Elimination of Chemical and Spectral Interferences in Measurement of Trace Elements in Urine and Blood by Combined Electrodeposition-Electrothermal Atomic Absorption Spectrometry

Mashkouri Najafi [+], *Nahid*

Chemistry Research Center, University of Shahid Beheshti, Tehran, I.R. Iran

ABSTRACT: *A combined electrodeposition-ETAAS technique has been applied to the analysis of Pb, Cd, Co, Ni, Cr and Mn in biological samples in order to overcome interferences and to minimize sample pretreatment. It requires minimal sample preparation with the electrolysis process aiding partial decomposition of the organic matrix, adequate for the release and deposition of trace elements. In an initial electrodeposition step, the graphite furnace is coated by Pd. Analytes are then electrodeposited in situ from the sample at optimized applied voltage and time onto the Pd-coated furnace, followed by removal of spent electrolyte and atomization. The high background signals due to matrix components of urine and blood were eliminated by the technique and the sensitivity of determination was improved up to five-fold with respect to conventional ETAAS technique. A good precision of 1-3 %RSD (depending on the element and sample matrix) has been obtained. The recovery of added analytes is close to 100%, based on comparison with conventional ETAAS of aqueous samples. The accuracy of the technique was established by analyzing the urine standard reference material, SRM2670. The results of determinations of the elements tested are within the range of certified values given by the NIST with an acceptable %RSD.*

KEY WORDS: *Blood and urine analysis, ETAAS, Trace element analysis, Electro deposition.*

INTRODUCTION

The elements such as Mn, Mo, Co, Cr, V, Ni, Se and Sn are considered as essential and Cd, As and Pb as toxic ultratrace elements[1]. One of the major problems and challenging task in trace analysis techniques is accurate determination of essential and toxic elements in the biological and environmental samples. The essential elements are present at extremely low concentrations[2], maintained within a narrow margin of optimal concentration, between states of deficiency and toxicity. The toxic elements such as Pb and Cd exert toxic effects at any concentration. These concentrations are often near or below the detection limit of most of the analytical techniques. There are perhaps only three analytical methods with sufficient sensitivity for such determinations: neutron activation analysis, mass spectrometry and electrothermal atomic absorption spectrometry (ETAAS). The two for-

+ *E-mail:n-najafi@cc.sbu.ac.ir*

1021-9986/02/2/ 80 7/$/2.70

mer techniques are not widely available in most laboratories and the third one unfortunately is the most susceptible to the matrix interference in real samples.

Urine and blood are known to be some of the most troublesome samples to analyze by atomic spectrometric techniques because of the high and variable concentration of organic and inorganic components, especially high chloride content [3-9]. Chemical and spectral interferences affecting these complex samples are the main contributors to systematic errors in such analyses.

However, preconcentration process and separation of analyte from the interfering concomitants prior to thermal programs in the ETAAS could solve these problems and enable the easier determination.Electrochemical deposition of trace elements is commonly used as preconcentration and separation step followed by ETAAS [10-15].

For urine and blood analysis the concerns always are to develop a method which would minimize manual manipulations of samples preventing the contamination and improving the performance for the trace analysis in these samples. Therefore, extreme care must be used to develop a precise, accurate and interference-free ETAAS based methodology for this purpose that can provide full analytical confidence to the clinical studies.

A new approach has been developed [16] by coupling the electrodeposition technique with ETAAS for in situ matrix elimination in ETAAS in order to analysis of Cd and Pb in high-salt matrices. In an initial electrodeposition step, the graphite furnace is coated by Pd. Analytes are then electrodeposited in situ from the sample onto the Pd-coated furnace, followed by removal of spent electrolyte and atomization. A modified autosampler were used incorporating a Pt/Ir delivery tube doubling as an electrode for electrodeposition step. Programming of the autosampler to remove spent electrolyte after the electrolyses made it possible to remove the bulk of interfering matrix prior or atomization. Chemical pretreatment outside the furnace or adding another potentially contaminating reagent to control the furnace chemistry often cause more contamination[17]. In situ electrodeposition of analyte inside the furnace precoated with purified electrodeposited Pd as the modifier considerably reduced contamination risk.

This work utilizes the reported in situ electrodeposition-ETAAS technique to the analysis of Pb, Cd, Co, Ni, Cr and Mn in urine and blood samples in order to fulfil the requirements for these analysis, to overcome interferences and to minimize sample pretreatment.

EXPERIMENTAL

Instrumentation

A GBC932AA double beam atomic absorption spectrometer with deuterium lamp background correction equipped with GF3000 furnace and PAL3000 autosampler were used throughout this work.

The construction of modified autosampler is similar to an early design [18], in which the last section of PTFE sample delivery tube is replaced with a 6 cm Pt/Ir tube. The furnace and Pt/Ir tube were connected to a d.c. power supply (0-12V) via a multimeter (0-150 mA) indicating the deposition current.

Thermal programs for the GF3000 furnace with sampler programs for each steps of electrodeposition are applied and controlled automatically by the external computer.

Reagents

Ultrapure water was obtained by passing distilled water through milli-Q ion exchange and membrane filtration system. All acid used, namely HNO_3 HCl, and H_2SO_4 were of Aristar (BDH) quality and were all diluted by milli-Q water.

Sodium chloride solutions were prepared from high purity grade (BDH) Anala R or analytical reagent (Univar). Stock Pd solution (100ppm) was made by dissolving $PdCl_2$ (BDH) in 1-5% Aristar HNO_3 and was diluted to 10ppm working solution with milli-Q water. All analyte standard solutions were prepared in 1% HNO_3 or 1%HCl by diluting stock solutions of 1000ppm (BDH) standard solution for AAS. All solutions were stored in high density polyethylene containers previously soaked in 1% HNO_3 for a long time.

All urine and blood samples were collected using IUPAC guidelines[2] for sample collection for trace elements in blood and urine, and were stored in refrigerator at < 5°C for blood and 5°C for urine samples in plastic tubes or bottles pre-washed with acid. All urine samples were analysed within three hours after collection. The Reference Standard Material SRM2670 was obtained

from National Institute of Standard and Technology NIST, USA.

Procedure

Experiments involved both conventional sample introduction and sample electrodeposition into the pyrolytic graphite furnace coated by electrodeposited Pd modifier. In the conventional way a standard autosampler-furnace program was used to deposit the sample into the furnace (with electrodeposited Pd modifier), followed by a furnace program.

For electrodeposition and AAS determination of elements such as Co, Ni, Cr, and Mn, a typical program is shown in Table 1. Solutions of 25 l of 0.1M NaCl, urine, blood or dilute acid solutions (0.01% H_2SO_4 for Cr, 0.25-0.5M ammonia and 0.25-0.18M sulfuric acid mixture solutions corresponding to pH= 4.0-10 for the remaining elements), containing 1-20 ng/ml of the elements or urine and blood samples were electrolysed at 4.0-6.0V (5-10 mA) for 60-80s in the furnace pre-coated with electrodeposited Pd modifier, the spent electrolyte removed by autosampler and a furnace program initiated.

Table 1: Autosampler-furnace program for AAS determinations of electrodeposited Co, Ni, Cr and Mn in the pyrolytic graphite furnace pre-coated with electrodeposited Pd modifier.

Graphite furnace parameters						
Step	Final temp. (°C)	Ramp Time (s)	Hold Time (s)	Gas Type	Read	Signal Graphics
1	110	20.0	30.0	Inert	Off	Off
2	20	20.0	5.0	None	Off	Off
3	110	20.0	30.0	Inert	Off	Off
4	140	5.0	5.0	Inert	Off	Off
5	1300	8.0	1.0	Inert	Off	Off
6	400	4.0	1.0	Inert	Off	Off
7	400	1.0	1.0	None	Off	Off
8	Variable	1.0	2.0	None	Off	On
9	2500	1.0	1.5	Inert	Off	Off

**Co= 2300, Ni & Mn = 2400, Cr =2500*

Autosampler parameters

Solutions	Inject at step	Injection mode	Volume (μl)	Rinse	Current	Current Time, (s)
Modifier	1	Electro Dep.	40	No	Yes	40.0
Sample	3	Electro Dep.	25	No	Yes	80.0

RESULTS AND DISCUSSION

Background reduction in ED-ETAAS

Removal of the spent electrolyte after electrodeposition of analyte in the Ed-ETAAS technique ensured the efficient removal of interfering concomitants. Hence, spectral and non-spectral interferences are expected to exist under the experimental conditions for determination of the elements in any kind of matrices. The decision as to whether an absorption signal is interfered with or not can only be made with a display of absorption and background curves. Fig.1a shows the AA and background signals for urine sample which was analysed by the conventional ETAAS technique for Cd. Although an optimized ashing program is used for removing the interfering matrix before the atomization step, the AA signal is overlapped by a very high and broad background signal from the matrix. It is apparently impossible to distinguish Cd signal from the high noise produced by the high background signal. In addition, the loss of analyte due to molecular compound formation takes place in this step. However, the ED-ETAAS technique for the same sample was quite successful in removing the highly concentrated interfering matrix and separating traces of Cd from the bulk of sample. Thus, background signal was reduced to a very low level as it is shown in Fig.1b. The same experiments were carried out for Pb, Cr, Ni, Co and Mn in the urine and blood samples and the similar results were

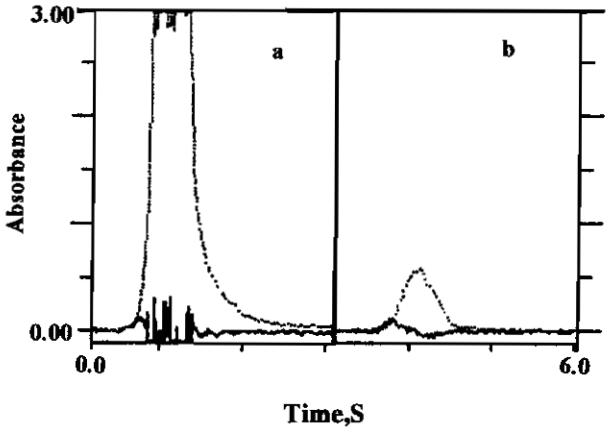

Fig. 1: Typical AA signals (—) and background (...), measured at Cd 228.8 nm, for a urine sample by : a) conventional ETAAS , b) ED-ETAAS.

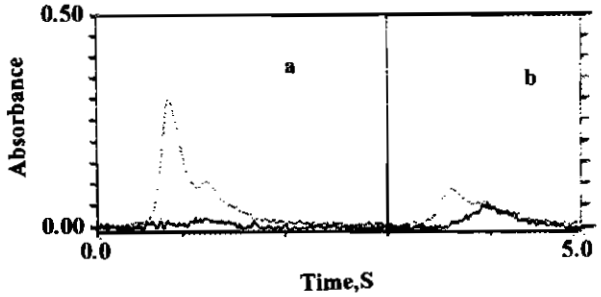

Fig. 2: Typical AA signals (—) and background (...), measured at Ni232.0 nm, for a blood sample by :a) conventional ETAAS, b) ED-ETAAS.

achieved for enhancing the AA signals and drastic reduction in background signals by using ED-ETAAS comparing to conventional ETAAS technique.

The AA and background signals for Ni in blood sample is shown as an example in Fig.2 which is analysed by conventional ETAAS and ED-ETAAS (a and b respectively). The optimized thermal program and in situ ET digestion process with 10% HNO_3 and 10% H_2SO_4 could not remove the interfering matrix completely and AA signal was suppressed by the overlapped background signal in conventional ETAAS (Fig.2a). However, The AA signal was enhanced when the analyte was successfully separated from the interfering matrix by ED-ETAAS technique and the background signal reduced to a very low level as is shown in Fig.2b. The results appear to offer the best solution for the problem of highly concentrated and variable matrix in urine and blood samples that have made the analysis of these samples so difficult.

Analytical performance

The ED-ETAAS technique was evaluated for precision by measuring Pb, Cd, Cr, Ni, Co and Mn in different matrices. The results of measurements as peak height and peak area for each group of replicates also shown in Table 2 together with %RSD calculated for each group of replicates. As the peak area absorbance showed a better reproducibility for Mn measurements than the peak height absorbance, the former is listed in Table 2 for this element.

In Table 3, Dls for measurement of Pb, Cd, Cr, Ni, Co and Mn by conventional ETAAS are compared with those by ED-ETAAS in different matrices. The results show that for most of the elements, ED-ETAAS has a lower Dls than conventional ETAAS in exactly the same experimental conditions.

The linearity of spiked calibration curves were investigated over the expected range of analytes[2], in the urine and blood samples for Pb, Cd, Cr, Co, Ni and Mn by the ED-ETAAS technique. The results for Pb and Cr electrodeposited from a urine and blood sample prior to

Table 2: Precision of measurements for Pb, Cd, Cr, Ni, Co and Mn by ED-ETAAS in different matrices.

Rep-locates	Pb A	Cd A	Cr 6^+ A	Cr 3^+ A	Ni A	Co A	Mn* A.s	Mn** A.s	Mn*** A	Mn**** A
1	0.522	0.660	0.783	0.637	0.443	0.630	0.397	0.525	0.821	1.05
2	0.520	0.685	0.772	0.637	0.440	0.628	0.386	0.515	0.744	1.04
3	0.517	0.650	0.789	0.636	0.450	0.632	0.395	0.522	0.767	0.936
4	0.524	0.692	0.780	0.640	0.468	0.618	0.385	0.520	0.801	0.936
5	0.506	0.693	0.781	0.635	0.446	0.620	0.391	0.522	0.750	1.04
6	0.514	0.667	0.778	0.642	0.470	0.625	0.388	0.518	0.810	0.942
7	0.506	0.670	0.784	0.638	0.454	0.630	0.393	0.520	0.782	1.03
X	0.515	0.674	0.781	0.637	0.450	0.625	0.390	0.520	0.782	0.996
%RSD	1.4	2.4	0.7	0.4	2.3	0.8	1.1	0.7	3.5	5.1

A) peak height absorbance.
A.s) peak area absorbance.
**) cathodic deposition (Mn^{2+} → Mn) from ammonia-sulfuric acid solution (pH=4).*
***) cathodic deposition (Mn^{2+} → Mn) from a urine sample.*
****) anodic deposition (Mn^{2+} → MnO_2) from ammonia-sulfuric acid solution (pH=8).*
*****) anodic deposition (Mn^{2+} → MnO_2) from ammonia-sulfuric acid solution (pH=4).*

Table 3: Comparison of detection limits (DL) for conventional ETAAS and ED- ETAAS measurements in different matrices.*

Element	DL conventional ETAAS In acid/ammonia Solution** ppb	DL ED-ETAAS From Acid/ammonia Solution** ppb	DL ED-ETAAS From 0.1M NaCl ppb
Pb	1.0	0.6	0.8***
Cd	0.2	0.15	0.1***
Cr	0.3	0.04 - 0.08	0.05
Ni	0.2	0.2 - 0.3	0.2
Co	0.9	0.3 -0.6	0.8
Mn	0.2	0.2 # 0.2 ▷	0.2 # 0.1▷

**) ($DL = \frac{3S_b}{m}$), Sb is standard deviation for the blank, and m is calibration curve slope.*
***) 1%HNO_3 for Pb and Cd, 0.01% H_2SO_4 for Cr, ammonia-sulfuric acid solution for Ni, Co and Mn.*
****) 0.5M NaCl.*
#) Mn, anodic deposition ($Mn^{2+} \rightarrow MnO_2$).
▷) Mn, cathodic deposition ($Mn^{2+} \rightarrow Mn$).

measurement by ETAAS are shown in Figs. 3 and 4. The corresponding correlation coefficient r is also shown. Very good linear curves were obtained for the elemental concentration range of 1-10 ppb, with a correlation coefficient better than 0.99 even in a complex matrix of urine and blood.

The reliability of the analyses was further assessed through recovery studies. This was done by performing triplicate determination of the Pb, Cd, Cr, Co, Ni and Mn by the ED-ETAAS technique in different analyte-spiked aliquots of two real urine and blood samples. The results gave close to 100% average recovery (98-102) for added analytes. Under the conditions of electrodeposition specific to an element, the slopes of analytical curves for both analyte in interference-free solution and the analyte added to a urine or blood samples were the same within the experimental error, implying the absence of non-spectral interference in the ED-ETAAS technique and permitting the use of either calibration graphs or the standard addition method for qualification. This also indicates the

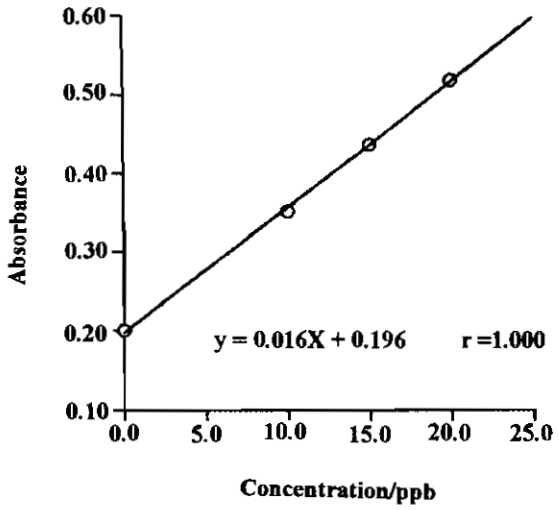

Fig.3: Calibration curve for spiked-Pb electrodeposited from a urine sample onto a Pd-coated furnace at E_d = 3.0V, measured at Pb 283.3 nm (peak height).

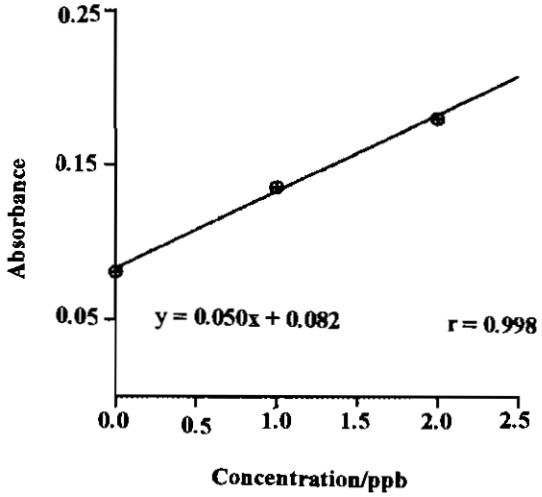

Fig.4: Calibration curve for spiked-Cr^{3+} electrodeposited from a blood sample onto a P_d-coated furnace at Ed = 8.0V, measured at Cr 357.9 nm (peak height).

capability of the ED-ETAAS technique to separate the bulk of the analyte from the interfering matrix in the urine or blood sample and perform an interference free determination with full recovery of added analytes. Typical results are shown in Figs.5-6 for the measurement of Mn in a urine and Ni in a blood sample.

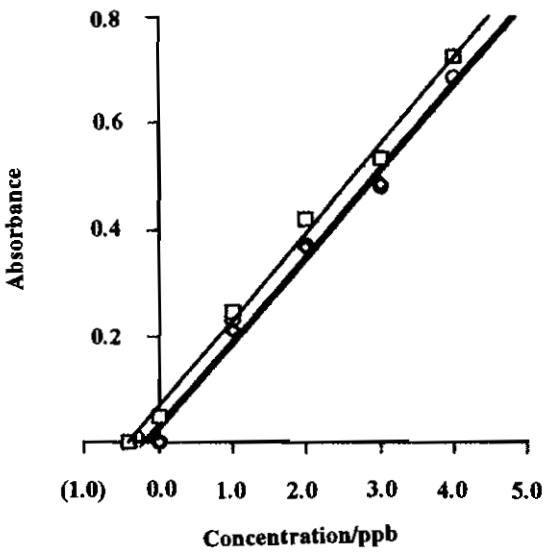

Fig.5: Calibration curves for Mn electrodeposited (cathodically) from 0.1 M NaCl (o) and conventionally deposited in ammonia buffer solution (◊). The standard addition plot is for Mn electrodeposited from urine (❑).

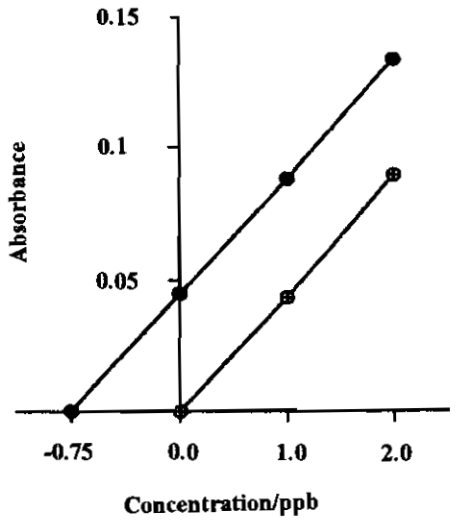

Fig.6: Calibration curves for Ni conventionally deposited in ammonia buffer solution (⊗) and the standard addition plot is for Ni electrodeposited from a blood sample (●).

Use of certified standards

Accuracy of an analytical determination and thus of a new proposed technique can be established in one of two ways. The first would be to analyse the same samples by two or more independent methods. Since many laboratories do not have access to two independent methods for the same determination, the second way may be analysing standard reference materials. These are materials whose analyte content has been established by two or more independent methods, and whose matrix should be nearly identical to that of the analysed samples.

The latter procedure is especially important for the analysis of ultra-trace analytes in a large series of similar complex samples. A number of organizations and laboratories have produced and established the analyte concentrations in a variety of biological reference materials. The recent one is that of the National Institute of Standard and Technology. Gaithersburg, MD (NIST) in USA. In the area of biomedical standards for use in clinical laboratories.

The NIST has used neutron activation analysis widely for certification of SRMs involving elemental trace analysis together with other sensitive and modern spectroscopic techniques such as inductively coupled plasma spectrometry, isotope dilution thermal (or spark) source mass spectrometry, and d.c. plasma emission spectrometry.

The analysis of reference materials of blood, serum, packed cells or urine, certified for trace elements at concentrations similar to those present in the samples, is a prerequisite for verifying the results and providing assurance of the proposed analytical technique(2).

Veillon[19] also recommends verification and evaluation of analytical data and / or the accuracy of the method for analysis of biological sample types by using SRM or independent methods.

Two NIST *Standard Reference Materials* for urine samples (SRM 2670 normal and elevated level) were used to validate the ED-ETAAS method for Pb, Cd, Cr, Ni and Mn determination.

The results are also summarized in Table 4. A very good agreement betwean the ED-ETAAS data and the certified values was achieved for all the analyte elements, as shown in this table. In addition, the precision based on 6-7 replicates is quite promising for this technique considering the difficult sample matrix and trace levels of analyte elements.

The results are in a very good agreement with those published [20] recently by using sensitive technique of ICP-MS for the SRM 2670 reference material.

Table 4: ED-ETAAS analysis of NIST Urine SRM 2670

Element	Normal level Concentration, ppb			Elevated level Concentration, ppb		
	Experimental	%RSD n = 7	Certified range*	Experimental	%RSD n = 7	Certified range*
Pb	10.3	3.8	(10)	113	5.6	109±4
Cd	0.45	5.2	(0.4)	84	1.2	88±3
Mn	29.2	5.4	(30)	310	2.8	(330)
Cr	12.4	1.8	(13)	83	2.5	85±6
NI	68.4	2.3	(70)	282	3.5	(300)

* *The values in parentheses are not certified by NIST because they are not based on the results of either a reference method of known accuracy or on two or more independent methods. They are included for information only [21].*

CONCLUSIONS

Based an the findings outlined, it may be concluded that in situ electrodeposition of analytes onto the Pd-coated fu method overcomes most of the difficulties. Encounted in ultrace analysis of the elements mentioned.

The results for analytical performance of the ED-ETAAS technique indicate a good precision with 1-3%RSD. Low detection limits even for highly concentrated salt solutions and similar characteristic masses in complex matrices to those of the conventional ETAAS were obtained for the tested elements. The wide linear working range and complete recovery for analytes added to the complex matrix samples was demonstrated. Excellent accuracy was established by analysing the standard reference material (SRM 2670).

It is therefore concluded that the technique presented possesses the capability of making accurate and precise measurements of the elements tested in complex biological and environmental samples. The analytical performance of this technique needs to be studied further for a wider range of elements and samples than reported in the present work.

Received: 7th July 2001 ; Accepted: 14th May 2002

REFERENCES

[1] Frieden, E., *J. Chem. Edu.* **62**, 917 (1985).

[2] Cornelis, R., Heinzow, B., Herber, R.F.M., Christensen, J.M., Poulsen, O.M., Sabbioni, E., Templeton, D. .M., Thomassen, Y., Vahter, M. and Vesterberg, O. International Union of Pure and Applied Chemistry, IUPAC, **67**, 1575 (1995).

[3] Guthrie, B.E., Wolf, R.W. and Veillon , C. *Anal. Chem.* **50**, 1900 (1978).

[4] Hall, D.J. and Fell, G.S., *J. Anal. Atom. Spectrom.* **1**, 135 (1986).

[5] Dungs,K. and Neidhart, B. *Analyst*, **109**, 877 (1984).

[6] Chen, T. and Littlejohn, D., *Analyst* **118**, 541 (1993).

[7] Feitsma, K.G., Franke, J.P. and de Zeeuw, R. A., *Analyst*, **109**, 789 (1984).

[8] Yin, X., Schlemmer, G. and Welz, B., *Anal. Chem.* **59**, 1462 (1987).

[9] Bertholf, R.L. and Renoe, B.W., *Anal. Chem. Acta* **139**, 287 (1982).

[10] Jensen, F.O., Dolezal, J. and Langmyhr, F.J. *Anal. Chem. Acta*, **72**, 245 (1974).

[11] Batley, G.E., and Matousek, J. P. Anal. Chem. 49, 2031 (1977).

[12] Habib, M.M. and Salino, E.D., *Anal. Chem.* **57**, 2055 (1985).

[13] Matusiewicz, A., Fish, J. and Malinski, T. *Anal. Chem.* **59**, 2264 (1987).

[14] Pretty, J. R., Evans,E.H., Blubaugh, E.A., Shen, W., Caruso, J. A. and Davidson, T.M. *J. Anal. Atom. Spectrom.* **5**, 437 (1990).

[15] Beinrohr, *E. Mickrochim. Acta* **120**, 39 (1996).

[16] Matousek, J.P. and Powell, H. K. J. *Spectrochim. Acta* **50B**, 857 (1995).

[17] Taylor, A. *Mickrochim. Acta*, **123**, 251 (1996).

[18] Matousek, J.P. and Powell, H.K. J., Talanta, 40, 1829 (1993).

[19] Veillon, C. *Anal. Chem.* **58**, 851A (1986).

[20] Vollkopf,U.and Barnes,K. *Atomic Spectroscopy*, **16**, 19 (1995).

[21] NIST, "Certificate of analysis", SRM 2670, toxic metals in freeze-dried urine, (1994).