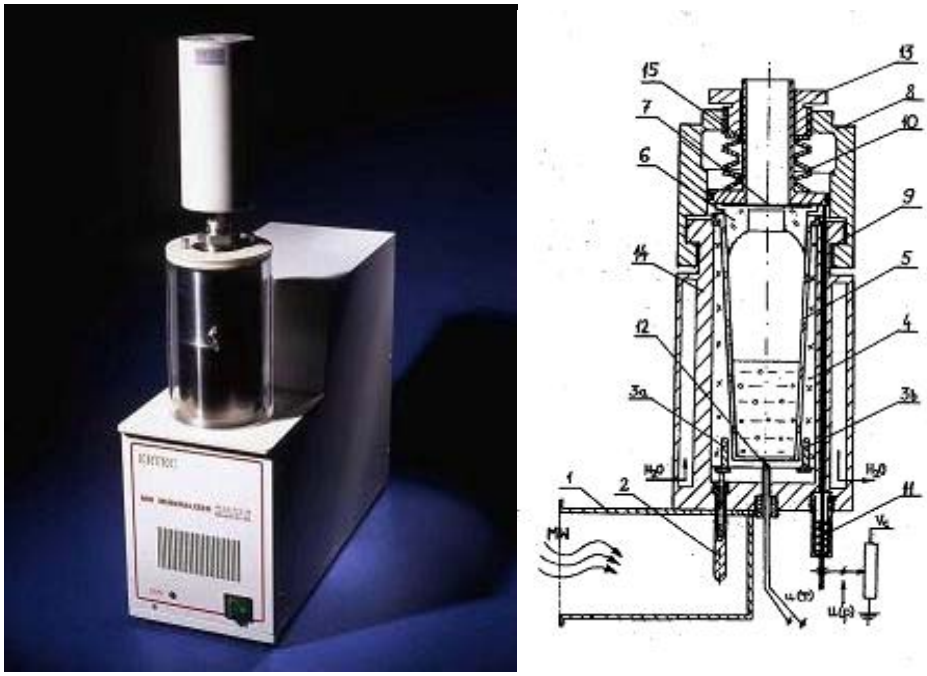


Fig. 1. The new microwave device Ertec-Magnum with cross-section trough the vessel

The operator controls the digestion unit through the operator console or by using a personal computer. Both PC and the operator console enable setting and storing the digestion programs with automatic switching off-and on of the generator featuring all the necessary user's control over the instrument. Main advantage of PC control is the possibility to check the course of pressure, temperature and level of microwave energy inside the vessel with a sample. The course of mineralization can be displayed and stored (Fig. 2) and can further be used either for optimizing the mineralization process or as a record of mineralization performed in closed system.

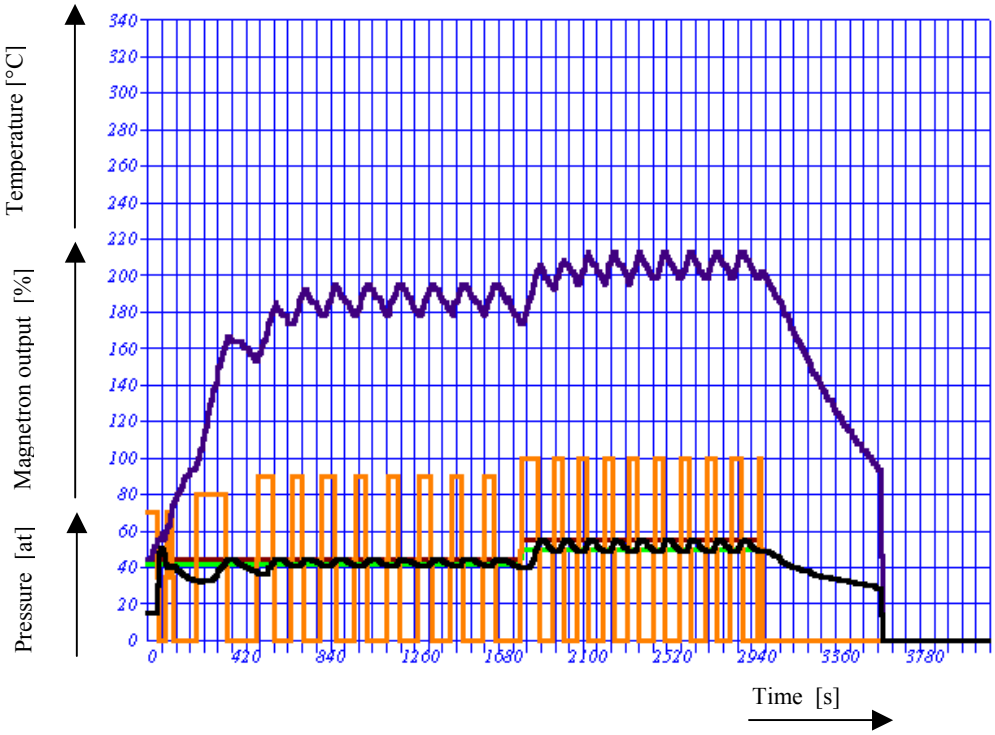


Fig. 2. Flour digestion / PC monitoring chart

Main advantages of this system is a very fast and reliable measurement of the pressure resulting in the possibility to digest extremely high weights of samples and short times of decomposition (Tab. 1), and possibility of conversion to whether pressurized or atmospheric pressure systems. The application of water cooling immediately after decomposition enables short times of cooling the samples down after processing (typ.10 min).

Tab. 1. Examples of decomposition procedures

Sample	Sample mass	Digestion procedure	Pressure (max-min)	Solvents
Vitamine pills	0.1g	15 min/100%	45-42 at	2ml HF 2ml H_2O_2
Blood	0.5g	7 min/100%	45-42 at	7ml HNO_3,
Flax seed	0.5g	7 min/100%	45-42 at	6ml HNO_3
Sečet flag rhizome	0.5g	7 min/100%	45-42at	6ml HNO_3
Bovine liver	0.2g	8 min/80%	45-42 at	3ml HNO_2
Child's hair	1.0g	Step 1) 1 min/50%, 30 sec/0% Step 2) 1 min/60%, 30 sec/0% Step 3) 2 min/70%, 30 sec/0% Step 4) 2 min/80%	20-17 at 30-27 at 35-32 at 45-42 at	7ml HNO_3 3ml H_2O
Horse hoof	0.2g	15 min/100%	45-42 at	6ml HCl 3ml $H2O_2$
Bovine bone	0.2g	25 min/100%	45-42 at	6ml HCl, 3ml H_2O_2
Water, waste	50ml	15/100%	45-42 at	7ml HNO
Bovine meat, wet	5.0g	Step 1) 2 min/80%, Step 2) 1 min/60%, 30 sec/0% Step 3) 2 min/70%, 30 sec/0% Step 4) 2 min/80%	20-17 at 30-27 at 35-32 at 45-42 at	10ml HNO_3
Fat sausage	1.0g	6 min/100%	45-42 at	6ml HNO_3
Cottage cheese	1.0g	10min/100%	45-42 at	6ml HNO_3
Hard cheese	1.0g	10min/100%	45-42 at	6ml HNO_3
Butter	1.0g	Step 1) 2 min/60% Step 2) 2 min/70% Step 3) 2 min/80% Step 4) 5 min/90%	20-17 at 30-27 at 35-32 at 45-42 at	6ml HNO_3
Pork fat	1.0g	10min/100%	45-42 at	6ml HNO_3
Fly	0.2g	5min/80%	45-42 at	1ml H_2O_2

Striping chronopotentiometry

Trace concentrations of all toxicologically interesting elements incl. anions and some organic compounds can easily be determined by electro-analytical methods, which are not only much cheaper as compared to other commonly used methods but may also be more resistant against the influences of salts and organic species. Beside the extremely low running costs another advantage of electroanalysis is the ease of its use. Modern electroanalytical methods such as stripping chronopotentiometry (SCP) enable determination of elements beginning at concentration down to the levels of ca. 0.1 μg/L.

Galvanostatic stripping chronopotentiometry is a two-step analytical method. In the first step, the analyte species are collected at the working electrode, which is set to a suitable deposition potential or is supplied with an appropriate deposition current. After a short quiescence period the deposit is stripped by a constant current whereas the change of the potential of the working electrode is registered. The dependence of potential vs. time resembles of an oxidation-reduction titration curve. However, the change of the potential is evaluated according to the memory mapping technique, which converts the original S-shaped dependence to a peak-like signal containing the stripping peaks of the deposited species. The integrated value of a stripping peak called chronopotentiometric transition time τ, is straight proportional to the concentration of the corresponding elements in the sample, the peak potential is a function of the redox potential and reversibility of the element in the actual sample matrix.

The above described principles were used in construction of EcaFlow 150 GLP, a compact laboratory instrument which is controlled by an IBM compatible PC. The control unit in the instrument contains precise current controller, control processor with the dual channel analyser, fast A/D and D/A converters and a switchable potentiostat/galvanostat. The compact flow system is controlled by the control unit and operates fully automatically. It contains computer controlled electromagnetic valves for switching either to the sample or electrolyte solutions or to a standard solution to realize the standard addition procedure. The solutions are driven by a peristaltic pump. The heart of the system is the patented compact electrochemical cell with porous flow-through working electrode made of suitable inert material. (Fig. 3)

The guaranteed accuracy of the method checked against reference materials is better than 10 % for the ppb concentration region. The precision in the same region is between 1 and 5 % depending on the element determined. The detection limits for 5 ml sample volumes are about 0.1 ppb (Cd, Pb, Hg), 0.3 ppb (Se, As, Cu), 1 ppb (Zn), 5 ppb (Mn, Sn, Bi) and 10 ppb (Fe, Cr, iodine, sulphide). However, these values can be improved by using purified reagent solutions and larger sample volumes. The concentration range without sample dilution expands from the sub-ppb region up to 10-100 ppm, i.e. at least 5 orders of concentration range magnitude.

Fig. 3. EcaFlow 150 GLP – flow-through coulometric analyzer

Main advantages of this contemporary analytical system are: automatic analysis including the calibration, high sample throughput, some applications in the calibration-less mode, large dynamic concentration range, no need of technical gases for sample deairation.. The system is variable, the hardware and software enables the use of other modes such as direct coulometry, voltammetry, potentiometry, conductometry as well as other electrode systems.

Conclusions

Striping chronopotentiometry with microwave enhanced sample preparation is a suitable analytical method for determination of trace elements in various matrices. Low cost of analysers and low running costs make it possible to built versatile and reliable systems for analysis of trace elements

in laboratories which are not able to afford purchasing of much more expensive equipment which use spectrometric methods like ETA AAS or ICP. Broadening of progressive electroanalytical methods could lead to better environmental and hygienic control of waters, soil, plants and animal materials, food and beverages and clinical samples as well as to a better control of industrial processes.

References

[1] Matusiewicz H.: Development of high pressure closed-vessel systems for microwaves-assisted sample digestion. *Microwave-enhanced chemistry fundamentals.* American chemical society 1997
[2] Parosa R., Reszke E.: System UniClever - nowa idea konstrukcyjna wysokosprawnych mineralizatorów mikrofalowych. *Research Report 3/97* Plazmatronika, Wroclaw 1997
[3] Helán V., Reszke E., Parosa R.: Mikrovlnná technika pro přípravu vzorků. *Anorganická analýza v životním prostředí,* 2 THETA, Český Těšín 1998
[4] Krakovská E., Kuss H.M.: *Rozklady v analytickej chémii,* Vienala, Košice 2001
[5] Reszke E.: *Int. report,* ERTEC Poland 2005
[6] Reszke E., Oziembłowski M., Trziszka T.: Application of high energy densities in laboratory practice: sterilization, digestion and ashing of analytical samples. *Aktuální problematika hygienických laboratoří,* 2 THETA, Český Těšín 2005
[7] Beinrohr E., Cakrt M., Dzurov J., Kottas P., Kozakova E.: Calibrationeless determination of mercury by flow-through stripping coulometry. Fresenius J. Anal. Chem. 356:253 (1996)
[8] Beinrohr E., Dzurov J.: Prietoková coulometria. *Elektroanalytické metody,* 2 THETA, Český Těšín 2001
[9] Beinrohr E., Dzurov J.: EcaFlow: flow-through coulometry. ISTRAN Ltd., Bratislava 2003
[10] Beinrohr E., Manová A., Střelec M.: Nové možnosti stanovenia kovov i nekovov prietokovou coulometriou. *Aktuální problematika hygienických laboratoří,* 2 THETA, Český Těšín 2005

Determination of Thallium in Fungal Biomass by ETAAS

Milan Kališ, Martin Urík, Slavomír Čerňanský, Ján Medveď
Comenius University in Bratislava, Faculty of Natural Sciences, Geological Institute, Mlynská dolina G, 842 15 Bratislava 4, Slovak Republic

Abstract

Thallium is highly toxic element that may be immobilized in the environment by fungal biomass. Two types of fungal biomass originally isolated from locality Pezinok - Kolársky vrch, later identified as *Neosartorya fischeri* (Type A) and *Aspergillus* section *Nigri* (Type B) were used for preparation of pelletized fungal biomass by dynamic cultivation. The analytical conditions for thallium determination in fungal biomass by electrothermal atomic absorption spectrometry were studied and optimized. Optimal temperature conditions for pyrolysis and atomization of thallium from aqueous standards and matrix-matched standards were selected. The precision and accuracy of thallium determination by described method for fungal biomass was acceptable. The limit of detection of the used method was around 0.1 μg.L^{-1} Tl determined in 100 mL solution of decomposited fungal biomass.

Key words: *Electrothermal AAS; Thallium; Fungal Biomass*

1. Introduction

Thallium was detected spectroscopically by Sir William Crooks, an English chemist, in 1861. Crooks had obtained the sludge left over from the production of sulfuric acid from a friend. After removing all the selenium, he inspected it with a device known as a spectroscope to look for sign of tellurium. Rather than seeing the yellow spectral lines produced by tellurium, he observed a bright green line thallium, after the Greek word for „green twig“, thallos. The next year he isolated samples of thallium.

Thallium enters to the biosphere from natural and anthropogenic sources. Natural sources of thallium are less bioavailable and therefore of less concern to regulators than anthropogenic sources of thallium. It's more toxic to mammals then mercury, cadmium, lead, silver, copper or zinc [1-3].

Nowadays, biosorption is widely used to reduce amount of toxic compounds, as a very effective method for bioremediation of contaminated waters. Biosorption represents uptake of heavy metal by microbial biomass (dead or alive) through physico-chemical interactions, such as adsorption or ion exchange [4,5]. Biosorption by fungal biomass is primarily realized by fungal cell wall that represents active polyelectrolyte with amino, carboxyl, phosphate and sulfate groups [5].

Thallium is almost always determined as total metal, rather than specific thallium compounds. Among the wide range of techniques that can be used to measure thallium are spectrophotometry (SP), mass spectrometry (MS), differential pulse anodic stripping voltammetry (DPASV), neutron activation

analysis (NAA), inductively coupled plasma atomic emission spectrometry (ICP AES) and X-Ray fluorescence (XRF) [6]. The direct aspiration atomic absorption analysis is the most used and straightforward method for thallium determination, electrothermal atomic absorbtion (ETAAS) analysis is used for very low analyte levels [7-8]. Usually for the sample digestion for thallium determination in biological samples by ETAAS an oxidizing acid mixture, such as nitric:perchloric:sulfuric acid mixture 3:1:1 (v/v/v) is used [9]. Alternatively, thallium can be extracted from biological samples such as blood or urine by chelating agents such as diethylthiocarbamate in methylisobutylketone and measured by atomic absorbtion analysis [10]. Additionally, analytical procedures of preconcentration are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

The scope of the present work was:

- optimization of work conditions of ETAAS for thallium determination in biomass,
- identification and elimination of interference effects caused by used extracting agents and effects of sample matrix variability on accuracy of analytical results,
- verification of reliability of obtained analytical results by analysis of certified reference materials and by standard additions technique,
- application the proposed analytical procedure for the determination of thallium in fungal biomass.

2. Experimental

Instrumentation

A Perkin-Elmer (Überlingen, Germany) Model Zeeman 3030 atomic absorption spectrometer equipped with a HGA 600 graphite furnace and an AS-60 autosampler was used.

Reagents

All employed reagents were of the analytical grade (HNO_3 was purified by the sub-boiling distillation in quartz apparatus). Standard Tl solutions: Standards were prepared by dilution of 1000 mg L^{-1} Tl stock solution (Merck, Darmstadt, Germany) with redistilled water to a required concentration with the HNO_3 addition (3 mL conc. HNO_3 in 100 mL solution), and with the same reagents, which were used for extraction. The calibration was checked periodically after every 25 measurements with a 30 μg L^{-1} Tl solution. Matrix modifier solutions*:* 1000 mg L^{-1} Pd, [Pd in 1% HNO_3] (Analytika, Czech Republic); 1000 mg L^{-1} $Mg(NO_3)_2$, [$Mg(NO_3)_2.6H_2O$ in H_2O] (Analytika, Czech Republic); 10.0 g L^{-1} ascorbic acid in H_2O (Analytika, Czech Republic). The mixed palladium nitrate and magnesium nitrate modifier contained 1000 mg L^{-1} Pd and 600 mg L^{-1} $Mg(NO_3)_2$ [7-10]. Working Pd solution 1000 mg L^{-1} Pd and 1% ascorbic acid solution was used in order to obtain reduced Pd [8].

Certified reference materials (CRM): NIST 1643c (Trace elements in water) from the National Institute of Standards and Technology (USA) and CRM PB 12-2-03 (Lucerna-alfalfa) from the Slovak Institute of Metrology - SIM Bratislava (Slovakia).

Preparation of fungal biomass by dynamic cultivation

Two 1 L conical flasks were filled with 500 mL of Sabouraud culture medium (HiMedia, Mumbai) and inoculated by 10 mL of spore suspension of two different types of fungal propagules. Prepared systems were cultivated on shaker (90 shakes.min^{-1}) under laboratory conditions. After 4-day cultivation there were fungal pellets with spherical shape 1 - 3 mm in radius in these systems. Prepared different types of fungal biomass (type A and type B) in pelletized form were then separated by filtration and used in following experiments.

250 mL beakers were filled with 45 mL of redistilled water and 5 mL of $TlNO_3$ solution with concentration of 1 $mg.L^{-1}$ Tl or 5 $mg.L^{-1}$ Tl was added. Into each beaker was then added 2 g of wet weight of fungal biomass type A or B. After an hour's sorption were both types of fungal biomass isolated from solution by filtration and analyzed for content of biosorbed thallium.

Fungal biomass type A and type B was later identified as *Neosartorya fischeri* and *Aspergillus* section *Nigri*, respectively.

Decomposition and determination of the thallium in fungal samples

The total thallium concentrations in the fungal samples (1-10g) were determined after their high-pressure digestion in closed vessels with concentrated nitric acid (5 mL), respectively, at temperatures up to 160 ^oC/4 hours. The solutions were transferred into a 50 mL calibrated flask, diluted to volume with redistilled water. Then, 25 mL of solution was transferred into glass beaker, 5 mL of conc. sulfuric acid was added. This mixture was evaporated 4 hours and after cooling was transferred into 25 - 50 mL calibrated flask and diluted to volume. The instrumental settings and the heating programmes used for Tl determination using pyrolytically coated graphite tubes with integral platforms are shown in Table 1. Integrated absorbance was measured. All measurements were made with at least four replicates. The blank solution was prepared by the same procedure.

Table 1. Optimal instrumental and working parameters for thallium ETAAS determination

Spectrometer					
Wavelenght	276.8 nm				
Bandwidth	0.7 nm				
Lamp	Perkin-Elmer EDL system (7 watts)				
Platform	L´vov coated				
Carrier gas	Argon				
Background correction	Zeeman				
Signal mode	Peak height				
Scale expansion	None				
Read time	4 sec				
Sample volume	20 µL				
Modifier volume I. (Pd solution – 1000 mg L^{-1} Pd)	8 µL (8 µg mass of Pd)				
Modifier volume II. (Ascorbic acid - 1% solution)	5 µL (50 µg mass of ascorbic acid)				
Temperature programme					
Step	**Temperat. /^oC**	**Rmap/s**	**Hold/s**	**Ar flow rate/mL min^{-1}**	**Read**
Drying	90	10	20	250	
Drying	120	10	20	250	
Pyrolysis	900	20	10	250	
Atomization	1800	0	5	0	On
Cleaning	2400	1	3	250	

3. Results and discussion

Optimization of thermal programme and selection of optimal matrix modifier

The sensitivity of thallium determination is strongly dependent to experimental conditions. The pyrolysis and atomization temperatures for Tl determination were optimized in the absence and in the presence the mixtures of a 10 μL Pd + $Mg(NO_3)_2$ [0.05% m/v Pd + 0.03% m/v $Mg(NO_3)_2$], and 8 μL Pd (8 μg mass) + 5 μL ascorbic acid (50 μg mass), respectively as matrix modifiers in redistilled water and fungal matrix solution. Redistilled water and fungal matrix solution contained 50 ng mL^{-1} Tl.

Integrated absorbance was used for signal quantification. The pyrolysis and atomization curves for Tl in prepared solutions are presented in Fig. 1 (50 ng mL^{-1} Tl in redistilled water) and Fig. 2 (50 ng mL^{-1} Tl in fungal biomass solution). The experimental results show that the thallium absorbance decreased in presence of Pd + $Mg(NO_3)_2$ matrix modifier for both aqueous and fungal biomass thallium solutions. The optimal drying, pyrolysis and atomization temperatures and the choice of suitable modifier (Pd + ascorbic acid) were selected from the course of temperature curves for Tl determination. The optimal ETAAS temperature programme is summarized in Table 1.

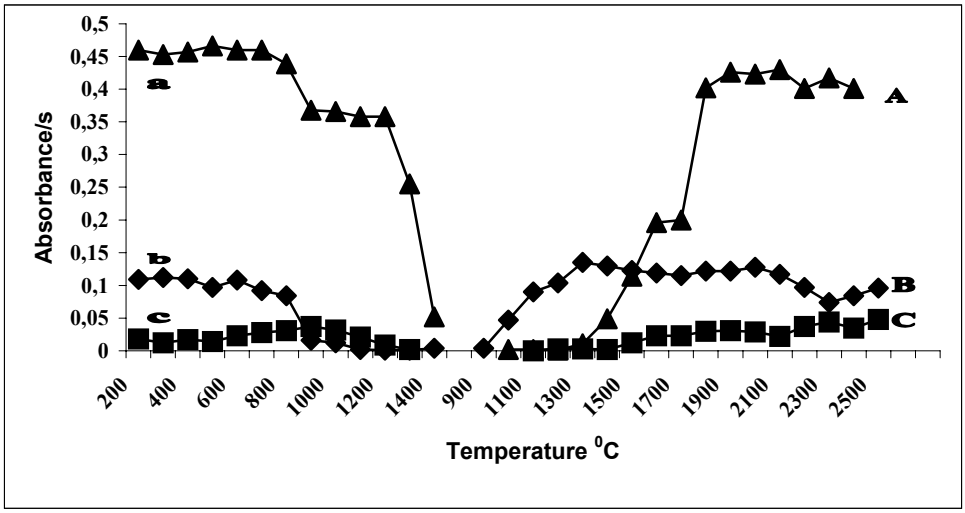


Figure 1. Pyrolysis (a, b, c) and atomization (A, B, C) curves for Tl in redistilled water containing a 50 ng mL^{-1} Tl: a, A - with modifier Pd + ascorbic acid; b, B – without modifier; and c, C – with modifier Pd + $Mg(NO_3)_2$

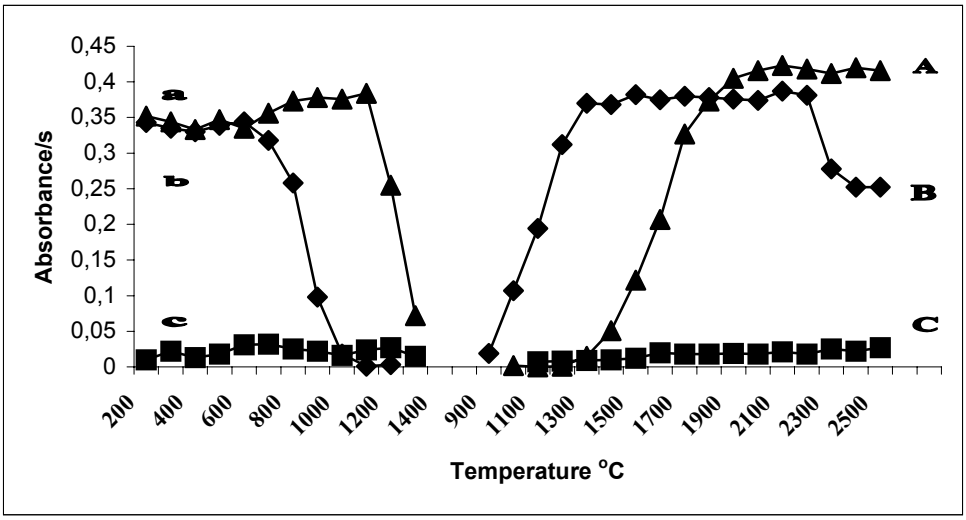


Figure 2. Pyrolysis (a, b, c) and atomization (A, B, C) curves for Tl in spiked fungal biomass solution containing a 50 ng mL^{-1} Tl: a, A - with modifier Pd + ascorbic acid; b, B – without modifier and c, C - with modifier Pd + $Mg(NO_3)_2$

Influence of macro-components variability

The influence study of macro-components present in biological samples on Tl reliable determination was evaluated by examination of its recovery on CRM of water and plant and studied real fungal sample solution. The 0.030 mg L^{-1} Tl was added to each solutions. The results are summarized in Tab. 2 and they show that the Tl determinations are not affected by macro-components present in the studied samples.

Table 2. Recovery of thallium added to CRM and real fungal sample (n = 5)

Sample	Determined (mg L^{-1})	Added (mg L^{-1})	Estimated (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
Water NIST 1643c	0.0073 ± 0.001	0.030	0.0373	0.0354 ± 0.006	94.9
Lucerne 12-2-03 (mg.kg^{-1})	0.047 ± 0.0035	0.030	0.077	0.074 ± 0,0038	96.1
Real fungal - sample[a]	0.052 ± 0.004	0.030	0.082	0.080 ± 0.0048	97.6

[a] determined in 100 mL solution of decomposited fungal biomass

Limit of detection, accuracy, and precision

The analyses were performed using a calibration curve and the method of standard additions. The accuracy of analytical results for Tl in fungal biomass was checked by the analysis of total Tl concentrations in environmental CRMs NIST 1643c - Trace elements in water and SIM 12-2-03 - Lucerne Alfalfa and by additions of 0.030 mg L^{-1} Tl to studied CRMs (Tab. 2). The obtained Tl contents were in a good agreement with the recommended values. The calibration curve was linear at least up to 100 ng mL^{-1}. The limit of detection (LOD) based on 3 σ definition for the used method was around 0.1 μg.L^{-1} determined in 100 mL solution of decomposited fungal biomass. Precision of studied elements determination expressed by relative standard deviation (RSD) varied in a range from 2.5 % to 17.3 %.

Application of the proposed analytical procedure for the determination of thallium in fungal biomass samples

Fungal biomass as part of biogeochemical barriers is widely used as biosorbent in ecological mechanism of bioremediation of waters. Determination of concentration of biosorbed thallium on fungal biomass may help to determine which type of biomass (fungal species) or which mechanism of chemical or physical pretreatment of biomass is most effective for biosorption. In this work we have used two different common fungal types (fungal species) to determine which fungal type more effectively sorbs thallium. Type A (*Neosartorya fischeri*) immobilized on its surface in average 2.72 μg.L^{-1} Tl (determined in 100 mL solution of decomposited fungal biomass) from systems with concentration of 0.1 mg.L^{-1} Tl, while the sorption activity of biomass Type B (*Aspergillus* section *Nigri*) was much lower (0.63 μg.L^{-1} Tl). Results are shown in Tab. 3. From systems with concentration of 0.5 mg.L^{-1} Tl (Tab. 4) immobilized fungal biomass Type A and Type B in average 18.08 μg L^{-1} Tl and 1.88 μg.L^{-1} Tl, respectively.

Table 3. Concentration of immobilized thallium in two types of fungal biomass (biosorption) from aqueous solution of $TlNO_3$ with concentration of 0.1 mg L^{-1} Tl (n = 4)

Type of fungal biomass	Concentration of Tl ± SD in biomass*** (μg.L^{-1})	RSD (± %)
type A*	2.53 ± 0.37	14.62
type A*	2.84 ± 0.51	17.92
type A*	2.79 ± 0.6	21.51
type B**	0.67 ± 0.18	26.87
type B**	0.61 ± 0.13	21.31
type B**	0.61 ± 0.21	34.42

* pelletized biomass from fungal species identified as *Neosartorya fischeri*
** pelletized biomass from fungal species identified as *Aspergillus* section *Nigri*
*** determined in 100 mL solution of decomposited fungal biomass

Tab. 4. Concentration of immobilized thallium in two types of fungal biomass (biosorption) from aqueous solution of $TlNO_3$ with concentration of 0.5 mg.L^{-1} Tl (n = 4)

Type of fungal biomass	Concentration of Tl ± SD in biomass*** (μg.L^{-1})	RSD (± %)
type A*	20.10 ± 2.81	13.98
type A*	16.52 ± 1.57	9.5
type A*	17.62 ± 1.57	8.88
type B**	2.13 ± 0.87	40.84
type B**	2.05 ± 0.53	25.85
type B**	1.46 ± 0.38	26.0

* pelletized biomass from fungal species identified as *Neosartorya fischeri*
** pelletized biomass from fungal species identified as *Aspergillus* section *Nigri*
*** determined in 100 mL solution of decomposited fungal biomass

4. Conclusion

An ETAAS method for determination of Tl in biological (fungal biomass) samples was studied and optimized. The potential matrix interferences in biological samples were studied and eliminated by application of mixture of 8 μg Pd + 50 μg ascorbic acid as matrix modifier, Zeeman background correction, pyrolytically coated graphite tubes with integral platforms, standard additions technique and matrix-matched calibration standards with fungal biomass solutions. The detection limit, precision and accuracy thallium determination by the described method was acceptable for studied materials.

Acknowledgements

This work was financially supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic, Grant VEGA No. 1/3555/06, VEGA No. 1/3462/06 and Grant Agency of the Comenius University No. 132.

References

[1] Lee, A.G.: The chemistry of Thallium. Pergamon Press, London, 1971
[2] Nriagu, O.J. (Ed.): Thallium in the environment. Wiley, New York, 1998

[3] Sharma, J., Sharma, R.L., Singh, H.B., Satake, M.: Hazards and analysis of thallium. A review, Toxicol. Environ. Chem., 1986, 11, 93-116
[4] Šimonovičová, A., Ševc, J., Iró, S.: Trichoderma viride Pers. ex Gray as biosorbent of heavy metals (Pb, Hg and Cd).Ekologia, 2002, 21, 298-306
[5] Gadd, G.M., White, C.: Microbial treatment of metal pollution – a working biotechnology? Trends Biotechnol., 1993, 11, 353-359
[6] Adriano, D.C.: Trace Elements in Terrestrial Environments – Biochemistry, Bioavailability and Risk of Metals (2nd ed.), Springer, New York, 2001
[7] Rušová, V., Kolihová, D.: A contribution to the determination of thallium in waters. Proceedings of XXIX conference „New analytical methods in water chemistry - Hydrochémia ´92“, Bratislava, 1992 210-220
[8] Villar, M., Alava, F., Lavilla, I., Bendicho, C.: Operational speciation of thallium in environmental solid samples by electrothermal atomic absorption spectrometry according to the BCR sequential extraction scheme. J. Anal. At. Spectrom., 2001, 16, 1424-1428
[9] Kneip, J., Crable, M.: Metals in blood or tissue – method 118. In: Method for biological monitoring. Washington, D.C.: American Public Health Association, 1988, 221 - 228
[10] Angeger A., Schaller F.: Analysis of hazards substances in biological materials. Volume 1, Weinheim, FRG:VCH, 1985, 201 – 208

Determination of Lead in Blood by Electrothermal Atomic Absorption Spectrometry - Long-Term Participation in External Quality Control for Lead in Blood

Lucie Kašparová, Jana Knotková
National Institute of Public Health, Centre of Occupational Health, Šrobárova 48, 100 42 Praha 10, Czech Republic

Abstract

The analytical method for the determination of lead in whole blood was tested and verified by successful long-term participation in the external quality control program United Kingdom National External Quality Assessment Service (UK NEQAS). Blood samples after microwave digestion were analyzed by electrothermal atomic absorption spectrometry with a transversely heated graphite atomizer and a longitudal Zeeman background correction system. Two samples a month with various content of lead were analyzed in the UK NEQAS. The concentration range of blood lead samples from July 2003 to March 2006 (n=54) was from 0.10 to 3.50 µmol.l^{-1} (from 20 to 720 µg.l^{-1}). Recovery was within the range 92.2%-108.9% and expanded uncertainty was 9.0% (coverage factor 2).

Key Words: *lead – whole blood – electrothermal atomic absorption spectrometry – external quality control*

Introduction

Lead is well established occupational and environmental pollutant with a broad spectrum of serious health effects [2]. The recent research unequivocally demonstrated its irreversible effects on central nervous system in children encountered at surprisingly low blood lead levels without an apparent treshold [1,8]. Blood lead level has become the most frequently used biomarker of exposure to lead [3].

The most common and widespread method for lead quantitative analysis in various biological samples is electrothermal atomic absorption spectrometry (ETAAS) [6,7], which is used for determination of low concentrations approaching the limit of determination. This is typical scenario when issues of accuracy and reliability of the measurement is of great interest.

The external quality control system is one of the most important tools in quality assurance [4,9]. Our experience shows that the regular participation in an external quality control program is unavoidable. Otherwise, accuracy, reliability, and comparability of results cannot be guaranteed.

Materials and methods

Our laboratory has been participating in the international quality program "United Kingdom National External Quality Assessment Service" (UK NEQAS) ensured by Wolfson EQA Laboratory in Birmingham for more than 25 years. The Varian SpectrAA30 atomic absorption spectrometer with Zeeman background correction has been used since 1992 and GBC Avanta UZ atomic absorption spectrometer equipped with a transversely heated graphite atomizer and a longitudal Zeeman background correction (GBC, Australia) since 2000. Each participating laboratory analyses two samples of blood per month.

Liquid sonicated whole blood haemolysates (prepared from fresh equine blood anticoagulated with EDTA), sent to UK NEQAS participants, are ready to use. Blood samples (in duplicate) were prepared for analysis by microwave mineralization with HNO_3 and H_2O_2. We used microwave oven MEGA 1200 (Milestone, Italy) equipped with an evaporation rotor FAM 40 Module. Sample blanks and reference materials (after reconstitution according to directions) were prepared in the same way.

Ultra-pure nitric acid (65% v/v) prepared by subboiling distillation in our lab (SubPUR, Milestone, Italy) and hydrogen peroxide (30% v/v Suprapur, Merck, Germany) were used for samples preparation.

Reagent $NH_4H_2PO_4$ (Suprapur, Merck), $Mg(NO_3)_2.6H_2O$ (Suprapur, Merck) and the Palladium matrix modifier solution (10 $g.l^{-1}$, Merck) were used for preparing chemical modifier. Calibration solutions were prepared by successive dilution of standard solution 1000 ± 2 mg $Pb.l^{-1}$ (CertiPUR, Merck). All water in use was prepared in water purification system Simplicity (Millipore, France).

Certified reference materials ERM®-CE 194 – 196 (IRMM, Belgium) and Seronorm™ Trace Elements Whole Blood Level 1-3 (SERO AS, Norway) were used for quality control.

Lead content was determined by ETAAS Avanta UZ with Pb superlamp (Photron). Optimized conditions and parameters of the measurement are given in Tab.1.

Table 1. Optimized conditions and parameters of the measurement for Avanta UZ

AAS Avanta UZ	
Wavelength	217.0 nm
Slit	0.8 nm
Pyrolytic temperature	1200°C
Atomization temperature	2200°C
Modifier	0.2% Pd 0.1% $HN_4H_2PO_4$ 0.1% $Mg(NO_3)_2$
Type of calibration	Standard addition technique
Limit of detection	5 µg $Pb.l^{-1}$ (whole blood)
Repeatability	2.0%
Reproducibility	4.0%

Due to difference between the aqueous standard calibration and the standard addition technique or the blood matrix-matched calibration the use the aqueous standard calibration was not possible.

We also participated in the external quality program German External Quality Assessment Scheme (G-EQUAS), Erlangen, Germany. We analyzed two samples in occupational medical field and two samples in environmental medical field per year (2003-2005).

Result and discussion

Accuracy of ETAAS method was checked by the analysis of reference materials with certified values ERM®-CE 194 – 196 (bovine blood). Results are in Table 2.

Table. 2. Test of accuracy

Material	Certified value µg Pb.l^{-1}	Found µg Pb.l^{-1}	Recovery %
ERM-CE194	126 ± 4	130 ± 4	102.8
ERM-CE195	416 ± 9	438 ± 26	105.2
ERM-CE196	772 ± 11	814 ± 36	105.5

Reference materials Seronorm™ Trace Elements Whole Blood Level 1, 2 or 3 were used for day-to-day quality control.

The method for determination of lead in blood was verified it in the external quality control program UK NEQAS. Average number of participating laboratories in determination of lead in blood in the UK NEQAS from July 2003 to March 2006 was 53 (44-59). The concentration range in blood lead samples was from 0.10 to 3.50 µmol.l^{-1} (from 20 to 720 µg.l^{-1}).

Our results from determination of lead in blood in the UK NEQAS from July 2003 to March 2006 along with target values are given in Fig. 1 and the distribution of bias depending on concentration is given in Fig. 2.

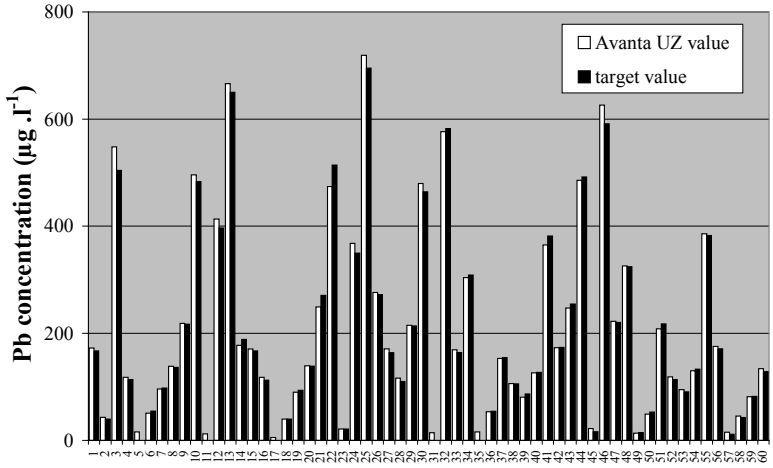


Fig. 1. Results – determination of lead in blood in the external quality control UK NEQAS (July 2003 - March 2006) along with target values
The UK NEQAS did not evaluate target values of the samples No. 5, 11, 17, 31, and 35. Lead content in these samples was very low. Only our results are presented.

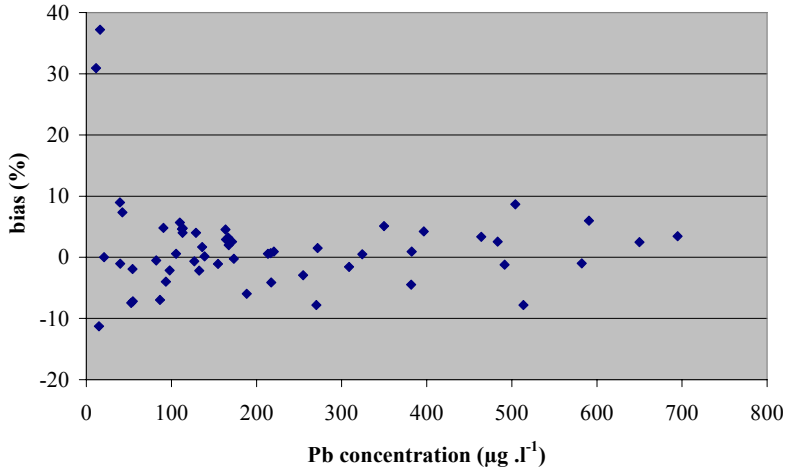


Fig. 2. Bias distribution depending on lead concentration

Average recovery and expanded uncertainty [5] for range 20-720 µg Pb.l^{-1} (n=54) were 100.7% (range 92.2%-108.9%) and 9.0% (coverage factor 2), respectively. The agreement of the Avanta UZ data in the UK NEQAS program was always within 10 µg Pb.l^{-1} below 20 µg Pb.l^{-1} and within 10% above that level.

We also participated in the external quality program German External Quality Assessment Scheme. In the G-EQUAS program, the Avanta UZ data did not differ from the target value by more than 10 µg Pb.l^{-1} below 100 µg Pb.l^{-1} or by more than 10% above that level.

Figures 3 and 4 summarize the data from the UK NEQAS (July 2003 – March 2006) and the G-EQUAS (2003-2005).

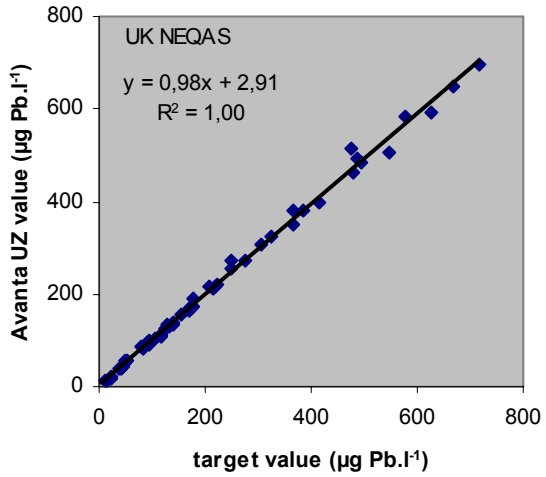


Fig. 3. Data for the UK NEQAS

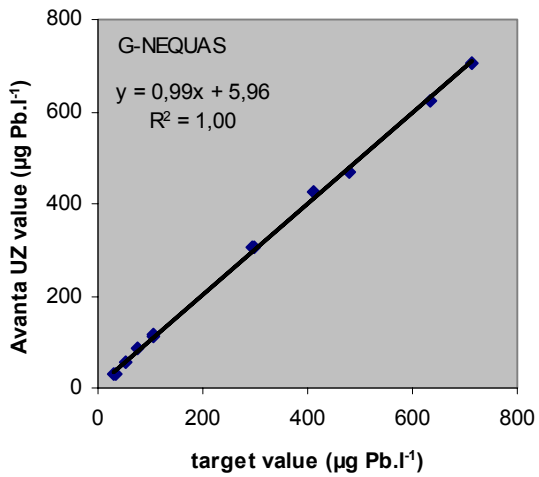


Fig. 4. Data for the G-EQUAS

Conclusion

The method for the determination of lead in whole blood was tested and verified by successful long-term participation in the external quality control program United Kingdom National External Quality Assessment Service. Besides, we verified this method in the external quality control program German External Quality Assessment Scheme.

Our experience shows that the regular and frequent participation in an external quality control program is important to ensure that the procedures and calibrations remain under good control and for clinical laboratory or for laboratory doing blood lead screening is unavoidable.

References

[1] Ahamed M., Verma S., Kumar A., Siddiqui M.K.J.: Environmental exposure to lead and its correlation with biochemical indices in children. *Sci. Total. Environ. 346, 2005, 48-55*

[2] Bencko V., Cikrt M.: *Toxické kovy v životním a pracovním prostředí člověka.* GRADA Publishing, Praha, 1995

[3] Cikrt M., Šmerhovský Z., Svačinka P., Černá M., Knotková J., Kašparová L., Spěváčková V., Šubrt P., Hejlek A.: Využití plumbémie pro hodnocení expozice olovu a kontrolu rizika. *České pracov. lék., 3, 2000, 172-178*

[4] Kašparová L., Knotková J.: Stanovení olova v krvi metodou atomové absorpční spektrometrie s elektrotermickou atomizací – mezilaboratorní kontrola kvality, *České pracov. lék. 5, 2004, 14-17*

[5] *Kvalimetrie 11. Stanovení nejistoty analytického měření.* EURACHEM/CITAC 4. EURACHEM-ČR, Praha, 2001

[6] Parson P.J., Geraghty C., Verostek M.F.: An assessment of contemporary atomic spectroscopic techniques for the determination of lead in blood and urine matrices. *Spectrochim. Acta Part B 56, 2001, 1593-1604*

[7] Parsons P.J., Slavin W.: A rapid Zeeman graphite furnace atomic absorption spectrometric method for determination of lead in blood. *Spectrochim. Acta 48B, 1993, 925-939*

[8] Soldin O.P. Hanak B., Soldin S.J.: Blood lead concentrations in children: new ranges. *Clin. Chim. Acta 324, 2003, 109-113*

[9] Taylor A., Angerer J., Claeys F., Kristiansen J., Mazarrasa O., Menditto A, Patriarca M., Pineau A., Schoeters I., Sykes C., Valkonen S., Weykamp C: Comparison of procedures for evaluating laboratory performance in external quality assessment schemes for lead in blood and aluminium in serum demonstrates the need for common quality specifications. *Clin. Chem. 48, 2002, 2000-2007*

GC-MS Investigation of Interaction of Pesticides with Soil with Regard to their Bioavailability

Attila Kiss, Diána Virág, Zoltán Murányi, Szabolcs B. Tóth
Eszterházy Károly University, Regional Academic Knowledge Center, Leányka str. 6, H-3300, Eger, Hungary

Abstract

Determination of the bioavailability of widely used pesticides is necessary for environmental risk assessment and proper agricultural application, as well as for reducing human toxicological problems. During our studies we developed and adapted experimental systems to model and compare bioavailability of different pesticides, and determine maximally available amounts of pesticides for plants. The two pesticides examined were simazine (triazine herbicide) and carbendazim (benzimidasole fungicide). For the examination a sandy and a browny soil were used. After the sorption of the pesticides to different type of soils, their desorption was examined with seven kinds of model system. The extracted amount of the pesticides was determined by GC-MS and HPLC technique. The studied extracting solvents showed diverse efficiency in extracting pesticides. For further investigations 80% methanol, acetate-buffer and humic acid solutions proved to be suitable to model the bioavailable amount of pesticides.

Key words: *pesticides, bioavailability, 7 distinctive extraction patterns, soil adsorption, GC-MS technique*

Introduction, objectives

The components being present in environmental systems are available for organisms to various extent thus they have diverse effect on the ecosystem. The chemical waste in soil, sediment, and water may influence, or even regulate, bioremediation efficiency [6]. Bioavailability can be defined as the amount of present contaminant that can be degraded and readily taken up by microorganisms [4]. The characterization of pesticide bioavailability, particularly in aged soils, could be important to be taken into consideration because this information is necessary for environmental risk assessment [5].

The objective of this study was to use different solvent extraction methods to model the bioavailability of some widely used pesticide [1]. The two pesticides examined, which have diverse chemical structures and reaction mechanisms, are simazine (triazine herbicide) and carbendazim (benzimidasole fungicide). Two types of soils were chosen for the experiments, one sandy and one browny soil. Plough layer (0-20cm) was examined major characteristics were precisely determined.

Behaviour of [^{14}C]-hydroxy-simazine in a parabraun soil was examined to estimate its residues at different incubation time [3]. Samples were analyzed according to two extraction methods at four different times of the incubation period. 62 days after beginning conversion the fulvic acid and humin fractions contained up to 85 % of the labelled ring carbon applied.

Aged and bound herbicide residues in soil and their bioavailability were studied [2] by applying [Carbonyl-^{14}C]-methabenzthiazuron to growing winter wheat. 140 days after application the 0-2.5 cm soil layer was examined. 0.01 M $CaCl_2$ solution and organic solvents were used for the extractions. About 20% of the radioactive parent compound was found in the leaves and residues of pesticide were detected from the soil at the end of the plant experiment as well.

To establish chemical extraction procedures for predicting bioavailability of butachlor and myclobutanil in soil, several solvent systems (methanol, methanol–water (9:1), methanol–water (1:1), acetone–water (5:3), petroleum ether and water) were assessed for to determine the extractability of the target compounds from soil samples [7]. According to the experimental data these extraction procedures may be efficient for predicting bioavailability of the two pesticides.

Influence of soil aging on sorption and bioavailability of simazine was studied by Regitano et al. [5]. Solvent extraction methods were applied to correlate simazine residue bioavailability in aged soils to simazine mineralization using a simazine-mineralizing bacterium. Soil samples were treated with UL-ring-labeled [^{14}C] simazine and extracted with 0.01 M $CaCl_2$, or extracted with aqueous methanol (80:20 v/v methanol/water). 0.01 M $CaCl_2$/methanol solution was proved to be suitable to estimate bioavailable residues of simazine in aged soils.

Materials and methods

The preparation of soils: two types of soils were chosen for the experiments, a sandy and browny soil. The soil samples were totally cleaned from plant parts and pebbles, same in size and homogenous.

The examined pesticides (simazine, carbendazim) (higher than 99% HPLC purity) and other applied chemicals were purchased from Aldrich. The pesticides were dissolved in butanol, the concentration of the solutions was 200 ppm.

Soil preparation examinations were carried out to establish the optimal amount of the solvent in which the maximum pesticide amounts were dissolved. 100.0 μg pesticide was dissolved in 2.5 – 5.0 – 10.0 ml butanol. Soil samples were treated with these solvents, and then extracted with 30 ml chloroform, centrifuged. 25 ml of the supernatant was vacuum rotary evaporated. The obtained sample was redissolved in 1.65 ml chloroform and analyzed by GC-MS to detect the optimal amount of solvent.

To model the bioavailable amount of pesticides according to the literary we used aqueous methanol (80:20 $^{v}/_{v}$ methanol/water), Na-acetate - acetic acid buffer (pH=5.6), $CaCl_2$ solution (0.1 M, 0.01 M) and humic acid solution in two different concentrations (0.5 and 1.0 ml SERA humic acid extract/l) was used for the extraction.

10 g of each soil sample contained 5 ml of each solution of pesticide, so 0.1 mg active ingredient was on 1 g soil. The soil samples containing pesticides were dried at 105ºC for 2 hours, than powdered, and the same amount of them (1 g) were extracted with 30 ml of the extracting solvents mentioned above for 16 hours. The soil samples were then centrifuged for 10 minutes and the supernatants were removed. 25 ml of them were extracted with 15 ml of chloroform for three minutes twice, then vacuum rotary evaporated. Dry extracts were redissolved in 1.65 ml of chloroform in case of simazine and 0.5 ml of methanol in case of carbendazim. The extracted amount of the basic component of simazine was detected by using GC-MS technique and HPLC in case of carbendazim.

The GC separations and the mass spectrometric measurements were performed by using a GC-GC/MS QP-2010S Shimadzu under the next measuring conditions: column: HP-5MS (30 m x 0.25 mm x 0.25 um), column oven temperature: 110°C, injector temperature: 230°C, injection mode: split, split ratio:10.0, carrier gas: He (1 ml/min), temperature program: t_1=110°C → 240°C (15°C/min), 240°C → 290°C (35°C/min), detector: GC/MS QP-2010S, ionization mode: EI (70 eV), interface temperature: 230°C, ionsource temperature: 200°C, inject volume: 1 μl.

Quantitative analyses were performed on a Shimadzu LC-10 AD VP Liquid Chromatograph and a Shimadzu SPD-10A VP UV-VIS Detector. The column used was a Discovery C18 (250 mm x 4.0 mm, 5 μm). Chromatographic separation was carried out using an acetonotrile-ammonia-solution (0.6%) (15:85). Injected volume was 20 μl, flow rate was 1 ml/min; wavelength: 288 nm.

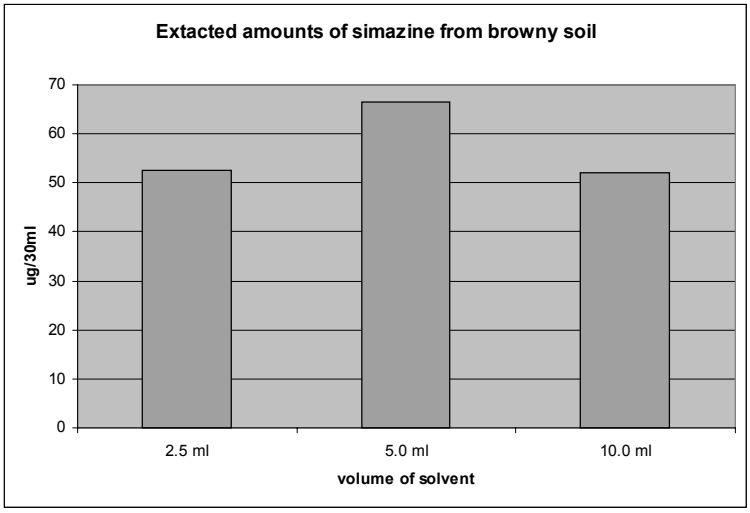
Results and discussion

Two types of soils were chosen for the experiments, a sandy and browny soil. The plough layer was examined, (0-20 cm). Both types of soil samples were characterized using traditional methods, measuring for example pH, specific conductivity, humus content and granulometric composition. Results are summarized in Table 1.

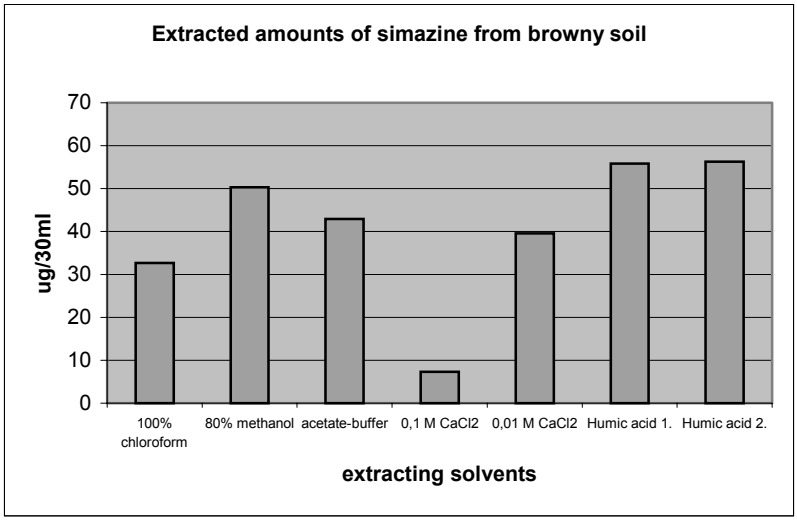
Table 1. Physical and chemical properties of soil samples

Soil type	Layer (cm)	Sand (m%)	Rock-flour (m%)	Mud (m%)	Clay (m%)	pH	Humus-content (%)	Spec. conductivity (μs/cm)	Ca^{2+} (mg/100g soil)	TPH (mg/kg)
Sandy	0-20	94.8	5.2	0.0	0.0	5.10	1.58	64.2	24	<5
Browny	0-20	6.0	41.0	31.0	22.0	7.26	2.32	111.0	5380	<10

Throughout the sample preparation procedure three distinctive solvent volumes were applied. It was apparent (Graph 1) that application of 5 ml solvent proved to be the most efficient in respect of achieving maximum extracted amount of pesticide therefore it was used for further experiments.



Graph 1. Extracted amounts of simazine from browny soil



Graph 2. Extracted amounts of simazine from browny soil

In case of the investigation of modeling the bioavailable amount of simazine from browny soil the least efficient extracting solvent was found to be 0.1 M $CaCl_2$ solution, rather than chloroform and 0.01 M $CaCl_2$ solution (Graph 2). For the further investigations, due to the previous findings we neglected these extractants and 80% methanol, acetate-buffer and humic acid solutions were applied to model the bioavailable amount of pesticides. The largest amount of simazine could be obtained with humic acid solutions, more than with aqueous methanol as well as acetate buffer.

In case of sandy soil there was hardly any difference among the extracting solvents (Figure 1). With aqueous methanol it was possible to extract more simazine from the soil than with acetate-buffer and two different humic acid solutions, as it is reflected by peak area of GC-peak: 7.426. When comparing the four chromatograms not more than 5 % alterations might be observed in peak areas of different samples.

In case of carbendazim the extraction solvents displayed almost the same efficiency in extracting carbendazim. Aqueous methanol and acetate-buffer were found to be slightly more efficient extractants than $CaCl_2$ solution and humic acid solutions, however large differences have not occurred (Table 2).

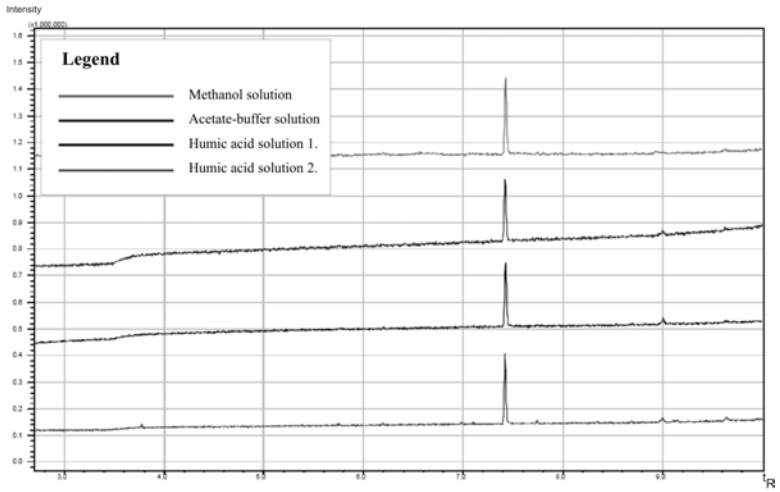


Figure 1. Comparison of GC-chromatograms of simazine samples extracted from sandy soil

Table 2. Extracted amounts of simazine and carbendazim from sandy soil

	Extracted amounts of pesticides (%)				
Extracting solvents	Methanol solution	Acetate-buffer	Humic acid solution 1.	Humic acid solution 2.	M $CaCl_2$ solution
Simazine sandy soil	54.26	49.37	50.65	49.63	-
Carbendazime sandy soil	57.50	58.52	55.15	41.41	53.96

It might be stated that in case of both pesticides and soil types involved in our studies approximately 50% of original pesticide amount was to be obtained by the applied extraction models.

Adsorption coefficients (1) were also calculated for the examined samples. The results are summarized in Table 3.

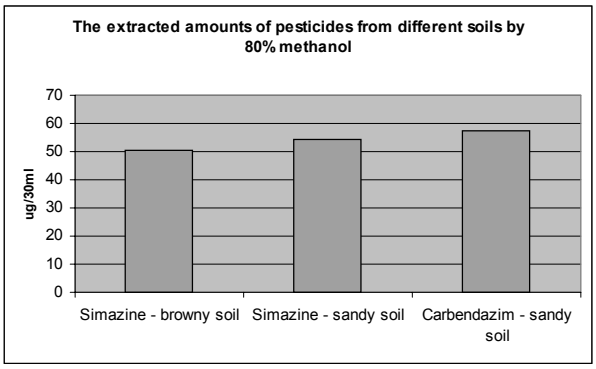
$$K_d = C_i/C_e \quad (1)$$

C_i – initial concentration (μg/ml)
C_e – extracted concentration (μg/ml)

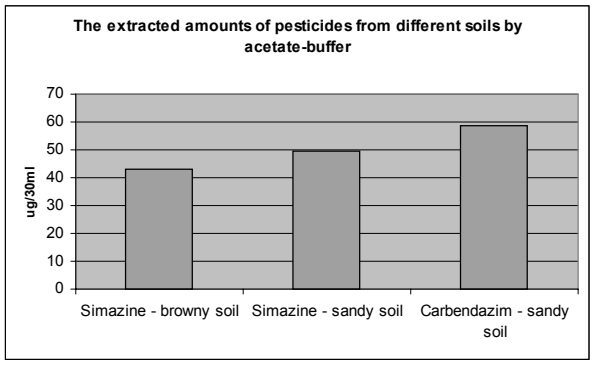
Table 3. K_d values of the examined samples

	K_d values				
Extracting solvents	Methanol solution	Acetate buffer	Humic acid solution 1.	Humic acid solution 2.	0.01 M $CaCl_2$ solution
Simazine browny soil	1.99	2.33	1.79	1.77	2.53
Simazine sandy soil	1.84	2.03	1.97	2.01	-
Carbendazime sandy soil	1.74	1.71	1.81	2.41	1.85

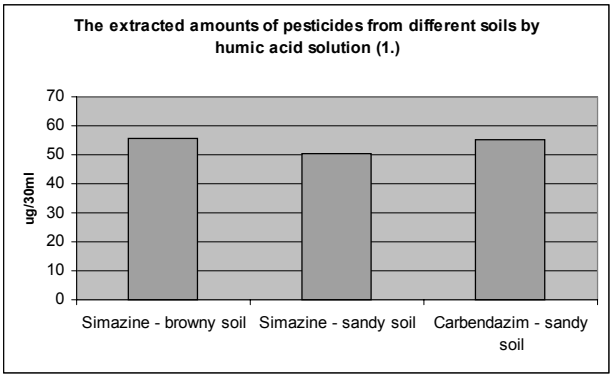
It is evident from the comparison of the efficiency of the used extracting solvents that with aqueous methanol and acetate-buffer more pesticide was extracted from the sandy soil than from the browny one. It could be explained by the lower sorption property of the sandy soil. Out of the studied extracting solvents 3 proved to be suitable to model the bioavailable amount of pesticides in case of different type of soils, namely: aqueous methanol, acetate-buffer and humic acid solution (Graph 3-4-5).



Graph 3. Extracted amounts of pesticides from different soils by 80% methanol

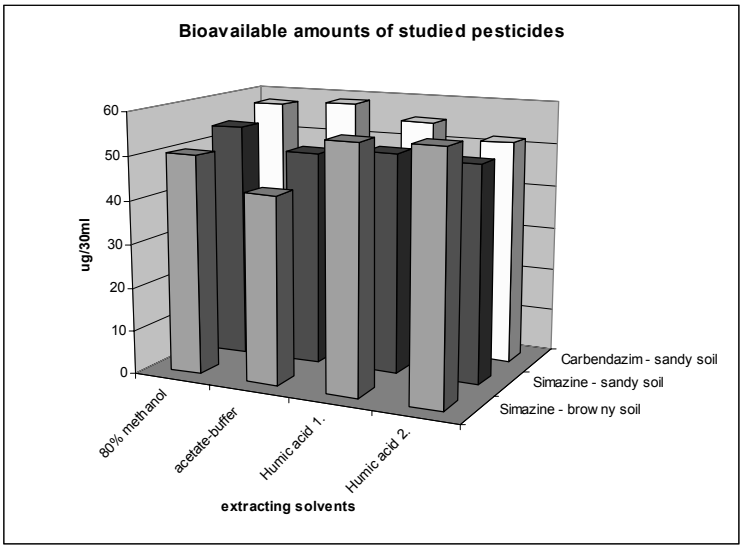


Graph 4. Extracted amount of pesticides from different soils by acetate-buffer



Graph 5. Extracted amount of pesticides from different soils by acetate-buffer

Comparison of bioavailability of distinctive pesticides when using different soil-types might be surveyed in graph 6. In case of sandy soil samples aqueous methanol was found to be the most efficient extracting solvent, while in case of the browny soil the most efficient extraction could be performed with the humic acid solutions. Prior to performing our experiments more significant differences were expected when comparing adhering characteristics of browny and sandy soils, as large alterations have not been experienced in bioavailable amounts of studied pesticides. It was established that the sorption of pesticides to sandy soil is nearly equally efficient as to the browny soil.



Graph 6. Bioavailable amouns of studied pesticides

Summary

Determination of the bioavailability of widely used pesticides is necessary for environmental risk assessment and proper agricultural application, as well as for reducing human toxicological problems.

During our studies we developed and adapted experimental systems to model and compare bioavailability of different pesticides, and determine maximally available amounts of pesticides for plants. The two pesticides examined were simazine (triazine herbicide) and carbendazim (benzimidasole fungicide). For the examination a sandy and a browny soil were used. After the

sorption of the pesticides to sandy and browny soils their desorption were examined with distinctive model systems. The extracted amounts of the pesticides were determined by GC-MS and HPLC techniques.

The studied seven types of extracting solvents showed diverse efficiency in extracting simazine from browny soil. The most efficient extracting solvents (80% methanol, acetate-buffer and humic acid solutions) were chose to estimate the bioavailable amounts of simazin from sandy soil. In this case there was hardly any difference among the extracting solvents. In case of carbendazim five kinds of extracting solvents were applied, which showed almost the same efficiency: aqueous methanol and acetate-buffer were found to be slightly more efficient extractants than $CaCl_2$ solution and humic acid solutions. It might be stated that in case of both pesticides and soil types involved in our studies approximately 50% of original pesticide amount was to be obtained by the applied extraction models. Adsorption coefficients were also calculated for the examined samples. Large alterations have not occurred in bioavailable amounts of studied pesticides. It was established that the sorption of pesticides to sandy soil is nearly equally efficient as to the browny soil.

References

[1] Fomsgaard, I.S.: The Influence of Sorption on the Degradation of Pesticides and other Chemicals in Soil. *Danish Institute of Agricultural Sciences*

[2] Kloskowski R., Fuhr F.: Aged and bound herbicide residues in soil and their bioavailability. Part 2: Uptake of aged and non-extractable (bound) [carbonyl-14C]methabenzthiazuron residues by maize. *J Environ Sci Health* B. 1987 Dec; 22(6):623-42

[3] Kloskowski, R., Führ, F.: Behaviour of [ring-14C] hydroxy-simazine in a parabraun soil. *Chemosphere,* Volume 14, Issues 11-12, 1985, P. 1913-1920

[4] Maier, R.: Bioavailability and its importance to bioremediation. In: Valdes, J.J. (ed.). *International Society for Environmental Biotechnology: Environmental Monitoring and Biodiagnostics*. Kluwer Academic Publishers*,* 2000, 59-78

[5] Regitano J.B., Koskinen W.C., Sadowsky M.J.: Influence of Soil Aging on Sorption and Bioavailability of Simazine. *J Agric Food Chem.* 2006 Feb 22; 54(4):1373-1379

[6] Southgate, D., Johnson, I. and Fenwick G.R.: Nutrient Availability: Chemical and Biological Aspects. Royal Society of Chemistry, Cambridge, UK. 1989

[7] Yu, Y.L., Wu, X.M., Li, S.N., Fang, H., Tan, Y.J., Yu J.Q.: Bioavailability of butachlor and myclobutanil residues in soil to earthworms. *Chemosphere,* Volume 59, Issue 7, May 2005, P. 961-967

Determination of Cadmium by Atomic Absorption Spectrometry after Electrodeposition

Jan Knápek, Josef Komárek, Pavel Krásenský, Jan Vondra
Department of Analytical Chemistry, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
e-mail: komarek@chemi.muni.cz

Abstract

For the atomic absorption spectrometry determination of traces of cadmium in the samples with high concentration of salts a separation step is usually needed for the elimination of chemical and spectral interferences. In this work preconcentration and separation of cadmium in the flow mode was studied. Various electrode materials and designs of electrochemical cell were investigated. Conditions for the electrodeposition, such as pH of solution, the deposition potential, the current and the concentration of electrolyte were optimised. Electrodeposition with injection of dissolved deposit into electrothermal atomizer was applied for the determination of cadmium in the samples of water.

Key words: *atomic absorption spectrometry, electrodeposition, cadmium, separation*

Introduction

Cadmium is an important contaminant in the environment and its determination in the samples with high amount of salts is not easy. To reduce of the interferences many procedures, including Zeeman-effect background correction, improved furnace and platform designs, use of effective chemical modifiers and careful optimisation of the temperature programme have been proposed. Electrochemical deposition serves as one of many techniques for preconcentration and separation of analyte from the matrix. During electrolysis the analyte ions are separated from the sample matrix and can be also preconcentrated onto the surface of the electrode. In connection with atomic absorption spectrometry used electrodes can be manufactured from different materials, such as high melting metals, graphite or mercury. [1] After the electrolysis the deposit can be treated in several manners. By the in situ electrolysis in the optical path of the spectrometer the working electrode is directly the graphite atomizer [2]. Electrolysis in the off-line mode is performed outside the spectrometer on commercially available or specially constructed electrodes. The electrode with the deposit is transferred into the atomizer [3,4]. Alternatively, the deposit can be dissolved and subsequently injected into the atomizer as solution [5,6].

Electrolytic cells are constructed in stationary or flow-through arrangement. The electrolysis can be accelerated by rigorous mixing of the solution and by maximising the ratio of the electrode surface to sample volume. This can be achieved in a flow system by passing the sample solution through a porous working electrode. However, for these cells a large volume of the eluent is required to wash out metals. Therefore the cells with porous electrodes are used often for on-line sampling of solution in the flame or the plasma [7].

In this work preconcentration and separation of cadmium in the flow mode was studied. The various electrode materials and designs of electrochemical cell were investigated. Conditions for the electrodeposition, such as pH of the solution, the deposition potential, the current and the concentration of electrolyte were optimised. Electrodeposition with injection of dissolved deposit into electrothermal atomizer was applied for the determination of cadmium in the samples of water.

Experimental

The measurements were carried out using a Perkin-Elmer 3030 AA spectrometer with HGA-400 graphite furnace or flame instrument Perkin-Elmer 306. A Cathodeon cadmium hollow-cathode lamp was operated at 8 mA (228.8 nm). A deuterium lamp was used for background correction. An autosampler AS-1 was used for the solution sampling to the graphite furnace.

The flow arrangement for electrolysis consisted of the peristaltic pump, the automatic injection valve and the flow-through electrochemical microcell. The connections were made of 0.8 mm inner diameter PTFE tubes. In this work two flow-through electrochemical microcells were applied. The power supply Radelkis OH 404 was used.

The first two-electrode electrochemical microcell (Fig. 1) was made of transparent Plexiglas. Working electrodes in this cell were two rods from the spectral graphite SU (Elektrokarbon Topolčany) with 5 mm diameter. The electrodes were inserted into the cell face to face. The distance between the electrodes provided the volume of the cell. Electric contacts were fixed at the ends of the graphite rods. Graphite rods with mercury, platinum or bismuth coating of the surface were also used as the cathode material. This cell was used in connection with ETAAS.

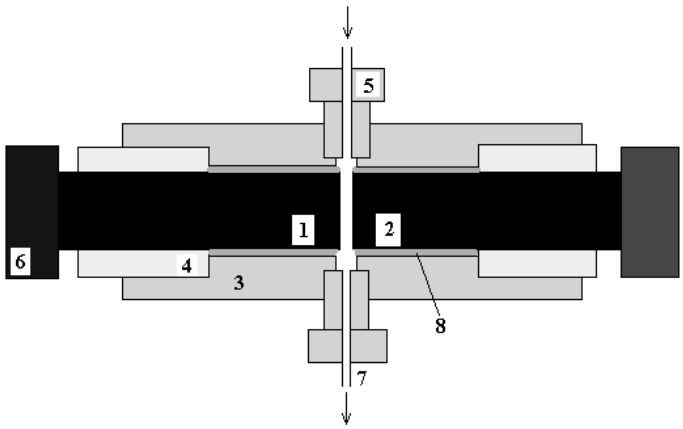


Fig. 1: Scheme flow-through electrochemical microcell (1 – cathode, 2 – anode, 3 – Plexiglas 4 – Epoxy resin, 5 – screw, 6 – electric contacts, 7 – PTFE tube, 8 – seal)

The second cell was two- or three-electrode electrochemical microcell (Fig. 2). Transparent Plexiglas was also used for construction of this cell. The cathodic space was separated from anodic space with cation-exchange membrane Nafion. Through the anodic space the solution of nitric acid circulated. Working electrode was made of reticulated vitreous carbon (RVC). Auxiliary electrode was from graphite rod, as reference electrode was used calomel electrode. This cell was used in connection with FAAS.

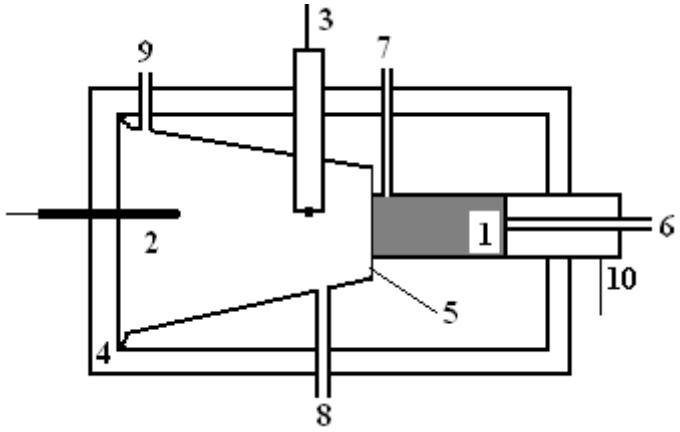


Fig. 2: Scheme electrochemical cell with porous working electrode (1 – working electrode (RVC), 2 – auxiliary electrode, 3 – referent electrode, 4 – Plexiglas body, 5 – membrane Nafion, 6 – sample in, 7 – sample out, 8 – electrolyte in, 9 –electrolyte out, 10 – graphite contact)

The analytical procedure consisted of four steps:

- electrodeposition – the sample solution was pumped by the peristaltic pump and the deposition current or potential was applied to the electrodes
- rinsing – the electrodes with deposit were rinsed by bidistilled water to get rid of the residues of the sample matrix
- elution – deposit was dissolved by 0.1 mol l^{-1} nitric acid and injected to the graphite tube (40 μl) or flame (100 – 500 μl)
- Cd signal measurement by ETAAS or FAAS

Results and discussion

Optimization of conditions for cadmium electrodeposition

The parameters of electrodeposition were tested using the on-line connection of flow-through cell with FAAS. The parameters for the first cell were optimized by step by step method. The distance between electrodes was changed in the range from 0.09 to 1 mm. The optimum distance was 0.13 mm. The current used during electrolysis influenced the efficiency of electrochemical process. The current lower than 0.5 mA was not effective. Good results between 1 – 2 mA were observed. Optimum current of 1 mA was selected. The process of electrodeposition depends on the solution composition and its pH. The optimum pH values for electrodeposition of Cd were in the interval pH = 4.0 – 5.5. The pH 4.5 was used in this work, by using 1 mol l^{-1} sodium acetate for adjustment of pH. The influence of the deposition time was studied in connection with ETAAS in the range from 1 to 15 min. The dependence of the absorption signal on the deposition time is linear in this range (Fig. 3)

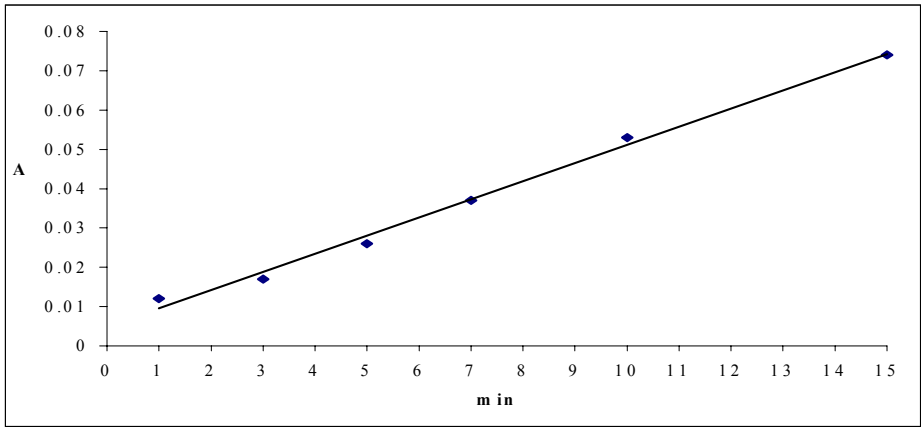


Fig. 3: Dependence of Cd absorption signal on the time of electrolysis

The second flow-through electrochemical cell was optimised by central composit design. The optimized parameters were: the deposition current, potential, flow rate by porous electrode and pH of sample solutions.

Treatment of the electrode surface

The quality of the electrode surface significantly influences the process of electrodeposition. The electrodes in arrangement one were ground with emery paper and polished by dense filter paper. This procedure was repeated, when the efficiency of the cell was deteriorated. Another possibility is the use of the electrode surface coating with the thin layer of metals. In this study the coverage of the surface of spectral graphite with mercury, bismuth or platinum film was tested.

The mercury film electrode was formed during co-deposition of Cd^{2+} in the presence of $2.5x10^{-5}$ mol l^{-1} Hg^{2+}. However, mercury and the mercury salt employed for the preparation of mercury film electrode (MFE) are extremely toxic. Recently, Wang's research group has introduced a new type of working electrode [8]. It is a bismuth film electrode (BiFE) that represents an attractive alternative to traditionally used mercury film electrodes. The most significant advantage of BiFE is that they are environmentally friendly. The toxicity of bismuth and its salts is negligible. The analytical properties of BiFE are analogous to MFE. BiFE was formed by co-deposition of Cd^{2+} in the presence of Bi^{3+} ions in solution. The dependence of Cd signal on the amount of Bi is in the Fig. 4.

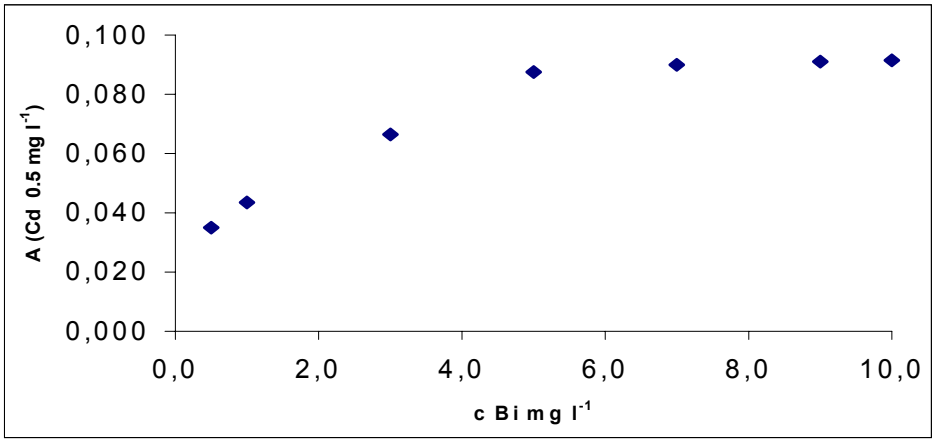


Fig. 4: Dependence of the Cd signal on the concentration of Bi in solution after electrodeposition (FAAS)

It is shown in Fig. 4 that the concentration of Bi in the solution should be 10x higher than the amount of analyte. The BiFE was formed at pH 4.5 the same as Cd. It is advantageous, because this pH is not optimal for deposition of mercury film. The platinum was predeposited on the surface of graphite cathode by electrolysis from 6 ml of 4 % hexachloroplatinum acid solution, but the sensitivity with this working electrode is too low.

Conclusion

Electrodeposition in connection with atomic absorption spectrometry is powerful tool for elimination of spectral and chemical interferences, because the matrix of sample is removed.

This method can be used for the analysis of seawater, drinking water or surface water and biological fluids (e.g. urine).

References

[1] E. Beinrohr: Electrolytic sample pretreatment in atomic spectrometry: a review, Mikrochim. Acta 120 (1995) 149–154
[2] J.P. Matousek, H.K.J. Powell: Coupled in situ electrodeposition – electrothermal atomic absorption spectrometry: a new approach in quantitative matrix free analysis, Spectrochim. Acta, Part B: Atom. Spectrosc. 50 (1995) 857–872
[3] A. Vraná, J. Komárek: Determination of cadmium and copper with ET-AAS after electrochemical deposition on a graphite electrode, Fresenius'J. Anal. Chem. 355 (1996) 321–323
[4] M. Veber, S. Gomišček, V. Streško: Electhrothermal atomic absorption spectrometry of elements after electrochemical deposition on graphite electrodes, Anal. Chim. Acta 193 (1987) 157–167
[5] E. Bulska, M. Walcerz, W. Jedral, A. Hulanicky: On-line preconcentration of lead and cadmium for flame atomic absorption spectrometry using a flow-through electrochemical microcell, Anal. Chim. Acta 357 (1997) 133–40
[6] J. Knápek, J. Komárek, P. Krásenský: Determination of cadmium by electrothermal atomic absorption spectrometry using electrochemical separation in a microcell, Spectrochim. Acta, Part B 60 (2005) 393–398
[7] E. Bulska: Analytical advantages of using electrochemistry for atomic spectrometry, Pre Appl. Chem. 73 (2001) 1–7
[8] J. Wang, J.M. Lu, S.B.Hočevar, P.A.M. Farias, B. Ogorevc: Bismuth-coated carbon electrodes for anodic stripping voltammetry, Anal. Chem. 72 (2000) 3218-3222

Determination of Gold by Electrothermal Atomic Absorption Spectrometry after Electrochemical Preconcentration

Marie Konečná and Josef Komárek
Department of Analytical Chemistry, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
e-mail: komarek@chemi.muni.cz

Abstract

Gold was determined by electrothermal atomic absorption spectrometry after electrochemical preconcentration on the graphite ridge probe used as a working electrode. The surface of the probe was modified with Pd, Re and the mixture of both. The metals were electrochemically predeposited on the probe surface in one step electrodeposition. On the probe surface permanently modified with Pd, Re or the mixture of both maximum pyrolysis temperature of 1200°C can be use for the determination of gold from the samples containing high salt matrix.

Key words: *gold, electrothermal atomic absorption spectrometry, electrodeposition, modifier*

Introduction

Traces of gold in different kinds of samples (environmental, geological and biological) are usually determined by electrothermal atomic absorption spectrometry (ET-AAS). In the view of very low concentration and complicated sample matrix, different preconcentration and separation methods are required. Sorption, ion exchange, coprecipitation and electrochemical deposition can be used before ET-AAS determination of gold [1-3].

The electrodeposition is advantageous for both – the preconcentration and the separation. The connection of the electrodeposition with ET-AAS can be done in on-line, in-situ and off-line arrangements. In on-line arrangement the analyte is deposited on an electrode in a flow-through electrochemical microcell. The deposited elements are dissolved by short-circuiting the electrodes and flushing the cell with diluted acid or electrochemically in the presence of an appropriate stripping reagent [4-8]. The advantage is the direct sampling of dissolved metal into the atomizer. By in-situ arrangement analyte electrodeposition is proceeded on the inner surface of the graphite atomizer placed directly in the optical path of the atomic absorption spectrometer [9]. In off-line arrangement of electrolysis in a flow-through or batch performance the sample amount is limited only with analysis time, similarly as in the on-line arrangement. For the determination of heavy metals in off-line arrangement in batch performance, the electrodeposition on the graphite disk electrode and the graphite probe of various designs was proposed [10,11]. Especially, the shape of graphite commercial ridge probe calls directly upon to the use of the probe as electrode in batch performance.

With regard to the volatility of gold and the matrix effects especially with the high salt content the chemical modifiers should be used. Gold in biological fluids was determined by using the mixture of rhodium and rhenium [12]. Noble metals (Pd, Rh, Ir) in their elemental form were successfully applied for the determination of various elements and their permanent performance was studied [13,14]. Electrochemically deposited palladium on the graphite tube surface was applied for the determination of gold in river waters [3].

In this work different metals (Pd, Re and the mixture of both) were used for the modification of the graphite ridge probe. Gold was electrodeposited on the modified probe surface and determined by ET-AAS. The long term performance of the modified surfaces and the options of gold determination in different sample matrix were studied.

Experimental

Measurements were carried out on an ATI UNICAM SOLAAR 939 atomic absorption spectrometer with deuterium background correction. The instrument was equipped with a graphite furnace GF 90 and with an autoprobe assembly AP 90. Au hollow cathode lamp (UNICAM) was operated at 10 mA (242.8 nm).

A stationary cell was constructed for electrodeposition of the modifier and gold. The cathodic space was separated from the anodic space with cation-exchange membrane Nafion. Through the anodic space the solution of nitric acid circulated. The amount of electrolysed solution was 20 ml. The probe was wrapped with Teflon strip for the area definition where the metal was deposited. The one step electrodeposition of the modifier was done at the current of 10 mA for 10 min from the solution containing 4 mg of metal, in the case of the mixture 2 mg of each metal. The electrodeposition of gold was carried out at the current of 0.5 mA. The stability of gold solution was obtained by adding 0.1 mol l^{-1} HCl and 0.5 g l^{-1} NH_4SCN [3, 12]. The temperature program for gold determination on the modified ridge probe shows Table 1.

To simulate the saline matrix the solution consisted of sodium chloride at the concentration of 25 g l^{-1} and magnesium sulphate at the concentration of 7 g l^{-1} [15].

Table 1: The temperature program for the determination of gold after electrodeposition on the modified probe surface

	Temperature [°C]	Ramp [s]	Hold [s]
Drying	150	20	20
Pyrolysis	1000	10	20
Atomization	2500	0	3
Cleaning	2600	0	2

Results

The long term performance of the modifiers (Pd, Re and the mixture of both) was studied by means of the relative standard deviation (RSD) of gold determination. The RSD was measured directly after the one step electrodeposition of the modifier, after 150 atomisation cycles and after 250 atomisation cycles. These results were obtained for the determination of gold from aqueous solutions and from the samples with model matrix of sea water. For all the modified surfaces the differences between RSD in different stages of the probe lifetime was not statistically significant. Table 2 shows the RSD values measured on the surface modified with the mixture of Pd+Re.

Table 2: The relative standard deviation of gold determination on the surface modified with the mixture of Pd+Re (n=8, 2 μg l^{-1} Au, 2 min, 0.5 mA)

	RSD after electrodeposition of the modifier (%)	RSD after 150 atomization cycles (%)	RSD after 250 atomization cycles (%)
Peak height	7.1	7.1	7.8
Peak area	4.1	4.5	5.0
Average value	5.6	5.8	6.4

The influence of the salts was studied by using the model matrix of sea water containing sodium chloride and magnesium sulphate. Maximum pyrolysis temperature of 1200°C can be use for the determination of gold after electrodeposition on the modified probe. The pyrolysis curves are shown in Fig. 1. The best detection limit of gold after its electrodeposition from the high salt matrix was found for the probe modified with Re 38 ng l^{-1} (for 2 min electrodeposition at the current of 0.5 mA).

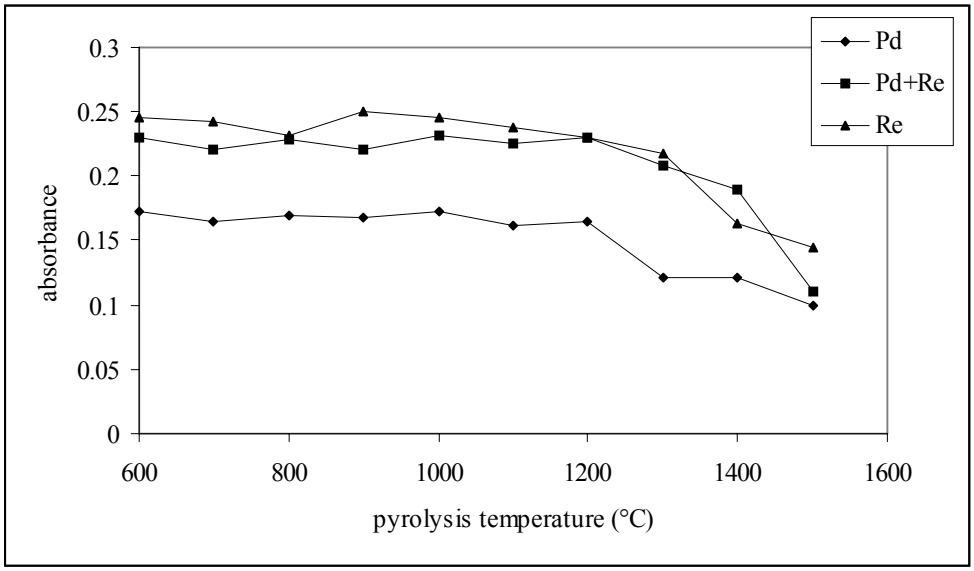


Fig. 1: Pyrolysis curves of Au for the probe surfaces modified with Pd, Re and Pd + Re from the model matrix of sea water (2 μg l^{-1} Au, 0.5 mA, 2 min)

Conclusion

The probe surfaces modified in one step electrodeposition with Pd, Re and the mixture of both can be use for electrodeposition of gold prior to its ET-AAS determination. By this way gold can be determined in the samples of natural water.

Acknowledgement

This work was supported by project MSM 0021622412 of Ministry of Education, Youth, and Sports of the Czech Republic. The authors wish to thank the Department of Preventive Medicine, Faculty of Medicine, Masaryk University for enabling them to work on AA spectrometer ATI UNICAM.

References:

[1] J. Medved, M. Bujdos, P. Matus, J. Kubova: Determination of trace amounts of gold in acid-attacked environmental samples by atomic absorption spectrometry with electrothermal atomization after preconcentration, Anal. Bioanal. Chem. 379 (2004) 60-65

[2] R. Cidu, L. Fanfani, P. Shand, W. M. Edmunds, L. Van't dack, R. Gijbels: Determination of gold at the ultratrace level in natural waters, Anal. Chim. Acta 296 (1994) 295-304

[3] J. Komárek, P. Houserová: Determination of gold by electrothermal atomic absorption spectrometry after electrodeposition on a graphite tube, Spectrochim. Acta Part B 58 (2003) 1525-1530

[4] E. Bulska, M. Walcerz, W. Jedral, A. Hulanicki: On-line preconcentration of lead and cadmium for flame atomic absorption spectrometry using a flow-through electrochemical microcell, Anal. Chim. Acta 357 (1997) 133-140

[5] E. Bulska, W. Jedral: ETAAS determination of lead with on-line preconcentration using a flow-through electrochemical microcell, At. Spectrosc. 18 (1997) 202-204

[6] E. Beinrohr, M. Rapta, M-L. Lee, P. Tschöpel, G. Tölg: On-line electrochemical preconcentration of manganese for graphite furnace atomic absorption spectrometry using a flow-through electrochemical microcell, Microchim. Acta 110 (1993) 1-12

[7] E. Beinrohr: Electrolytic sample pretreatment in atomic spectrometry: a Review, Microchim. Acta 120 (1995) 39-52

[8] B. Godlewska-Żylkiewicz, M. Zaleska: Preconcentration of palladium in a flow-through electrochemical cell for determination by graphite furnace atomic absorption spectrometry, Anal. Chim. Acta 462 (2002) 305-312

[9] J.P. Matousek, H.K.J. Powell: Coupled in situ electrodeposition-electrothermal atomic absorption spectrometry: a new approach in quantitative matrix free analysis, Spectrochim. Acta Part B 50 (1995) 857-872

[10] A. Vraná, J. Komárek: Determination of cadmium and copper with ET-AAS after electrochemical deposition on a graphite electrode, Fresenius J. Anal. Chem. 355 (1996) 321-323

[11] J. Komárek, J. Holý: Determination of heavy metals by electrothermal atomic absorption spectrometry after electrodeposition on a graphite probe, Spectrochim. Acta Part B 54 (1999) 733-738

[12] N. S. Thomaidis, E. A. Piperaki, C. E. Efstathiou: Comparison of chemical modifieres for the determination of gold in biological fluids by electrothermal atomic absorption spectrometry, J. Anal. At. Spectrom. 10 (1995) 221-226

[13] E. Bulska, K. Liebert-Ilkowska, A. Hulanicki: Optimization of electrochemical deposition of noble metals for permanent modification in graphite furnace atomic absorption spectrometry, Spectrochim. Acta Part B 53 (1998) 1057-1062

[14] M. Piascik, E. Bulska: Performance of electrodeposited noble metals as permanent modifiers for the determination of cadmium in the presence of mineral acids by electrothermal atomic absorption spectrometry, Spectrochim. Acta Part B 56 (2001) 1615-1623

[15] S. Griesel, U. Reus, A. Prange: Electro-deposition as a sample preparation technique for total-reflection X-ray fluorescence analysis, Spectrochim. Acta Part B 56 (2001) 2107-2115

On Line Electrochemical Preconcentration of Trace Metals for GFAAS

Alena Manová, Ernest Beinrohr, František Čacho, Milan Střelec
*Institute of Analytical Chemistry, Slovak University of Technology, **Radlinského 9,** 812 37 Bratislava, Slovakia*
e-mail: alena.manova@stuba.sk

Abstract

Arsenic is determined by graphite furnace atomic absorption spectrometry after the preconcentration of As (III) on gold working electrode in a flow system coupled on-line to the AAS instrument. The porous electrode is made of glassy carbon particles coated with gold. Water samples were analyzed with this method. The method enables it to determine As in the concentration range of 0.01 to 5 μg.dm^{-3}.

Key words: *preconcentration of As – GFAAS -- electrochemical cells -- water samples*

Introduction

Waste waters may contain toxic element e.g. arsenic and arsenic compounds and therefore it is important to have reliable and simple methods for determine their content. There are few methods enabling the determining of traces of arsenic [4-12]. Most of them are based on atomic absorption spectrometry and make use of the easy reduction and evaporation of arsenic to its atomic form. These analyzers chemically convert the different arsenic species present in the sample solutions to elementary arsenic that is then stripped by a gas to the absorption tube for measurements. The measurement step is simple but the sample preparation procedure may be rather tedious. Arsenic can be easily preconcentrated on gold electrode, which is successfully used for its electrochemical determination [1-3]. This principle may significantly simplify the procedure owing to the fact that the reduction of As species and the determination can be done with the same electrode. The chemical reduction step can be omitted. The main advantages of the use of flow-through cell are simplicity, high speed and low costs. However, like other methods, this method is also vulnerable to matrix interferences and electrode fouling. The aim of this paper is to show the utility of preconcentration determinations of As in waters by making use of a flow system coupled on-line to the AAS as measurement principle.

Experimental

Analytical grade purity reagents and deionized boiled cooled water were used throughout the experiments. The carrier electrolyte solution contained 0.1 mol/ dm^{-3} of HCl and 2 mol.dm^{-3} HNO_3 for the deposition and stripping/AAS measurement, respectively.

Standard solution 1.00 μg.dm^{-3} of As (III) was used. The flow-through cell for preconcentration was prepared from perspex body. The working electrode of diameter and length of 8 mm and 4 mm resp. was prepared from reticulated vitreous carbon RVC of 100 pores per inch porosity (Electrosynthesis Co., Inc., East Amherst, NY, USA) crushed to particles of 10 to 100 μm size. The surface of the working electrode was plated with a thin layer of gold. The cell was filled with 0.0002 mol/ dm^{-3} H[$AuCl_4$] in 0.02 mol/ dm^{-3} HCl and voltage of –2000 mV was applied to the electrodes. The flow system (Fig.1) was constructed. The sampling valve was a 6-way one (Latek, Eppelheim, Germany). A peristaltic pump drove the liquids. AAS instrument SP9 with graphite furnace atomizer (Pye Unicam) coupled on-line to the flow system was used to measure the preconcentrated As species.

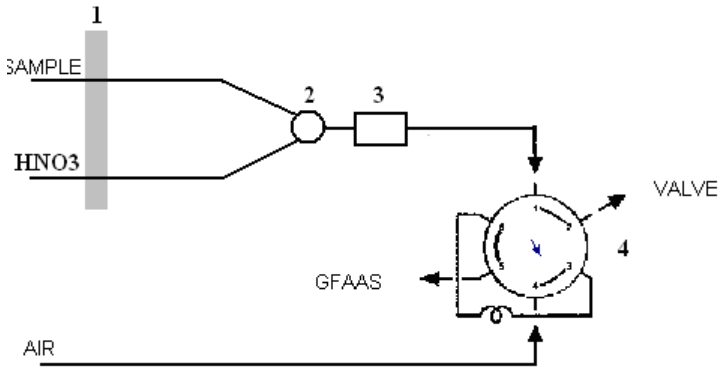


Fig. 1. Scheme of the flow-through system
Legend: 1 - pump, 2 - valve, 3 - preconcentration cell, 4 - the 6-way valve with sampling loop calibrated to the sampling volume 57.2 μl

Results and discussion

Arsenic can easily be determined in various samples by GFAAS after the preconcentration of As (III) species on gold working electrode. However, the porous working electrode used in this technique may rapidly be clogged when analyzing wastewater with high content of colloidal particles. Moreover, dissolved gases may be liberated from waters, which tend to form bubbles in the pores of the working electrode, decreasing its active surface and efficiency. Hence, for direct analysis of such samples an oxygen scavenger is desirable. Preliminary measurements have demonstrated the utility of a cell with a gold working electrode. To minimize the clogging of the electrode, a membrane filter was used for the samples inlet to the target cell. Most waters, especially wastewaters, contain organic matter, which may reduce arsenic to its elementary form. However, elementary arsenic cannot be preconcentrated electrochemically. To determine the total As content, the sample should be treated first to transform all As species into an electrochemically active, ionic form. Flow-through systems enable a simple and elegant on-line sample pre-treatment just by mixing a suitable modifier solution to the flowing sample. The flow system used in this work enabled it to mix the sample solution with a carrier electrolyte just through segmenting the sample and carrier electrolyte flows by switching the valve periodically once to the sample and then to the electrolyte. The carrier electrolyte contained diluted hydrochloric acid. The preconcentration parameters for the electrochemical process were optimised. The deposition runs at a constant current of -2500 μA and the optimum stripping current was found to be 100 μA.

The deposition was made from a hydrochloric acid solution, which was then on-line replaced by a diluted nitric acid facilitating the AAS measurement of As. The advantage of the used preconcentration system was in an automatic and reliable matrix exchange facility. Optimum electrolyte concentrations were found to be 0.1 mol.dm^{-3} HCl and 2 mol.dm^{-3} HNO_3 for the deposition and stripping/AAS measurement, respectively.

Samples with low conductivity can also be analyzed since they are mixed in the flow system with the carrier electrolyte to form a conductive solution.

Figures of merit are shown in Table 1.

Table 1. Linear ranges, LOD and LOQ for different feeding volumes As (III)

Preconcentrated volume [ml]	Linear Range [μg·dm^{-3}]	LOD [μg.dm^{-3}]	LOQ [μg.dm^{-3}]
1	0.20 – 5.0	0.06	0.2
5	0.06 – 2.0	0.02	0.06
10	0.01 – 0.5	0.004	0.01

Conclusion

Graphite furnace atomic absorption spectrometry in connection with flow-through electrochemical cell provides a useful tool for determination of arsenic in water samples. The main advantage of the method lies in its simplicity.

References

[1] Vydra F., Stulik K., Julakova E.: Electrochemical stripping analysis. Ellis Horwood, Chichester, 1976
[2] Jagner D., Aren K.: Anal. Chim. Acta, 1982,141, p.157
[3] Gil E.P., Ostapczuk P.: Anal. Chim. Acta, 1994, 293, p.55
[4] Burguera M., Burguera. J.L.: Talanta, 1997, 44, 1581–1604
[5] Torgov V.G., Vall G.A., Demidova M.G.: Chemical Geology, 1995, 124, 101-107
[6] Iglesias M., Antico E., Salvado V.: Talanta, 2003, 59, 651-657
[7] Latva S., Hurtta M., Peräniemi S., Ahlgrén M.: Anal. Chim. Acta, 2000, 418, 11–17
[8] Shemirani F., Baghdadi M., Ramezani M.: Talanta, 2005, 65, 882–887
[9] Gonzalez M.M., Gallego M., Valcarcel M.: Talanta, 2001, 55, 135–142
[10] Hung D.O., Nekrassova O., Compton Richard G.: Talanta, 2004, 64, 269-277
[11] Alonso E.V., de Torres A.G., Cano Pavón J.M.: Talanta, 2001, 55, 219-232
[12] Man-Ching Hsiang, Yu-Hsiang Sung, Shang-Da Huang: Talanta, 2004, 62, 791–799

Evaluation of the Novel Spectrometric Tandem Technique Optimization

Mikuláš Matherny and Silvia Ružičková
Technical University of Košice, Metallurgical faculty, Department of Chemistry, Letná 9, SK-042 00 Košice
e-mail: mikulas.matherny@tuke.sk

Abstract

The tandem methods, whereas they are latter version of the spectroscopic techniques, need a complex optimization because of finding of their acquisition in term of figures of merit. It is proper to use the determination of information theory parameters for verification of complex experimental optimization. The experimental optimization is partially based on the one-dimensional exploratory analysis but mainly on the calculation of the parameters of analytical calibration, which represents atomic specifically values.

Key words: *exploratory analysis, information theory, analytical calibration*

Introduction

The exploratory analysis is based on the measurement of repeated ($N \in \langle 20, 100 \rangle$ intensity values of the matrix with constant concentration [1,2]. The result of the exploratory analysis is the determination of the arithmetical mean, their standard deviation, the median and their standard deviation, the modus and the halfsum. Further is determined the asymmetry and the excess of the input value. On this characteristics with testing it is possible too determined the normality and the homogeneity of the tested group. The calculation of the information theory parameters [3,4] need the knowledge of the limiting values $c(X)_{min}$, $c(X)_{max}$, the absolute standard deviation of the method s(X), and the limit of detection $c(X)_L$. Similarly it is necessary choose the satisfactory tolerance value. Upon this value it is possible to obtain the following characteristic values: the information content $I(p, p_o)$, the information efficiency $E(p, p_o)$, and after summation for all tested elements the mass of information content $MI(p, p_o)$, and the mass of the information efficiency $ME(p, p_o)$.The separate calculation based on the following cardinal equations:

$$I(p, p_o) = \ln \frac{c(X)_{max} - c(X)_{min}}{s(X)} * \frac{\sqrt{n}}{2t(\alpha, F)} \qquad (1)$$

The value of n presents the number of repeated measurements by the determination of standard deviation s(X), and the value $t(\alpha, F)$ represents the correction factor of Students distribution. Where α is the significance level, and F is the degree of freedom ($F = N - 1$). For the determination of information efficiency it is firstly necessary the numerical determination of two partial efficiency coefficient $e(X)_1$ and $e(X)_2$. The determination of first partial efficiency coefficient based on the ratio

of tolerance standard deviation and actual standard deviation. If $s(X) \leq s(X)_T$ the coefficient is conventionally equal to unity, but if $s(X) > s(X)_T$ the partial coefficient is necessary to compute on the hand of ratio this values. Thru multiplication of e_1 with e_2 coefficient we obtain the final efficiency coefficient E(X), The same case is with the second partial coefficient.

Experimental

In the given case the new tandem method [5] of the powder sample evaporation and excitation was tested. Evaporation overshoots in the separate quartz cell for aerosol production. Created sample aerosol is transported by the carrier Ar gas into Marinković [6] plasma source where the atoms of the evaporated sample are excitated. Experimental conditions of the applied equipment are in the Table 1.

Table 1. Experimental conditions

General conditions	
Spectrometer	LECO – 750, simultaneous, Germany
Grating	2400 lines per mm
Linear dispersion	0.55 nm per mm
Spectral range	220 – 766 / nm
Evaporating conditions	
Evaporation source	controlled DC arc, generator DCA-301, Spectral sources Ltd., Germany
Current intensity of the DC arc	20 A
Ar flow in the evaporation cell	1.6 dm^3 min^{-1}
Carrier electrode	SW 380, Elektrokarbon, Slovakia
Counter electrode	SU 206, Elektrokarbon, Slovakia
Distance between electrodes	1 mm
Sample character	oxide mixture or Si-carbides
Sample amount	10 mg
Exposition time	42 s
Excitating conditions	
Excitation source	Marinković plasma source
Current intensity	11 A
Ar flow in the Marinković plasma source	2.8 dm^3 min^{-1}
Evaluating conditions	
Software	LECO-SPECTRUMAT, Germany

The used spectral lines with their characteristic parameters are summarized in the Table 2. The evaporation process is in detail discussed in the reference [7]. As result the current intensity of 20 A, and 42 s exposition time were used as an optimal values. During this exposition time was the used graphite matrix totally burned up. The values of the limits of detection (LOD) were determined in the conformity with the 3σ criterion.

For the stabilization of evaporation and excitation it was used the AgCl buffer (spectrochemical active additive). The AgCl was prepared by addition of 10 μL $AgNO_3$ (cca 10 mg cm^{-3}) and 10 μL of conc HCl direct into the carrier electrode. By the reaction of the given reagents originated in the electrode the needed AgCl. Then the electrodes were dried 1 hour at 105 ^oC and they were filled with 10 mg analyzed sample. In this way modificated electrodes were used for all experiments.

Table 2. Parameters of the used elements and spectral lines

Element	Used oxidation form	T_b / °C	λ / nm	E_e / kJ M^{-1}
Al	Al_2O_3	2980	396.152	577.61
B	H_3BO_3	1750	249.773	800.77
Ca	CaO	2850	393.367	589.87
Cr	Cr_2O_3	4000	425.433	652.00
Fe	Fe_2O_3	3000	238.207	759.50
Mg	MgO	3600	280.270	737.84
Ni	NiO	2732	349.296	736.78
V	V_2O_5	1750	318.540	652.51

T_b – boiling temperature, λ - wavelength, E_e – excitation energy

Results and Discussion

At first, the results of the exploratory analysis were evaluated. The values of arithmetical mean, the medium, the modus and the halfsum are identical for all tested elements in the framework of measuring errors. The value of the parameter A(x), the s.c. asymmetry, was identical with the zero and the value of excess, parameter E(x), was identical with the tested value of 3. On the ground of these tests it is possible to determine that the tested sets have normal and homogeneous distribution.

Further, obtained parameters of the calibration process were compared with the tolerance parameter (Table 3). This value is derived from the convention that represents level of c(X) = 0.001 % which is the interface between the trace and ultra trace concentrations. From the comparison of the determined values of information contents it is evident that the differences are negligible. The elements Cr, Fe, Mg, and Ni have similar behavior like B, and Ca like V. Statistical parameters for Ca and V represent from the standpoint of the efficiency and detectability the less suitable parameters.

Table 3. Comparison of the tolerance and experimental calibration parameters

Element			B	Ca
Character of parameter		**Tolerance parameter**	**Experimental parameter**	
Minimal concentration	$c(X)_{min}$	0.001	0.0005	0.0010
Maximal concentration	$c(X)_{max}$	0.01	0.0100	0.0400
Standard deviation	s(X)	0.50	0.19	3.23
Information content	$I(p, p_o)$	1.02	1.08	1.02
Limit of detection	$c(X)_L$	0.001	0.0009	0.0600
Efficiency coefficient	E(X)	1.00	1.00	0.16
Information efficiency	$E(p, p_o)$	1.02	1.08	0.17

The narrowing of the concentration ranges (Table 4) only inconsequentially wrote down the information content. This procedure has completely different influence on the information efficiency, mainly values of this evaluation parameter got close. It is the synergic effect of the interaction of the precision and contemporary the detectability.

Table 4. Comparison of the Ni results of two concentrations ranges

Element		Ni	
Character of parameter		**Experimental parameter**	
Minimal concentration	$c(X)_{min}$	0.0005	0.001
Maximal concentration	$c(X)_{max}$	0.0100	0.045
Standard deviation	s(X)	1.12	2.02
Information content	$I(p, p_o)$	3.17	2.08
Limit of detection	$c(X)_L$	0.0005	0.001
Efficiency coefficient	E(X)	0.20	0.10
Information efficiency	$E(p, p_o)$	0.74	0.21

Conclusion

The checking of the selection of the spectra lines is most efficient with the determination of the parameters of the one-dimensional exploratory analysis. Finally it can be stated that extension of the concentration range of calibration in the direction to the lower concentration, as c(X) < 0.001 %, improved all evaluation parameters of the analytical calibration. The extension of the concentration range in direction to the higher concentration, as c(X) > 0.01 %, is always connected with degradation of the evaluation parameters.

Acknowledgement

This work was supported by the DAAD project 11/2005, APVV project No. 20-009404 and the VEGA project No 1/3149/06. The authors are obliged to express their gratitude for this support.

References

[1] Kupka K.: QC-Expert^{TM} 2.5 – Statistical software. TriloByte. Pardubice 2002
[2] Meloun M., Militký., Flórián M.: *Chemometrics for Analytical Chemistry.* Elis Horwood, Chichester, 1992
[3] Matherny M., Eckschlager K.: *Chem. Zvesti* 1984, **38,** 479
[1] Flórián K., Matherny M.: *Fresenius Z. Anal. Chem.* 1986, **324,** 525
[2] Ružičková S., Jankovská E., Koller L., Matherny M.: *Chem. Pap.* 2003, **57,** 204
[3] Marinković M., Antonijević V.G.: *Spectrochim. Acta, Part B,* 1980, **35,** 129
[4] Ružičková S., Koller L., Matherny M.: *Chem. Pap.* 2006, **60,** 116

State of the Art in Determination, Speciation Analysis and Fractionation of Aluminium in Environmental Samples by Spectrochemical Analytical Methods

Peter Matúš
Comenius University in Bratislava, Faculty of Natural Sciences, Geological Institute, Mlynská dolina, 842 15 Bratislava 4, Slovakia, e-mail: matus@fns.uniba.sk

Abstract

The direct determination of total Al concentration and concentration of various Al fractions and species is a difficult analytical task. A number of different procedures have been developed in order to distinguish between the various Al chemical forms in environmental samples. The separation techniques often employed in the fractionation and speciation analysis of Al can be divided up by their principles, e.g. methods based on size or mass exclusion, kinetic and/or binding strength discrimination, chromatographic and electroseparation techniques and computer simulations. The spectrometry detection techniques coupled with these separation methods played an important role in the fractionation and speciation analysis of Al during the last ten years. Atomic and molecular spectrometry, mass spectrometry and X-ray spectrometry are often used for this purpose. In this research the various results are discussed and some general comments and remarks on sample storage and pretreatment, and separation and spectrometry detection methods used for Al prior to its determination, fractionation and speciation analysis are pointed out.

Key words: *aluminium -- determination -- fractionation -- speciation analysis -- spectrochemical analysis*

Introduction

Aluminium occupies a somewhat anomalous position among the elements, in that it is a very common and important constituent of many inorganic materials of the biosphere, but is quite rare and usually an unimportant component of living matter. Although aluminium is found typically in low amounts in plants and animals, the aluminium content of most soils is quite high. Such Al is usually not readily available and is contained primarily in the structures of primary minerals and aluminosilicate clays. Because of its low solubility in solution under neutral conditions, Al was regarded as a non-toxic element and its environmental and biological effects were not investigated until recently. As soils become acidified through weathering and/or possible anthropogenic activity and the leaching of nonhydrolyzable cations, these sources of aluminium solubilize, releasing aluminium in its readily available chemical species. During the last two decades it has been confirmed

that acid deposition leads to considerable increases of dissolved Al concentrations in acidified soils and surface waters [1-4].

The differentiation of the various species of aluminium in environmental samples is especially difficult. These species have ranges of extractability in solution because of a wide range in particle size, crystallinity, and ionic composition. Aluminium is a strongly hydrolyzing metal and is relatively insoluble in the neutral pH range (6.0 to 8.0). Under acidic (pH < 6.0) or alkaline (pH > 8.0) conditions, and/or in the presence of complexing ligands, the solubility of Al is enhanced, making it more available for biogeochemical transformations. The extent of complexation depends on the availability of soil Al, solution pH, concentrations of complexing ligands, ionic strength, and temperature. Aqueous Al may be redeposited to free-soil pools, assimilated by living biomass or transported from the system [1,2].

The speciation of Al in soil solution (i.e. its physico-chemical forms) is important because this factor controls the toxicity of Al to plants and living organisms. High aluminium amounts limit the input and the transport of the nutriments and influence negatively the cell division and cell-walls of the plants. The chronical effect of Al compounds is often connected with mortality of some animals and Alzheimer or some other human neurodegenerative diseases. It has been claimed that free aqua monomeric complexes of Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$ and some polymeric species (e.g. Al_{13}) are the most toxic species. The toxicity of $AlSO_4^{+}$ is not always accepted [1,2].

Some spectrometric methods for the determination of Al in various sample types were earlier presented by Tikhonov [5] and Frech and Cedergren [6]. The Al fractionation and speciation analysis methods were reviewed by many authors before [7-14] and some new review reports have appeared in last six years [15-23]. The comparison of distribution of published papers on the determination, fractionation and speciation analysis of Al and on the determination, fractionation and speciation analysis of Al by spectrometry techniques during the period 1985-2005 is pointed in Fig. 1. Up to 35 % of all scientific works on quantitative analysis of aluminium is equivalent to Al fractionation and speciation analysis (Fig. 1a). In the case of spectrometric quantitative analysis this number decreases to 28 % (Fig. 1b). The spectrometry techniques are employed in a greater extent in determination of total Al concentration than in fractionation and speciation analysis of Al.

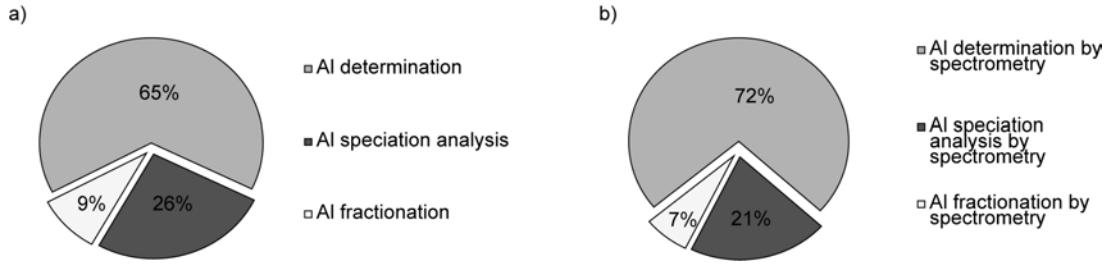


Fig. 1. The distribution of published papers during the period 1985-2005 a) on the determination, fractionation and speciation analysis of Al and b) on the determination, fractionation and speciation analysis of Al by spectrometry methods; total number of publications: a) 4293, b) 873 (Science Citation Index Expanded, Web of Science, ISI Web of Knowledge[SM])

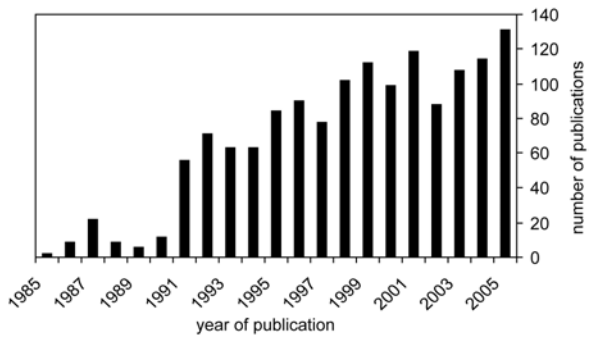


Fig. 2. The development of literature published about fractionation or speciation analysis of Al during the period 1985-2005; total number of publications: 1438 (Science Citation Index Expanded, Web of Science, ISI Web of Knowledge[SM])

Fig. 2 shows the development of literature published about fractionation or speciation analysis of Al during the period 1985-2005. It is evident that the scientific literature concerning this topic has increased in last fifteen years, since 1991.

The contemporary separation and spectrometry detection techniques utilized in the determination, fractionation and speciation analysis of Al are listed in Table 1. It is important to realize that all fractionation and speciation analysis methods, perhaps with exception of potentiometric and direct spectrometric techniques (e.g. NMR), will alter the speciation of sample Al during measurement [2].

Table 1 The contemporary separation and spectrometry detection techniques utilized in the determination, fractionation and speciation analysis of Al

Separation techniques	Spectrometry detection techniques
The size or mass exclusion techniques	*The atomic spectrometry techniques*
Filtration and ultrafiltration	Flame atomic absorption spectrometry (F AAS)
Centrifugation and ultracentrifugation	Graphite furnace atomic absorption spectrometry (GF AAS)
Dialysis	Direct current plasma optical emission spectrometry (DCP OES)
Size exclusion chromatography (SEC)	Inductively coupled plasma optical emission spectrometry (ICP OES)
Gel electrophoresis	Electrothermal vaporization inductively coupled plasma optical emission spectrometry (ETV ICP OES)
The kinetic and/or binding strength discrimination techniques	Optical emission spectrometry (OES) with lasers
Extraction methods	
Ion exchange	*The molecular spectrometry techniques*
Fluoride ion-selective electrode (ISE) potentiometry and other electrochemical measurements	Ultraviolet (UV) and visible (VIS) spectrophotometry
Procedures based on the rates of reaction with complexing agents	Fluorimetry
Flow injection analysis (FIA)	Infrared (IR) spectrometry
Continuous flow analysis (CFA)	Nuclear magnetic resonance (NMR)
	Mass spectrometry techniques
The chromatographic and electroseparation techniques	Inductively coupled plasma mass spectrometry (ICP MS)
High performance liquid chromatography (HPLC)	Electrospray mass spectrometry (ES MS)
Fast protein liquid chromatography (FPLC)	Electrospray tandem mass spectrometry (ES MS MS)
Ion exchange chromatography (IEC)	Secondary ion mass spectrometry (SIMS)
Reverse phase chromatography (RPC)	Accelerator mass spectrometry (AMS)
Immobilized metal affinity chromatography (IMAC)	Time-of-flight mass spectrometry (TOF MS)
Isotachophoresis (ITP)	Laser microprobe mass spectrometry (LM MS)
Capillary zone electrophoresis (CZE)	
	X-ray spectrometry techniques
Other techniques	X-ray flourescence spectrometry (XFS)
Computer simulation	Energy disperzive X-ray microanalysis (EDXM)

This concise summary paper includes recent significant contributions in the field of sample storage and pretreatment, separation and spectrometry detection methods used for Al prior to its determination, fractionation and speciation analysis.

Sample storage and pretreatment

Generally, the choice of an appropriate procedure for sample storage and pretreatment is of great amportance in the determination, fractionation and speciation analysis of all elements. Water samples should be stored with HNO_3 addition for determination of total Al (this ensures that Al remains in

solution) and without acidification for Al fractionation and speciation analysis. Prior to analysis the solid samples (soil, sediment, biological tissue, food) must be homogenized and extracted or digested [24]. Blood and urine samples may be treated by the centrifugation and diluted by H_2O and HNO_3 or if appropriate, analysed directly without pretreatment [2,6].

Any changes in concentrations of monomeric Al were observed by Seip et al. [25] after two months storage of natural water samples. On the contrary, Sullivan et al. [26] found the time-dependent changes in the speciation of Al in stored water samples. The samples with low content of reactive Al, high levels of organic matter and pH > 5 seem to be most sensitive to modification of Al speciation [27]. It is also believed that microbiological activity can alter the organic matter in samples, and thereby affect their influence on Al speciation [9]. The material of containers used for sample storage is also important (high-density polyethylene is more suitable than polypropylene) [9]. Andrén [28] describes the changes in concentration of labile Al species in stream water after 1-2 months storing at temperature 4 °C. According to Derome et al. [29] the freezing of soil solutions is unsuitable because of the decreasing of total Al. The authors [29] found that the storage of samples at 4 °C affected the concentration of ion-exchange monomeric Al species, but acidification of samples to pH < 3.7 allowed the conservation of ion-nonexchange, e.g. organically bound Al. Drying and freezing have the significant influence to speciation of organically bound Al in soil samples [30]. Peréz et al. [30] accept only the application of moist soil samples or short-term (no more than 15 days) sample storing at 4 °C.

Determination of Al

Currently, the vast majority of Al determinations in environmental samples is carried out by using atomic and mass spectrometry methods [6,8,10], see Table 1. Most common used atomic spectrometry techniques are graphite furnace atomic absorption spectrometry (GF AAS) and inductively coupled plasma optical emission spectrometry (ICP OES). Flame atomic absorption spectrometry (F AAS) using the nitrous oxide/acetylene flame is unsuitable for determination of Al at low concentration level because of its low sensitivity.

GF AAS is characterized by the high analytical sensitivity (16 pg/0.0044 absorbance unit) and consequently low detection limit (1-3 $\mu g\ l^{-1}$), relatively short analysis time, small sample size and relatively simple pretreatment of analyte [8]. The main difficulties experienced in using this method for determination of Al, e.g. matrix interferences of chlorine and sulfur, formation of carbonaceous residues in the graphite tube and worse precision of measurement, have been overcome with the using of modern GF AAS instrumentation (pyrolytically coated high density graphite tubes, thorium-treated and other types of platforms) and the application of various matrix modifiers and pretreatment procedures [8,10]. These improvements are combined with other developments in the graphite furnace technique (e.g. solid sampling technique) and incorporated in the so called STPF (stabilized temperature platform furnace) concept [6]. The general conditions of Al determination by GF AAS are detailed discussed by Welz and Sperling [31]. Srinivasan et al. [32] examined the different furnace programs and matrix modifiers for GF AAS determination of Al in drinking water. GF AAS measurements of Al in blood-serum were reviewed by Beinrohr et al [33].

The advantages of ICP OES include the easy atomization of Al and lack of chemical interferences (only significant matrix effect is due to changes in sample viscosity) at high temperatures in Ar plasma (10 000 K), and although its detection limit for Al (10 $\mu g\ l^{-1}$) is not always sufficient, the combination of ICP OES with electrothermal volatilization and/or previously performed analyte separation and preconcentration, preferably in on-line system, can mitigate such drawback [8]. However, the spectral interferences of Ag, B, Mn and Ca can be significant due to the overlap of emission lines, increase of background and detection limit for Al [6,10]. Various types of continuously operated nebulizers are employed to increase the efficiency of analyte nebulization, e.g. glass-frit, ultrasonic or direct-injection nebulizer. Sensitivity of this technique can be improved by use of electrothermal vaporizer coupled with ICP OES. Electrothermal vaporization inductively coupled

plasma optical emission spectrometry (ETV ICP OES) gives the detection limit around 1-2 μg l^{-1} for Al in serum [6].

Ultraviolet (UV) and visible (VIS) spectrophotometry is most often used for the Al determination, but main disadvantages of this method is the narrowness of pH range of the reaction of Al with agent, instability of the formed Al complex, low selectivity and sensitivity [2]. Fluorimetric methods are generally very sensitive, but for complex matrices the selectivity is poor [6]. Infrared (IR) spectrometry and nuclear magnetic resonance (NMR) are appropriate analytical methods for the quantification of Al at per par million levels [23].

ICP can also be combined with a mass spectrometer to further increase the sensitivity of the method. ICP MS offers the ability to measure isotope ratios [2]. With this technique, the above mentioned spectral interferences observed with ICP OES are circumvented. On the other hand, ICP MS suffers from isobaric overlap and sample matrix interferences of magnesium and molecular nitrogen [6]. Another improvements of analytical characteristics have been achieved by use of electrospray mass spectrometry (ES MS) and electrospray tandem mass spectrometry (ES MS MS). Aluminium concentrations in human brain can be investigated by laser multipoint microprobe mass analysis (LAMMA) using focused laser ionization with time-of-flight mass spectrometry (TOF MS) [2].

X-ray flourescence spectrometry (XFS) is not sensitive enough to measure trace levels of Al in environmental samples. The general lack of sensitivity limits the use of this method to data requirements in the percentage range. This shortcoming is largely related to low atomic weight of Al, but is still useful for Al determination in soils, sediments or rocks, obviating the need of chemical dissolution or digestion of sample [10].

Speciation analysis and fractionation of Al

In present it seems that the coupled methods play an important role in the speciation analysis of Al. Mostly they include an efficient separation unit coupled with sensitive detector. In most cases it is based on the combination of liquid chromatography techniques and spectrometry detection [21]. The chromatographic separation methods coupled on-line or off-line with a specific element spectrometry detection techniques (see Table 1) create an extremely powerful analytical tool for the direct speciation analysis of various elements [34-39]. The reverse phase and ion exchange chromatography are the most common applied forms of high performance liquid chromatography (HPLC) or fast protein liquid chromatography (FPLC) in Al speciation analysis [17]. They have the capability to separate various free and complex Al species in environmental samples. The electroseparation methods (isotachophoresis and capillary zone electrophoresis) are utilized in a lesser extent for this purpose [9,11,40]. Most common used spectrometry detection systems in Al speciation analysis are atomic (GF AAS, ICP OES) and mass (ICP MS, ES MS and ES MS MS) spectrometry techniques. These techniques have very low detection limit for Al, a wide linear calibration ranges are achieved and they are time-saving. The interfaces between HPLC or FPLC, respectively, and GF AAS, ICP OES, ICP MS, ES MS or ES MS MS, respectively, are fundamental to the success of the on-line device arrangement. Recent developments in HPLC/FPLC-GF AAS/ICP OES interfaces include the volatilization of analyte effluent into an aerosol in a heated silica tube. This aerosol then enters the furnace/plasma through a vitreous graphite tube. Another advance is the flash evaporation of analyte effluent to an aerosol before it enters to the furnace [17].

The extensive development of various separation methods coupled with atomic and mass spectrometry techniques have been achieved by work group of Radmila Milačič [19,22,23,41-55]. The authors employed the microcolumn chelating ion-exchange chromatography (MCC) with ICP OES and GF AAS detection to separate and determine aqua Al^{3+} and hydroxo, sulfato and fluorocomplexes of Al in soil extracts [41] and environmental water samples [42]. A strong cation- and anion-exchange FPLC column was used for separation of various monomeric Al species (e.g. Al^{3+}, $Al(OH)^{2+}$,

$AL(OH)_2^+$, $Al(OH)_4^-$, $AlSO_4^+$, AlF_2^+, AlF^{2+}, Al-oxalate, Al-citrate, Al-aconitate, Al-malate) [43-55]. The solution of NH_4NO_3 was used in a linear gradient elution of analyte. The technique of size exclusion chromatography (SEC) was employed in separation of Al-humate and Al-fulvate complexes [50], separation of complexed Al-serum proteins with high molecular mass (HMM) from low molecular mass (LMM) Al compounds (e.g. citrate, phosphate) [53] and separation of LMM complexes of Al and Al-binded milk proteins [55]. In most works the concentration of Al was determined by GF AAS, ICP OES, ICP MS, ES MS and ES MS MS. Z-spray ion source as the interface in FPLC-ES MS [48] and FPLC-ES MS MS [49,51,54,55] techniques was employed in some author's works. The coupled methods were applied to different samples (biological fluids, human serum, dialysis concentrates, natural and percolating waters, soil extracts, plants, tea infusions, milk). The combination of these techniques indicated good agreement of results and also provided more comprehensive information on various Al species present in investigated samples.

The chromatographic separations coupled with spectrometry methods were employed also by the team of Alfredo Sanz-Medel [8,13,19,56-63]. Alonso et al. [56] used anion exchange HPLC to study the protein binding of Al. HPLC separation of serum proteins followed by GF AAS determination of Al species was carried out by Sanz-Medel and Fairman [57]. The authors investigated the Al speciation in human serum by a hybrid HPLC/gel electrophoresis-GF AAS [57]. Anion exchange HPLC offers important advantages compared with traditional size exclusion chromatography (SEC) [19,58]. The coupling of FPLC-GF AAS was used for the quantitative studies of aluminium binding species to desferrioxaminein (DFO) in human uremic serum [59]. The comparison of element-specific detection using HPLC-ICP MS with an established HPLC-fluorimetric method for aluminium speciation in waters was described in paper [60]. The increased selectivity of ICP MS over molecular fluorescence measuring was shown in the reliable quantification of Al^{3+} and AlF^{2+} ions in a range of tap and natural water samples. The speciation of basal aluminium in human serum by FPLC with ICP MS detection was studied also [61,62]. The Al detection was carried out on-line using both quadrupole ICP MS and double-focusing ICP MS systems. The latter detector proved to be adequate for this detection [61]. The separation of the two Al species (AlF^{2+} and Al^{3+}) in drinking and sea water samples by chromatographic system with ICP MS detection was shown in paper of Bayon et al. [63].

Andreas Seubert and his colleagues worked also at Al speciation studies [64-68]. Borrmann and Seubert [64,65] investigated the speciation of Al fluoride, oxalate and citrate complexes at different pH values by employing HPLC coupled on-line to ICP OES. The complete speciation of the above mentioned Al species in synthetic solution was obtained within 20 min by the combination of cation- and anion-exchange column [65]. HPLC using a combined size exclusion and cation exchange column was applied to the determination of aluminum and its fluoro, oxalate and citrate species [66,67]. The Al species were detected by post-column reaction with a Tiron based solution, followed by UV photometry as well as by on-line coupling to ICP OES. The dependence of the degree of disintegration and therefore the applicability of ion chromatography for the speciation of aluminium fluoride species was examined for two different column geometries, a standard bore and a microbore column [67]. The procedure for separation of three anionic Al citrate complexes in plant sap by anion exchange HPLC-ICP OES within 5 min using isocratic conditions was developed by Happel and Seubert [68].

HPLC/GF AAS hybrid technique allowing an adequate separation of the proteins and of the inorganic/organic Al species of interest was developed by van Landeghem et al. [69]. Because of high sensitivity of used chromatographic system, the method can be used at clinically relevant concentrations and was applied successfully to study aluminum binding to serum transferrin [70].

The soluble complexes of Al, including fluorides, citrates, acetates, were separated and detected using gradient elution cation exchange HPLC with the fluorescence detection [71]. The same chromatographic system was used in the study of Al binding to humic substances in both laboratory and field samples by Sutheimer and Cabaniss [72].

Hils et al. [73,74] developed procedure for the speciation of aluminium in percolating water of forest soil by online coupling of HPLC to an element selective and sensitive ICP MS detection system. While the inorganic Al species were retained on a cation exchange column, the aluminium complexed

to organic components passed through the column and could be measured [73]. This method led to low detection limits of 0.3-0.6 μg l^{-1} and high precision and reproducibility (1.2-3.5 % RSD) of the speciation procedure [74].

To study aluminum speciation after the administration of deferoxamine, high-performance liquid chromatography (HPLC) and ultrafiltration techniques were used by Canteros-Picotto et al. [75]. The detection of Al was performed by ultraviolet (UV) spectrophotometry and atomic absorption spectrometry. Unknown species of aluminum other than aluminoxamine were found in the early elution fractions.

The chemical forms of co-existing Al bound to human serum transferrin were studied by combined on-line HPLC and high-resolution ICP MS (HPLC-HR ICP MS) by Nagaoka and Maitany [76-78]. The samples were subjected to HPLC equipped with an anion exchange column and quadrupole ICP MS [76]. The effects of sialic acid residues of transferrin on the binding with aluminum were investigated by the same tandem technique [77,78].

The cation exchange high-performance liquid chromatography with high resolution inductively coupled plasma mass spectrometric detection (CE HPLC-ICP MS) was developed for the speciation of aluminum in environmental samples [79]. Three types of aluminum species ($AlL(X)^{<2+}$, $Al(X)^{2+}$, Al^{3+}) were separated from one another. The present system showed better sensitivity for aluminum than CE HPLC with fluorimetric detection. The liquid chromatography coupled with electrospray tandem mass spectrometry (LC-ES MS MS) [80] and atmospheric pressure chemical ionisation tandem mass spectrometry (LC-APCI MS MS) [81] were used for the determination of fosetyl-aluminium fungicide in lettuce [80] and hop cones [81].

The complexation of aluminium with polyphenol antioxidant purpurogallin has been investigated using nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography-mass spectroscopy (LC-MS) and Fourier transform infrared (FT IR) spectroscopy [82]. H-1 NMR was used to determine the coordination site of the aluminium ion and LC-MS to determine the stoichiometry and molecular weight of the major complex formed in solution. FT IR spectral comparisons were used to corroborate the proposed chelating moiety.

A possible method for the speciation of KCl and water extractable Al in forest soils was suggested by the use of cation exchange HPLC with UV detection after post-column derivatization [83-84]. This approach enables the separation of various Al species according to the value of their positive charge. Species Al^{3+} were the most abundant Al forms in the KCl extracts (around 93 %). Prevailing Al forms (more than 70 %) in water extracts were $AI(X)^{+}$, i.e. $Al(OH)_2^{+}$, $Al(SO_4)^{+}$, AIF_2^{+}, $Al(oxalate)^{+}$, $Al(H\text{-}citrate)^{+}$ species.

In work [85] the different hyphenated techniques have been employed for the speciation of aluminium. Aluminium hydroxide, citrate, phosphate and lactate complexes were investigated in neuroblastoma cells exposed to Al lactate by separation system of size-exclusion or reverse phase HPLC and capillary electrophoresis with ICP MS detection (SE(RP)HPLC(CE)-ICP MS). The isolated low molecular weight Al species did not produce a signal in electrospray mass spectrometry (ES MS).

A novel method has been developed to purify and characterize aluminium binding ligands from fungi exudate solutions using the immobilized metal affinity chromatography (IMAC), reversed phase chromatography (RP HPLC) and electrospray ionization mass spectrometry (ES MS) in positive and negative ion modes by Baldwin et al. [86]. The fungal exudates exhibit a strong binding capacity for Al ions, allowing their selective enrichment and collection using IMAC method. RP HPLC separation and elemental analysis of the IMAC eluent indicate that both Al and exudate ligands elute from the column but they are not bound in a complex.

Conclusion

The determination and speciation of Al directly by coupled or hyphenated techniques is the primary tool in present. It has many potential applications in the field of Al speciation for plant and animal biology and biochemistry, nutrition, ecological and clinical toxicology. The reduction in cost, increasing of the robustness and more simplification of these methods are still necessary. In general, the problems with redistribution of Al species and verification of analytical results play the important role also in these methodologies.

Acknowledgements

The work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-010204 and Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences under the contracts Nos. VEGA 1/2439/05 and VEGA 1/3561/06.

References

[1] G. Sposito (Ed.), The Environmental Chemistry of Aluminium, second ed., CRC Press, Boca Raton, Florida, 1996
[2] H. Habs, B. Simon, K.U. Thiedemann, P. Howe, Environmental Health Criteria Volume 194: Aluminium, World Health Organization, Geneva, 1997
[3] P. Dlapa, A. Kopcová, J. Kubová, Influence of reactive aluminium species on soil-vegetation relationship, Phytopedon (Bratislava) 1 (2002) 86-93
[4] J. Kubová, P. Matúš, M. Bujdoš, J. Medveď, Influence of acid mining activity on release of aluminium to the environment, Anal. Chim. Acta, 547 (2005) 119-125
[5] V.N. Tikhonov, Analytical Chemistry of Aluminium, Wiley, New York, 1973
[6] W. Frech, A. Cedergren, Aluminium, in: M. Stoeppler (Ed.), Techniques and Instrumentation in Analytical Chemistry, Vol. 12: Hazardous Metals in the Environment, Elsevier, Amsterdam, 1992, pp. 451-473
[7] J.P. Boudot, T. Becquer, D. Merlet, J. Rouiller, Aluminum toxicity in declining forests – a general overview with a seasonal assessment in a silver fir forest in the Vosges mountains (France), Ann. Sci. For. 51(1994) 27-51
[8] A. Sanz-Medel, B. Fairman, K. Wróbel, Aluminium and Silicon Speciation in Biological Materials of Clinical Relevance, in: S. Caroli (Ed.), Element Speciation in Bioinorganic Chemistry, Wiley, New York, 1996, pp. 223-254
[9] N. Clarke, L.-G. Danielsson, A. Sparén, Analytical methodology for the determination of aluminium fractions in natural fresh waters (Technical Report), Pure & Appl. Chem. 68 (1996) 1597-1638
[10] P.M. Bertsch and P.R. Bloom, Aluminum, in D.L. Sparks (Ed.), Methods of Soil Analysis, Part 3, Chemical Methods, Soil Science Society of America and American Society of Agronomy, Madison, 1996, pp.517-550
[11] G.F. van Landeghem, M.E. de Broe, P.C. D'Haese, Al and Si: Their speciation, distribution, and toxicity, Clin. Biochem. 31 (1998) 385-397
[12] Ph. Quevauviller, Method Performance Studies for Speciation Analysis, Royal Society of Chemistry, Cambridge, 1998, pp. 172-180
[13] A. Sanz-Medel, The chemical speciation of aluminium and silicon in human serum, Analusis Magazine 26 (1998) M76-M80
[14] P.T. Srinivasan, T. Viraraghavan, K.S. Subramanian, Aluminium in drinking water: An overview, Water SA 25 (1999) 47-55
[15] J. Kubová, Utilization of selective extraction techniques for element speciation in soils and sediments – Critical evaluation of the contemporary state, Slovak Geol. Mag. 6 (2000) 43-52

[16] K. Pyrzyńska, S. Guçer, E. Bulska, Flow-injection speciation of aluminium, Water Res. 34 (2000) 359-365
[17] S.P. Bi, X. Yang, F. Zhang, X. Wang, G. Zou, Analytical methodologies for aluminium speciation in environmental samples – a review, Fresenius J. Anal. Chem. 370 (2001) 984-996
[18] P. Matúš, J. Kubová, Speciation of aluminium in waters and soil solutions, Chem. Listy 96 (2002) 174-181
[19] A. Sanz-Medel, A.B. Soldado Cabezuelo, R. Milačič, T. Bantan Polak, The chemical speciation of aluminium in human serum, Coord. Chem. Rev. 228 (2002) 373-383
[20] J. Kubová, V. Streško, P. Matúš, Analytical methodologies for aluminium speciation in environmental samples – a review, Slovak Geol. Mag. 9 (2003) 125-127
[21] H.Z. Lian, S.P. Bi, L.Q. Tian, Reliability of aluminum speciation by high performance liquid chromatography, Chin. J. Anal. Chem 32 (2004) 1677-1682
[22] R. Milačič, Speciation of Aluminium, in: R. Cornelis, J. Caruso, H. Crews, K.G. Heumann (Eds.), Handbook of Elemental Speciation II: Species in the Environment, Food, Medicine and Occupational Health, Wiley, Chichester, 2005, pp. 7-19
[23] J. Ščančar, R. Milačič, Aluminium speciation in environmental samples: a review, Anal. Bioanal. Chem., in press
[24] J. Medveď, V. Streško, J. Kubová, J. Polakovičová, Efficiency of decomposition procedures for the determination of some elements in soils by atomic spectroscopic methods, Fresenius J. Anal. Chem. 360 (1998) 219-224
[25] H.M. Seip, L. Müller, A. Naas, Aluminium speciation: comparison of two spectrophotometric analytical methods and observed concentrations in some acidic aquatic systems in Southern Norway, Water Air Soil Pollut. 23 (1984) 81-95
[26] T.J. Sullivan, H.M. Seip, I.P. Muniz, A comparison of frequently used methods for the determination of aqueous aluminium, Intern. J. Environ. Anal. Chem. 26 (1986) 61-75
[27] M. Berdén, N. Clarke, L.-G. Danielsson, A. Sparén, Aluminium speciation: variations caused by the choice of analytical method and by sample storage Water Air Soil Pollut. 72 (1994) 213-233
[28] C. Andrén, Aluminium speciation; Effects of sample storage, Water Air Soil Pollut. 85 (1995) 811-816
[29] K. Derome, J. Derome, A.-J. Lindross, Techniques for preserving and determining aluminium fractions in soil solution from podzolic forest soils, Chemosphere 36 (1998) 1143-1148
[30] D.V. Pérez, R.C. de Campos, N.A. Meneguelli, Effects of soil sample storage treatment on the composition and Fe, Al, and Mn speciation of soil solutions obtained by centrifugation, Water Air Soil Pollut. 151 (2004) 195-214
[31] B. Welz, M. Sperling, Atomic Absorption Spectrometry, hird ed., Wiley-WCH, Weinheim, 1999
[32] P.T. Srinivasan, T. Viraraghavan, K.S. Subramanian, Method development for drinking water aluminum measurement using a graphite furnace atomic absorption spectrophotometer, Am. Lab. 32 (2000) 76-91
[33] E. Beinrohr, J. Mocák, X. Svobodová, O. Ľosová, Analysis of aluminium in blood-serum by electrothermal atomic absorption spectrometry, Chem. Listy 85 (1991) 131-141
[34] B. Welz, Speciation analysis: where is it going? An attempt at a forecast, Spectrochim. Acta, Part B 53 (1998) 169
[35] A. Kot, J. Namiesńik, The role of speciation in analytical chemistry, Trends Anal. Chem. 19 (2000) 69-79
[36] A.K. Das, M. de la Guardia, M.L.Cervera, Literature survey of on-line elemental speciation in aqueous solutions, Talanta 55 (2001) 1-28
[37] A. Seubert, On-line coupling of ion chromatography with ICP-AES and ICP-MS, Trends Anal. Chem. 20 (2001) 274-287
[38] J. Szpunar, R. Łobiński, A. Prange, Hyphenated techniques for elemental speciation in biological systems, Appl. Spectrosc. 57 (2003) 102A-112A
[39] K.A. Francesconi, M. Sperling, Speciation analysis with HPLC-mass spectrometry: time to take stock, Analyst 130 (2005) 998-1001
[40] H. Klöppel, W. Kördel, S. Schmid, W. Klein, Development and Improvement of Analytical Methods for Specification Scheme of Aluminium in the Mobile Soil Phase, in: J.A.C. Broekaert,

Ş. Güçer, F. Adams (Eds.), NATO ASI Series, Vol. G 23: Metal Speciation in the Environment, Springer, Berlin, 1990, pp 447-460
[41] N. Kožuh, R. Milačič, B. Gorenc, Comparison of two methods for speciation of aluminium in soil extracts, Annali di Chimica 86 (1996) 99-113
[42] N. Kožuh, R. Milačič, B. Gorenc, O. Abollino, C. Sarzanini, Speciation of aluminium in environmental water samples employing microcolumn chelating ion-exchange chromatography ETAAS, Intern. J. Environ. Anal. Chem. 67 (1997) 27-40
[43] B. Mitrović, R. Milačič, B. Pihlar, Speciation of aluminium in soil extracts by employing cation-exchange fast protein liquid chromatography inductively coupled plasma atomic emission spectrometry, Analyst 121 (1996) 627-634
[44] T. Bantan, R. Milačič, B. Pihlar, Possibilities for speciation of Al-citrate and other negatively charged Al complexes by anion-exchange FPLC-ICP-AES, Talanta 46 (1998) 227-235
[45] T. Bantan, R. Milačič, B. Pihlar, Quantitative determination of trace amounts of Al-citrate by anion-exchange FPLC-ETAAS, Talanta 47 (1998) 929-941
[46] R. Milačič, N. Kožuh, B. Mitrović, Combination of three analytical techniques for speciation of Al in environmental samples, Mikrochim. Acta 129 (1998) 139-145
[47] B. Mitrović, R. Milačič, B. Pihlar, P. Simončič, Speciation of trace amounts of aluminium in environmental samples by cation-exchange FPLC-ETAAS, Analusis 26 (1998) 381-388
[48] T. Bantan, R. Milačič, B. Mitrović, B. Pihlar, Combination of various analytical techniques for speciation of low molecular weight aluminium complexes in plant sap, Fresenius J. Anal. Chem. 365 (1999) 545-552
[49] T. Bantan, R. Milačič, B. Mitrović, B. Pihlar, Investigation of low molecular weight Al complexes in human serum by fast protein liquid chromatography (FPLC)-ETAAS and electrospray (ES)-MS-MS techniques, J. Anal. At. Spectrom. 14 (1999) 1743-1748
[50] B. Mitrović, R. Milačič, Speciation of aluminium in forest soil extracts by size exclusion chromatography with UV and ICP-AES detection and cation exchange fast protein liquid chromatography with ETAAS detection, Sci. Tot. Environ. 258 (2000) 183-194
[51] T.B. Polak, R. Milačič, B. Mitrović, M. Benedik, Speciation of low molecular weight Al complexes in serum of CAPD patients, J. Pharm. Biomed. Anal. 26 (2001) 189-201
[52] T.B. Polak, R. Milačič, B. Pihlar, B. Mitrović, The uptake and speciation of various Al species in the Brassica rapa pekinensis, Phytochem. 57 (2001) 189-198
[53] B. Kralj, J. Sčančar, I. Križaj, M. Benedik, P. Bukovec, R. Milačič, Determination of high molecular mass Al species in serum and spent CAPD fluids of dialysis patients combining SEC and anion-exchange FPLC with ETAAS detection, J. Anal. At. Spectrom. 19 (2004) 101-106
[54] T. Bantan-Polak, R. Milačič, B. Mitrović, The use of fast protein liquid chromatography with ICP-OES and ES–MS–MS detection for the determination of various forms of aluminium in the roots of Chinese cabbage, Anal. Chim. Acta 540 (2005) 83-89
[55] B. Kralj, I. Križaj, P. Bukovec, S. Slejko, R. Milačič, Speciation of aluminium in tea infusions by use of SEC and FPLC with ICP-OES and ES-MS-MS detection, Anal. Bioanal. Chem. 383 (2005) 467-475
[56] J.I.G. Alonso, A.R. García, J.P. Parajón, E.B. González, A.S. Medel, J.B. Cannata, High-performance liquid-chromatography methods for studying protein-binding of aluminium in human serum in the absence and in the presence of desferrioxamine, Clin. Chim. Acta 189 (1990) 69-80
[57] A. Sanz-Medel, B. Fairman, Aluminium speciation: clinical and environmental aspects, Mikrochim. Acta 109 (1992) 157-160
[58] K. Wróbel, E.B. González, K. Wróbel, A. Sanz-Medel, Aluminium and silicon speciation in human serum by ion-exchange high-performance liquid chromatography-electrothermal atomic absorption spectrometry and gel electrophoresis, Analyst 120 (1995) 809-815
[59] A.B.S. Cabezuelo, E.B. González, A. Sanz-Medel, Quantitative Studies of Aluminium Binding Species in Human Uremic Serum by Fast Protein Liquid Chromatography Coupled With Electrothermal Atomic Absorption Spectrometry, Analyst 122 (1997) 573-577

[60] B. Fairman, A. Sanz-Medel, P. Jones, E.H. Evans, Comparison of fluorimetric and inductively coupled plasma mass spectrometry detection systems for the determination of aluminium species in waters by high-performance liquid chromatography, Analyst 123 (1998) 699-703
[61] A.B.S. Cabezuelo, M.M. Bayón, E.B. González, J.I.G. Alonso, A. Sanz-Medel, Speciation of basal aluminium in human serum by fast protein liquid chromatography with inductively coupled plasma mass spectrometric detection, Analyst 123 (1998) 865-869
[62] M.M. Bayón, A.B.S. Cabezuelo, E.B. González, J.I.G. Alonso, A. Sanz-Medel, Capabilities of fast protein liquid chromatography coupled to a double focusing inductively coupled plasma mass spectrometer for trace metal speciation in human serum, J. Anal. At. Spectrom. 14 (1999) 947-951
[63] M.M. Bayón, A.R. García, J.I.G. Alonso, A. Sanz-Medel, Indirect determination of trace amounts of fluoride in natural waters by ion chromatography: a comparison of on-line post-column fluorimetry and ICP-MS detectors, Analyst 124 (1999) 27-31
[64] G. Borrmann, A. Seubert, Aluminum speciation by liquid chromatography concerning hydro- and geo-chemical aspects, Anal. Chim. Acta 332 (1996) 233-239
[65] G. Borrmann, A. Seubert, Aluminum speciation by means of anion chromatography and coupled anion/cation chromatography, Anal. Chim. Acta 386 (1999) 77-88
[66] M. Busch, A. Seubert, Influence of column temperature on the ion chromatographic separation of aluminum species, Anal. Chim. Acta 399 (1999) 223-235
[67] M. Busch, A. Seubert, Influence of column geometry on the ion chromatographic separation of aluminium species, Fresenius J. Anal. Chem. 366 (2000) 351-355
[68] O. Happel, A. Seubert, Characterization of stable aluminium-citrate species as reference substances for aluminium speciation by ion chromatography, J. Chromatogr. A 1108 (2006) 68-75
[69] G.F. van Landeghem, P.C. D'Haese, L.V. Lamberts, M.E. De Broe, Quantitative HPLC/ETAAS hybrid method with an on-line metal scavenger for studying the protein-binding and speciation of aluminum and iron, Anal. Chem. 66 (1994) 216-222
[70] P.C. D'Haese, G.F. van Landeghem, L.V. Lamberts, M.E. De Broe, HPLC-AAS hybrid technique for studying the speciation of trace metals (Al, Fe, Si, Hg) in biological fluids: a review of applications, recent experiences and perspectives, Mikrochim. Acta 120 (1995) 83-90
[71] S.H. Sutheimer, S.E. Cabaniss, Aqueous Al(III) speciation by high-performance cation-exchange chromatography with fluorescence detection of the aluminum-lumogallion complex, Anal. Chem. 67 (1995) 2342-2349
[72] S.H. Sutheimer, S.E. Cabaniss, Aluminum binding to humic substances determined by high performance cation exchange chromatography, Geochim. Cosmochim. Acta 61 (1997) 1-9
[73] A. Hils, E. Janssen, M. Grote, Procedures for aluminium speciation in percolating water of forest soil, J. Eichhorn, Agribiol. Res. 51 (1998) 89-95
[74] A. Hils, E. Janssen, M. Grote, J. Eichhorn, Speciation of trace amounts of aluminium in percolating water of forest soil by online coupling HPLC-ICP-MS, Fresenius J. Anal. Chem. 364 (1999) 457-461
[75] A. Canteros-Picotto, J.L. Fernandez-Martin, J.B. Cannata-Andia, Use of ultrafiltration and chromatography to assess aluminum speciation in serum after deferoxamine administration, Amer. J. Kidney Dis. 36 (2000) 969-975
[76] M.H. Nagaoka, T. Maitani, Binding patterns of co-existing aluminium and iron to human serum transferrin studied by HPLC–high resolution ICP-MS, Analyst 125 (2000) 1962-1965
[77] M.H. Nagaoka, T. Maitani, Effects of sialic acid residues of transferrin on the binding with aluminum and iron studied by HPLC/high-resolution ICP-MS, Biochim. Biophys. Acta 1526 (2001) 175-182
[78] M.H. Nagaoka, T. Maitani, Binding affinity of aluminium to human serum transferrin and effects of carbohydrate chain modification as studied by HPLC/high-resolution ICP-MS - Speciation of aluminium in human serum, J. Inorg. Biochem. 99 (2005) 1887-1894
[79] K.I. Tsunoda, T. Umemura, K. Ohshima, S.I. Aizawa, E. Yoshimura, K.I. Satake, Determination and speciation of aluminium in environmental samples by cation exchange high-performance

liquid chromatography with high resolution ICP-MS detection, Water Air Soil Pollut. 130 (2001) 1589-1594
[80] F. Hernandez, J.V. Sancho, O.J. Pozo, C. Villaplana, M. Ibanez, S. Grimalt, Rapid determination of fosetyl-aluminium residues in lettuce by liquid chromatography/electrospray tandem mass spectrometry, J. AOAC Intern. 86 (2003) 832-838
[81] J. Poustka, J. Hajslová, K. Holadová, K. Nováková, Potential of liquid chromatography-atmospheric pressure chemical lonisation tandem mass spectrometry for determination of fosetyl-aluminium residues in dried hops, Czech J. Food Sci.22 (2004) 24-28
[82] M. O'Coinceanainn, C. Astill, B. Baderschneider, Coordination of aluminium with purpurogallin and theaflavin digallate, J. Inorg. Biochem. 96 (2003) 463-468
[83] O. Drábek, L. Borůvka, L. Mládková, M. Kočárek, Possible method of aluminium speciation in forest soils, J. Inorg. Biochem. 97 (2003) 8-15
[84] O. Drábek, L. Mládková, L. Borůvka, J. Száková, A. Nikodem and K. Němeček, Comparison of water-soluble and exchangeable forms of Al in acid forest soils, J. Inorg. Biochem. 99 (2005) 1788-1795
[85] K. Połeć-Pawlak, P. Zambenedetti, J. Szpunar, R. Łobiński, P. Zatta, Investigation of the aluminium binding in Al(III)-treated neuroblastoma cells, J. Anal. At. Spectrom. 19 (2004) 41-45
[86] C. Baldwin, J. Cumming, A.T. Timperman, Isolation of naturally occurring aluminium ligands using immobilized metal affinity chromatography for analysis by ESI-MS, Analyst 130 (2005) 318-324

Fractionation of Aluminium by Coupled Separation and Spectrometry Detection Techniques

Peter Matúš
Comenius University in Bratislava, Faculty of Natural Sciences, Geological Institute, Mlynská dolina, 842 15 Bratislava 4, Slovakia, e-mail: matus@fns.uniba.sk

Abstract

The suggested research deals with the fractionation of aluminium in acid soil, sediment, rock and water samples by five different separation procedures (single and sequential extractions, membrane filtration, method based on kinetic strength discrimination and solid phase extraction with utilization of chelating ion-exchangers). The selectivity of these procedures was investigated with the relation to the Al plant availability, i.e. the relationship between the Al concentrations in the individual soil fractions and the Al concentrations in the grass stems samples was studied. The selectivity of obtained results decreases with the increased efficiency of the used methods. The concentration of Al was measured by flame atomic absorption spectrometry (FAAS), UV/VIS spectrophotometry and optical emission spectrometry with inductively coupled plasma (ICP OES).

Key words: *aluminium -- fractionation – separation -- spectrometry*

Introduction

The concentration of some individual Al species is difficult to determine directly. For that reason these species are determined in one or more fractions. Generally, these fractions are defined operationally or functionally, respectively, and they are termed according to the used separation method, e.g. the reactive species fraction, or the function of the isolated analyte, e.g. bioavailable species fraction [1].

The operational and functional nature of the Al fractionation means that it is very hard to compare results obtained by different laboratories using different protocols [1-10]. Therefore, harmonization and standardization are needed to enable wider comparison studies for specific Al fractions. The current drawbacks of proposed procedures are not always adequate selectivity, robustness and potential redistribution and readsorption of analyte during the fractionation. The verification of aluminium fractionation results is influenced by various factors closely connected with the Al speciation in sample. At present no suitable reference materials with certified operationally defined fractions of Al exist. Therefore, only the interlaboratory tests with utilization of harmonized fractionation procedures applied on reference materials and the comparison of experimental results obtained by various analytical methods and their different combinations offer the sufficient evaluation of Al fractionation reliability.

Sometimes the sequential arrangement of several analytical techniques (single extraction, filtration, solid phase extraction) is needed for the efficient fractionation of Al in soils. In the agreement with IUPAC (International Union of Pure and Applied Chemistry) rules it can be designate as sequential fractionation of aluminium. Analogically with sequential extractions this approach allows the separation and quantification of some Al fractions by their physical and chemical properties after consecutive application of various analytical procedures with different selectivity. Ideally, the individual analytical procedures can mutually complete with aim to achieve the operationally defined separation of analyte with requested selectivity [9].

The selectivity of used fractionation procedures was investigated with the relation to the Al plant availability, i.e. the relationship between the Al concentrations in the individual soil fractions and the Al concentrations in the grass stems samples was studied. The concentration of Al was measured by flame atomic absorption spectrometry (FAAS), UV/VIS spectrophotometry and optical emission spectrometry with inductively coupled plasma (ICP OES).

Evaluation of Al fractionation in acid soil, sediment, rock and water samples

The use of single extraction procedures by H_2O, KCl, NH_4Cl, $CaCl_2$, $BaCl_2$, NH_4F agents and diluted acetic acid leaching (first step of optimized BCR three-step sequential extraction) is a simple and selective approach for monitoring of aluminium mobility in acidified systems [5-7,10], see Table 1 and Table 2. The complexing extractants NTA, EDTA and DTPA show the lower selectivity from aspect of aluminium bioavailability of separated fractions [6,7,10]. The reducible agents $(NH_4)_2C_2O_4$ and $Na_2S_2O_4$ extract mainly aluminium forms bounded to oxocompounds [5-7,10]. But the reducible Al fraction separated by $NH_2OH \cdot HCl$ in second step of optimized BCR sequential extraction contains higher Al amount [5-7,10]. In spite of some literature readings [1] the single extractions by $CuCl_2$, $LaCl_3$ a $Na_4P_2O_7$ do not allow the effective separation of Al organic forms in studied samples with low pH values [6,7,10]. The better approach is a direct oxidation of organic matter by H_2O_2 used in third step of optimized BCR three-step sequential extraction [5-7,10]. The dilute HCl extracts mainly Al species connected with anthropogenic contamination of the environment. The single extraction procedures with $(NH_4)_2C_2O_4$ and HCl can supersede the optimized BCR three-step sequential extraction for the separation of mobile or mobilizable Al fraction [5-7,10]. The nonmobilizable Al fraction obtained from fourth step of optimized BCR three-step sequential extraction and decomposed only by hot mixture of strong acids (HF, HNO_3, $HClO_4$) and H_2O_2 contains mostly the primary and secondary aluminosilicate minerals [5-7,10].

Table 1 The experimental conditions of the single extraction procedures

Extracting agent	H_2O	KCl	NH_4Cl	NH_4F	$CaCl_2$	$BaCl_2$	$CuCl_2$	$LaCl_3$	$(NH_4)_2C_2O_4$
Concentration (mol l^{-1})		1	1	0.5	0.01	0.1	0.5	0.3	0.2
Ratio volume/weight	5/1	10/1	10/1	10/1	10/1	10/1	10/1	10/1	20/1
Extraction time (h)	24	1	1	1	2	1	1	1	1

Extracting agent	$Na_2S_2O_4$	$Na_4P_2O_7$	NTA	EDTA	DTPA	HCl	HQN	Salicylic acid
Concentration (mol l^{-1})	0.2	0.1	0.005	0.005	0.005	0.5	0.1-1 %	0.2-2.9 %
Ratio volume/weight	10/1	10/1	10/1	10/1	10/1	20/1	10/1	10/1
Extraction time (h)	1	1	1	1	1	1	1	1

HQN, 8-hydroxyquinoline in 0.2-2 % acetic acid

Table 2 The experimental conditions of the optimized BCR three-step sequential extraction

Step	Fraction	Chemical reagents and analytical conditions
1	Acid extractable	0.11 mol l^{-1} CH_3COOH
2	Reducible	0.5 mol l^{-1} $NH_2OH{\cdot}HCl$ (in 0.05 mol l^{-1} HNO_3)
3	Oxidizable	H_2O_2 followed by 1.0 mol l^{-1} CH_3COONH_4 adjusted to pH 2.0 by HNO_3
4	Residual	HF, HNO_3, $HClO_4$, H_2O_2

The specific fraction precision, repeatability and accuracy of the optimized BCR three-step sequential extraction was tested on sixteen reference materials [5-7,10], see Table 3. The standard deviation (S.D.) and relative standard deviation (R.S.D.) were calculated from four replicates. The precision with the most R.S.D. values less than 5 % except a few cases (R.S.D. from 5 to 10 %) was very good; see Fig. 1 and Fig. 2. The specific fraction repeatability of the used optimized BCR three-step sequential extraction was tested on SRM 2710 (Fig. 1). The optimized BCR three-step sequential extraction was performed five times within two years (from august 2003 to august 2005). The repeatability of the results is acceptable (R.S.D. of the grand mean was less than 4 % for the steps 1 and 4, overall and total concentration). The greater R.S.D. values (< 10 %) were found for the steps 2 and 3, which can be connected with the increasing number of the sample manipulations and the possible analyte readsorption and redistribution [1]. The accuracy of the used sequential extraction valuated for three reference materials (2710, 2711, 483) with the indicative values [11,12] of Al specific fractions is satisfactory (Fig. 2).

Table 3 The reference materials [13] used in this study

Reference material	Description
SRM 2710 (NIST)	Highly contamined soil collected from Montana area
SRM 2711 (NIST)	Moderately contamined agricultural soil collected from Montana area
CRM 483 (BCR)	Sewage sludge amended soil collected from Northampton
CRM 701 (BCR)	Freshwater sediment collected from lake Orta, Piemonte
RSS SO-2 (CANMET)	Ferro-Humic Podzol soil sampled in the Montmorency Forest north of Quebec City
RSS SO-4 (CANMET)	Black Chernozemic soil sampled northeast of Saskatoon, Saskatchewan
CRM SA-B (HPS)	Sandy soil obtained from Johns Island, Charleston County, South Carolina
SRM RTH 912 (WEPAL)	Loess soil from a forest in Switzerland
NMCRM 025-050 (RTC)	Soil from a moderately contamined site located in the Western United States
CRM GBW 07103 (NRCCRM)	Rock
CRM GBW 07304 (NRCCRM)	Stream sediment
CRM GBW 07401 (NRCCRM)	Podzolitic soil
CRM GBW 07405 (NRCCRM)	Yellow-red soil
CRM GBW 07407 (NRCCRM)	Laterite soil
RM Bentonit 1 (SMÚ)	Natural bentonite
RM Zeolit 1 (SMÚ)	Natural zeolite

NIST, National Institute of Standards and Technology
BCR, formerly Community Bureau of Reference, now Institute for Reference Materials and Measurements or Standards, Measurements and Testing Programme
CANMET, Canada Centre for Mineral and Energy Technology
HPS, High-Purity Standards
WEPAL, Wageningen Evaluating Programs for Analytical Laboratories
RTC, Resource Technology Corporation
NRCCRM, National Research Centre for Certified Reference Materials
SMÚ, Slovak Institute of Metrology

The accuracy of the results was also evaluated by the comparison of the total Al concentrations with the sum of four Al individual fractions (the overall Al concentrations). The recovery values of the overall Al concentrations calculated from total (determined by ourselves) and from certified Al total concentrations are in the all cases close to 100 %. As aluminium is strongly bound in the alumino-

silicate lattice, neither the aqua regia extraction protocol according to the ISO Norm 11466 (3HCl + HNO_3 reflux) nor the EPA Method 3050 (HNO_3 and H_2O_2 reflux) used in cited works [11,12] are not suitable for the determination of the residual or total Al concentrations in the samples because of the used reagents (HCl, HNO_3 and H_2O_2) do not completely destroy the alumino-silicate lattices [14].

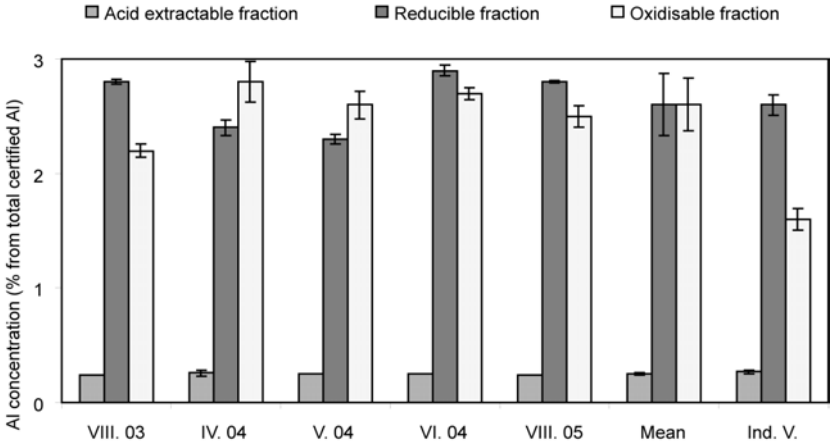


Fig. 1 The optimized BCR three-step sequential extraction fraction specific Al concentrations for testing of method precision, repeatibility and accuracy on SRM 2710 during two years
(Ind. V., indicative values from Sutherland and Tack [11])

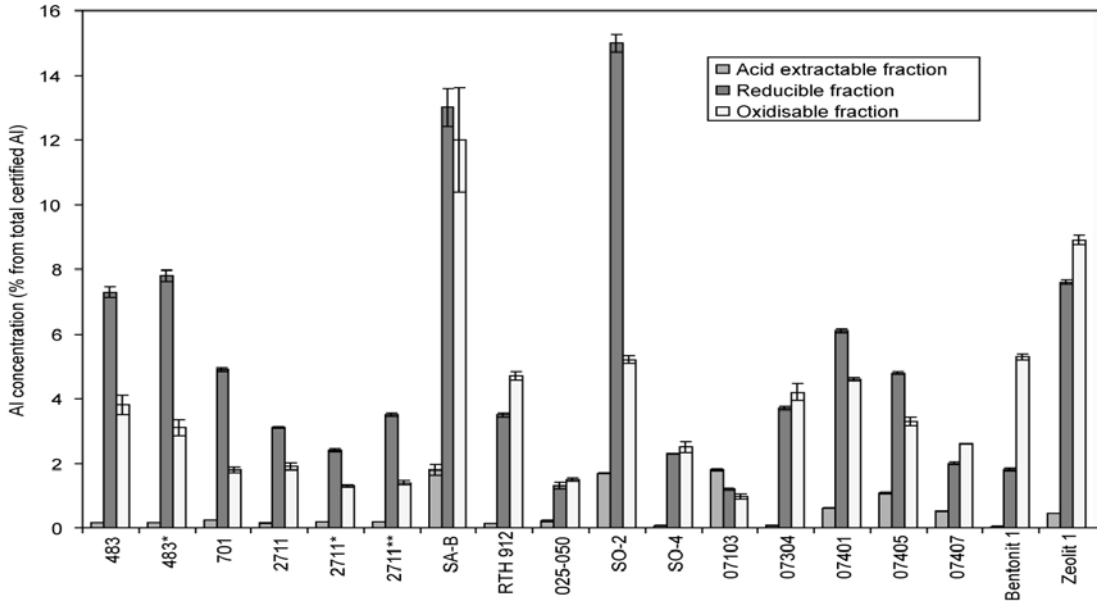


Fig. 2 The optimized BCR three-step sequential extraction fraction specific Al concentrations for testing of method precision and accuracy on the other reference materials
(*, indicative values from Sutherland and Tack [11]; **, indicative values from Larner et al. [12])

The chelating agents 8-hydroxyquinoline and salicylic acid were used in different fractionation protocols (single extraction, solid phase extraction and oxine method based on kinetic strength discrimination). They separate the fraction of nonstabile and reactive Al species, i.e. mainly inorganic monomeric Al, which is responsible for aluminium toxic effects on living organisms [2-10,15].

The single extractions by 8-hydroxyquinoline and salicylic acid were applied to all investigated solid samples [9]. The separation efficiency of 8-hydroxyquinoline decreases and the selectivity of obtained results increases with increased concentration of 8-hydroxyquinoline in solution. Reversibly, in the case of salicylic acid the extraction efficiency increases and the selectivity decreases with increased concentration of salicylic acid functional groups.

The complexation of aluminium on chelating resins Iontosorb Oxin and Iontosorb Salicyl (see Fig. 3) by solid phase extraction also allows the separation of nonstabile and reactive Al species in liquid samples [3-10,15]. For the analysis of solid samples the sequential procedure with preliminary included H_2O single extraction and filtration (under low vacuum in a polysulfone apparatus with a cellulose nitrate membrane filter of nominal pore size 0.40 μm) steps is needed.

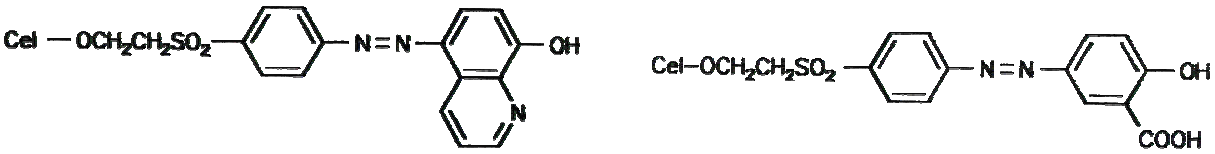


Fig. 3 The functional groups of 8-hydroxyquinoline and salicylic acid covalently bound on cellulose resin Iontosorb Oxin and Salicyl

The oxine method based on kinetic strength discrimination applied on soil samples utilizes the reaction of nonstabile and reactive Al species with 8-hydroxyquinoline solution in diluted acetic acid at pH 5 and their consecutive extraction to methyl isobutyl ketone (MIBK). This procedure in sequential arrangement with H_2O single extraction and membrane filtration (0.40 μm) allows the sufficient selectivity with respect to aluminium bioavailability and toxicity [2,3]. The selectivity of this method is given at first and foremost by the short reaction time (5 s), analogically with the complexation time for solid phase extraction (1-20 min) and extraction time for the single extraction (1 h). From used time intervals and obtained results it is obvious that the oxine kinetic method reaches the best selectivity, which is given by high reactiveness of 8-hydroxyquinoline in H_2O phase. The single extraction by 8-hydroxyquinoline or salicylic acid solutions and the solid phase extraction with 8-hydroxyquinoline or salicylic acid fixed on resins are probably less selective because of slower kinetics of reaction between nonstabile and reactive Al species and agent functional ligands. However, the rate of this reaction between liquid and solid phases is influenced by many different sorption and diffusion effects.

Under the certain conditions of aluminium fractionation in solid samples the single extraction with 8-hydroxyquinoline or salicylic acid solutions can supersede both the solid phase extraction and the oxine kinetic method procedures which require the application of extraction and filtration steps for the obtaining of soluble Al fraction. In the case of filtered liquid samples it is favourable to use the solid phase extraction or the oxine kinetic method. The listed separation procedures can be also applied in the field with utilization of given extractant or resin filled plastic syringes with the possibility of sample membrane filtration (0.40 μm) [9].

Conclusion

All used methods for fractionation of aluminium are relatively simple, rapid and without the need of high-cost instrumentation. These properties predetermine proposed procedures for the routine monitoring of mobility, bioavailability and toxicity of aluminium in the environment. The current drawbacks of listed procedures are not always adequate selectivity, robustness and potential redistribution and readsorption of analyte during the fractionation. The verification of aluminium fractionation results is influenced by various factors closely connected with the Al speciation in sample. At present no suitable reference materials with certified operationally and/or functionally defined fractions of aluminium exist. The interlaboratory tests with utilization of harmonized fractionation procedures applied on reference materials and the comparison of experimental results obtained by various analytical methods and their different combinations are often used for the evaluation of fractionation reliability. Both approaches were used in this study.

Acknowledgements

The work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-010204 and Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences under the contracts Nos. VEGA 1/2439/05 and VEGA 1/3561/06.

References

[1] J. Kubová, Utilization of selective extraction techniques for element speciation in soils and sediments – Critical evaluation of the contemporary state, Slovak Geol. Mag. 6 (2000) 43-52
[2] P. Dlapa, A. Kopcová, J. Kubová, Influence of reactive aluminium species on soil-vegetation relationship, Phytopedon (Bratislava) 1 (2002) 86-93
[3] P. Dlapa, J. Kubová, P. Matúš, V. Streško, Chemical and physical aluminium speciation in soil solutions, Fresenius Environ. Bull. 11 (2002) 626-630
[4] P. Matúš, J. Kubová, V. Streško, Utilization of chelating ion exchange for aluminium speciation, Chem. Pap. – Chem. Zvesti 57 (2003) 176-178
[5] P. Matúš, J. Kubová, M. Bujdoš, V. Streško, J. Medveď, Chemical partitioning of aluminium in rocks, soils, and sediments acidified by mining activity, Anal. Bioanal. Chem. 379 (2004) 96-103
[6] P. Matúš, J. Kubová, M. Bujdoš, J. Medveď, Determination of operationally defined fractions of aluminium in reference materials and acid attacked environmental samples, Anal. Chim. Acta 540 (2005) 33-43
[7] J. Kubová, P. Matúš, M. Bujdoš, J. Medveď, Influence of acid mining activity on release of aluminium to the environment, Anal. Chim. Acta, 547 (2005) 119-125
[8] P. Matúš, J. Kubová, Complexation of labile aluminium species by chelating resins Iontosorb - a new method for Al environmental risk assessment, J. Inorg. Biochem. 99 (2005) 1769-1778
[9] P. Matúš, J. Kubová, Complexation efficiency of differently fixed 8-hydroxyquinoline and salicylic acid ligand groups for labile aluminium species determination in soils- comparison of two methods, Anal. Chim. Acta 573-574 (2006) 474-481
[10] P. Matúš, J. Kubová, M. Bujdoš, J. Medveď, Free aluminium extraction from various reference materials and acid soils with relation to plant availability, Talanta, in press
[11] R.A. Sutherland, F.M.G. Tack, Determination of Al, Cu, Fe, Mn, Pb and Zn in certified reference materials using the optimized BCR sequential extraction procedure, Anal. Chim. Acta 454 (2002) 249-257
[12] B.L. Larner, A.J. Seen, A.T. Townsend, Comparative study of optimised BCR sequential extraction scheme and acid leaching of elements in the certified reference material NIST 2711, Anal. Chim. Acta 556 (2006) 444-449
[13] J. Medveď, V. Streško, J. Kubová, Reference materials in geology and geochemistry, Mineralia Slovaca 27 (1995) 278-282
[14] J. Medveď, V. Streško, J. Kubová, J. Polakovičová, Efficiency of decomposition procedures for the determination of some elements in soils by atomic spectroscopic methods, Fresenius J. Anal. Chem. 360 (1998) 219-224
[15] J. Polakovič, J. Polakovičová, J. Kubová, Application of thermodynamics in a specific force field to the adsorption of Al^{3+} ions on the chelating resins Ostsorb Oxin, Ostsorb Salicyl, Spheron Oxin, and Spheron Salicyl - Mathematical analysis of sorption isotherms using nonlinear mean square software, Anal. Chim. Acta 488 (2003) 123-132

Fractionation and Determination of Thallium in Soil Samples by ETAAS

Ján Medveď, Milan Kališ, Peter Matúš, Marek Bujdoš, Jana Kubová
Comenius University in Bratislava, Fuculty of Natural Sciences, Geological Institute, Mlynská dolina G, 842 15 Bratislava 4, Slovak Republic

Abstract

Electrothermal atomic absorption spectrometry was used to the determination of extractable thallium in soils following the application of the modified BCR three steps sequential extraction scheme. The potential interferences from cations and anions commonly present in soil solutions and reagents used for leaching were observed. Optimal temperature conditions for atomisation of thallium from aqueous and matrix-matched standards with extractants were selected. The utilization of $Pd(NO_3)_2$ + ascorbic acid and $Pd(NO_3)_2$ + $Mg(NO_3)_2$ as a matrix modifier were investigated and compared. The precision and accuracy of thallium determination by the described method for soils was acceptable. The limit of detection of thallium for the used method was around 50 $\mu g.kg^{-1}$. The proposed analytical procedure was applied for the determination of thallium in soils from area polluted by mining activity.

Key words: *thallium, ETAAS, soils, modified BCR sequential extraction scheme*

1. Introduction

Thallium is a heavy metallic element, which occurs in earth's crust in an estimated abundance from 0.1 to 0.5 $mg.kg^{-1}$ and in the environment is mainly combined with other elements (primarily oxygen, sulphur, halogens, K and Rb) in inorganic compounds. Thallium is quite stable in the environment, since it is neither transformed nor biodegraded. Compounds of thallium are generally soluble in water and the element is found primarily as the monovalent ion (Tl^+). Thallium tends to be sorbed to soils and sediments [1] and to bioconcentrate in aquatic plants, in vertebrates, and fish [2]. Terrestrial plants can also absorb thallium from soil [3]. The main sources of pollution nowadays come from antropogenic emissions from refineries, coal-fired power stations, mining activities, metal smelters and the cement industry [4]. Humans may be exposed to thallium by ingestion, inhalation, or dermal absorption. However, the general population is exposed most frequently by ingestion of thallium-containing foods, especially home-grown fruits and green vegetables. Toxicity of thallium can be compared with cadmium and mercury as far as the soil contents, i.e. a potential risk for humans can arise at levels around 1 $mg.kg^{-1}$ [5]. The lethal dose for humans is approximately 15-20 mg/kg [6]. Several methods have been proposed for determination of thallium in environmental and biological samples, such as anodic stripping voltammetry [7], spectrophotometry [8], spectrofluorimetry [9], X-ray fluorescence spectrometry [10], electrothermal, hydride generation and flame atomic absorption spectrometry [11-13] and inductively coupled plasma mass spectrometry [14]. Knowledge of the chemical forms of potentially toxic metals binding in environmental solid samples (e.g. soil, sediment)

is very important issue in the monitoring of environmental pollution. Although the total metal concentration in such samples may provide relevant information about the degree of contamination, it has been recognised that knowledge of the metal fraction associated with significant phases is essential requirement for assessing the mobility and bioavailability of the metal, which is directly related to toxicological effects. In order to discover the distribution patterns of trace metals among specific solid phases sequential extraction schemes (SES) with 3 – 6 stages are commonly used [15-17].

The scope of the presented work was the optimization of work conditions of electrothermal atomic absorption spectrometry method (ETAAS) for the determination of thallium in soils and soil extracts after using modified BCR tree-step sequential extraction scheme (SES) [16], verification of reliability of obtained analytical results by analysis of certified reference materials and application of optimized analytical procedure for the determination of thallium in acid attacked soil samples and soil extracts from polluted area in the Banská Štiavnica – Šobov (Slovakia).

2. Experimental

Instrumentation

A Perkin-Elmer (Überlingen, Germany) Model Zeeman 3030 atomic absorption spectrometer equipped with a HGA 600 graphite furnace and an AS-60 autosampler was used. The instrumental settings and the heating programmes employed for Tl determination using pyrolytically coated graphite tubes with integral platforms are shown in Table 1. Integrated absorbance was measured. All measurements were made with at least four replicates.

Horizontal laboratory shaker LT 2 Kavalier (Czech Republic) was used for the extraction of soil samples.

A centrifuge type MPW – 360 (Poland) was used for separation of the solid residue from the extraction liquid after each stage of the BCR SES.

Table 1. Optimal instrumental and working parameters for thallium ETAAS determination

Spectrometer					
Wavelength				276.8 nm	
Bandwidth				0.7 nm	
Lamp				Perkin-Elmer EDL system	
Lamp power				7W	
Sample volume				20 μL	
Modifier volume I. (Pd solution – 1000 mg L^{-1} Pd)				8 μL (8 μg mass of Pd)	
Modifier volume II. (Ascorbic acid - 1% solution)				5 μL (50 μg mass of ascorbic acid)	
Temperature programme					
Step	**Temperat. /°C**	**Rmap/s**	**Hold/s**	**Ar flow rate/mL min^{-1}**	**Read**
Drying	90	10	20	250	
Drying	120	10	20	250	
Pyrolysis	900	20	10	250	
Atomization	1800	0	5	0	**On**
Cleaning	2400	1	3	250	

Reagents

All employed reagents were of the analytical grade (HNO_3 was purified by the sub-boiling distillation in quartz apparatus). Standard Tl solutions: Standards were prepared by dilution of 1000 mg L^{-1} Tl stock solution (Merck, Darmstadt, Germany) with redistilled water to a required concentration with the HNO_3 addition (3 mL conc. HNO_3 in 100 mL solution), and with the same reagents, which were used for extraction. The calibration was checked periodically after every 25 measurements with a 30 μg L^{-1} Tl solution.

Matrix modifier solutions*:* 1000 mg L^{-1} Pd, [Pd in 1% HNO_3] (Analytika, Czech Republic); 1000 mg L^{-1} $Mg(NO_3)_2$, [$Mg(NO_3)_2.6H_2O$ in H_2O] (Analytika, Czech Republic); 10.0 g L^{-1} ascorbic acid in H_2O (Analytika, Czech Republic). The mixed palladium nitrate and magnesium nitrate modifier contained 1000 mg L^{-1} Pd and 600 mg L^{-1} $Mg(NO_3)_2$ [17-19]. Working Pd solution was 1000 mg L^{-1} Pd and 1% ascorbic acid solution was used [16, 17]. Extraction reagents: acetic acid (Merck), hydroxylamine hydrochloride (Merck), ammonium acetate (Merck), and 33% v/v hydrogen peroxide (Merck). Residue decomposition reagents*:* 65% m/m HNO_3 (Merck), 48% m/m HF (Merck), 70% m/m $HClO_4$ (Merck) and 33% v/v H_2O_2 (Merck).

Certified reference materials (CRM): NIST SRM 2710 and NIST SRM 2711 (Montana Soils) from the National Institute of Standards and Technology (USA), CRM GBW 07103, and CRM GBW 07405 (Soils) from the National Research Center for Certified Reference Materials (China), CRM SA-B (Sandy Soil) from High-Purity Standards Cherleston (USA

Determination of the total and residual thallium concentrations

The total and residual thallium concentrations in the certified reference materials and samples were determined after decomposition by the HF, HNO_3, $HClO_4$ and H_2O_2 mixture in the open system [20].

Sequential extraction scheme

Operating conditions used in the modified BCR tree-step SES were described in detail elsewhere [16] (Table 2) and were applied to five certified reference materials and samples. All the selected CRMs are only certified for total Tl content, but can be useful for assessing the Tl extractability. The solid residue after third step of this SES was decomposed by mixture of HF, HNO_3, $HClO_4$ and H_2O_2 in the open system [20].

Tab. 2. Reagents and working conditions applied for the modified BCR three-step SES proposed by the Rauret et al. [16]

Step	Fraction	Reagent	Treatment
1	Acid soluble (e.g. carbonates)	20 mL CH_3COOH 0.11 mol L^{-1}	Shaking for 16 h at 22±5 ^oC
2	Reducible (e.g. Fe-Mn oxides)	20 mL NH_2OH-HCl 0.5 mol L^{-1} (in 0.05 mol L^{-1} HNO_3)	Shaking for 16 h at 22±5 ^oC
3	Oxidisable (e.g.organic matter)	5 mL H_2O_2 8.8 mol L^{-1} 5 mL H_2O_2 8.8 mol L^{-1} 25 mL CH_3COONH_4 1.0 mol L^{-1} adjusted to pH 2.0 by HNO_3	Manual shaking for 1h at 25 ^oC Continue the digestion for 1 h at 85±2 ^oC Shaking for 16 h at 22±5 ^oC
Residual		HF, HNO_3, $HClO_4$, H_2O_2	[20]

3. Results and discussion

Selection of matrix modifier and optimal thermal programme

The optimal experimental ETAAS conditions were selected by using only a reducible hydroxylamine hydrochloride fraction of the modified BCR tree-step SES spiked with Tl solution (Tl concentration was 100 ng ml^{-1}). The pyrolysis and atomization temperatures for Tl determination was optimized in the absence and in the presence of a 10 μL Pd + $Mg(NO_3)_2$ [0.05% m/v Pd + 0.03% m/v $Mg(NO_3)_2$], and 8 μL Pd (8 μg mass) + 5 μL ascorbic acid (50 μg mass) as matrix modifiers. Integrated absorbance was used for signal quantification. The pyrolysis and atomization curves for Tl in prepared solution are presented in Fig. 1. From these curves the optimal drying, pyrolysis and atomization temperatures and the choice of suitable modifier Pd + ascorbic acid was determined for Tl. The optimal ETAAS temperature programme is summarized in Table 1. The thermal conditions and selection of suitable matrix modifier for determination of Tl in other fractions of the modified BCR SES and for determination of the total Tl content in the residual fraction were intensively studied by Villar et al. [17].

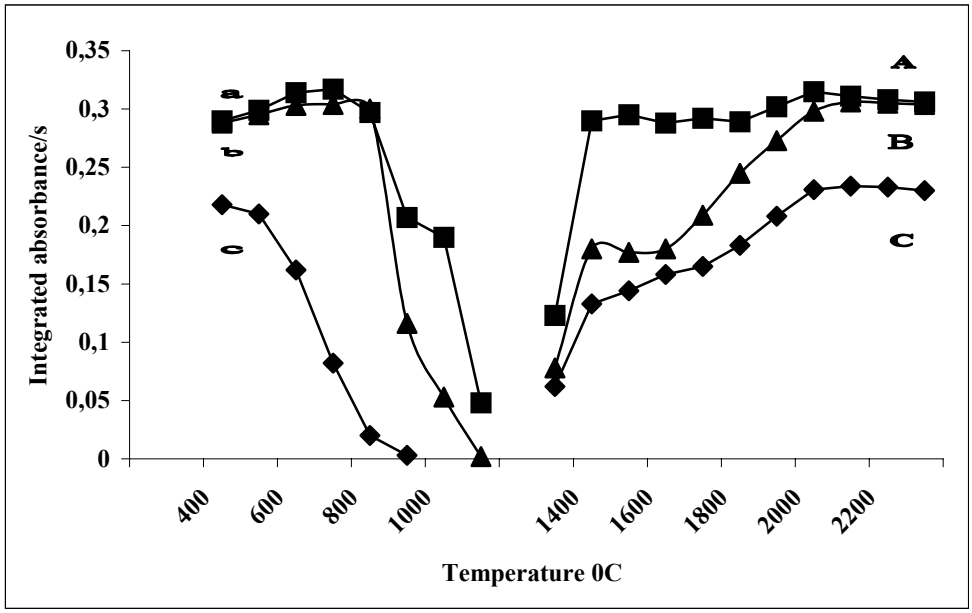


Figure 1. Pyrolysis (a, b, c) and atomization (A, B, C) curves for Tl in spiked hydroxylamine hydrochloride extractant, corresponding to the optimized BCR tree-step SES. Spiked extracts containing a 100 ng mL^{-1} Tl; a, A - with modifier Pd + ascorbic acid ; b, B – with modifier Pd + $Mg(NO_3)_2$ and c, C - without modifier

Accuracy, precision limit and of determination

The accuracy of analytical results for Tl in soils was checked by the analysis of total Tl concentrations in soil CRMs NIST 2710, NIST 2711, GBW 07103, GBW 07405 and HPS SA-B (Tab. 3). The obtained Tl contents were in good agreement with the certified values. The calibration curve was linear at least up to 100 ng mL^{-1}. The results are summarized in Tab. 3 and they show that the Tl values with selected working conditions are not affected by macro-components present in the various soil CRM samples.

The limit of detection (LOD) based on 3 σ definition for the used method was around 0.050 $mg.kg^{-1}$, and the limit of determination (LOQ) based on 10 σ definition was 0.15 mg kg^{-1} Tl. Precision of studied elements determination expressed by relative standard deviation (RSD) varied in a range from 2.5 % to 17.3 %. The standard additions technique was needed for accurate quantification of Tl in the all fractions.

Table 3. Analytical results (mg.kg^{-1}) for thallium following application of modified BCR SES to soils CRMs

Soil CRMs	Certified value	Found total value	Amount extracted (mg kg^{-1})			Rezidue IV	Σ I-IV	Recovery[c] (%)
			Step I	Step II	Step III			
NIST 2710	0.63	0.60±0.06[a]	< LOQ[b]	< LOQ	LOQ	0.59±0.05	0.59	93.7
NIST 2711	2.47±0.40	2.40±0.06	< LOQ	0.19±0.02	20±0.03	2.02 ±0.35	2.41	97.7
GBW 07103	1.93±0.26	1.89±0.05	16±0.02	< LOQ	< LOQ	1.69±0.20	1.69	95.8
GBW 07405	1.60±0.40	1.50±0.12	< LOQ	0.33±0.03	< LOQ	1.20±0.07	1.53	95.5
HPS SA-B	58.0±2.00	58.16±2.2	32.5±1.8	22.8±3.2	1.96±0.26	0.42±0.05	57.7	99.5

[a]Average value ± standard deviation (n = 4)
[b]Tl content is below the LOQ ($LOQ = x_{b1} + 10s_{b1}$ where x_{b1} is the mean of the blank measurements and s_{b1} is the standard deviation of the blank measurements).
[c]Recovery was calculated as the following ratio: (Σ I – IV value / certified value) x 100.

Application of the modified BCR SES on the real soil samples

The fractional Tl in real weathered earth and soil samples collected in the Šobov region (open quartzite mine, mine dump, the meadows below the mine dump) affected by the acification (a product of pyrite oxidation) was determined by using the modified BCR SES and developed ETAAS method. The composition of the soil samples is presented in Fig. 2. According to the extraction results shown (Fig. 2), a low mobility is to be expected for Tl from studied soil samples.

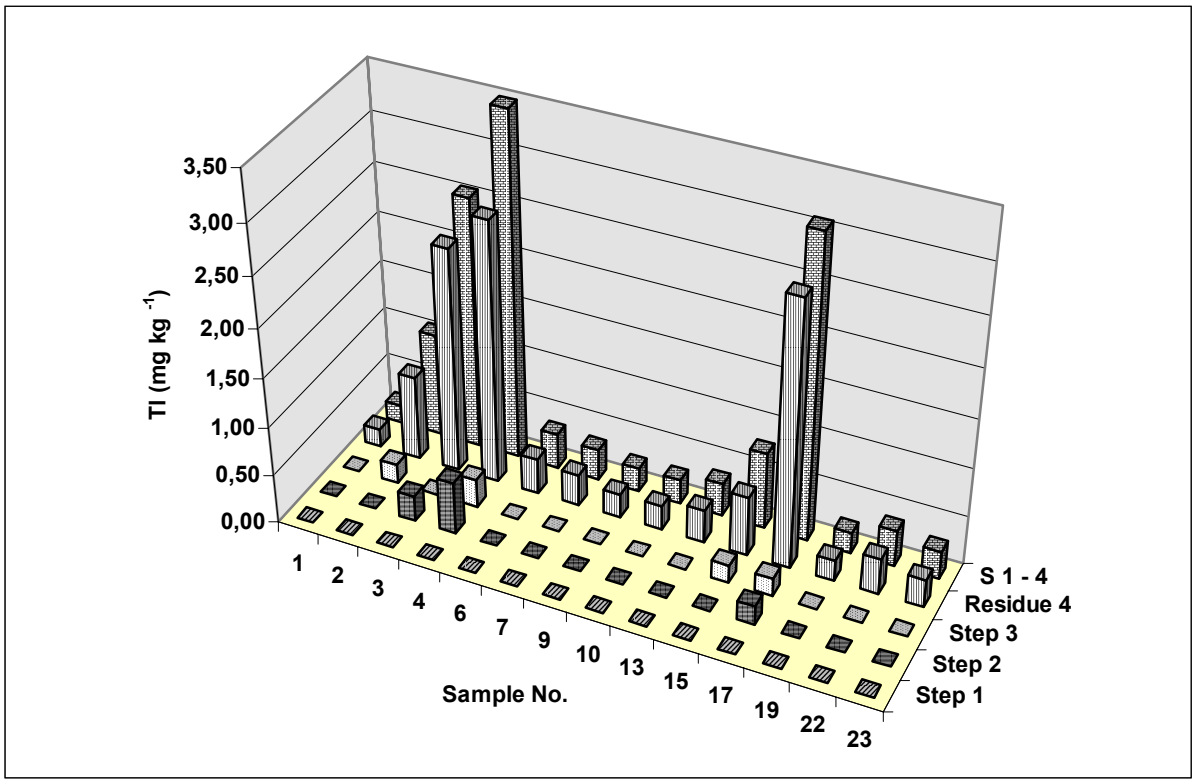


Fig. 2. Concentrations of fractional thallium in real samples obtained by the optimized BCR tree-step SES

4. Conclusion

An ETAAS method for determination of fractional Tl according of modified BCR SES to soil samples was studied and optimized. The strong interferences for soils mainly in hydroxylamine hydrochloride and acid extracts (reducible step II of SES and residue) by application of 8 μg Pd + 50 μg ascorbic acid as matrix modifier, Zeeman background correction, pyrolytically coated graphite tubes with integral platforms, standard additions technique and matrix-matched calibration standards with extractant solutions were eliminated. The precision and accuracy of fractional thallium determination by the described method for soils was acceptable. The limit of detection for the proposed method was around 0.050 mg kg^{-1} Tl. The developed method was applied for fractional thallium determination in weathered earth and soils affected by the acidification from open quartzite mine in Šobov region (middle Slovakia).

Acknowledgements

This work was financially supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences VEGA No. 1/3555/06 and UK Grant No. 132.

References

[1] Frantz, G., Carlson, R.M.: Division S-2-soil chemistry effects of rubidium, caesium and thallium on interlayer potassium release from transual vermiculite. Soil Science Society of America Journal, 1987, 51, 305-308
[2] Barrows, M.E.: Biocencentration and elimination of selected water pollutants by bluegill sunfish. In: Haguer, ed.: Dynamics, exposure and hazard assessment of toxic chemicals. Ann Arbor, Ann Arbor Publishers, 1978, 379-392
[3] Ewers, U.: Environmental exposure of thallium. Sci. Total Environ.,1988, 71, 285-292
[4] Kemper, F.H., Bertram, H.P.: In Metals and their Compounds in the environment, (ed. E. Merian), VCH, Weinheim, 1991
[5] Merian, E. (ed.): Metals and their compounds in the environment. Occurrence, analysis and biological relevance. VCH Weinheim, 1991
[6] Boyarsky I., Crisan A.D.: Toxicity, Thallium, Medicine, Department of Emergency Medicine, Magee-Women's Hospital of the University of Pittsburgh Medical Center, Pittsburgh 2006
[7] Lukaszewski, Z., Karbowska, B., Zembrzuski, W.: Determination of mobile thallium in soil by flow injection differential pulse anodic stripping voltammetry. Electropanalysis, 2003, 15, 480-483
[8] Deb, M.K., Agnihotri, P.K., Thakur, M., Mishra, R.K.: Fractionation and spectrophotometric determination of thallium with N,N´-diphenylbenzamidine, brilliant green and cetylpyridinium chloride. Chemical Speciation and Bioavailability, 1998, 10, 53-60
[9] Ensafi, A.A., Rezaei, B.: Speciation of thallium by flow injection analysis with spectrofluorimetric detection. Microchemical journal, 1998, 60, 75-83
[10] Adriano, D.C. Trace Elements in Terrestrial Environments – Biochemistry, Bioavailability and Risk of Metals (2nd ed.), Springer, New York, 2001
[11] Cvetkovic, J., Arpadjan, S., Karadjova, I., Stafilov, T.: Determination of thallium in wine by electrothermal atomic absorption spectrometry after extraction preconcentration. Spectrochim. Acta Part B, 2002, 57, 1101-1106
[12] Zhu, D., Xu, S.K.: Enhancement of thallium response by flow injection hydride generation AAS using palladium and rhodamine B. Atomic Spectroscopy, 2000, 21, 136-142
[13] Lin, T.S., Nriagu, J.O.: Thallium speciation in river waters with Chelex-100 resin. Anal. Chim. Acta, 1999, 395, 301-307

[14] Carlsson, S., Bäckström, M., Lifvergren, T.: Distribution of thallium in a contaminated lake. ICP Information Newsletter, 2003, 28, 556-557
[15] Sager, M.: Speciation of Thallium in River Sediments by Consecutive Leaching Techniques. Microchim. Acta, 1992, 106, 241-251
[16] Rauret, G., López-Sánchez, J.-F., Sahuquillo, A. et al.: Application of a modified BCR sequential extraction (three-step) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil reference material (CRM 438), complemented by a three-year stability study of acetic acid and EDTA extractable metal content. J. Environ. Monit., 2000, 2, 228-233
[17] Villar, M., Alava, F., Lavilla, I., Bendicho, C.: Operational speciation of thallium in environmental solid samples by electrothermal atomic absorption spectrometry according to the BCR sequential extraction scheme .J. Anal. At. Spectrom., 2001, 16, 1424-1428
[18] Welz, B., Schlemmer, G., Mudakavi, J.R.: Palladium nitrate-manganesium nitrate modifier for electrothermal atomic absorption spectrometry, Part 2: Determination of arsenic, cadmium, copper, manganese, lead, antimony, selenium and thallium in water, J. Anal. At. Spectrom. 1988, 3, 695-701
[19] Tsalev, D.L., Slaveykova, V.I., Mandjukov, P.B.: Chemical modification in graphite-furnace atomic absorption spectrometry, Spectrochim. Acta Rev. 1990, 13, 225-274
[20] Krakovská, E., Kuss, H.M.: Decompositions in analytical chemistry – recent state and trends (in Slovak), Vienala, Košice, 2001

Determination of Lanthanides in Environmental Samples by Source Excited EDXRF Method

Visnja Orescanin, Luka Mikelic, Vibor Roje, Stipe Lulic
Rudjer Boskovic Institute, Department for Marine and Environmental Research, Bijenicka cesta 54, 10002 Zagreb, Croatia (vobescan@irb.hr)

Abstract

A new analytical procedure for determination of lanthanides in environmental samples after chemical separation from major matrix elements on DOWEX 50W-X8 and TRU (EiChrom) resins followed by preconcentration with chelating agent ammonium pirrolidine dithiocarbamate (APDC) and analyses of thin targets by energy dispersive X-ray fluorescence (EDXRF) method using ^{109}Cd as the source of excitation was presented. Characteristic Lα X-ray lines of the lanthanides were used for calculations of the net peak area and mass concentrations. The influence of pH value of the solution and addition of organic matter on the complexation was investigated. Percentages of recovery of each lanthanide after separation on resins were also determined.

Keywords: *Lanthanides-- Preconcentration--APDC—EDXRF*

Introduction

Lanthanides are used in industry due to their metallurgical, optical and electronic properties. Also, they are constantly being used in the nuclear power and nuclear weapon industries for both practical and experimental purpose.

Many analytical techniques are used for determination of rare earth elements in various sample matrices. Among them the most common are: inductively coupled plasma mass spectrometry (ICP-MS) [5,7,9,10,12], instrumental neutron activation analysis (INAA) and liquid chromatography (LC) [8].

Only few papers presented analysis of lanthanides using energy dispersive X-ray fluorescence method (EDXRF) [4]. Overlapping of the characteristic L X-ray lines of the lanthanides present in the environmental samples in relatively low concentrations with respective K X-ray lines of more abundant elements like Ti, V, Cr, Mn, Fe represents the major problem in determination of lanthanides with tube excited EDXRF method.

In order to avoid interferences of major elements, different methods have been developed to separate matrix elements from lanthanides [1-3,6,8,11-14].

This paper deals with the development of analytical procedure for determination of lanthanides in iron rich matrices after chemical separation from the major matrix elements on DOWEX 50W-X8

resin [4] and TRU spec. resin (EiChrom) followed by preconcentration of lanthanides with chelating agent ammonium pirrolidine dithiocarbamate (APDC) and analyses by EDXRF method as thin targets.

Experimental

All solutions were prepared using analytical reagents grade chemicals and distillated Milli-Q water. Heavy metals and lanthanides stock solutions (concentration of each element 1 mg/L) were prepared from MERCK 1000 mg/L standard solutions of each element (NH_4VO_3, $Mn(NO_3)_2$, $Fe(NO_3)_3$, $Ni(NO_3)_2$, $Zn(NO_3)_2$, $La(NO_3)_3$, $Ce(NO_3)_3$, Pr_2O_3, Nd_2O_3, Sm_2O_3, Gd_2O_3 and Dy_2O_3 in 0.5M HNO_3). APDC solution was prepared daily by dissolving APDC (Aldrich) in distilled water to produce 1% (w/v) solution. To separate heavy metals from lanthanides DOWEX 50W-X8 and EiChrom TRU extraction (100 – 150 μm) resins were used.

100 mL of solution containing 1 mg/L of each lanthanide (La, Ce, Pr, Nd, Sm, Gd, Dy) was adjusted to pH values 3-11 by the addition of hydrochloric acid and ammonium hydroxide in order to estimate the effect of pH on the recovery of all lanthanides. All pH measurements were made with a Mettler Toledo digital pH meter. After the pH adjustment 2 mL of 1% (w/v) APDC was added into each flask. After the complexation lasted for 20 min, the suspension was filtered through a Millipore HAWP filter (pore size-0.45 μm; diameter-25 mm). A Millipore micro filtration system was used for that purpose. Prepared thin targets were air dried, protected by thin mylar foil (2 μm), inserted into a plastic carrier and placed 0.5 mm above the X-ray source of the X-ray spectrometer.

A batch equilibrium method was employed for the determination of distribution coefficients of V^{5+}, Mn^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+} and Dy^{3+} on TRU resin. The effect of different concentrations nitric acids and distilled water was studied using the following procedure. A 200 mg of the resin was suspended in 20 mL of 200 μg/L heavy metals and lanthanides solution at different concentrations of HNO_3 (10M, 6M, 4M, 1M) and distilled water, and was equilibrated for 24 h with constant shaking. After equilibration 1 mL of the solution from each flask was analyzed for the metal content by ICPMS. The measurements were carried out by high resolution inductively coupled plasma mass spectrometer Element 2 (Thermo Finnigan, Bremen, Germany). An autosampler (ASX 510, Cetac Technologies, USA) and sample introduction kit consisting of a conical nebulizer (Thermo Finnigan, Bremen, Germany) and quartz spray chamber (Thermo Finnigan, Bremen, Germany) were employed to transport the analyzed solutions into plasma. Before the analyses, the measurement conditions of the HR-ICP-MS were optimized using a multielemental solution at a concentration of 1 μg/L.

It has been done external calibration with the series of the multielemental standard solutions (0, 1, and 10 μg L^{-1}) for the transition metals as well as for the rare earth elements. The internal standard was In (1 μg L^{-1})

The distribution coefficients, K_d, were calculated using this formula:

$$K_d = \left(\frac{c_0 - c_s}{c_s} \right) \cdot \left(\frac{V_s}{m} \right)$$

where $c_0 - c$ is concentrations of cations absorbed on the known weight of the resin, m, and c_s is concentration in known volume, V_s, of the solution.

Lanthanides separation from heavy metals using DOWEX 50W-X8 resin was performed according to the procedure described in Djingova and Ivanova [4]. An eluations behavior of selected heavy metals and lanthanides on the TRU resin was investigated by filling the column (φ = 1 cm, h = 30 cm) with 2 g TRU resin. For that purpose 20 mL of 10M HNO_3 sample stock solution containing 1 mg/L of all elements was passed through the column with TRU resin (50 – 100 μm) pre-equilibrated with 10M HNO_3. V^{5+} and Ni^{2+} were eluted with 20 mL of 6M HNO_3. Zn^{2+} was eluated with 20 mL of 4M HNO_3 and Fe^{3+} with 20 mL of 1M HNO_3. The lanthanides were eluted with 100 mL H_2O.

Obtained solution of metals in each fraction from both resins were preconcetrated with 2mL of APDC solution (1w/v) at pH 3 and lanthanides fraction at pH 8. After the complexation lasted for 20 min thin targets are prepared as is described above. The same procedure was repeated six times and the results were expressed as mean values of these six measurements. Obtained mean values were divided with initial concentrations and multiplied with 100 in order to calculate the percentage of the recovery of each lanthanide.

All targets were analyzed by energy dispersive X-ray fluorescence (EDXRF). Samples were irradiated by X-rays generated from the ^{109}Cd annular source. The incident angle was 49.76°. Detection of characteristic X-ray radiation from the sample was conducted with a Si(Li) detector (Canberra) cooled with liquid nitrogen with the following characteristics: detector size =30 mm^2, Si thickness = 3 mm. Be window = 25 μm, FWHM for 5.9 keV ^{55}Fe 165 eV. The emerging angle was 74.05° and the distance was 1.5 cm. Spectra were collected by Genie – 2000 software (Canberra, Meriden, CT USA). Spectral data were analyzed by WinAxil software version 4.5.2 (Canberra Eurisys Benelux, Belgium) using characteristic L_α lines of the elements. In order to obtain a good counting statistic, collecting time for all targets was 10 000s. Calibration file (model) for the quantitative analyses was created on the basis of the measurements of the standard solution (Merck) containing 1 mg/L of La, Ce, Pr, Nd, Sm, Gd, Dy preconcentrated with APDC at pH=8 which was found as an optimum pH value for the chelation of the majority of the tested lanthanides. Quantitative analysis was done by the "Compared method" from the WinFund package, version 4.5.2 (Canberra Eurisys Benelux, Belgium).

Results and discussion

The effect of pH's between 3 and 11 on the relative recovery of each lanthanide was presented in Table 1. Relative recovery was calculated by dividing the net peak area (N) of characteristic L_α X-ray lines obtained for each lanthanide in each target with WinAxil software by maximum net peak area (N_0), which was obtained from the target prepared at pH 8.

Table 1. The effect of pH between 3 and 11 on the relative recovery of each lanthanide

Recovery percentage (%)							
pH	La	Ce	Pr	Nd	Sm	Gd	Dy
3	13	15	5	1	0	1	1
4	19	18	23	21	17	20	3
5	7	25	34	34	38	40	26
6	0	21	37	39	44	49	49
7	8	19	34	36	45	46	53
8	45	100	100	100	100	100	100
9	100	91	95	88	79	81	77
10	88	98	86	84	73	75	73
11	70	83	82	77	70	75	74

The elements Pr, Nd, Sm, Gd and Dy showed very similar recovery patterns with more or less linear increase from pH 3, with no or minimum recovery (app. 1%), to pH 6 (with app. 40% relative recovery). At pH 7 complexation of all mentioned elements with APDC decreased significantly while the optimum results were obtained at pH=8 and decreased slightly toward pH 11. La and Ce showed more irregular patterns compared to other five elements. Obtained recovery for La was less than 20% at pH=3 and 4 and slightly decreased to 0 at pH=7, reaching maximum value at pH=9 and slightly decreased to 70% at pH=11.

Distribution coefficients (K_d) of the TRU resin with heavy metals and lanthanides were determined at various concentrations of nitric acid (Table 2). Results showed that lanthanides were strongly bound to the resin in the nitric acid with concentrations from 1M to 10 M while heavy metals only in the solutions with higher acid concentrations. Neither heavy metals nor lanthanides are bounded onto the resin in pure aqua solution.

Table 2. Distribution coefficients (K_d) of heavy metals and lanthanides for TRU resin in H_2O an various concentrations of HNO_3

	V	Cr	Mn	Fe	Ni	Zn	La	Ce	Pr	Nd	Sm	Gd	Dy
TRU(H2O)	24.8	5.9	8.8	17.8	11.3	23.15	27.7	27.0	24.5	24.4	22.3	16.1	22.1
TRU(0.1M)	22.0	7.6	10.0	18.7	13.0	35.6	38.6	40.1	40.0	36.9	35.4	30.8	35.6
TRU(1M)	21.3	11.8	19.3	22.1	17.7	90.9	690.1	885.4	907.7	872.5	751.6	642.7	454.4
TRU(2M)	28.5	15.0	22.5	23.5	26.7	117.2	732.8	940.5	954.0	896.1	790.2	710.5	513.1
TRU(4M)	43.0	23.7	22.0	43.6	36.0	119.5	686.2	862.0	965.3	900.3	799.3	711.5	610.4
TRU(6M)	86.3	56.1	62.9	52.3	74.8	231.8	746.6	896.3	972.3	901.2	967.5	780.3	902.3
TRU(10M)	172.3	56.6	68.8	100.5	78.5	243.5	756.5	900.2	978.3	905.3	970.2	792.3	1210.7

Figure 1 presents the percentage of the recovery of lanthanides after separation on DOWEX 50W-X8 and TRU resins, preconcentration with APDC at pH 8 and measurement of thin targets by EDXRF.

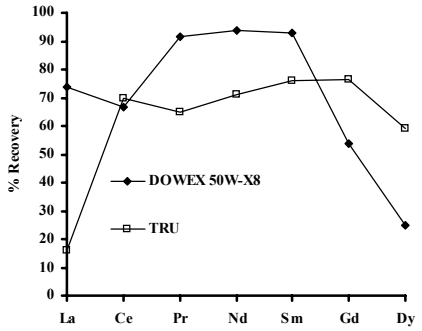


Fig. 1. Recovery percentage of lanthanides after separation on DOWEX 50W-X8 and TRU resins

The best recovery was found for the elements Pr, Nd and Sm using both resins while the least satisfying results were obtained in the case of Dy (24.9%) and Gd (54%) using DOWEX 50W-X8 resin. Recovery for Dy and Gd using TRU resin were 59.3% and 76.4% respectively. 74% of La and 66.6% of Ce were recovered after separation using DOWEX 50W-X8 resin and 15.8% and 64.1% using TRU resin.

Conclusion

Results showed that the selected lanthanides made stable complexes with APDC in the basic medium with the maximum recovery at pH=8. The exception was La which reached maximum recovery at pH=9. At pH values ranging from 9 to 11 recoveries varied from 98 to 70%. The complexation with APDC was irregular at all other pH values.

Recovery of the elements after separation on DOWEX 50W-X8 resin and preconcentration with APDC at pH=8 varied from 91.4% (Pr) to only 24.9% in the case of Dy and on TRU resin from from 76.4% (Gd) to 15.8% in case of La.

References

[1] Arai T., Wei Y., Kumagai M., Horiguchi K.: Separation of rare earths in nitric acid medium by a novel silica-based pyridinium anion exchange resin. *J Alloys Compd*, 2006, 408-412, 1008-1012
[2] Bühn B., Rankin A.H.: Composition of natural, volatile-rich Na-Ca-REE-Sr carbonatitic fluids trapped in fluid inclusions. *Geochim Cosmochim Acta*, 1999, 63, 3781-3797
[3] De Vito I.E., Masi A.N., Olsina R.A.: Determination of trace rare earth elements by X-ray fluorescence spectrometry after preconcentration on a new chelating resin loaded with thorin. *Talanta*, 1999, 49, 929-935
[4] Djingova R., Ivanova J.: Determination of rare earth elements in soils and sediments by inductively coupled plasma atomic emission spectrometry after cation-exchange separation. *Talanta*, 2002, 57, 821-829
[5] Jarvis K.E.: Inductively coupled plasma mass spectrometry: A new technique for the rapid or ultra-trace level determination of the rare-earth elements in geological materials. *Chem Geol*, 1988, 68, 31-39
[6] Larsen L.M., Fitton J.G., Pedersen A.K.: Paleogene volcanic ash layers in the Danish Basin: compositions and source areas in the North Atlantic Igneous Province. *Lithos*, 2003, 71, 47-80
[7] Lichte F.E., Meier A.L., Crock J.G.: Determination of the rare-earth elements in geological materials by inductively coupled plasma mass spectrometry. *Anal Chem*, 1987, 59, 1150-1157
[8] Na C., Nakano T., Tazawa K., Sakagawa M., Ito T.: A systematic and practical method of liquid chromatography for the determination of Sr and Nd isotopic ratios and REE concentrations in geological samples. *Chem Geol*, 1995, 123, 225-237
[9] Ochsenkühn M., Lyberopulu Th., Parissakis G.: Direct determination of lanthanides, yttrium and scandium in bauxites and red mud from alumina production. *Anal Chim Acta*, 1994, 296, 305-313
[10] Ochsenkühn-Petropulu M., Ochsenkühn M., Luck J.: Comparison of inductively coupled plasma mass spectrometry with inductively coupled plasma atomic emission spectrometry and instrumental neutron activation analysis for the determination of rare earth elements in Greek bauxites. *Spectrochim Acta, Part B*, 1991, 46, 51-65
[11] Padilla R., Van Espen P., Godo Torres P. P.: The suitability of XRF analysis for compositional classification of archaeological ceramic fabric: A comparison with a previous NAA study. *Anal Chim Acta*, 2006, 558, 283-289
[12] Pasinnli T., Eroğlu A.E., Shahwan T.: Preconcentration and atomic spectrometric determination of rare earth elements (REEs) in natural water samples by inductively coupled plasma atomic emission spectrometry. *Anal Chim Acta*, 2005, 547, 42-49
[13] Sun Y.: Detailed study on simultaneous separation of rare earth elements by capillary electrophoresis. *J Chromatogr A*, 2004, 1048, 245-251
[14] Suzuki T., Itoh K., Ikeda A., Aida M., Ozawa M., Fujii Y.: Separation of rare earth elements by tertiary pyridine type resin. *J Alloys Compd*, 2006, 408-412, 1013-101

Trends in Ion-Beam Based Spectroscopic Techniques

[1]Márius Pavlovič, [1]Róbert Hinca, [1]Ivan Strašík, [1]Štefan Krnáč, [2]Erich Griesmayer, [3]Thomas Schreiner

[1]Slovak University of Technology in Bratislava, Faculty of Electrical Engineering and Information Technology, Department of Nuclear Physics and Technology, Ilkovičova 3, 812 19 Bratislava, Slovak Republic, marius.pavlovic@stuba.sk
[2]University of Applied Sciences Wiener Neustadt, Johannes Gutenberg-Strasse 3 and Fotec – Research and Technology Ltd., Viktor Kaplan-Strasse 2, A-2700 Wiener Neustadt, Austria
[3]Fotec – Research and Technology Ltd., Viktor Kaplan-Strasse 2, A-2700 Wiener Neustadt, Austria

Abstract

Ion-beam based spectroscopic techniques. **Ion beams offer several spectroscopic techniques that are called "Ion Beam Analysis" – IBA-techniques. Wide range of beam parameters can be used for IBA and various types of analyses can be performed. The IBA techniques including activation studies are reviewed with emphasis on the results achieved at the Slovak University of Technology. Corresponding accelerator and spectroscopic technology is described.**
Research possibilities at medical accelerators. **Spectroscopic techniques that could be in principle available at medical accelerators are described. The research possibilities and beam parameters of these accelerators are analysed. The main attention is paid to the MedAustron project as well as to a cyclotron facility under development in Slovakia.**

Key words: *ion beam analysis--residual activation--medical accelerator--synchrotron--cyclotron--ion spectroscopy*

Introduction

Ion beams offer several spectroscopic techniques that are called "Ion Beam Analysis" – IBA-techniques. Various types of analyses can be performed by means of IBA. Typical examples are trace-element analyses, measurements of concentration profiles, activation studies. Under special conditions like channelling or blocking, structure of matter can also be investigated. In addition to the techniques that use the primary ion beam directly as an ion probe for sample analysis, another group of analyses can be recognised that uses radiation probes generated by ion beams. Typical examples are neutron activation, neutron diffraction and synchrotron radiation facilities. Neutrons can be produced in nuclear reactors or by ion beams bombarding a suitable target. Synchrotron radiation facilities run electron storage rings with an electron beam of ≈1 to 2.5 GeV to produce a so-called synchrotron light. Synchrotron light exhibits several unique physical properties that are suitable for numerous kinds of materials analysis. In this paper, the main attention is paid to analytical techniques using primary ion beam directly as a probe.

The ion beam analyses exhibit typically high sensitivity, good depth resolution, non-destructive character and work for small amounts of sample material. These properties can be advantageous

compared to other analytical tools. However, some of them require rather sophisticated and expensive instrumentation including large accelerator installations. That is why, research possibilities of accelerators built for another primary purpose – medical applications – are discussed in this paper, too.

Basic principles of ion beam analyses

The working principle of ion beam analyses is schematically shown in Fig. 1. The primary beam particles interact with the target atoms. The interaction products and their characteristics like energy, mass, etc. are analysed by a spectroscopic apparatus. In a typical case, the resulting outcome of the spectroscopic apparatus is an energy spectrum or mass spectrum of a certain specific interaction product. Information of interest is obtained by analysing the spectrum. Tab. 1 lists the basic IBA methods.

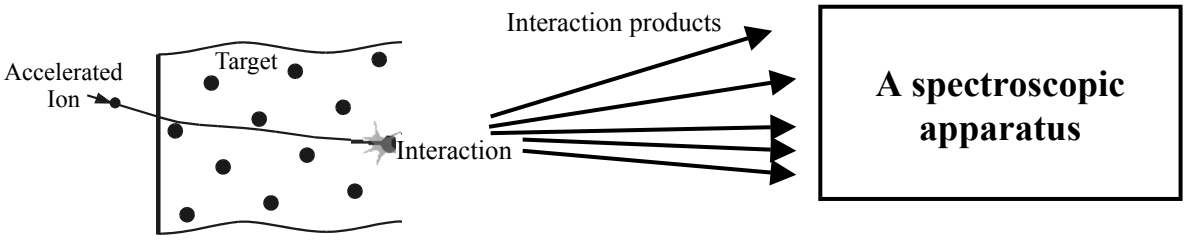


Fig. 1. Physical principle of the ion beam analyses. The primary beam particles interact with the target atoms. The interaction products are detected and analysed by a spectroscopic apparatus

Tab. 1. Classification of IBA methods
RBS = Rutherford Backscattering Spectrometry, PG = Protonography, MEIS = Medium Energy Ion Scattering, LEIS = Low Energy Ion Scattering, PES = Proton Elastic Scattering, ERDA = Elastic Recoil Detection Analysis, NRA = Nuclear Reaction Analysis, PIXE = Proton Induced X-Ray Emission, PIGE = Particle Induced Gamma-Ray Emission, CMS = Conventional Mass Spectrometry, SIMS = Secondary Ion Mass Spectrometry, AMS = Accelerator Mass Spectrometry

Technique	Ion Beam Usage		Beam Energy [keV]				Beam Scattering		Interaction with	
	Probe	Sample	1	10	100	1000	Elastic	Non-elastic	Nuclei	Electrons
RBS	✓					✓	✓		✓	
PG	✓				✓		✓		✓	
MEIS	✓				✓		✓		✓	
LEIS	✓		✓				✓		✓	
PES	✓					✓	✓		✓	
ERDA	✓					✓	✓		✓	
NRA	✓				✓	✓		✓	✓	
PIXE	✓					✓		✓		✓
PIGE	✓					✓		✓	✓	
CMS		✓	✓							
SIMS	✓	✓	✓	✓						
AMS		✓				✓				

Under special circumstances, the IBA can be applied to some structure-related problems using channelling regime as illustrated in Fig. 2.

Among the types of IBA methods using the primary ion beam as a probe, the Rutherford Backscattering Spectrometry (RBS), Proton Induced X-ray Emission (PIXE) and Nuclear Reaction Analysis (NRA) are most widely used. Physical principle of RBS and PIXE is shown in Fig. 3. More detailed description of other IBA methods can be found in Ref. [3].

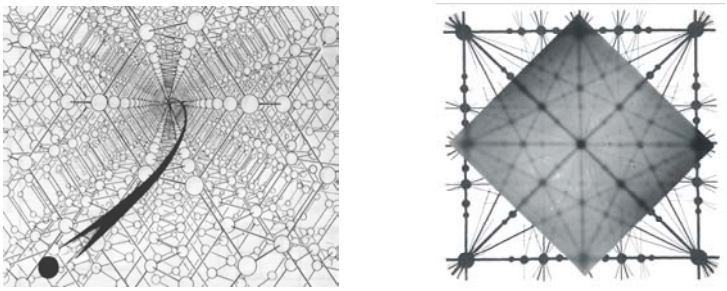


Fig. 2. An illustration of ion-channelling (left) and a crystal lattice pattern produced by protonography (right). An experimental protonogram is combined with its theoretical prediction

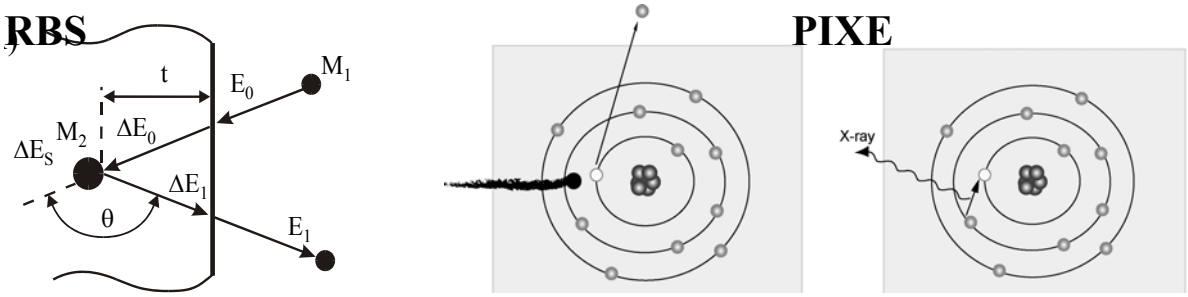


Fig. 3. Principle of the RBS (left) and PIXE (right) analyses

The RBS is based on spectrometry of backscattered primary ions that underwent an elastic collision with a target nucleus. The energy of the backscattered ion immediately after the collision, E_{AFTER} is given:

$$E_{AFTER} = KE_{BEFORE} \qquad (1)$$

where K is the kinematic factor and E_{BEFORE} is the ion energy immediately before the collision. The kinematic factor depends only on the mass of ion, M_1, the mass of the scattering nucleus, M_2 and the scattering angle, θ. The energy-loss related to the nuclear scattering is $\Delta E_S = E_{BEFORE} - E_{AFTER}$.

The mass of ion M_1 is known and the scattering angle θ is defined by the measurement geometry, i.e. by location of the detector with respect to the direction of the primary beam supposing that there is only one elastic nuclear collision on the ion trajectory in the sample. The mass of the scattering nucleus M_2 can be determined by measuring the energy of the scattered ions E_1:

$$E_1 = E_0 - \Delta E_0 - \Delta E_S - \Delta E_1 = E_{AFTER} - \Delta E_1 = KE_{BEFORE} - \Delta E_1 = K(E_0 - \Delta E_0) - \Delta E_1 \qquad (2)$$

where ΔE_0 and ΔE_1 represent the energy-loss of the ion from the surface to the scattering nucleus and from the scattering nucleus back to the surface, respectively, E_0 is the initial ion energy.

The final energy of the backscattered ion depends not only on the mass of the scattering nucleus, but also on its position in depth. This makes the measurement of concentration profiles possible. Standard RBS is performed with ≈2 MeV He ions.

The PIXE technique uses different principle. A proton beam with typical energy of few MeV is used to ionise the target atoms. Vacancies occur in the electron shell-structure of the ionised atoms. These vacancies are filled in by another electron, which is accompanied by emission of a characteristic X-ray. The energy spectrum of emitted X-rays contains information about the elements present in the sample. PIXE is widely used for analysis of environmental samples, archaeological and geological objects, objects d'art etc. An example is given in Fig. 4 that shows a theoretical PIXE-spectrum and a real PIXE-spectrum of four sand-samples. Similar character of all four spectra indicates the same origin of pollution.

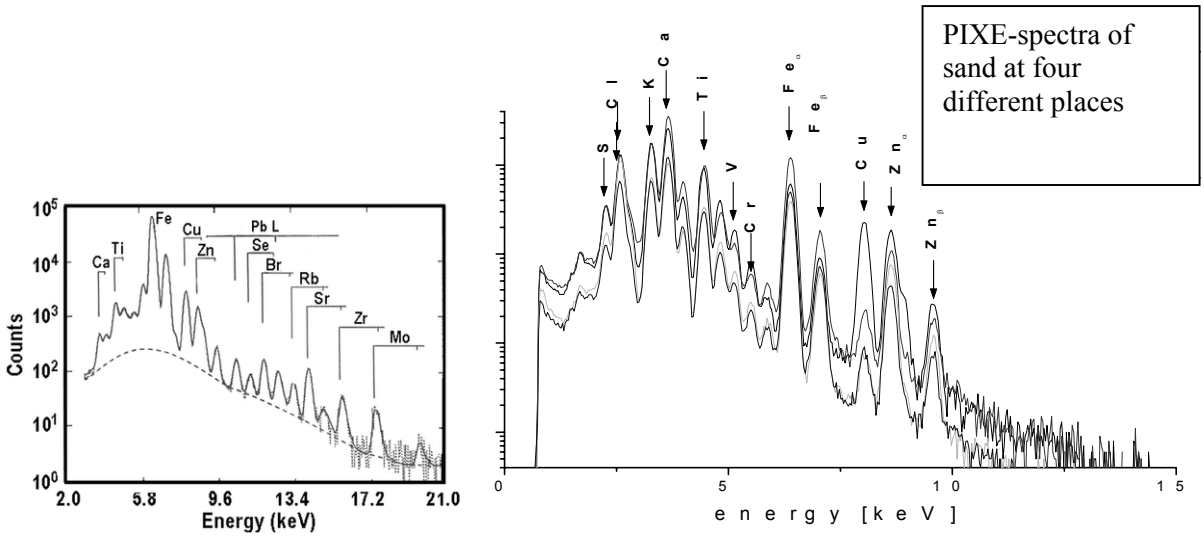


Fig. 4. Theoretical (left) and real (right) PIXE spectra. An example is given for PIXE-spectra of sand collected at four different places. Similar character of the spectra indicates a common origin of pollution

Standard PIXE is performed with a few MeV protons. However, Hahn-Meitner-Institute Berlin reports interesting PIXE analyses using considerably higher energies (68 MeV) [2].

Nuclear reaction analysis (NRA) uses spectroscopy of characteristic products of specific nuclear reactions. Some nuclear reactions exhibit a sharp resonance maximum of cross section at certain particular energy. This technique is called RNRA – Resonance Nuclear Reaction Analysis. The resonance nuclear reactions are available at energies of few hundreds keV, as listed in Tab. 2. Primary beam particles can be protons or deuterons. Reaction products can be gamma-photons, alpha particles or neutrons. Depth-resolving analysis is possible with RNRA because of continuous energy loss of primary beam in the sample. The reaction of interest takes place only in depth where the primary beam has the resonance energy. However, in contrast to RBS, the depth profiling requires manipulations with the initial energy of the incoming beam. The higher the initial energy, the deeper sample slice is analysed.

The accelerator technology for IBA is dictated by required energies that range typically from ≈100 keV up to several MeV. This energy interval is a domain for compact electrostatic systems that are nowadays produced as tandem accelerators. Electrostatic accelerators are relatively simple to operate, do not require large installation rooms and serve DC-beams. Tandems provide an easy access to and exchange of an ion source that is on the ground potential. That is why different particle species can be accelerated in tandems. The output beam energy depends basically on the applied voltage and the charge-exchange ratio at the stripping foil. Other types of accelerators like RFQ, LINAC and

cyclotron are also possible. However, they are more complicated to operate because of inclusion of an RF-system. In the energy region of ≈10 MeV, cyclotrons may reach an extremely compact design. The output beam energy is limited by the magnetic beam rigidity given as the ratio of particle momentum and charge. As an example, the CYCLONE18/9 cyclotron installed at BIONT, a.s. Bratislava is designed to accelerate protons and deuterons. The maximum energy for protons is 18 MeV. Deuterons can only have 50% of the velocity of protons in order to have the same momentum and magnetic rigidity. Their total kinetic energy can therefore be 9 MeV only (half of the proton velocity, double of proton mass) when assuming the non-relativistic energy-to-velocity relation.

Tab. 2. Selected (p, γ) resonance nuclear reactions used for IBA

Reaction	E_{proton} [keV]	Eγ [MeV]	Reaction	E_{proton} [keV]	Eγ [MeV]
$^{7}Li(p,\gamma)^{8}Be$	441	17,65	$^{24}Mg(p,\gamma)^{25}Al$	226	2,06
		14,75			1,56
$^{9}Be(p,\gamma)^{10}B$	330	6,15			0,95
		5,15	$^{19}F(p,\alpha\,\gamma)^{16}O$	224	7,12
		4,75			6,72
		2,15		340	6,13
		1,7		483,6	6,13
		1,43		597	6,13
		1,02		672	6,13
		0,72	$^{18}O(p,\gamma)^{19}F$	630	8,5
		0,41	$^{15}N(p,\alpha\,\gamma)^{12}C$	360	4,43
$^{11}B(p,\gamma)^{12}C$	163	16,11		429	4,43
		11,68	$^{14}N(p,\gamma)^{15}O$	278	6,8
		4,43		700	8,0
	675	12,15	$^{13}C(p,\gamma)^{14}N$	550	8,06
		4,43	$^{12}C(p,\gamma)^{13}N$	459	2,36

Activation studies

Ion beams can also be used for activation studies that are needed for design of future high-power accelerator installations. An STU-team took part in activation studies for the GSI Future Facility. Copper and stainless steel samples were irradiated by ^{238}U ions at 500 MeV/u and 950 MeV/u [5]. Irradiated samples were analysed by gamma-ray spectrometry in a depth-resolving mode achieved by the stacked-foil sample arrangement. This technique allows distinguishing between activation products and projectile fragments. Fig. 5 and 6 show the difference in gamma-spectra of target activation (upstream the range of primary particles) and projectile fragments (downstream the range of primary particles). Example is given for copper irradiated by 500 MeV/u uranium beam.

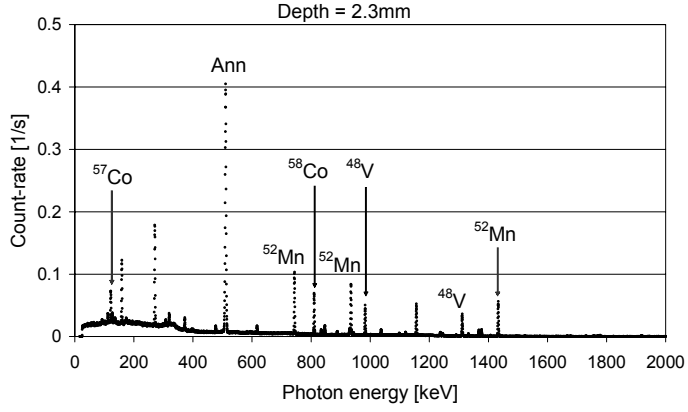


Fig. 5. A gamma-spectrum of residual activity in the target activation region upstream the range of primary beam Copper, uranium beam, 500 MeV/u

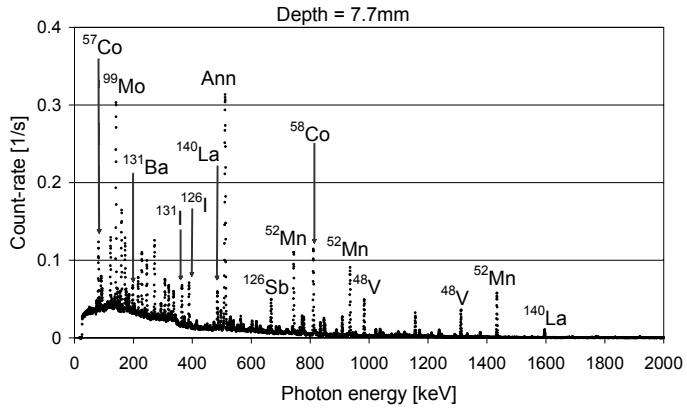


Fig. 6. A gamma-spectrum of residual activity in the projectile fragment region downstream the range of primary beam.
Copper, uranium beam, 500 MeV/u

Activation studies require large synchrotron-based accelerator installations and high-resolution spectroscopic equipment. Alternative methods of spectra evaluation based on the WSP – Whole Spectrum Processing approach can be applied [4].

Accelerator technology for cancer therapy

There are two current medical accelerator projects in the central Europe: (1) Cyclotron Centre in Bratislava, Slovakia and (2) MedAUSTRON in Wiener Neustadt, Austria.

The cyclotron centre will be equipped with a DC72 isochronous cyclotron produced in Joint Institute for Nuclear Research in Dubna, Russia. Its main characteristics and application areas are collected in Tab. 3. Further technical details can be found in Ref. [7]. The maximum energy of proton beam (72 MeV) is dictated by the proton therapy of eye. In addition, the cyclotron can be used for production of some medical isotopes as well as for applied and fundamental research depending on the equipment of the target stations.

Tab. 3. The planned main applications and corresponding beam parameters of the DC-72 cyclotron

Application	Particle species	Energy [MeV/u]	Intensity [eμA]
^{123}I production	proton	30	50
^{87}Rb production	proton	30	30
^{67}Ga, ^{201}Tl, ^{111}In production	proton	30	100
Proton therapy	proton	72	0.05
Fast neutron therapy	proton	66–72	30 – 35
Applied research	Li – Xe	2.8 – 2.7	5 – 1
Mass-spectrometry	C – Kr	8.6 – 2.8	20 – 2
Physics research	Li– Xe	2.8 – 2.7	5 – 1

Technical concept of the MedAUSTRON accelerator has been thoroughly described in Ref. [1]. It is a synchrotron-based dedicated cancer therapy facility using proton and carbon-ion beams. The choice of particle species is motivated by combination of low-LET (protons) and high-LET (carbon ions) treatment modalities. The energy is dictated by desirable penetration range in the patient body, which is set to cover 3.5 cm to 27.5 cm in water. This translates into beam-energies from 60 MeV to 220 MeV for protons and 120 MeV/A to 400 MeV/A for carbon ions. The maximum magnetic beam rigidity corresponds to 400 MeV/A carbon beam and reaches 6.346 Tm. This rigidity allows accelerating other particle species too with the maximum energy per nucleon $E_{kA} = E_k / A$ [6]:

$$E_{kA} = E_{rA}\left(\sqrt{\left[\frac{(B\rho)c}{10^6 E_{rA}}\cdot\frac{n}{A}\right]^2 + 1} - 1\right) \qquad (3)$$

where $B\rho$ is the maximum beam rigidity allowed in the machine, n is the particle charge-state, c is the speed of light in vacuum, A is the number of nucleons (mass number) and E_{rA} is the rest energy per nucleon in [MeV]. In order to simplify interpretation of Eq. (3), the rest energy per nucleon, which varies slightly for different ions, can be assumed constant. Under this assumption, particles with the same n/A ratio will have the same kinetic energy per nucleon at the same magnetic beam rigidity.

Exact calculation has been performed for ions of interest. Results are shown in Tab. 4.

Tab. 4. Maximum kinetic energy of different ions of interest corresponding to the magnetic beam rigidity of 6.346 Tm

	protons	**$^{4}He^{2+}$**	**$^{7}Li^{3+}$**	**$^{9}Be^{4+}$**	**$^{10}B^{5+}$**	**$^{11}B^{5+}$**	**$^{12}C^{6+}$**	**$^{14}N^{7+}$**	**$^{16}O^{8+}$**	**$^{19}F^{9+}$**	**$^{20}Ne^{10+}$**
A/n	1.00	2.00	2.33	2.25	2.00	2.20	2.00	2.00	2.00	2.11	2.00
E_{kA} MeV/A	1183	400	306	326	400	339	400	400	400	365	2.00

Except for the maximum magnetic beam rigidity, other technical limits may come from the RF-system and gamma-transition energy [6].

Ion therapy is a demanding application requiring many specific beam-control features that may be very attractive for research experiments, too. The most important ones are the following:

- ***Fast and fine energy-variation.*** The energy can be varied pulse-to-pulse providing the range step of 1 mm.
- ***Adjustable beam spot-size.*** The spot-size of the static beam can be varied from 4 mm to 10 mm (FWHM) in 1 mm steps.
- ***Large-area active beam-scanning.*** The beam can be scanned orthogonally by means of a pair of scanning magnets over a large area, typically 30 cm x 30 cm.
- ***An advanced control system.*** Medical operation requires extreme machine reliability and beam stability. This is achieved by sophisticated machine-design and an advanced control system. The operation of the machine is foreseen for hospital environment by medical staff in a user-friendly manner.
- ***Slowly extracted beam.*** The beam can be extracted in spills with adjustable duration typically from 1 s to 10 s. Beam intensities (for therapy) are 10^{10} protons per spill and $4\cdot10^{8}$ carbon ions per spill.

Conclusions

Medical accelerators have a potential to serve beams for non-clinical research with parameters and features discussed in the paper. Among many possible applications, materials research using ion-beam based spectroscopic techniques is possible. The beam parameters are directly suitable for high-energy PIXE (≈70 MeV protons), other techniques would require reduction of the beam energy, which can be done by passive energy-degrading devices. Activation studies could also be possible with beam energies of about 400 MeV/A. In addition to this, modification of materials by high-energy beams with different ion species and adjustable penetration range can be performed. MedAUSTRON facility is planned to serve beams for non-clinical research, too and will become a unique cancer therapy and ion-beam research centre in the central Europe.

Acknowledgement

This work was carried out in the framework of the MedAustron Interreg IIIA Project that is funded by the European Union, Republic of Austria and Country of Lower Austria and GSI-INTAS 03-54-3588 Project that is supported by European Community.

References

[1] Auberger Th., Griesmayer E. (eds.): *Das Projekt MedAustron.* Forschungs- und Technologie-transfer GmbH, Wiener Neustadt, 2004

[2] Denker A., Opitz-Coutureau J., Campbell J.L., Maxwell J.A., Hopman T.: High-energy PIXE: quantitative analysis. *Nucl. Instr. And Meth. B, 2004, B 219-220, 130-135*

[3] Kováč P., Pavlovič M., Stanček S.: Analysis of materials by ion beams. In: Konjevic N., Petrovic Z.Lj., Malovic G.: *The Physics of Ionized Gases.* SFIN year XIV Series A, Institute of Physics, Belgrade, 2001, 193-212

[4] Krnáč Š., Povinec P.P.: Factor analysis of semiconductor γ-ray spectra. *Appl. Radiat. Isot., 1996, 47/9-10, 905-910*

[5] Mustafin E., Iwase H., Kozlova E., Schardt D., Fertman A., Golubev A., Hinca R., Pavlovič M., Strašík I., Sobolevskiy N.: Measured residual activity induced by U ions with energy 500 MeV/u in Cu target. *Proceedings of the 10th European Particle Accelerator Conference EPAC2006, Edinburgh, Scotland, 26-30 June 2006, 1834-1836*

[6] Pavlovič M., Griesmayer E., Schreiner Th.: Research possibilities of medically-optimised accelerator complexes. In: Weis M., Vajda J. (eds.): *Proceedings of the 12th International Conference on Applied Physics of Condensed Matter APCOM2006, 21-23 June 2006, Malá Lučivná, Slovakia, 15-18*

[7] Solivajs D., Borisov O.N., Gáll A., Gulbekian G.G., Keníž J., Kliman J., Králik S., Pavlovič M.: A study of charge-exchange beam extraction from the multi-purpose isochronous cyclotron DC-72, *Journal of Electrical Engineering, 2004*, *55/7-8, 201-206*

Uncertainties Sources in the Fractionation Analysis of the Gravitation Dust Sediment

[1]Dagmar Remeteiová, [2]Daniela Mackových, and [2]Henrieta Šoltýsová
[1]*Technical University of Košice, Faculty of Metallurgy, Department of Chemistry, Letná 9, 042 00 Košice, Slovak Republic, e-mail: dagmar.remeteiova@tuke.sk*
[2]*Geological Survey of Slovak Republic, Geoanalytical Laboratories, Markušovská cesta 1, 052 40 Spišská Nová Ves, Slovak Republic*

Abstract

The basic parts of fractionation analysis are extraction and determination of elements in the extracts. These parts the most affect of the total uncertainty value of the fractionation analysis (the combined standard uncertainty u_C). The fractionation analysis of the gravitation dust sediment was evaluated by the determination of the standard uncertainty of the type A (the uncertainty of measurement in 1 extract – u_{AM} and the uncertainty of extraction – u_{AE}) and the standards uncertainty of type B (the uncertainty of dilution – u_{BD}, the uncertainty of purity of chemicals – u_{BCH}, the uncertainty of preparation of calibration solutions – u_{BC}, and the uncertainty of weighting of the sample for extraction – u_{BW}). For isolation of various forms of Cd, Cu, Pb, and Zn the single-step extraction by 0.05 mol dm^{-3} Na_2EDTA and 2 mol dm^{-3} HNO_3 were used. For quantification of elements in extracts the flame atomic absorption spectrometry (FAAS) method was applied.
Key words: *gravitation dust sediments, fractionation analysis, single-step extraction, FAAS, standards uncertainties*

Introduction

The total content of elements in the environmental samples is not a suitable indicator of biological availability, toxicity, and mobility of elements in environment [1]. The chemical, biological and toxicological properties of the element are critically dependent on the form, in which the element occurs in the sample [2]. The fractionation analysis is often used for evaluation of ecotoxicity and bioavailibility physical-chemical element forms. This method enables classification (isolation and quantification) of element forms according to their different physical or chemical properties [3]. One of the methods of the isolation of element forms is the single-step extraction. For the quantification of elements in the extracts is possible to use of some adequate spectral method. In the fractionation analysis of the soil samples the single-step extraction by 0.05 mol dm^{-3} Na_2EDTA and 2 mol dm^{-3} HNO_3 is often applied [4, 5]. These extracting reagents enable the isolation of the mobilizable (EDTA) and the maximal potential mobilizable element forms (HNO_3) from soils. With the aim of isolation of these element forms from dust sediment, which may contaminate soils, the single-step extracting procedures, after optimalization of some parameters, were applied [6 - 9]. The extraction is the longest part of fractionation analysis with amount of sectional procedures hence is possible to assume that the biggest source of uncertainties in the fractionation analysis wills the extraction.

The value of combined standard uncertainty (u_C) involves:

- uncertainties of type A obtained from statistical evaluation of repeated measurements or procedures;
- uncertainties of type B dedicated by different way that statistical.

The sources of uncertainties of type A in the fractionation analysis may be:

- analytical method used on the determination of elements content in extracts;
- extraction procedure.

The sources of uncertainties of type B are:

- dilution of solutions;
- purity of used chemicals;
- preparation of calibration solutions;
- weighting of the sample for extraction.

In this paper we present the values of uncertainties native from different operations carried out in process of the fractionation analysis of gravitation dust sediment samples.

Experimental

The sample of the gravitation dust sediment from locality Košice – city was collected. Individual single-step extraction procedures were carried out by techniques used for a soil sample. For the isolation of different mobile element forms 0.05 mol dm^{-3} Na_2EDTA (pH = 7, treated by NH_4OH) and 2 mol dm^{-3} HNO_3 (pH = 0.7) were used. The ratio value of the sample mass to volume of extracting solution (m / V) was constant, 0.5 g / 75 cm^3 for all experiments [6, 7]. Others extraction conditions (time of extraction – 1 hour for EDTA, 6 hours for HNO_3, temperature – 20 ± 2 °C) and the experimental procedures in accordance with validated extraction procedures of soil samples were unbroken [4, 5]. For the determination of Cd, Cu, Pb, and Zn in extracts the atomic absorption spectrometry with flame atomization (SPECTR AA 400 Varian) was applied.The combined standard uncertainty was calculated using the formula

$$u_C = \sqrt{u_A^2 + u_B^2} \qquad (1)$$

The standard uncertainty of the type A (u_A), which includes the uncertainty of the measurement by the FAAS method (u_{AM}) and the uncertainty of the extraction (u_{AE}), was calculated as

$$u_A = \sqrt{u_{AM}^2 + u_{AE}^2} \qquad (2)$$

The uncertainty of the measurement by statistical evaluation of 10 repeated measurements of elements content in one extract was defined. The uncertainty of the extraction by statistical evaluation of measurements of elements content in 10 extracts was defined. Both standard uncertainties represent percentage relative standard deviations calculated according to formula [10]

$$RSD / \% = \frac{s}{\bar{x}} 100 \qquad (3)$$

where s means standard deviation, $\bar{x}$ expresses mean value of concentrations.

The standard uncertainty of the type B (u_B) involves uncertainties of dilution (u_{BD}), purity of chemicals (u_{BCH}), preparation of calibration solutions (u_{BC}), and weighting of the sample for extraction (u_{BW}), it was calculated as

$$u_B = \sqrt{u_{BD}^2 + u_{BCH}^2 + u_{BC}^2 + u_{BW}^2} \quad (4)$$

Results and Discussion

From the values of uncertainties of type A corresponding the measurement by the FAAS method (u_{AM}) in the EDTA (Table 1) and HNO_3 extracts (Table 2) results that used method is not suitable for determination of Cd. The high values of RSDs for determination of Cd are caused by very low content of Cd in extracts. The values of RSDs for determination of Cu, Pb, and Zn in HNO_3-extract are lower than for determination in EDTA-extract.

Table 1. Statistical evaluation of 10 repeated measurements of contents of chosen elements in the EDTA-extract

Element	Cu	Zn	Pb	Cd
$\bar{x}$ / μg cm^{-3}	16.475	3.899	0.811	0.012
s	0.310	0.073	0.027	0.006
u_{AM} (RSD) / %	1.88	1.87	3.33	50.0

Table 2. Statistical evaluation of 10 repeated measurements of contents of chosen elements in the HNO_3-extract

Element	Cu	Zn	Pb	Cd
$\bar{x}$ / μg cm^{-3}	34.100	9.909	2.084	0.049
s	0.380	0.127	0.023	0.007
u_{AM} (RSD) / %	1.11	1.28	1.10	14.28

The values of RSDs obtained from 10 repeated extractions by EDTA (Table 3) and HNO_3 (Table 4) represent the uncertainty of extraction (u_{AE}). These RSD values, for both using extracting reagents and all chosen elements, were higher than the values representative of the uncertainties of measurement by the FAAS method. The highest values of uncertainties u_{AE} were determined for extraction of Cd-forms.

Table 3. Statistical evaluation of measurements of contents of chosen elements in the 10 EDTA-extracts

Element	Cu	Zn	Pb	Cd
$\bar{x}$ / μg cm^{-3}	15.566	3.773	0.768	0.011
s	0.497	0.178	0.074	0.009
u_{AE} (RSD) / %	3.19	4.72	9.64	81.82

Table 4. Statistical evaluation of measurements of contents of chosen elements in the 10 HNO_3-extracts

Element	Cu	Zn	Pb	Cd
$\bar{x}$ / μg cm^{-3}	34.420	10.519	2.078	0.029
s	0.470	0.589	0.049	0.014
u_{AE} (RSD) / %	1.37	5.60	2.36	48.28

Unlike uncertainties of type A, uncertainties of type B have no significant influence on the combined standard uncertainty value (Tables 5 and 6).

Table 5. Standard uncertainties of fractionation analysis (extracting reagent – EDTA)

Element	u_{AM} / %	u_{AE} / %	u_A / %	u_B / %	u_C / %
Cu	1.88	3.19	3.70	0.47	3.73
Zn	1.87	4.71	5.08	0.47	5.10
Pb	3.33	9.64	10.20	0.63	10.22
Cd	50.0	81.82	95.89	0.95	95.89

Table 6. Standard uncertainties of fractionation analysis (extracting reagent – HNO_3)

Element	u_{AM} / %	u_{AE} / %	u_A / %	u_B / %	u_C / %
Cu	1.11	1.37	1.76	0.58	1.85
Zn	1.28	5.60	5.74	0.47	5.76
Pb	1.10	2.36	2.60	0.63	2.68
Cd	14.28	48.28	50.35	0.95	50.36

Conclusion

From the results obtained in this work it can be stated that the main source of uncertainties in the fractionation analysis is the extraction process. The FAAS method is not suitable for determination of Cd in extracts of the fractionation analysis. The uncertainties of determination of Cu and Zn in the HNO_3-extract are lower than in the EDTA-extract. Contrariwise the uncertainties of Zn determination in the extract of HNO_3 are higher that in the extract of EDTA.

Acknowledgements

This research was supported by projects VEGA No. 1/3149/06 and APVV No. 20-0094-04.

References

[1] Broekaert J.A.C., Gücer S., Adams F.: *Metal Speciation in the Environment*. Springer-Verlag, Heidelberg, 1990

[2] Cornelis R., Caruso J., Crews H., Heumann K.: *Handbook of Elemental Speciation. Techniques and Methodology*. John Wiley & Sons Ltd, England, 2003

[3] Templeton D. M., Ariese F., Cornelis R., Danielsson L. G., Muntau H., Leeuwen H. P. van, Łobiński R.: Guidelines for therms related to chemical speciation and fractionation of elements. Definition, structural aspects, and methodological approaches. *Pure Appl. Chem., 2000, 72, 8, 1453-1470*

[4] Ure A.M., Davidson C.M., Thomas R.P.: Single and sequential extraction schemes for trace metal speciation in soil and sediment. *Quality Assurance for Environmental Analysis, ed. Elsevier Science B. V., ch. 20, 1995, 505-523*

[5] Makovníková J.: *Distribution of Cd, Pb, Cu, and Zn in Soil and its evaluation considering on potentions and barriers of metal transport to plants.* Research Institute of soil science and soil conservation, Bratislava, 2000

[6] Remeteiová D., Krakovská E., Tomko J., Sminčáková E., Vojteková V.: Extrakcia vzoriek prašných spadov EDTA. *Sborník konference "Mikroelementy 2002", Slapy, 2002, 124-128*

[7] Tomko J., Krakovská E., Remeteiová D. Sminčáková E.: Stanovenie mobilizovateľného obsahu niektorých kovov v prašnom spade 2M HNO_3, *Sborník konference "Mikroelementy2002, Slapy, 2002, 129-338*

[8] Remeteiová D., Sminčáková E., Krakovská E.: Application of the Fractionation Analysis at the Study of the Risk Elements Mobility from the Dust Sediment to Soils. *IV. International conference on inorganic environmental analysis, 59-61, 2005*

[9] Remeteiová D., Sminčáková E., Flórián K.: Study of the Chemical Properties of Gravitation Dust Sediments. *Microchim Acta, DOI 10.1007/s00604-006-0603-0, Springer-Verlag, 2006*
[10] Wenclawiak B.W., Koch M., Hadjicostas E.: *Quality Assurance in Analytical Chemistry.* Springer-Verlag Berlin Heidelberg, 2004

Evaluation of the Availability of the FAAS Method for Determination of Cd, Cu, Pb, and Zn in the Extracts of Fractionation Analysis of the Gravitation Dust Sediment

Dagmar Remeteiová and Radoslav Rusnák
Technical University of Košice, Faculty of Metallurgy, Department of Chemistry, Letná 9/A, 042 00 Košice, Slovakia, e-mail: ***dagmar.remeteiova@tuke.sk; radoslav.rusnak@tuke.sk***

Abstract

This work is focused to the evaluation of the availability of the FAAS method for determination of Cd, Cu, Pb, and Zn in extracts of fractionation analysis of the gravitation dust sediment by application of 0.05 mol dm^{-3} Na_2EDTA and 2 mol dm^{-3} HNO_3 as extracting reagents. The limit of detection (LoD), limit of quantitation (LoQ), precision (repeatability), and robustness were established. The LoD in the EDTA-extract is for Cu 0.07 μg cm^{-3}, Zn 0.16 μg cm^{-3}, Pb 0.15 μg cm^{-3}, and Cd 0.04 μg cm^{-3}. The LoD in the HNO_3 is for Cu 0.03 μg cm^{-3}, Zn 0.22 μg cm^{-3}, Pb 0.12 μg cm^{-3}, and Cd 0.02 μg cm^{-3}. The repeatability of the determination in the EDTA extract expressed as relative standard deviation is for Cu 1.88 %, Zn 1.87 %, Pb 3.32 %, and Cd 52.70 %. For determination in the HNO_3 extract the repeatability is for Cu 1.10 %, Zn 1.28 %, Pb 1.10 %, and Cd 15.06 %. The method is few robust concerning changes of extracting time (EDTA, Cu, Pb) and changes of extracting ratio (HNO_3, Cu).

Key words: fractionation analysis, gravitation dust sediment, single-step extraction, FAAS, LoD, LoQ, repeatability, robustness

Introduction

The fractionation analysis is analytical process applicable for the classification of various forms of elements in the environmental samples. This process consists from isolation of elements forms on the base of similar physical or chemical properties and consecutive quantification of the element in the isolated fraction [1]. The fractionation analysis is often applied for soils and sediments samples. Application of fractionation analysis for samples of the gravitation dust sediments results from the requirement to monitor mobility of risk toxic elements forms from dust to the soils. For the isolation of different mobile forms of the elements in the fractionation analysis of soils is often used the single-step extraction by defined extracting reagents [2,3]. For the determination of elements content in the extracts methods of atomic spectrometry (AAS, OES) are applied.

At validation of the analytical method intended for determination of elements content in the extracts of fractionation analysis is necessary take account mainly absence of reference materials.

Contents of elements in the extracts are on the different concentration levels and depend on the way of elements binding in dust particles.

Experimental

A sample of the gravitation dust sediment from the locality Košice-city was used in experiments. The single–step extractions by using of 0.05 mol dm^{-3} Na_2EDTA (pH = 7, treated by NH_4OH) and 2 mol dm^{-3} HNO_3 (pH = 0.7) were carried out. Individual extractions procedures were realised by techniques used on a soil sample. To 0.5 g sample placed in 100 cm^3 polyethylene extracting vessel 75 cm^3 of extraction reagents was added in all experiments, besides of evaluation of robustness, and immediately shaken in mechanical shaker at temperature 20 ± 2 °C. At application of EDTA as extraction reagents the sample 1 hour was extracted and at application of HNO_3 the extraction time was 6 hours [4, 5]. The content of Cd, Cu, Pb, and Zn in the refiltered extract by flame atomic absorption spectrometer, the model SPECTR AA 400 Varian, was determined. For repeatability determination 10 repeated measurements of elements content in one extract were realized. For definition of limit of detection (LoD) and limit of quantitation (LoQ) the 10 repeated measurements of chosen elements in blank extract were carried out.

For the calculation of LoD and LoQ were used the formulas

$$x_{LoD} = \bar{x}_B + 3s_B \quad (1)$$

$$c_{LoD} = 3s_B / B \quad (2)$$

$$x_{LoQ} = \bar{x}_B + 10s_B, \quad (3)$$

$$c_{LoQ} = 10s_B / B \quad (4)$$

where $\boldsymbol{x_{LoD}}$ is the signal on the limit of detection, $\boldsymbol{c_{LoD}}$ is concentration on the limit of detection, $\boldsymbol{x_{LoQ}}$ is the signal on the limit of quantitation, $\boldsymbol{c_{LoQ}}$ is concentration on the limit of quantitation, $\boldsymbol{s_B}$ is the standard deviation of the blank, $\bar{x}_B$ is mean of blank, and $\boldsymbol{B}$ is slope of the calibration line.

The precision of the measurement by FAAS method was evaluated as repeatability and obtained from statistical evaluation of 10 repeated measurement of an analyte in the extract. The value of repetability represents the percentage relative standard deviations calculated according to formula [6]

$$RSD / \% = \frac{s}{\bar{x}} 100 \quad (5)$$

where $\boldsymbol{s}$ means standard deviation, $\bar{x}$ expresses mean value of concentrations.

At the testing of robustness of the FAAS method in the fractionation analysis was evaluated the effect of extracting time and extracting ratio on the recoveries of Cu and Pb in EDTA and HNO_3-extracts and the effect of pH EDTA solution too.

Results and Discussion

The values of LoD corresponding to the three-fold standard deviation of the blanks and LoQ corresponding to the ten-fold standard deviation calculated according to formulas 1 – 4 are listed in Table 1 and 2. The values of limits are lower for determination in HNO_3-extract, except of Zn.

The values of repeatability of measurement by FAAS method in extracts of both using extraction reagents are presented in Table 3. The high RSD values for determination of Cd in both

extracts refer to that the method of FAAS is not suitable on the determination of this element in extracts of the fractionation analysis.

Table 1. The values of LoD and LoQ calculated for determination in the EDTA-extract

Element	LoD		LoQ	
	x_{LoD} / μg.cm^{-3}	c_{LoD} / μg.cm^{-3}	x_{LoQ} / μg.cm^{-3}	c_{LoQ} / μg.cm^{-3}
Cu	0.0741	0.0142	0.1071	0.0473
Zn	0.1553	0.0263	0.2166	0.0878
Pb	0.1456	0.0816	0.3370	0.2723
Cd	0.0369	0.0480	0.1485	0.1599

Table 2. The values of LoD and LoQ calculated for determination in the HNO_3-extract

Element	LoD		LoQ	
	x_{LoD} / μg.cm^{-3}	c_{LoD} / μg.cm^{-3}	x_{LoQ} / μg.cm^{-3}	c_{LoQ} / μg.cm^{-3}
Cu	0.0341	0.0142	0.0671	0.0473
Zn	0.2232	0.0403	0.3173	0.1343
Pb	0.1193	0.0754	0.2946	0.2509
Cd	0,0163	0.0323	0.0915	0.1078

Table 3. The values of RSD representing repeatability of the FAAS method for determination of Cd, Cu, Pb, and Zn in EDTA and HNO_3 extracts

Element	Cu	Zn	Pb	Cd
Extraction reagents	Repeatability (RSD) / %			
EDTA	1.88	1.87	3.32	52.7
HNO_3	1.10	1.28	1.10	15.06

The robustness of the FAAS method for determination of Cu and Pb in the EDTA extract was tested by changes of the extraction time, the ratio value of the sample mass to volume of extraction solution (m : V), and pH of the extraction reagent. The results of this testing are presented in Table 4. For determination of Cu and Pb in HNO_3 the changes of extraction time and the ratio value of the sample mass to volume of extraction solution (m : V) were applied (Table 5).

Table 4. The effect of extraction time, extraction ratio, and pH of EDTA on the recovery of Cu and Pb in extracts

pH	Cu	Pb	Time/hours	Cu	Pb	m:V / g:cm^3	Cu	Pb
4	46.6	32.4	**1**	44.2	27.4	**0.5 : 25**	40.1	25.5
5	46.4	31.7	**3**	55.2	36.7	**0.5 : 50**	40.1	25.6
6	45.6	30.5	**6**	63.5	46.2	**0.5 : 75**	44.2	27.4
7	44.2	27.4						

Table 5. The effect of extraction time, and extraction ratio on the recovery of Cu and Pb in HNO_3 extracts

Time / hours	Cu	Pb	Ratio / g : cm^3	Cu	Pb
1	86.6	69.6	**2 : 20**	80.7	73.5
3	90.9	71.7	**0.5 : 25**	80.9	73.7
6	92.0	72.9	**0.5 : 50**	89.6	74.6
			0.5 : 75	92.0	72.9

Conclusion

In consideration of obtained results we can state that:

- The LoD and LoQ values for determination of Cd, Cu, and Pb in the HNO_3-extracts are lower than for their determination in the EDTA-extracts. The lowest LoD and LoQ values were determined for Cd in both extracts and the highest values of LoD and LoQ were calculated for Zn.
- On the base of the RSD values, which represent of repeatability of the FAAS method, it can be stated that this method is not suitable for the determination of Cd in the both extracts.
- The method is few robust concerning changes of extracting time (EDTA, Cu, Pb) and changes of extracting ratio (HNO_3, Cu).

Acknowledgements

This research was supported by projects VEGA No. 1/3149/06 and APVV No. 20-0094-04.

References

[1] Templeton D.M., Ariese F., Cornelis R., Danielsson L.G., Muntau H., Leeuwen H.P., van, Łobiński R.: Guidelines for therms related to chemical speciation and fractionation of elements. Definition, structural aspects, and methodological approaches. Pure Appl. Chem., 2000, 72, 8, 1453-1470

[2] Ure A.M., Davidson C.M., Thomas R.P.: Single and sequential extraction schemes for trace metal speciation in soil and sediment. Quality Assurance for Environmental Analysis, ed. Elsevier Science B. V., ch. 20, 1995, 505-523

[3] Makovníková J.: Distribution of Cd, Pb, Cu, and Zn in Soil and its evaluation considering on potentions and barriers of metal transport to plants. Research Institute of soil science and soil conservation, Bratislava, 2000

[4] Remeteiová D., Krakovská E., Tomko J., Sminčáková E., Vojteková V.: Extrakcia vzoriek prašných spadov EDTA. Sborník konference "Mikroelementy 2002", Slapy, 2002, 124-128

[5] Tomko J., Krakovská E., Remeteiová D. Sminčáková E.: Stanovenie mobilizovateľného obsahu niektorých kovov v prašnom spade 2M HNO_3. Sborník konference "Mikroelementy 2002", Slapy, 2002, 129-338

[6] Wenclawiak B.W., Koch M., Hadjicostas E.: Quality Assurance in Analytical Chemistry. Springer-Verlag Berlin Heidelberg, 2004

Influence of the Spectrochemical Additive on the Calibration Process at the Optimization of the Novel Spectrometric Tandem Technique

Silvia Ružičková and Mikuláš Matherny
Technical University of Košice, Faculty of Metallurgy, Department of Chemistry, Letná 9, 042 00 Košice, Slovakia, e-mail: silvia.ruzickova@tuke.sk

Abstract

Calibration possibilities of the novel spectrometric tandem technique with aerosol evaporation in the Marinković plasma source were investigated. It was worked with model oxide samples in the graphite matrix of the some chosen elements (Al, B, Ca, Cr, Fe, Mg, Ni, V), which have their environmental importance. Efficiency of the new tandem method was found out by the exploratory statistical analysis of the one-dimensional input data [3] and statistically evaluated by the statistical software QC. Expert^{TM} 2.5. Calibration without and with spectrochemical additives was studied. AgCl has shown as optimal spectrochemical additive. Importance of the concentration range, in term of linearity and precision, by using of synthetic matrix was studied too.

Key words: *tandem method, optimization, statistical evaluation, calibration*

Introduction

Optimization process of each new method requires to perform complete investigation of the calibration too. Calibration as one of the most important processes of the validation of method has two main aims: 1. determine mathematical function - calibration dependance; 2. obtain statistical information about analytical system (sensitivity, precision of the method,…). The given paper has the aim to focus attention on the investigation of the calibration possibilities of the novel spectrometric tandem technique, which is based on the sample evaporation in the controlled high-energy direct current (DC) arc in the special quartz cell and following excitation in the stabilized Marinković plasma source [1,2]. Using of high-energy and computer-controlled DC arc as evaporation source enables to achieve on the top of the carrier electrode a temperature of about 4000 ^{o}C. This temperature provide for destruction and total combustion (evaporation) of the powder sample. From this reason, first experiments were oriented to the calibration without spectrochemical additives.

Experimental

The experiments were carried out using an LECO-750 simultaneous spectrometer with quartz-fibre optic, a computer-controlled DC arc DCA-301 evaporation source with optimized ramping of the

arc current and Marinković plasma excitation source. Experimental arrangement is in detail described in several previous publications [1, 2] as well as parameters of the used spectral lines [3]. Experimental conditions are summarized in Table 1.

Table 1. Experimental conditions

General conditions	
Spectrometer	LECO – 750, simultaneous
Grating	2400 lines per 1 mm
Spectral range	220 – 766 nm
Evaporating conditions	
Evaporation source	controlled direct current arc, generator DCA 301
Current of the DC arc	20 A, 15 A
Carrier electrode	SW 380, Elektrokarbon Topoľčany
Counter electrode	SU 206, Elektrokarbon Topoľčany
Distance between electrodes	1 mm
Concentration of the additive	9.3 mg cm^{-3} $AgNO_3$
Sample	0.06 % oxide mixture of the chosen elements
Sample amount	10 mg
Primary Ar flow	2.8 dm^3 min^{-1}
Secondary Ar flow	2 dm^3 min^{-1}
Excitating conditions	
Excitation source	Marinković plasma source
Current intensity	11 A
Display and evaluating conditions	
Display plasma area	r = 3.5 mm
Distance between lens and plasma	130 mm
Distance between optical fibre and lens	165 mm
Time of exposition	60 s, 42 s
Software	SPECTRUMAT

High-purity argon was used as optimal plasma transport gas. AgCl as spectrochemical additive was prepared directly in the carrier electrode following reaction:

$$AgNO_3 + HCl \rightarrow \mathbf{AgCl} + HNO_3 \quad (1)$$

Into each empty graphite carrier electrode of type SW 380 was added 10 μl of $AgNO_3$ (c = 9.3 mg cm^{-3}) and 10 μl of conc. HCl. After a one-hour drying at 105 ^oC were electrodes filled with 10 mg of 0.06 % oxide mixture of the above mentioned elements (J. Mathey, London) and graphite powder (Elektrokarbon, Topoľčany).

Results and discussion

It is necessary before investigation of the calibration experimentally verify the efficiency of the selected spectral lines and so find out performance of the novel tandem method. From this reason, the exploratory analysis of the one-dimensional input data of the obtained sets of intensities was performed by means of statistical software QC. Expert^{TM} 2.5. [4]. In most cases (Al, Fe, Mg, Ni, V) was confirmed normal and homogeneous distribution of intensities of the selected spectral lines and given spectral lines could be used for optimization of the calibration [3].

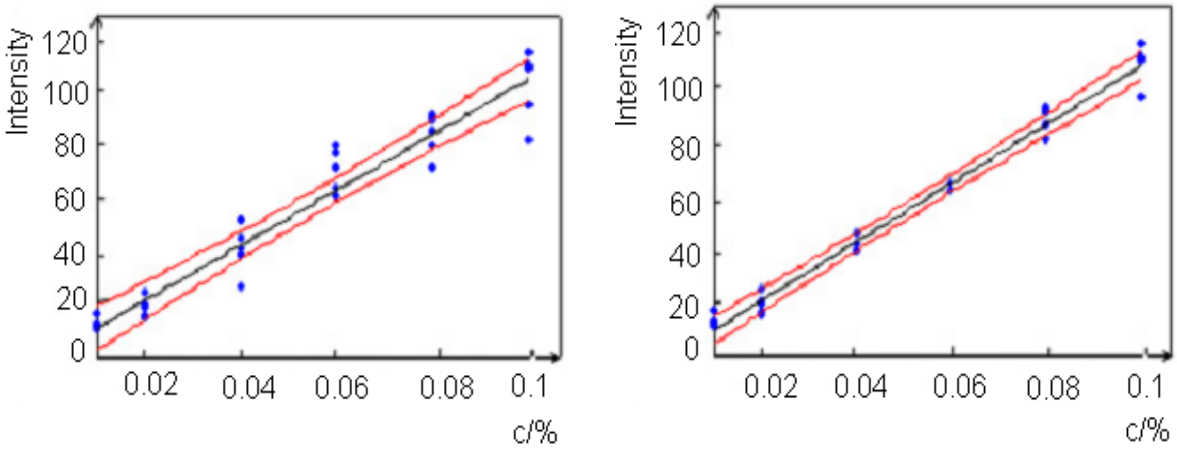
Analytical line was constructed by least-squares method by above mentioned statistical software and in accordance with data in literature [5]. At first, it was necessary to decide about concentration

range $\Delta c \in \langle c_{min}, c_{max} \rangle$ and then compare analytical lines, which were constructed from the original number as well as from the reduced number of the calibrating intensity values without leverages and outlayers. It were investigated two concentration ranges: $\Delta c \in \langle 0.01; 0.1 \rangle$ % and $\Delta c \in \langle 0.005; 0.1 \rangle$ %. The wider concentration range and exclusion of the leverages and outlayers proved obtained data summarized in Table 1. In comparison with original data it was improved precision of the method, LOD value, coefficient of determination R is rather high, about 98 % and they were achieved narrow confidence lines (Fig.1).

Table 1. Comparison of main figures of merit for calibration lines of Mg values

Element		Mg_0	Mg_1
Wavelength	λ / nm	280.270	280.270
Data number	N	29	21
Model		linear	linear
Fitness		suit	suit
Abs. member	A(x)	1.00	-1.05
St. deviation	$s_{A(x)}$	3.39	2.04
Test abs.	$t_{A(x)} = 0$	+	+
Sensitivity	B(x)	1058.59	1098.35
Min. conc.	c_{min} / %	0.010	0.005
Max. conc.	c_{max} / %	0.1	0.1
Limit of detection	c_L / %	0.009	0.005
Coef. deter.	$R = r^2 .100$	94.09	98.01

Mg0 – origin data; MgI – „reduced“ data



a) b)

Fig. 1. Calibration line for Mg a) origin set of I_{Mg} values, b) reduced set of I_{Mg} values

At first, the analytical calibration was performed at DC arc of 20 A and without spectrochemical additive for concentration range $\Delta c \in \langle 0.01; 0.1 \rangle$ %. In order to improve effect of evaporation and so intensity of signals, influence of spectrochemical additive was studied too. Because of prevention of complication with powder sample preparation (mixture with spectral buffer), investigation of the AgCl influence on the evaporation was preferred. Reaction (1) expresses creation of AgCl, which is during evaporation thermic decomposed into recombined atomic chlorine and silver. At spectra excitation was used not only „holder“ effect of AgCl but „chloric“ effect of released atomic chlorine too. Obtained results confirmed meaning influence of the AgCl on increasing of intensities of all spectral lines (Fig. 2). By this manner the volatility of the given elements has increased that finally has a positive influence not only on the limit of detection but on the precision of method too. All other

experiments were exclusively carried out with AgCl additive and original exposition time of 60 s was abbreviated to 42 s at DC arc of 15 A.

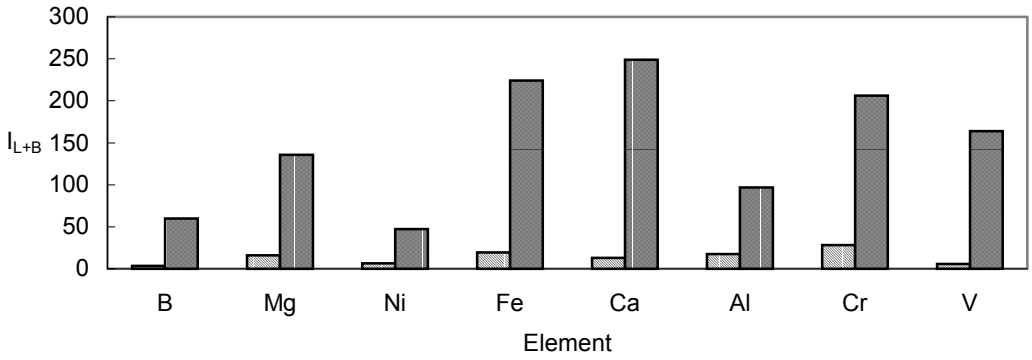
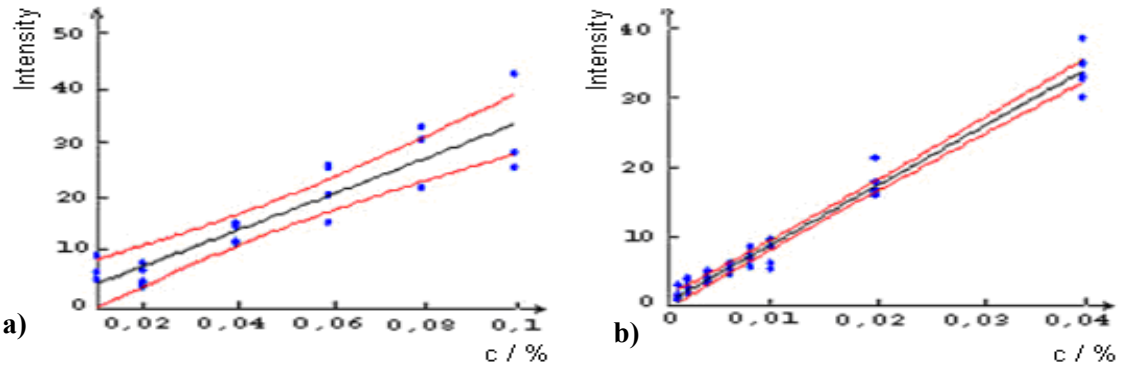


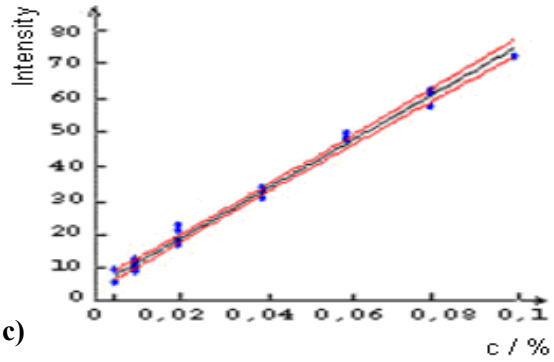
Fig. 2. Comparison of the influence of additive on the brutto signal of analytes; ▨ without AgCl and ▩ with AgCl additive

Since given method should be used for ceramics and environmental samples analysis, where is needed to determine trace concentrations of analytes, analytical calibration with AgCl additive was performed for two concentration ranges with lower concentrations: $\Delta c \in \langle 0.001; 0.04\rangle$ % and $\Delta c \in \langle 0.005; 0.1\rangle$ %. Comparison of some chosen figures of merit for calibration without and with AgCl additive is given in Table 2. Statistical evaluation of the analytical lines construction proved, that linear model was according to the literature [5] confirmed for all elements in the all three concentration ranges. Calibration was valid for whole concentration range and coefficient of determination R was in the interval $R \in \langle 96; 98\rangle$ % in the case of calibration with AgCl additive. LOD values as well as precision $s_{A(X)}$ values for $\Delta c \in \langle 0.005; 0.1\rangle$ % get worsen but area defined by the limits of confident was in the both cases wery narrow, that guarantees high precision of the calibration. Mentioned numeric data represent diagrams for Fe in Fig. 3. At majority of elements were achieved either more favourable or identical results, except from Ni and V, where the quadratic model was confirmed.

Table 2. Comparison of some figures of merit at the calibration

Calibration		
without AgCl	**with AgCl**	
$\Delta c \in \langle 0.01; 0.1\rangle$ %	$\Delta c \in \langle 0.001; 0.04\rangle$ %	$\Delta c \in \langle 0.005; 0.1\rangle$ %
linear model	linear model	linear model
$c_L \in \langle 0.009; 0.08\rangle$ %	$c_L \in \langle 0.0009; 0.01\rangle$ %	$c_L \in \langle 0.001; 0.005\rangle$ %
$s_{A(X)} \in \langle 0.20; 1.18\rangle$	$s_{A(X)} \in \langle 0.11; 0.67\rangle$	$s_{A(X)} \in \langle 0.25; 1.60\rangle$
$R \in \langle 82; 86\rangle$ %	$R \in \langle 96; 98\rangle$ %	$R \in \langle 96; 98\rangle$ %





c)

Fig. 3. Calibration lines for Fe; a) $\Delta c \in \langle 0.01; 0.1 \rangle$ % without AgCl; b) $\Delta c \in \langle 0.001; 0.04 \rangle$ % with AgCl; c) $\Delta c \in \langle 0.005; 0.1 \rangle$ % with AgCl

Conclusion

Using of spectrochemical additives is effective way of optimization of analytical calibration. In our particular case, using of AgCl, which was created only in the carrier electrode during sample evaporation, proved to be an effective stability factor. In the optimal concentration range $\Delta c \in \langle 0.001; 0.04 \rangle$ % was achieved needed linearity, matrix effect was uniform and coefficient of determination, which express how many calibrating data are in the tested range was about 98 %. Limit of detection value sometimes decreased to 0.0009 % but in average was in the interval $c_L \in \langle 0.005; 0.001 \rangle$ %.

References

[1] Ružičková S., Koller L., Matherny M.: *Chem. Pap., 2006, **60(2)**, 116-121*
[2] Ružičková S., Bajuszová J., Matherny M., Koller L.: *Chem. Listy, 2005, **100**, 348-356*
[3] Ružičková S., Matherny M.: *Trans. Univ. Košice, 2006, **1**, 41-45*
[4] Kupka K.: *QC. Expert™ Statistical software*. TriloByte, Pardubice, 2002
[5] Meloun M., Militký J.: *Statistické zpracování experimentálních dat,* Ars magna, Praha, 1998

Acknowledgement

This work was supported by the DAAD project No. 11/2005, grant project VEGA No. 1/3149/06 and APVV project 20-009404. The authors are obliged to express their gratitude for this support.

Surface-Enhanced Raman Spectroscopy on Single Molecule Level: Perspectives of Analytical Applications

B. Vlčková, M. Moskovits[2], I. Pavel[2], M. Sládková, K. Šišková, P. Šmejkal, M. Šlouf [3]
Department of Physical and Macromolecular Chemistry, Charles University, Hlavova 2030, 12840 Prague 2, Czech Republic
[2]Department of Chemistry and Biochemistry, University of California at Santa Barbara, Santa Barbara, CA 93106 USA
[3]Institute of Macromolecular Chemistry ASCR, Heyrovsky Sq.2, 16206 Prague 6, Czech Republic

Abstract

Recent challenging development in SERS and SERRS spectroscopy is associated with the discovery of its single molecule sensitivity. Achievement of single molecule level of SERS (Surface-Enhanced Raman Scattering) and/or SERRS (Surface-Enhanced Resonance Raman Scattering) spectral detection is conditioned by localization of detected molecules into strong nanoscale-localized optical fields (dubbed hot spots). Recent theoretical treatments predict SERS enhancement factors as high as 1 x 10^{15} for some chromophoric molecules located in the hot spot amidst two Ag nanoparticles. Owing to a combination of single molecule sensitivity with the fingerprint selectivity inherent to methods of vibrational spectroscopy, single molecule SERS spectroscopy offers unprecedented possibilities of analytical applications.

Keywords: *Surface-Enhanced Raman Scattering (SERS)--Surface-Enhanced Resonance Raman Scattering (SERRS)—single molecule SERS—Ag nanoparticle dimers—molecular linkers*

Introduction

Surface-Enhanced Raman Scattering (SERS) process takes advantage of a simultaneous interaction of electromagnetic radiation with plasmonic metal nanoparticles and/or nanostructures and with molecules located on their surfaces (Fig.1). The electromagnetic (EM) mechanism is the principal mechanism of SERS and is based on resonance Mie scattering of light by the plasmonic metal (such as Ag and Au) nanostructures and/or nanoparticles. In SERS, both the incident light and the light inelastically (Raman) scattered by molecules are enhanced by this process, provided that the wavelength of the incident light fulfills the Mie (dubbed also EM) resonance condition for the particular nanostructure and/or the nanoparticle assembly [1]. In this paper, we focus on systems with Ag nanoparticles which are most frequently employed in single molecule SERS. In such systems, the EM mechanism enhancement factors range between 10^4 and 10^{11}, in dependence on the morphology of Ag nanoparticle assembly, localization of molecules within the assembly and excitation wavelength selected for SERS experiment. The largest SERS enhancements are experienced by molecules located in special locations (dubbed hot spots) within assemblies of closely-spaced Ag nanoparticles, in particular large fractal aggregates [2,3] and/or dimers and small aggregates of Ag nanoparticles [4].

Molecular resonances contribute to the overall SERS enhancement provided that their resonance condition is fulfilled simultaneously with the EM resonance condition. Two types of molecular resonance contribution are usually considered. In the case of SERRS (Surface-Enhanced Resonance Raman Scattering), the target molecule is a chromophore with respect to the incident light wavelength. Nevertheless, when evaluating the molecular resonance contribution to SERRS, one has to take into account the actual structure of the chromophoric molecule in the SERS-active system, which can be perturbed by Ag surface-target molecule interaction. For example, free base porphyrins become frequently metallated when directly adsorbed on Ag nanoparticle surface [5,6]. Chemical mechanism contribution to the overall SERS enhancement is conditioned by formation of a Ag surface-target molecule surface complex and by the match between the wavelength of incident light and that of a photoinduced charge transfer transition excited in the surface complex. An example of such originally non-chromophoric molecule is 2,2'-bipyridine (bpy), which (when adsorbed on chloride-modified Ag nanoparticle surface) forms an Ag-bpy surface complex, in which a photoinduced charge transfer can be excited at ~540 nm [7].

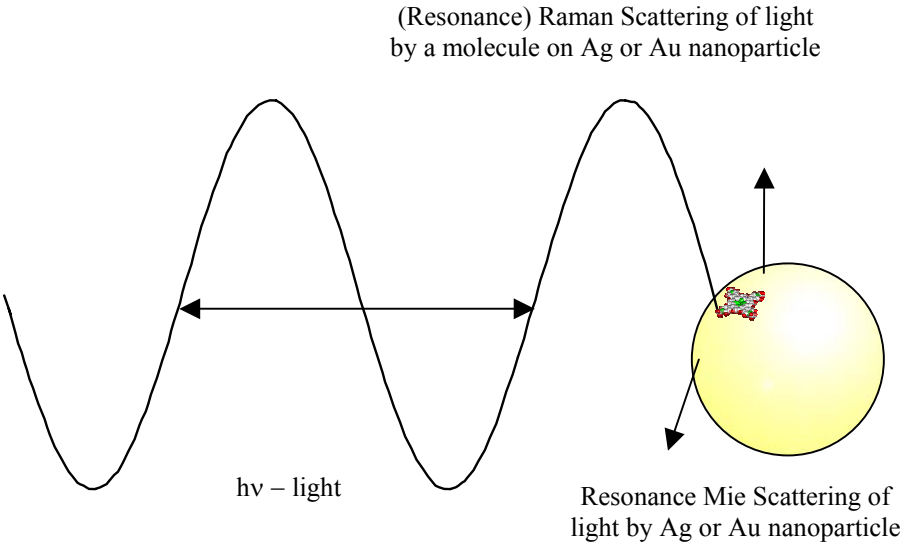


Fig. 1. Schematic depiction of surface-enhanced Raman scattering

SERS on single molecule level: theory and experiment

Single molecule SERS has been reported for the first time by K. Kneipp et al. [8]. In her experiments with highly diluted sol of small Ag nanoparticle aggregates and 10^{-14} M solution of cresyl violet she created a system in which there was on average 0.6 molecules per aggregate. She observed that the SERS signal from this system showed temporal fluctuations. When she made a histogram of signal intensities for a particular Raman band, she realized that instead of a Gaussian profile (obtained for more concentrated systems) she obtained a Poisson distribution of signal intensities, which was attributed to acquisition of the signal from a empty aggregate and from aggregates containing one, two or three molecules [8]. K. Kneipp's results immediately evoked a question whether single molecule SERS and SERRS are explicable by a combination of the EM and molecular resonance mechanisms, or whether there is some new mechanism which operates in these systems. The answer was provided chiefly through calculations of M. Kall and coworkers, who have shown that, indeed, for a molecule located in a hot spot amidst two Ag nanoparticles and excited by light polarized parallel to the dimer axis, the EM mechanism enhancement can be of 1×10^{11} [4]. In

addition to that, observation of temporal fluctuations of the SERS signal has been interpreted as manifestation of a dynamic behavior of a single, or of very few molecules. Localization of molecules into hot spots thus became an important task in single molecule SERS.

SERS from molecularly-bridged Ag nanoparticle dimers

Our research on this field [9] was driven by an idea that an efficient way how to localize molecules into hot spots is to make them to bridge two Ag nanoparticles. Our goal was to measure SERS signal form a selected single molecularly-bridged Ag nanoparticle dimer identified by transmission electron microscopy (TEM). The components from which the dimers were assembled were Ag nanoparticle stabilized by citrate ions and molecules with two amine groups in para-position, namely 4,4'-diaminoazobenzene and 4,4''- diaminoterphenyl. As the supporting surfaces for dimer assembling, we employed SiO_x coated finder grids for TEM, which we functionalized by 3-aminopropyltrimethoxysilane. Nanoparticle assembling was then accomplished in three steps: (1) attachment of particles to functionalized grid surface (2) edge-on adsorption of linker molecules (3) attachment of additional particles to free amine groups of the linkers. TEM images have shown a preferential formation of dimers and trimers (lying flat on the supporting surface). SERS spectral mapping was employed for localization of strong signals, TEM imaging for visualisation of nano-objects providing these signals. As examples, TEM image of a selected single dimer and SERS signal obtained from it are shown in Figs. 2 and 3, respectively. SERS signal collected from this particular dimer in 1s intervals at 514.5 nm excitation shows temporal fluctuations in which "signal on" – "signal off" pattern can be distinguished. The "on-signal" consists of characteristic SERS spectral bands of 4,4'-diaminoazobenzene, while the "off-signal is constituted by a broad background. The "on-signal" is tentatively attributed to the actual 4,4'-diaminoazobenzene-bridged dimer, while the off-signal can possibly correspond to the situation when one of the Ag-amine bond is broken. In that case, the interparticle distance is enlarged, and the conditions for the EM resonance may no longer be obeyed. Resuming of the Ag-amine bonding can then lead to the linker signal re-appearance [9].

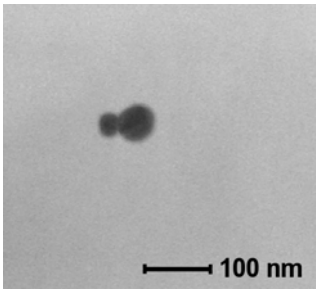


Fig. 2. TEM image of 4,4'-diaminoazobenzene-bridged Ag nanoparticle dimer

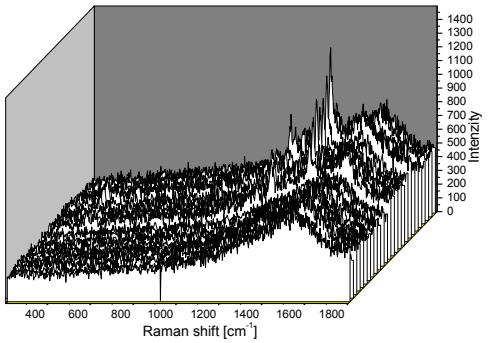


Fig. 3. Time-evolution of SERS-signal from the 4,4'-diaminoazobenzene-bridged Ag nanoparticle dimer depicted in Fig. 2

Perspectives of analytical applications

In single molecule SERS, we have, on one hand, a tremendous sensitivity, while, on the other, very high demands on the selectivity of detection. A problem frequently encountered in single molecule SERS is interference of graphitic carbon signal, which can originate from the thermal and/or photochemical decomposition of the target molecule(s). Since photochemistry is largely enhanced in

strong optical fields, photoproducts can be expected as contaminants preventing detection of target molecule signals. Future research on single molecule SERS will most probably be focused first on overcoming this as well as some other drawbacks by inventing new advanced approaches to Ag nanoparticle assembling and new pathways towards localization of molecules into hot spots. It is now probably too early to predict if taking the step from ensemble averaged systems (with improved performance based on the inventions mentioned above) to single molecule ones will actually be meaningful for practical spectroanalytical applications, as we know them these days. Nevertheless, it is quite probable that once we design and fabricate single molecule devices, we will have to detect a single molecule and monitor its function.

References

[1] Moskovits M.: Surface-enhanced spectroscopy. *Rev. Mod. Phys. 1985, 57, 783-826*
[2] Stockman M.I., Shalaev V.M., Moskovits M., Botet R., George T.F.: Enhanced Raman scattering by fractal cluster-scale-invariant theory. *Phys. Rev. B 1992, 46, 2821-2830*
[3] Zhang P., Haslett T.L., Douketis C., Moskovits M.: Mode localization in self-affine fractal interfaces observed by near-field microscopy. *Phys. Rev. B 1998, 57, 15513-15518*
[4] Xu H., Aizpurua J., Kall M., Apell P.: Electro-magnetic contributions to single-molecule sensitivity in surface-enhanced Raman scattering. *Phys. Rev. E 2000, 62, 4318-4324*
[5] Vlckova B., Matejka P., Simonová J., Pancoska P., Cermakova K., Baumruk V.: Surface-enhanced resonance Raman spectra of free-base 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin and its silver complex in systems with silver colloid: direct adsorption in comparison to adsorption via molecular spacer. *J. Phys. Chem.*, *1993, 97, 9719-9729*
[6] Sladkova M., Vlckova B., Mojzes P., Slouf M., Naudin, C., Le Bourdon G.: Probing strong optical fields in compact aggregates of silver nanoparticles by SERRS of protoporphyrin IX. *Faraday Discuss*. *2006, 132, 121-134*
[7] Srnova I., Vlckova B., Snoeck T.L., Stufkens D.J., Matejka P.: Surface-enhanced Raman scattering and surface-enhanced resonance Raman scattering excitation profiles of Ag-2,2'-bipyridine surface complex and of [Ru $(bpy)_3$] on Ag colloidal surfaces: manifestations of charge-transfer resonance contributions to overall surface enhancement of Raman scattering. *Inorg. Chem. 2000, 39, 3551-3559*
[8] Kneipp K., Wang Y., Kneipp H., Perelman L.T., Itzkan I., Dasari R. R., Feld, M.S.: Single molecule detection using surface-enhanced Raman scattering. *Phys.Rev.Lett. 78, (1997) 1667-1669*
[9] Vlckova B., Moskovits M., Pavel I, Sladkova M, Siskova K., Slouf, M.: Surface-enhanced Raman scattering from a molecularly-bridged silver nanoparticle dimer. *Langmuir-submitted*

Fast Monitoring of the Element Mobility Changes in Environmental Pollution

[1]Viera Vojteková, [2]Jarmila Nováková, [2]Daniela Mackových
[1]University of P. J. Šafárik, Institute of Chemical Science, Department of Analytical Chemistry, Moyzesova 11, SK - 041 67 Košice, Slovakia, e-mail: vojtekv@kosice.upjs.sk
[2]State Geological Institute of Dionýz Štúr, Geoanalytical Laboratories, Markušovská cesta 1, SK- 052 40 Spišská Nová Ves, Slovakia, e mail: mackovych@gsrcsnv.sk

Abstract

The mobility of elemental contaminants through the environment is basic information in terms of environmental protection. The evaluation of contaminant mobility in water solutions is realised through the isolation and identification of organic and inorganic elemental species, where it is possible to use complexing agents. For evaluation of the mobility and potential mobility of the elements in other environmental parts (e.g., soils, sediments, sediment dusts, etc.) fractionation analysis is usually used and obviously is realised in the form of sequential extraction. The extraction into a complexing agent could be a solution for the selective separation of elemental species and a simpler alternative for investigating the mobility or potential mobility of elements in the individual segments of the environment. Through single-step extraction into a suitably chosen strong complexing agent, it is theoretically possible to release the total non-residual fraction of the element from the source [1]. This method offers the possibility of combining (partially substituting) periodical tests of sedimentary and soil systems by sequential and simple extractions, into suitably selected complexing agents.

Keywords: *single and sequential extraction – fractionation – complexing agents – environmental pollution – sediments, spectro-analytical methods*

Introduction

Single-step extraction into a strong complexing agent could be used as an economical and time saving supplementary test to the sequential extraction procedures recommended and attested by IRMM (Institute for Reference Materials and Measurements). Thus, extraction into a complexing agent could be a rapid means of assessing sources and characterising contamination inputs into the environment (soils, sediments, water solution, biotope); a means of gathering preliminary information about the lithogenic or anthropogenic origin of the pollution; a way of checking the levels and simulation of the input of mobile pollutants in the water biota in a incomparably shorter time and with lower financial costs than an extraction sequence (by using single step extraction into complexing agent) [2-4].

Single and sequential extraction procedures were applied to sediment samples collected from an industrially polluted region. A sequential extraction procedure (SEP) recommended by the Institute for Reference Materials and Measurements (IRMM) was modified and used as a reference extraction

method. The single-step extraction with 0.05 mol dm^{-3} ethylenediaminetetraacetic acid (EDTA) was slightly modified for this study. The ability of "Na_2EDTA extraction" to remove elements was compared with the reference SEP. After optimization, the content of the elements extracted by Na_2EDTA were in good agreement with the sum of the first, second, third and alternatively fourth steps of the SEP for all studied elements [2-5]. In this paper the comparison of the analytical results of single and sequential extraction and comparison of these results with total content analyses (by XRF) for non-published macro-elements are presented. It deals about Ca, Mg and Fe. The details of the sample preparation were described in the former publications [6].

Experimental

Chemicals and reagents

All stock standards and chemicals were obtained from the Sigma-Aldrich group and Merck (Darmstadt, Germany) and Na_2EDTA was of analytical - reagent grade was used. Distilled water was cleaned by reverse osmosis and by ion exchanger.

Preparation of the extractant solutions

Single-step extraction procedure: 0.05 mol dm^{-3} solution of Na_2EDTA was prepared. The acidity of the solution was changed within pH 3 and 7 and was corrected directly in extraction vessel. The untreated 0.05 mol dm^{-3} Na_2EDTA solution had a pH = 4.7.

Sequential extraction procedure: During sequential extraction the following solutions were used: Solution A: 0.11 mol dm^{-3} acetic acid (HOAc); solution B: 0.1 mol dm^{-3} hydroxylammonium chloride ($HO\text{-}NH_2.HCl$); solution C: Hydrogen peroxide (H_2O_2): Concentrated H_2O_2 was acidified with nitric acid solution: water - 1:1 (v/v) to a pH value between 2 and 3. Solution D: 1 mol L^{-1} ammonium acetate ($NH_4\,OOC\text{-}CH_3$).

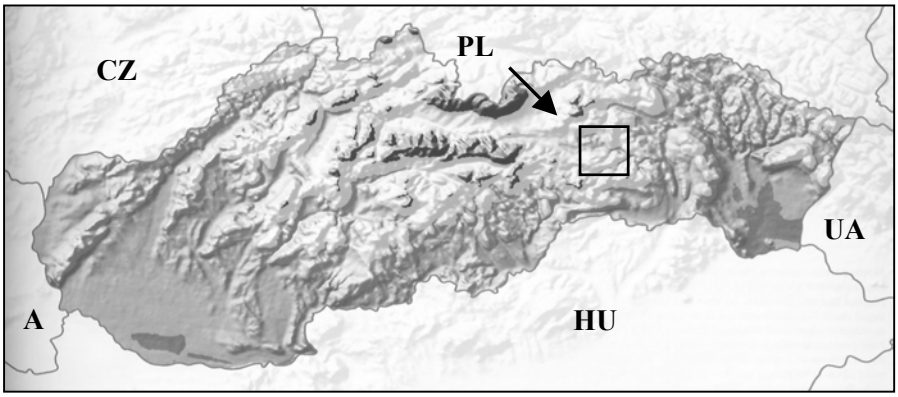


Fig. 1. Topographical location of the sampling area in Slovakia; CZ - Czech Republic, PL- Poland, UA- Ukraina, HU- Hungary, A- Austria

Collection and pretreatment of the sediment samples

Topographical identification of the chosen sampling region is given in Fig.1. There were chosen four sampling places (on the rivers Hornád, Hnilec and Poráččsky jarok) for collecting of four pilot sediment samples. The collection of the samples was done in accordance with the Methodical Instruction of the Slovak Ministry of Environment [7]. Sediments were dried at 40° C, and passed through a 0.125 mm sieve. Only the size fraction equal or lower than 0.125 mm was used for further mechanical treatment. It was milled in agate planetary treadmill to grain size under 0.09 mm. 0.5 g portions of homogenized samples were weighted for the assessment of extractable element content of studied elements.

Single-step extraction with 0.05 mol dm^{-3} Na_2EDTA: The original protocol for 0.05 mol dm^{-3} EDTA extraction of soils was optimised for sediments from the studied region. Optimised time was 6 hours, optimised solid sediment/solution - extraction ratio was 1:150, pH of the used extraction medium was 4.7. The original and modified extraction parameters are given in Table 1.

Table 1. Standard and modified procedure for single extraction into 0.05 mol dm^{-3} EDTA -salt

Standard procedure		Modification of standard procedure
extraction agent	$(NH_4)EDTA$	Na_2EDTA
time of extraction	1 hour	1-6 hours, without pH modification, pH = 4.7
temperature	20±2 °C	20±2 °C
extraction ratio	1:10	1:50, 1:100, 1:150
acidity of extraction agent	pH = 7 (modified with NH_4OH)	pH = 3 - 7 (1 hour- extraction during optimisation of pH, modified with HCl, NH_4OH)
Extraction vessel	250 cm^3	
Filtration	„paper with blue stripe“ Θ = 18.5 cm	

Procedure: All details of experimental treatment of the samples are described in the reference [6].

Sequential extraction procedure: Each step of the sequential extraction was performed in a mechanical end-over-end shaker for 16 hours (number of vibrations 200 min^{-1}, temperature 20±2° C). After finishing the extraction, the solution was centrifuged at 4000 rpm for 20 minutes. Solutions obtained from the individual extraction steps were saved in polyethylene vessels at a temperature of 4° C. Extractable portions of the chosen elements were determined form the solutions prepared in described way. Results were obtained as the average values of 5 repeated extractions. The extraction parameters of the sequential extraction are given in Table 2.

Procedure: Is analogical like in the reference [6].

Table 2. Applied sequential extraction scheme in comparison with original IRMM 3-step sequential extraction recommended for sediments

Fraction	Extracted phase	“IRMM extraction”	“Modified IRMM extraction”- applied
1	water-soluble	--	distilled water, pH≈6.7
2	Adsorbed, exchangeable and bound on carbonates	0.11 mol dm^{-3} CH_3-COOH; 16 hours	0.11 mol dm^{-3} CH_3-COOH; 16 hours
3	Bound on Fe and Mn oxides	mol dm^{-3} HO-NH_2. HCl; 25 % CH_3-COOH; pH=2; 16 hours	0.1 mol dm^{-3} HO-NH_2. HCl; pH=2 (pH corrected with HNO_3); 16 hours
4	Oxidisable-organic and sulphides	8.8 mol dm^{-3} H_2O_2; 85°C; 3 hours; 1 mol dm^{-3} CH_3-$COONH_4$; 16 hours	8.8 mol dm^{-3} H_2O_2; 85°C; 1 mol dm^{-3} CH_3-$COONH_4$; 16 hours
5	Residual	--	total decomposition – HNO_3, HF, $HClO_4$;

Analytical procedures and apparatus

Atomic emission spectrometry with inductively coupled plasma was used for the determination of calcium, magnesium and iron in each leached fraction. The measurements were carried out on a Varian spectrometer Liberty 200 with Cetac ultrasonic nebulizer. Experimental conditions of ICP OES measurements are listed in Table 3. X-ray fluorescence spectrometry was applied for the determination of total element contents in the studied fluvial sediments and measurements were performed on a Spectro X-LAB 2000 spectrometer. Experimental conditions of XRF measurements are described in followed references [8].

Table 3. Experimental conditions for ICP – OES measurements

Element	Wavelength (nm)	Correction
Ca	317.933	dynamic
Mg	279.079	dynamic
Fe	259.940	dynamic

Results and Discussion

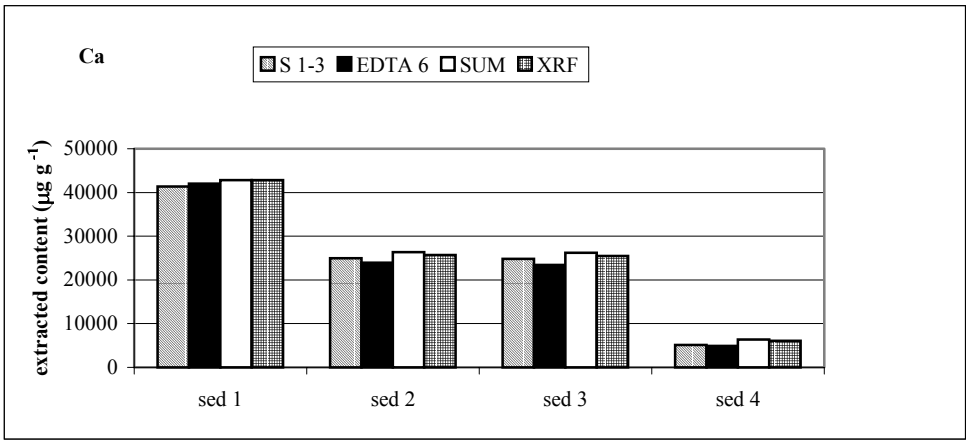
The determination of Ca, Mg and Fe in four pilot sediment samples could be discussed as follows: Testing of the accuracy of the Ca, Mg and Fe single and sequential extractions brought acceptable results. The obtained recoveries range between 80 and 105 %. This is documented in Table 4, which shows:

Table 4. Results of the total content analysis and accuracy testing of the analyses

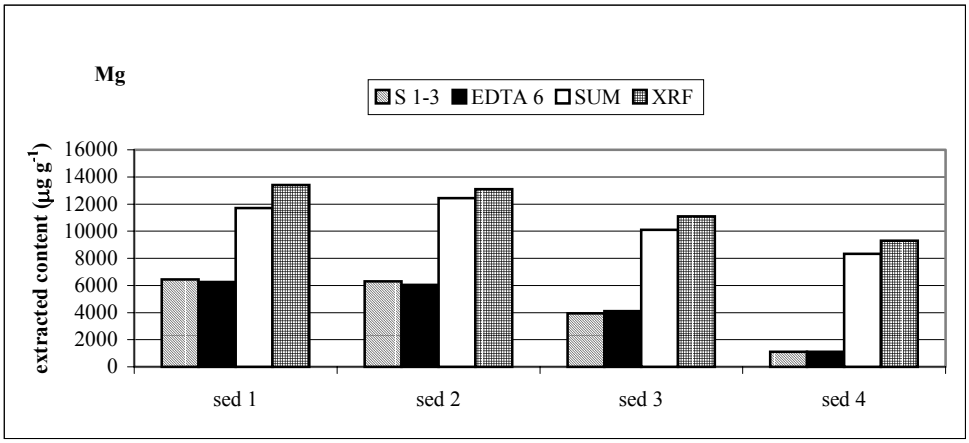
element		sediment 1	sediment 2	sediment 3	sediment 4
calcium	Sum (mg dm^{-3})	42811±550	26365±620	26222±620	6400±230
	EDTA Sum (mg dm^{-3})	42760±550	25450±620	25180±620	6361±230
	XRF (mg dm^{-3})	42800±190	25700±160	25500±160	6100±125
	Recovery XRF-SEP (%)	100	103	103	105
	Recovery XRF-EDTA (%)	100	99	99	104
magnesium	Sum (mg dm^{-3})	11701±390	12429±380	10096±370	8331±340
	EDTA Sum (mg dm^{-3})	11950±390	12750±380	9960±370	8790±340
	XRF (mg dm^{-3})	13400±210	13100±180	11100±190	9300±170
	Recovery XRF-SEP (%)	87	89	91	90
	Recovery XRF-EDTA (%)	89	97	90	95
iron	Sum (mg dm^{-3})	25396±635	48957±489	30740±769	45031±450
	EDTA Sum (mg dm^{-3})	27436±686	48310±483	32807±820	48155±482
	XRF (mg dm^{-3})	31700±159	48400±242	35700±178	50800±254
	Recovery XRF-SEP (%)	80	101	86	89
	Recovery XRF-EDTA (%)	87	100	92	95

a) The recoveries achieved by comparison of the sum of element contents obtained at the individual extraction steps of sequential extraction (labeled as "Sum") and total content analysis – realised by XRF. Recoveries are labeled as "Recovery XRF-SEP".

a)



b)



c)

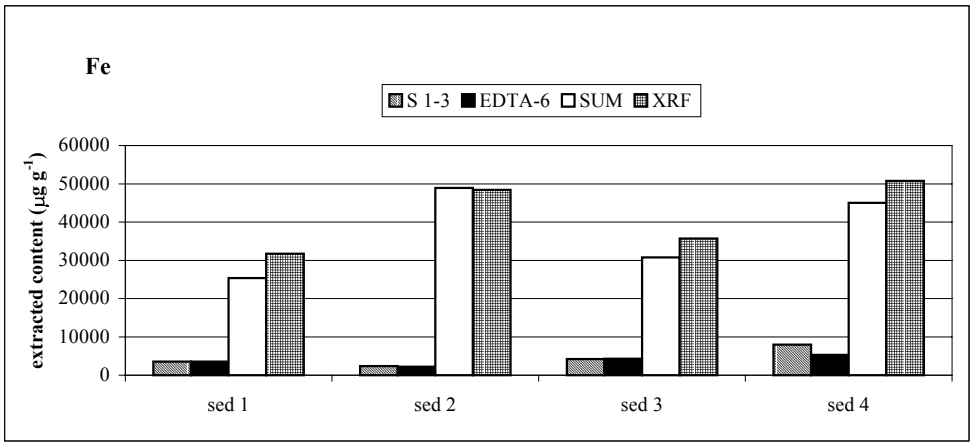


Fig. 2. Comparison single extraction, sequential extraction and total content analysis (by XRF) of Ca (a), Mg (b), and Fe (c)

S 1-3: sum of the 1st, 2nd and 3rd step of the five-step SEP;
EDTA-6: six hours extraction into 0.05 mol dm^{-3} Na_2EDTA;
SUM: sum of the 1st, 2nd, 3rd, 4th and 5th step of the five-step SEP;
XRF: total content analysis - control of accuracy of SEP

b) The recoveries achieved by comparison of the sum of the content extracted into Na_2EDTA and content present in the mineralised sediment residue after "Na_2EDTA extraction" and total content analysis – realised by XRF. Recoveries are labeled as "Recovery XRF-EDTA".

The determination of total element contents by X-ray fluorescence spectrometry was used like reference method (of total content determination) for the accuracy control. Results of the XRF analyses are given in the table 4. All results are presented with their expanded uncertainties, calculated as the sum of the uncertainty components with the coverage factor k=2.

$$U = 2\,u_c, \qquad u_c = \sqrt{u(p)^2 + u(q)^2 + \ldots} \tag{1}$$

Results of the optimisation of the single–step extraction were presented in the former papers [4,6] for heavy metals and they are analogical for macro-elements too: optimised extraction ratio is 1:150 and optimised extraction time is six hours, pH of the extraction medium was 4.7.

Regarding the comparison of single and sequential extraction procedures with respect to the removal ability of Na_2EDTA, it is possible to make the following statements: after optimisation, the single step extraction of all studied elements using Na_2EDTA is in good accordance with the sum of the first, second and minimally third steps of the SEP. For the studied sediment samples, extraction efficiency is element specific and depends on the composition of the sediment matrix. From the sediment matrix of the studied sampling area, the single step procedure (with 0.05 mol dm^{-3} Na_2EDTA) enables the extraction of potentially mobile forms corresponding to the sum of first three extraction steps of the SEP, see Fig. 2. For the extraction of the Fe (and Mn) portions responding to the fourth extraction step of the SEP, oxidation conditions are necessary for the release of the metal portions in their sulphide forms. Therefore, the Na_2EDTA extraction medium (under optimised conditions) is able to release the most environmentally important reducible sediment phase and could also release all elemental contaminants associated with Fe and Mn oxides. The extractability of Fe (and Mn) into 0.05 mol dm^{-3} Na_2EDTA in the studied region could reflect the mobility of other trace (all contaminating) elements of sediments and soils too. The behaviour of calcium, magnesium and iron in the extraction solution of the 0.05 mol dm^{-3} Na_2EDTA is analogical like behaviour of the other elements in sediment samples of this region [2-5,8]. Following these facts is possible to state, that single step extraction into 0.05 mol dm^{-3} Na_2EDTA is applicable as a screening control of the element mobility changes of majority of important metal contaminants of studied region.

Conclusions

With respect to the facts mentioned above and experiments published previously [2-5,8], it is possible to state that a single-step leaching procedure into a strong chelating agent (after optimisation of extraction conditions based on regional geological particularities) is able to release mobile and potentially mobile metal forms associated with specific phases of sediments and similar matrixes.

Specifically, in the case of Fe (and Mn) in the known conditions, it is possible to evaluate the extractability changes of the other contaminating elements present in the sediments on the basis of the changed extractability of iron and manganese. Extraction with 0.05 mol L^{-1} Na_2EDTA can be used for screening control of sediment and soil pollution under locally tested conditions - e.g., in the polluted regions of Eastern Slovakia. It is applicable as an economically viable and time saving supplementary test to the recommended and attested "IRMM" sequential extraction procedures and as a method of the fast monitoring of the element mobility changes in sedimentary systems.

Acknowledgements

This work is a part of the Grant Project VEGA No. 1/3149/06 and DAAD -11/2005. The authors would like to express their gratitude to the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences for financial support.

References

[1] Batley G.E.: Trace Element Speciation: Analytical Methods and Problems., CRC Press, Inc., Boca Raton, Florida 1990

[2] Vojteková V., Mackových D., Krakovská E., Remeteiová D., Tomko J.: Single and sequential extraction for element fractionation of sediments samples. *Chem. Pap., 2003, 57 (3), 179-184*

[3] Vojteková V., Krakovská E., Mackových D., Nováková J., Šoltýsová H.: Determination of removable portion of some elements in stream sediments of Spišsko-gemerské rudohorie Mountains. *Slovak Geol. Mag., 2003, 9 (2-3), 133-136*

[4] Vojteková V., Mackových D., Krakovská E.: Evaluation of As and Sb mobility in contaminated stream sediments. *Microchim. Acta, 2005, 150 (3-4), 261-268*

[5] Vojteková V., Krakovská E.: Fractionation Analysis of the Sediments – Limitations in the Selectivity of the Extractants. *Chem. Letters, 2006, in press*
[6] Vojteková V., Mackových D., Krakovská E.: Optimised extraction into chelating agent and determination of nonresidual mercury in sediment samples. *Transaction of the Universities of Košice*, *2004, 2, 38-46*
[7] Methodical Instruction of the Slovak Republic Ministry of Environment from 27th August 1998, No. 549/1998-2, for risk evaluations of contaminated fluvial and bed-load sediments
[8] Vojteková V.: *Fractionation study of the fluvial sediments of Spiš-Gemer region.* Ph.D. Thesis, Technical University of Košice, 2004

The Role and the Application of AAS Techniques in Speciation Analysis of the Elements in Environmental Samples

Mária Žemberyová, Ingrid Hagarová, Mária Závadská, Jana Barteková
Comenius University in Bratislava, Faculty of Natural Sciences, Department of Analytical Chemistry, Mlynská dolina CH-2, 842 15 Bratislava, Slovak Republic

Abstract

In the present work will be on some examples demonstrated the possibilities of using AAS techniques in speciation analysis: speciation of inorganic arsenic using simple speciation analysis, by which the differentiation of arsenite and arsenate, can be performed by careful control of reaction conditions in hydride generation atomic absorption detection; speciation of mercury and off-line combination with HPLC technique; speciation of chromium using solid-phase extraction, carried out by Lichrolut cartridges, using strong anionic exchanger (SAX) to separate Cr(VI) and Cr(III) for determination by FAAS; the methods most widely used to study the distribution of the metal in a solid phase, are based on extraction techniques with different chemical extractants, sequential extraction is now a well-established approach for the fractionation of trace metal content in solid samples, mainly soils and sediments.

Key words: *arsenic speciation--mercury speciation--chromium speciation--soils--sediments--sludges--fractionation*

Introduction

Speciation analysis of the elements, i.e., the determination of different compounds and/or oxidation states or of the fraction soluble under particular conditions instead of the total content, has found more and more attention over the past decades. The main reason is that the toxicity or bioavailability of an element can be several orders of magnitude different, depending on the chemical forms or the solubility [1]. Deduced from the number of publications, there is a steadily increasing in speciation analysis over the past years. Atomic absorption spectrometry (AAS) is a comparatively inexpensive, robust analytical technique that is available in most laboratories. It is characterized by a high selectivity and specifity. There is a variety of rather simple procedures available for the rapid determination of a relevant species or group of species, such as hydride-generation AAS under controlled pH conditions or in the presence of a buffer, and on-line solid phase extraction, coupled to graphite furnace AAS [2].

In the past years HPLC-ICP-MS shows the highest growth rate among the hyphenated techniques used for speciation analysis purposes, but as soon as the laboratories performing routine analysis, forced by legislation or quality control requirements, are involved in speciation analysis, ICP-MS is the most expensive detector for HPLC and other chromatographic systems. It is anticipated

that the simple, rapid and inexpensive procedures, based on AAS detection, will be most readily accepted when routine laboratories are involved in speciation analysis.

The need for speciation analysis arises from the necessity to determine the concentration of those species characterized by the highest toxicity, mobility etc. with respect to the other species of the same element. On these basis, it is possible to distinguish two different approaches: organometallic speciation, where metals whose inorganic and organic forms are characterized by different toxicity, mobility, etc. (mainly Hg, Pb and Sn); inorganic speciation, where metals whose different oxidation states are characterized by different toxicity, mobility, etc. (mainly Cr, As, Se and Sb) [3].

Organometallic speciation of mercury in environmental samples

It is well known that the toxicity and environmental fate of mercury strongly depends on its chemical forms. Under various natural conditions inorganic mercury may be converted to very toxic monomethylmercury MeHg compounds, which tend to bioaccumulate in the aquatic and/or terrestrial food chain. However, organomercury (II) compounds such as MeHg are more toxic to human beings than inorganic mercury and therefore speciation of the physicochemical forms in environmental samples is necessary. The presence of organomercury species in environmental at low concentration levels is promoting research of various approaches combining efficient and selective separation techniques with sensitive and selective detection techniques for their analysis. Coupling of liquid and gas chromatography and atomic spectrometry as a detection technique is frequent combination of choice. The most widespread analytical techniques used for the determination of total mercury in water are cold vapour atomic absorption technique (CV AAS) and CV AAS with preconcentration by amalgamation. Trace mercury analyser, TMA, single-purpose instrument is very useful for the determination from solid samples. In the contribution [4] a simple technique for speciation of mercury in water and sediment samples is described. The released methylmercury in distillate and mercury(II) in distillate rest in the samples were determined with TMA-254. The isolation of methylmercury from the model water samples by steam distillation was approved by HPLC, as an independent method. The details of chromatographic separation are given in [4].

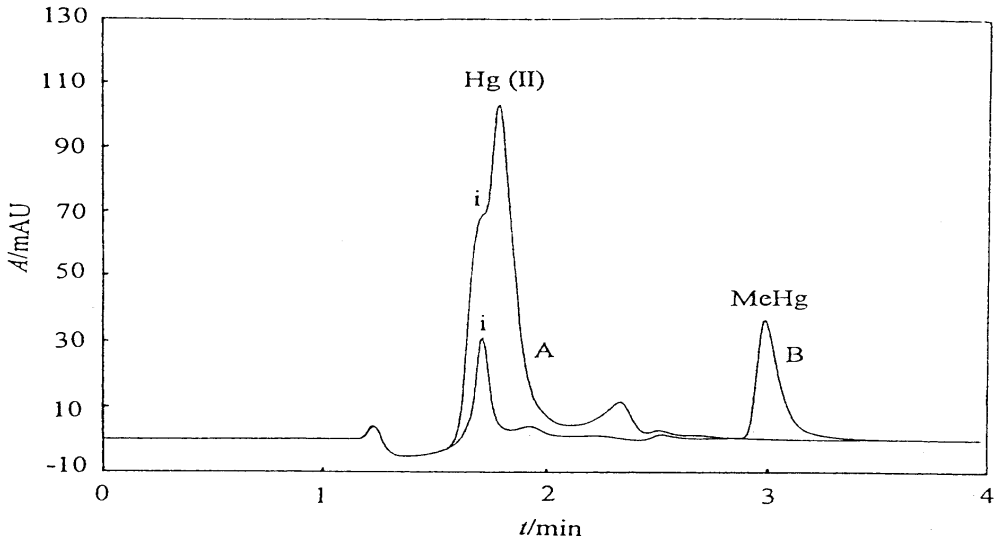


Fig. 1. Chromatographic separation of mercury and methylmercury after steam distillation of their mixture in solution at 1 μg cm-3 level each by RP-HPLC. A-chromatogram of distillate rest, B-chromatogram of distillate, Hg(II) represents position of mercury elution, MeHg represents position of methylmercury [4]

Chromatogram of separation of mercury after steam distillation in distillate and distillate rest as was detected at 254 nm is shown in Fig. 1. The accuracy of this isolation from stream sediments was verified by distillation of CRM 580 sediment (certified MeHg value 75.6±3.7; determined 71.0±7.5 ng g^{-1}). Detection limit as low as 0.25 ng MeHg was obtained.

Hyphenation between HPLC method and atomic absorption spectrometry detection was tested in off-line combination for speciation purposes described in [5]. RP HPLC system optimised for baseline separation of mercury (II), methylmercury and phenylmercury rendered fractions with volumes directly acceptable by TMA. The main problems of this combination via off-line interfacing i. e. volatility of organomercurials and lost of fractions due to overflow from porous nickel and/or platinum transfer boats were solved by addition of wetting agent (cetyltrimethylammonium bromide) and stabilized agent (dithizone) into the vessels prior to fractions collection. Chromatogram from separation of three mercury species as was detected at 254 nm is shown on Fig. 2.

Baseline separation of mercury (II) (A), methylmercury (B), and phenylmercury (C) is evident. Calibration curves were measured for Hg(II) and methylmercury within range from 25 ppb to 500 ppb. Detection limits achieved were around 0.3 ng for both mercury species. Matrix overlapped analytical signal of methylmercury and/or mercury can be evaluated by TMA measurement after HPLC separation and collection of suitable fraction of the eluent. Determination of mercury species by TMA is very selective. Practically no interferences are known for this determination. This is the main advantage of HPLC and TMA combination. Probably the first non-chromatographic speciation analysis of the mercury was the sequential determination of inorganic mercury and methylmercury in undigested biological samples by Magos [6]. In this procedure, inorganic mercury is reduced selectively, in the second step, methylmercury is reduced by the addition of cadmium and tin(II) chloride.

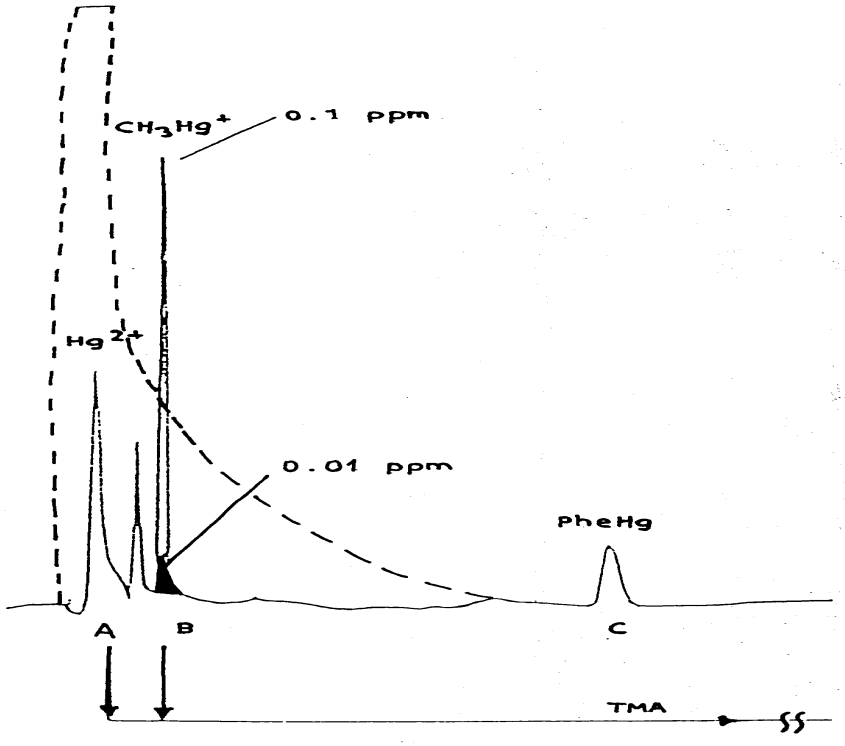


Fig. 2. Chromatographic separation of mercury (II), methylmercury and phenylmercury (PheHg) by RP HPLC Separation conditions are in [5]

Inorganic speciation of arsenic in waters by HG-AAS

A technique that can be applied directly for speciation analysis is hydride generation atomic absorption spectrometry (HG–AAS). It is well documented that, under controlled pH conditions, only the trivalent oxidation states of antimony and arsenic form a hydride. In addition, after a mild reduction, the total inorganic content of these elements may be determined, leaving organic compounds undetected. The toxicity of arsenic varies widely, ranging from highly hazardous inorganic arsenicals (arsine, arsenite As(III), and arsenate As(V)) to relatively harmless organic species (monomethylarsonate MMA and dimethylarsenate DMA). Indeed some organoarsenicals, such as arsenobetaine and arsenocholine, are effectively non-toxic to living organisms [7]. Among these arsenic compounds, of particular interest is arsenite, which is 10 times more toxic than arsenate and 70 times more toxic than the methylated species, DMA and MMA. These facts indicate why it would be of priority interest to develop a method for the selective determination of As(III). From the many techniques used for hydride generation, the most common is reduction with $NaBH_4$.

The reaction between $NaBH_4$ and an ion in solution is sensitive to pH and it appears that, for the reaction to proceed rapidly, the target species must not be present in solution as a negatively charged species. The most abundant arsenic species in waters are the inorganic species arsenate and arsenite. It therefore means that arsenite and arsenate must be fully protonated if they are to be converted to arsine. As pKa_1 for arsenic acid is 2.3, the reaction must therefore be carried out at very low pH. Arsenious acid, on the other hand, is protonated under most conditions ($pKa_1 = 9.2$) and will react with $NaBH_4$ under conditions, which are only mildly acidic [8]. Thus, the simple speciation analyses, such as the differentiation of arsenite and arsenate, can be performed by careful control of the reaction conditions.

This theory was applied for optimization of the analytical conditions to reliably determine As(III) in water samples, the river water sample (RWS) from the Slovak river Hron, the waste water sample (WWS) from the abandoned mining area near the Slovak town of Pezinok, and the synthetic water sample (SWS) Table 1, by continuous HG–AAS [9]. For the total inorganic arsenic determination, a complementary step of sample pre-reduction was needed to calculate the inorganic As(V) concentration as the difference between total inorganic arsenic and As(III).

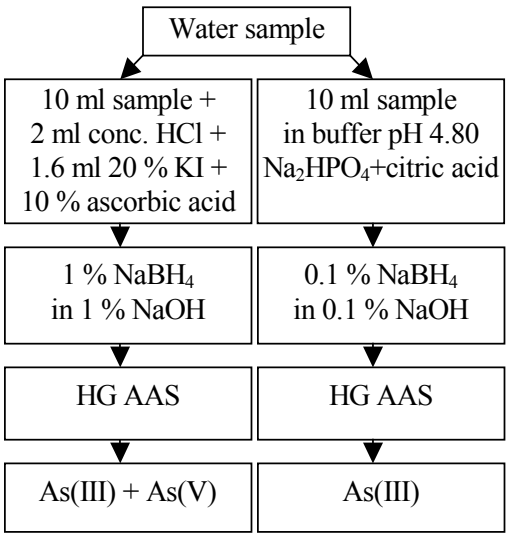


Fig. 3. Schematic procedures for As(III) and As(III)+As(V) selective determination in water samples by HG AAS

Table 1. Arsenic concentrations determined in water samples

Sample	Added As(III) (μg l^{-1})	Determined As(III) (μg l^{-1})	Determined total inorganic As (μg l^{-1})
SWS	-	-	9.68 ± 0.44
RWS	-	-	8.51 ± 0.28
RWS	10	9.92 ± 0.34	18.1 ± 0.24
WWS	-	1.22 ± 0.23	45.7 ± 0.72
WWS	10	11.3 ± 0.18	56.4 ± 0.43

The accuracy of the determination of total inorganic arsenic was verified using the reference material with the certified concentration of total inorganic arsenic "Trace Elements in Water" no. 12-3-10 (Slovak Institute of Metrology, Bratislava, Slovak Republic). The certified value ± U (k=2) was 21.0 ± 5.0 μg l^{-1}, while the mean determined value ± SD was 21.3 ± 1.8 μg l^{-1} (n = 12).

The limit of detection (3SD) for total inorganic arsenic determination was 0.22 μg l^{-1}.

There are several papers on the use of direct methods based on various reaction media to achieve a selective hydride generation of the different arsenic species. To obtain a selective arsine determination, different buffer solutions: acetic acid/sodium acetate, citric acid/sodium citrate, citric acid/sodium hydroxide [10] have been used to achieve a selective volatilization of arsenite in the presence of arsenate.

In the work [9], the determination of As(III) was made in Na_2HPO_4/citric acid buffer solution (pH 4.80).

The limit of detection (3SD) for As(III) determination was 0.28 μg l^{-1}.

Inorganic speciation and preconcentration of chromium in waters using solid-phase extraction and AAS

Toxicological studies have shown that the degree of toxicity of some elements depends on the chemical form in which the element is present. Chromium(III), for example, is considered an essential micronutrient for humans, whereas chromium(VI) is a potentially carcinogenic agent. It is therefore necessary to control the level of chromium in wastewater, natural water and drinking water. Several methods for speciation of chromium using atomic spectroscopy have been investigated. These methods include previous preparation of a sample by liquid-liquid extraction, solid-liquid extraction, or a direct coupling of liquid chromatography with atomic spectroscopy. The use of solid phase extraction permits simultaneous preconcentration and separation of Cr(III) and Cr(VI) [11]. Procedure for the speciation of chromium in water samples using atomic absorption spectrometry and solid phase extraction is described in [12] and schematically illustrated on Fig. 4. The solid phase extraction was carried out using Lichrolut cartridges (Merck) containing 500 mg of trimethylaminopropyl chloride. The procedure enables speciation of chromium and preconcentration of Cr(VI) in model and real water samples.

Table 2. Speciation of Cr (III) and Cr(VI) and preconcentration of Cr(VI) on anion exchange cartridge Lichrolut in the model water sample

Model sample	Added		Determined		Recovery
Volume	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(VI)
[ml]	[mg/l]		[mg/l]		[%]
25[a]	1.50	0.60	1.56	3.10	103
25[b]	1.50	0.60	1.61	2.74	91

[a]Procedure with HAc/Ac^- buffer – elution with 3 M HNO_3. [b]Procedure with HAc/Ac^- buffer – elution with 0.5 M NaCl. Factor of preconcentration was 5

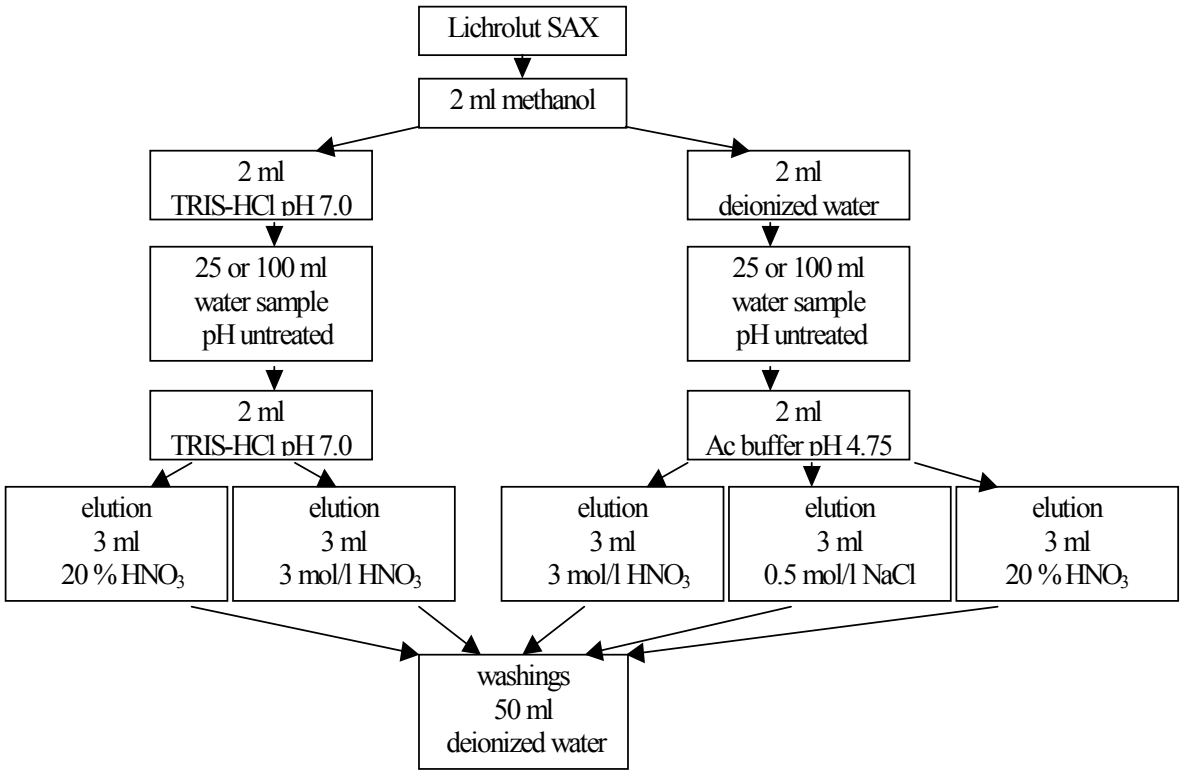


Fig. 4. Procedures for speciation of Cr(III) and Cr(VI) in water samples by SPE

The results for speciation of Cr (III) and Cr(VI) and preconcentration of Cr(VI) on anion exchange cartridge Lichrolut in the model water sample (using standard solution of chromium) and model river water sample using various elution procedures are in Tables 2 and 3.

Table 3. Speciation of Cr (III) and Cr(VI) and preconcentration of Cr(VI) on anion exchange cartridge Lichrolut in the model river water sample

Model river water sample Volume [ml]	**Added Cr(VI)** [μg/l]	**Determined Cr(VI)** [mg/l]	**Recovery Cr(VI)** [%]
100[a]	50	0,92	92
100[a]	10	0,21	103
100[b]	50	0,94	94
100[b]	10	0,20	100

[a]Procedure with TRIS-HCl – elution with 20 % HNO_3. [b]Procedure with HAc/Ac^- buffer – elution with 0.5 M NaCl. Factor of preconcentration was 20

Fractionation of the metals in solid samples

In order to assess the chemical forms of heavy metals in soils, sediments and sludges extraction procedures have been applied, both as single and sequential schemes. The use of sequential extraction furnishes detailed information about origin, mode of occurrence, mobilization and transport of trace metals. Many of the sequential extraction schemes employed are based on the five-stage procedure of

Tessier et al. [13]. The literature on metal speciation in solid matrices, taking into account its application in the analysis of soils is reviewed by Das et al. [14]. An improvement in analytical measurements for single and sequential procedures applied to soil and sediment analysis has been reported by Rauret et al. [15]. Two different approaches are usually applied in speciation studies for solid samples: single and sequential extraction procedures. Single step extraction procedures are usually applied to soil samples to identify the "bioavailable" fraction, using a number of different reagents able to extract all, or part of the metals from soil. The Measurement and Testing Programme - MAT (formerly BCR) - of the European Commission organized intercomparisons on determination of extractable trace elements. The results showed that the most suitable approach for certification of a soil sample was a single step procedures using EDTA / acetic acid to characterize the bioavailable fraction of metals [16].

In the contribution [17] was utilized single step extraction procedures validated by MTA, extraction with 0,05 mol l^{-1} EDTA and 0,43 mol l^{-1} acetic acid to three pedologically various soil reference materials, which represent basic soil types from Slovakia and are certified for total contents of the studied elements and to three Slovak reference materials of sludges. The determined values of bioavailable zink, copper, nickel, lead and cadmium in the studied soils and sludges could be recommended for hygienic and soil laboratories as indicative values for the certain type of soil. Determination of heavy metals in sewage sludge has received an increasing attention, since its agricultural use as fertiliser requires strict information about the metal composition of the sample. For assessing bioavailable metal fraction (and thus to estimate the related phyto-toxic effects) and the mobility of trace metals in soils, and to some extent also in sewage sludges, single extraction procedures are used. The accuracy of the procedure and determination was verified using CRM 483, certified for EDTA and acetic acid extractable contents of of Cd, Cr, Cu, Ni, Pb and Zn in sewage sludge amended soils. The choice of the CRM helped us to check the accuracy of the determination of the analytes in the matrices of reference materials of soils and sludges.

In the framework of BCR, a three-step sequential extraction procedure has been developed, which has been applied to a variety of matrices, including sediments, soils and sewage sludges [18]. The study has revealed some sources of uncertainty in application of the BCR three-stage sequential extraction procedure to sediments and has indicated modifications to the protocol. The modified extraction scheme is an operationally defined procedure [19] in which the reagent used at each stage is intended to release metals associated with particular soil phases (Table 4). The three-step sequential extraction procedure followed the modified BCR protocol was applied to Slovak soil reference materials (soil orthic luvisols, soil rendzina and soil eutric cambisol) with total certified values of the elements [20]. Hence we have established the extractable concentrations of six heavy metals in mentioned three soil reference materials for which the total concentrations have been established. The soil extracts were analysed by flame atomic absorption spectrometry (FAAS) or electrothermal atomic absorption spectrometry (ETAAS). The six elements determined in the extracts were cadmium, chromium, copper, nickel, lead and zinc.

Table 4. Modified BCR three-step sequential extraction procedure

Extraction step	Reagent(s)	Nominal target phase(s)
1	CH_3COOH (0.11 mol l^{-1})	Soil solutions, carbonates, exchangeable metals
2	$NH_2OH.HCl$ (0.5 mol l^{-1})	Iron/manganese oxyhydroxides
3	H_2O_2 (8.8 mol l^{-1}) then CH_3COONH_4 (1 mol l^{-1}), pH 2	Organic matter and sulfides
Residual	aqua regia	Remaining, non-silicate bound metals

The quality control of the procedure and the determination of Cu, Pb, Cd, Cr, Ni and Zn in the extracts was verified and compared using certified reference material CRM 701 (BCR, Brussels), with the certified extractable contents of Cd, Cr, Cu, Ni, Pb and Zn..

Percentage representation of extractable Cr, Cu, Ni, Pb, Cd and Zn in the extracts in all steps of sequential extraction procedure of soils S-SP, S-VM, S-MS in comparison to total contents-100% are shown in Fig. 5.

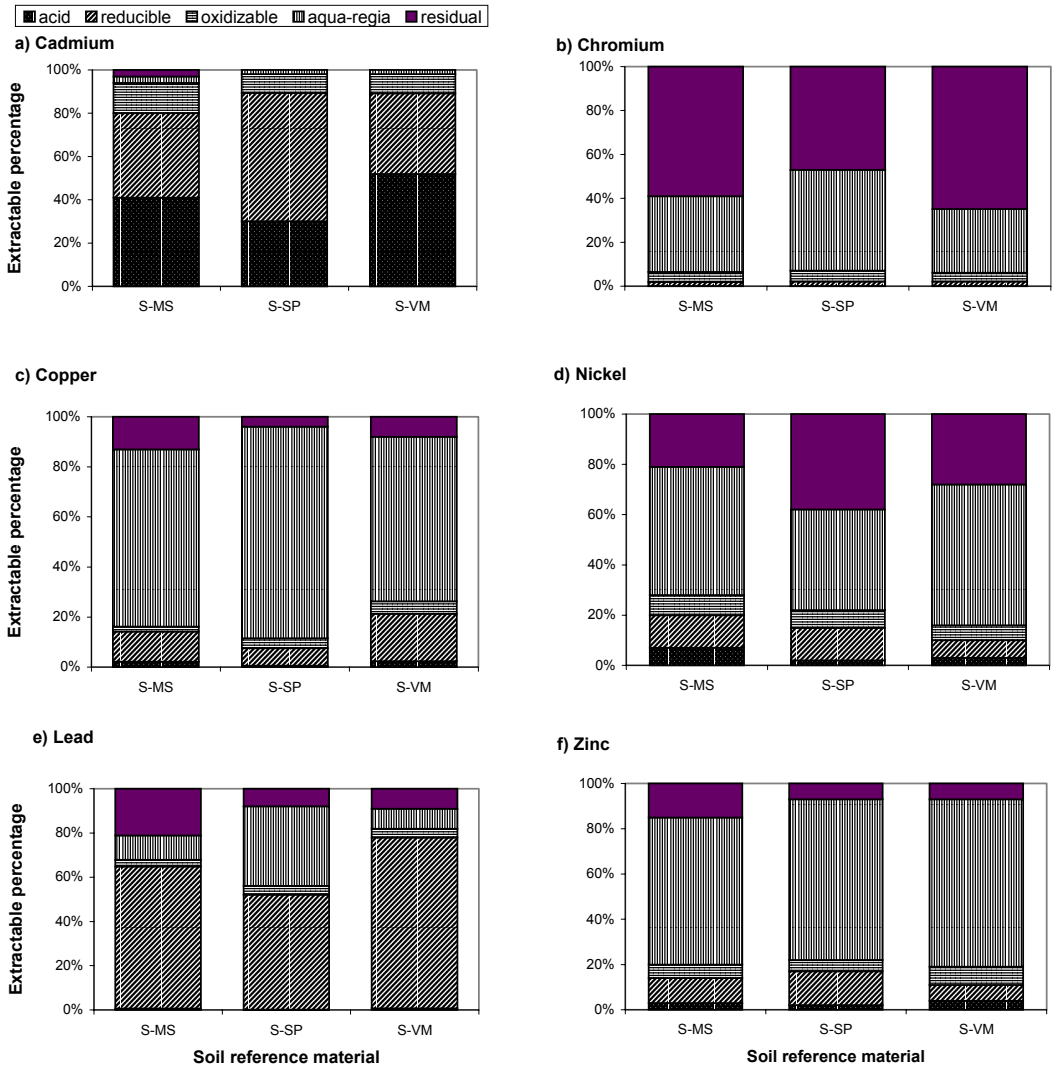


Fig. 5. Metal distribution obtained for the soils S-SP, S-VM, S-MS using the modified sequential extraction procedure

The used sequential extraction procedure permits the evaluation of the various chemical forms present in the soils. The applicability of the BCR method is easy, the method was found to be both, repeatable and sufficiently reproducible for environmental monitoring purposes.

Acknowledgements

This work was financially supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic VEGA 1/2466/05.

References

[1] Merian E. (ed.): Metals and their compounds in the environment: occurrence, analysis and biological relevance. VCH, Veinheim 1991

[2] Welz B.: Speciation analysis. The future of atomic absorption spectrometry. JAAS, 1998, 13, 413-417

[3] Morabito R.: Extraction techniques in speciation analysis of environmental samples. Fresenius J Anal Chem. 1995, 351, 378-385

[4] Závadská M., Žemberyová M., Hutta M., Gono M.: Determination of methylmercury in sediment samples by AAS. Chem. papers 2000, 54, 24-27

[5] Hutta M., Moskáľová M., Žemberyová M., Foltin M.: Off-line combination of RPHPLC and trace mercury analyser for organomercurial speciation in environmental samples. J.Radioanal Nucl. Chem. 1996, 208, 403-415

[6] Magos L.: Selective atomic-absorption determination of inorganic mercury and methylmercury in undigested biological samples. Analyst, 1971, 96, 847-853

[7] Shraim A., Chiswell B., Olsowy O.: Speciation of arsenic by hydride generation-atomic absorption spectrometry in hydrochloric acid reaction medium. Talanta 1999, 50, 1109-1127

[8] Howard A.C.: Borohydride techniques in trace element speciation. J. Anal. At. Spectrom. 1997, 12, 267-272

[9] Hagarová I., Žemberyová M., Shearman A., Chromá O.:Determination and speciation of arsenic in water samples by HG AAS. Hydrochémia 2004 "Nové analytické metódy v chémii vody", Bratislava, 2004

[10] Quináia S. P., Rollemberg M. C. E.: Selective reduction of arsenic species by hydride generation-atomic absorption spectrometry. J. Braz. Chem. Soc. 2001, 12, 37-41

[11] Adriá-Cerezo,D.M., Llobat-Estellés, M., Maurí-Aucejo A.R.: Preconcentration and speciation of chromium in waters using solid-phase extraction and atomic absorption spectrometry. Talanta 2000, 51, 531-536

[12] Žemberyová M., Diškancová M., Barteková J., Hagarová I.: Analytical procedures for the speciation of chromium in water samples and its determination bz AAS. Hydrochémia 2004 "Nové analytické metódy v chémii vody", Bratislava, 2004

[13] Tessier A., Campbell P.G.C., Bisson M.: Sequential extraction procedure for the speciation of trace metals. Anal.Chem., 1979, 51, 844-851

[14] Das A.D., Chakraborty R., Cervera M.L., de la Guardia M.: Metal speciation in solid matrices. Talanta 1995, 42, 1007-1030

[15] Rauret G., López-Sánchez J.F., Sahuquillo A., Rubio R., Davidson C., Ure A., Quevauviller Ph.: Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J. Environ. Monit. 1999, 1, 57-61

[16] Quevauviller Ph., Rauret G., Ure A., Bacon J., Muntau H.: Report EUR 17127 EN, Brussels,1997

[17] Žemberyová M., Barteková J., Závadská M., Šišoláková M.: Determination of bioavailable fractions of Zn, Cu, Ni, Pb and Cd in soils and sludges by atomic absorption spectrometry. Talanta, 2006, article in press

[18] Quevauviller Ph., Maier E.A., Griepink B. (eds.): Quality Assurance for Environmental Analysis, Elsevier Science, Amsterdam, 1995, pp. 505-523

[19] Rauret G., López-Sánchez J.F., Lück D., Yli-Halla M., Muntau H., Quevauviller Ph.: EUR Report, Nr. 19775, C. E. C., Brussels, 2001

[20] Žemberyová M., Barteková J., Hagarová I.: The utilization of modified BCR three-step sequential extraction procedure for the fractionation of Cd, Cr, Cu, Ni, Pb and Zn in soil reference materials of different origins. Talanta, 2006, article in press