Sensing in Electroanalysis

Vol. 7 (K. Kalcher, R. Metelka, I. Švancara, K. Vytřas; Eds.), pp. 57–69.

ISBN 978-80-7395-563-2 (printed); 978-80-7395-564-9 (on-line)

Detection of Heavy Metals in Biological Samples through Anodic Stripping Voltammetry

Vlad Buzea, Monica Florescu, and **Mihaela Badea***

Transilvania University of Brasov, Faculty of Medicine, Str. Nicolae Balcescu Nr.56, Brasov, Romania

---

Abstract: The toxicological aspects due to the presence of heavy metals in biological samples impose to have accurate and rapid methods for their detection. This paper is aimed to review approaches to anodic stripping voltammetry (ASV) determination of several heavy metals (lead, cadmium, copper, mercury, zinc) in biological matrices (blood, urine, saliva, tissue sample). Analytical performances (LOD, data linearity range, sensitivity) of the reviewed methods were presented for several electrochemical methods.

Keywords: Heavy metals, Lead, Cadmium, Copper, Mercury, Zinc, Electrochemical techniques / methods, Anodic stripping voltammetry, Samples, Blood, Urine, Saliva, Tissue.

---

*) Author to whom correspondence should be addressed. E-mail: mihaela.badea@unitbv.ro

Introduction

Heavy metals can be considered as non-biodegradable pollutants of variable toxicity depending on the type of metal, the chemical species they are constituted in, their attachment (or lack of) to stabile substrates. Cadmium, mercury and lead for instance are foreign to all organisms, in that they perform no biological function and are toxic for most organisms even in trace amounts. Given their non-biodegradable nature, the toxic burden of heavy metals tends to persist in an environment until they are eventually deposited in a chemically stable form (as a mineral [1]).

During their interaction with the biological samples heavy metals show toxic potential and this is a good reason to search for them in biological samples. Furthermore organisms accumulate these metals storing them in organic complexes thus concentrating them along food chains. So, they can be used as very relevant indicators of environmental contamination. The medical and toxicological aspect is perhaps the best reason to improve detection of heavy metals in biological samples. Because it seems that human activity is one source of heavy metal contamination, screening and diagnosis for heavy metal poisoning is often necessary.

Anodic Stripping Voltammetry (**ASV**) is an electrochemical method consisting of two fundamental steps and one intermediate period:

- *the pre-concentration time* - during which the analyte is electrochemically reduced and plated on the working electrode at potentials more negative than its half-wave potential;
- the *rest* (or *equilibrium*) *period* needed to let the high current drop to a measurable level (*Note:* Original intensity is being inappropriately high due to the faradic processes under convective transport, which is enabled by stirring the solution or, also, by rotating the working electrode itself);
- *the analysis time* is a period during which the analyte is oxidized off the electrode (the strip) through o potential sweep past its half-wave potential and the current is displayed as function of potential. The height of peak current is proportional to the analyte amount stripped of the electrode.

This method's potential has already been proven as an efficient tool in the detection of heavy metals being sensitive with lower limits of detection in the under-ppb level, having wide data ranges and when compared to other analytical methods such as flame atomic absorption spectrophotometry (FAAS), electrothermal atomic absorption spectrophotometry (EAAS) or inductively coupled plasma-mass spectrometry (ICP-MS) it is simpler and less costly [2,3,4].

In organic samples, however, ASV as well as other electrochemical methods is met with the challenge of numerous organic compounds that can complex heavy metal ions or be adsorbed onto the electrode surface thus fouling it and cause a decrease or total loss of signal. Another issue is that of oligoelement trace metals such as Cu, Zn, or Co that might interfere with foreign heavy metal detection [5,6,7]. Also solid tissue samples need to be rendered in liquid form before commencement of electrochemical analysis [8]. This paper is aimed at reviewing various approaches to ASV determination of heavy metals in biological matrices.

Heavy Metals in Blood Samples

Lead is the most frequent toxic heavy metal incriminated in human intoxication. In blood, of the total lead amount around 95-99% is bound to red blood cells [7]. The American Center for Disease Control provides some reference ranges for blood lead, 50µg/L for children and 100µg/L for adults, below these values no clinical consequence of lead poisoning is expected. Though plasma and serum are simpler matrices than whole blood because they have no cells and it may be tempting to use them in electrochemical determination as one could expect less interference, such a determination would be irrelevant because these blood components are much less concentrated in lead than whole blood, also there are no normal values for lead in either serum or plasma to compare with. Since ASV can detect multiple heavy metals including lead, a toxicological screening for heavy metals should be done in whole blood as it allows the detection of lead which is the most common heavy metal toxicants.

Before ASV can be performed pretreatment of the sample is required. Wet digestion in concentrated acids, if total, dissolves and oxidizes organic matter to simple inorganic compounds sometimes this nullifies the matrix effect [8] and releases metal ions from their bonded state making them available for reduction and then stripping at the working electrode. However some trace heavy metals are naturally present in blood, the normal range for Cu in human whole blood is around 1.1-1.5mg/L and double that amount in late pregnancy [9]. Copper is known to interfere with heavy metals at certain electrode setups like bismuth film covered electrodes [6].

In 1973, Searle used an Anodic Stripping Voltammeter, (ESA) Model 2014 (graphite mercury film plated electrode, Ag/AgCl reference Pt counter) to determine lead in capillary blood from 200 children [10]. The sample pretreatment consisted in digestion of blood in 70% perchloric acid for 30 minutes yielding a 1:80 dilution of blood lead in electrolyte reagent [10]. The method was compared with AAS and the average deviation between the two techniques was 0.9µg/L.

García-Fernández et al. [11] described a digestion method using a mixture of nitric/perchloric/sulfuric acids, 8:8:1, and thermal treatment until the digested sample is dry then dissolved in water (yielding a blood lead dilution of 1:50) and submitted for DPASV (Differential Pulse Anodic Striping Voltammetry) with a anodic stripping voltammeter (VA-647 workstation; Metrohm, Switzerland) electrode setup: working hanging mercury drop electrode, reference Ag/AgCl; KCl 3M and platinum counter. This method was used for detection of lead in blood harvested from wildlife as an indicator of environmental contamination and has been reproduced by other authors as well [12,13].

Dilution of blood (anticoagulated with EDTA) then acidification with concentrated hydrochloric acid, centrifugation and then adding reagent ($HgCl_2$/HCl solution) to the supernatant yielding a 1:21 dilution of blood lead (1.2M HCl and 9.2mg/L Hg^{2+}) was employed by Feldman et al. for square-wave anodic stripping voltammetry (SWASV) at a disposable carbon microarray (a dielectric covered carbon layer with 287 holes exposing as many carbon surfaces making for 287 working microelectrodes tied in parallel) with a Ag/AgCl reference and counter electrode plated ex situ with a mercury film [14]. The data range of the method is 3.2 to 50μg/L in blood derived solution. Results are compared with those obtained with graphite furnace atomic absorption spectrometry (GFAAS) and the deviations between the two ranges from -1μg/L to -7μg/L with the ASV constantly underestimating concentration. Mechanisms of interference described are complexation of lead and adsorption onto the electrode surface of fouling substances especially at bare carbon and $HgCl_2$ (from oxidation of Hg droplets on the microelectrode surface). These electrode fouling substances are probably albumins as nitrocellulose filtration removes the effect of Hg oxidation has on signal intensity.

Yantasee et al. [15] approached trace metal (Pb, Cd and Cu) detection in complex matrices (blood, urine and saliva) with a metal analyzer by ASV at a Hg film coated glassy carbon electrode under a turbulent flow of the diluted and pretreated sample. For blood the dilution was 1:10 and the pretreatment was identical as for urine or saliva: acidification to 1M HCl and ultrafiltration. The turbulent flow was obtained with Flow Injection Analysis (FIA) system designed so as the jet from the flow cell inlet would hit the Hg film. The analytical performance of the metal analyzer for blood samples was LOD (limit of detection) of 0.46 μg/L, data range 0-20 μg/L and sensitivity of 0.033μA/ppb for lead. Concerning interference from adsorption of blood proteins on the electrode, the jet-wall flow onto design of the cell and ultrafiltration seem to negate the matrix effect as the use of no blood lead spiked samples results in almost the same analytical parameters obtained on 1:10 blood dilution (0.035 slope and 0-20 range). Concerning lead complexation by blood proteins, it is studied on lead spiked samples that were allowed to incubate for 10 minutes. This time might not be enough for the degree of lead binding that would occur in real exposure situations, either chronic or acute, never the less based on the fact that Pb spiking after filtration or before it has no influence on the concentration detected leads to authors to the conclusion that the acidification to 1M HCl releases all lead bound to proteins.

Roda et al. [5] and Bannon et al. [16] used a common commercially available anodic stripping voltammeter of the past two decades, the ESA model 3010 (mercury film on graphite working electrode), to determine blood lead concentration.

The version A of the 3010 model was investigated by Roda et al. [5] and they found some short comings with the apparatus accuracy as it underestimated lead in blood below 400μg/L and overestimated it above this value also copper interference with lead is reported in this article. The authors obtain better accuracy by using Isotope Dilution-Mass Spectroscopy certified standards for the calibration of the anodic stripping voltammeter and by changing the analytical signal interpretation protocol from peak area to peak height. The lead copper interference is described as consequence of the stripping potential of the metals being very close copper's being slightly more anodic than lead's, also the manufacturer supplied reagent contained Cl^- ions which cause the cathodic slope of the Cu peak to be shallow thus causing peak overlap. The problem was addressed by altering the stair case striping potential parameters implemented by the manufacturer lowering the sweep rate making for better peak separation between Cu and Pb.

Bannon et al. [16] used a latter version the 3010B for lead determination in blood samples (anticoagulated on EDTA) from children and compared voltammetric with GF-AAS (Graphite Furnace Atomic Absorption Spectrometry) results and found them in accordance. The authors used their own recipe of reagent for the measurement, blood-reagent mixture accounted for 1:30 dilution of blood as would have been obtained for manufacturer supplied reagent both in the case of the A and B versions of the stripping voltammeter. The recipe employed here used chloride salts exclusively and no copper interaction is reported. Nickel chloride was added to reagent to free lead from its EDTA complex.

So far the cited articles describe the use of mercury electrodes, which perform very well even in the in the challenging matrix that is blood, having been extensively studied and used in commercial devices that were proven to be on par with AAS methods [5,10,16] as far back as almost 3 decades ago. Improvements made to these systems as far as analytic performance brought about a reduction in the quantities of Hg used. More recent articles reflect the tendency to abandon mercury altogether in favor of more environmentally friendly Au, Ag, bismuth film (BiFE), polymer, carbon nanotubes (CNT) modified electrodes.

Liu et al. [6] used a Nafion coated bismuth film electrode to determine Zn, Cd and Pb in blood samples via DPASV. The pretreatment of samples was an advanced oxidation process (AOP) that it took about one hour and was done in a photoreactor with UV irradiation, at the end the 0.5 mL blood sample was diluted 1:40. Then the blood reagent mixture was submitted for DPASV; the Nafion-coated electrode was plated *in situ* with 300μg/L Bi^{3+} at -1.3V. Analytical performance was good for all metals. The highest LOD was for Zn at 0.97 μg/L and comparison with AAS resulted in relative standard deviations of less

than 5% for all metals. Interference reported with copper at concentration ratio of copper-to-target analyte = 35:1, the peak for the said analyte is completely suppressed, the problem of copper interference is assumed to be due to competition for nucleation sites between Cu and Bi at the electrode surface and also by formation of inter-metallic compounds between Cu and Pb. The problem is addressed by adding $[Fe(CN)_6]^{4-}$ to the solution to complex Cu^{2+} ions.

Heavy Metals in Urine and Saliva Samples

Toxicology analysis for heavy metals usually requires blood samples because blood is a more reliable indicator of exposure to heavy metals. However sample collection for both urine and saliva is non- invasive and urine in particular reveals the plasma clearance of heavy metals, consequently samples of these two bodily fluids may hold some usefulness in monitoring chronically exposed individuals. In 1997, *Center for Disease Control* (CDC) [17] and its recommendations concerning the lead determination in urine point out to its relevance in lead excretion in a 8 hour period after a provocation test, namely the administration of $CaNa_2EDTA$ (lead chelating agent). When EDTA is present in urine the incorporation of Ni^{2+} salts into the supporting electrolyte is recommended to free Pb^{2+} as Ni has a higher affinity for EDTA than lead. For trace metal detection urine dilution has greater impact than in blood, as urine may vary in concentration on a physiological basis so spot urine samples have almost no relevance and it must be pooled over a time interval.

The urine matrix has been dealt with by means of acid digestion [18,19], UV irradiation [20] and ultrafiltration [15]. Mesaroš et al. [20] proved it was possible to use a mercury hanging drop semi-microelectrode (r = 40μm) in UV irradiated urine for determination of lead, without supporting electrolyte.

Lead determination in urine at a BiFE glassy carbon electrode through DP-ASV was attempted by Hop et al. [18] on 24 hours pooled urine samples from fishermen and net weavers that held small pieces of lead in their mouths while handling nets. Blood lead was determined by the same electro-analytical method as well and no correlation was found between urine and blood levels. Both urine and blood samples were also analyzed with GF-AAS and results pointed out that DP-ASV had a RSD of ±4.5% (vs. AAS).

Y. Bonfil et al. [19] used a rotating gold electrode and Subtractive Anodic Striping voltammetry (SAVS) to determine Hg^{2+} in urine down to a LOD of 4nM (0.8ppb). The authors subtracted the SWASV plot of a 0 s deposition time measurement from that of a 180 s

deposition time one, in order to attenuate background noise improving the quality of the striping curve and lowering the LOD by one order of magnitude. The urine sample had been pretreated by digestion in nitric and perchloric acids; the digestion procedure produced an increase in LOD from 0.4nM in supporting electrolyte to 4nM in the treated urine sample this was assumed to be due to impurities in the perchloric acid.

Khairy et al. [21] used screen printed electrodes modified with in situ Bi (1 ppm in sample solution) to detect Cd via SWASV in saliva, the pretreatment consisted in dilution of 1:100 and acidification to pH1 the LOD was 2.3µg/L but given the dilution of 1:100 this implies that Cd in actual samples could not be detected below 230µg/L.

Heavy Metals in Solid Tissue Samples

Before any type of electrochemical analysis can be performed on solid samples they must first be dissolved in the supporting electrolyte, so digestion becomes a necessary step in sample pretreatment. Digestion has the inherent disadvantages of contamination and loss of analytes so blank reagents and, spiked samples with recovery experiments become necessary [8].

Injang et al. [22] used Bi (0.15 ppm, plating time 180s) coated CNT (5%wt) modified SPCE (Screen Printed Carbon Electrode) to determine Pb, Cd and Zn in *Andrographis paniculata* leaf tissue (dried leafs and capsules mad from leafs). They experimented with two acid digestion methods namely dissolution in a mixture of $HNO_3/HClO_4$ (optimum ratio found was 3/2) the end result was 5g of leafs (digested and evaporated) dissolved in 5ml 1MHCl (recovery: 95%-105%); second method used HNO_3 and solid phase extraction. The SWASV measurement was done in a flow cell coupled to a system for sequential injection analysis (SIA). The results obtained were compared with ICP-AES measurements and found to be in accordance.

Meucci et al. [23] sought determination of Pb, Cd, Cu and Hg in Atlantic hake filets. Lead, cadmium and copper concentrations were measured at a SPCE with the working electrode coated by a cellulose derivative film with mercury salts, while in sample mercury was determined at a gold ink SPE (Screen Printed Electrode). Deposition potential was -1.1V vs. Ag/AgCl, 300s duration for the first three metals and 0.2V vs. Ag/AgCl for 200s for mercury, stripping was done by square wave. The authors experiment with five different recipes for fish tissue digestionworking on wet and dry muscle using 1M HNO_3, 65% HNO_3, $HNO_3/HClO_4$, H_2O_2/HCl varying time of digestion, temperature, and purification.

The optimal approach (the only method that produced acceptable SWASV signals) was digestion with H_2O_2 and HCl: 2 hours at 50^oC for H_2O_2 + 2 hours for HCl at 70^oC followed by carbon column (Superlean ENVI carbon column) to remove organic matter. This method yielded a recovery greater than 91% when used on certified reference material spiked with the analyses of interest.

Gaston et al. [24] used a gold rotatig electrode and subtractive square wave anodic stripping voltammetry (E deposition =0.1V vs. Ag/AgCl, deposition time =30s, E strip=1.15 vs. Ag/AgCl, step of 4mV, amplitude of 25mV and frequency of 15Hz)to detect Hg in human hair samples. The digestion method chosen was pressurized microwave digestion carried out in HNO_3/H_2O_2 2:1, the supporting electrolyte was 5mM H_2SO_4 with 10mM NaCl and the hair to final solution ratio was 200mg hair to 25mL solution. Various digest recipes were tested and the end result was judged based on the spectrophotometric measurement of absorption atλ=254nm (organic carbon absorbs at this wave length) as a degree to which the organic matrix had been destroyed. A type of digestion mixture that gave an even lower 254nm absorption than the above mentioned one was $HNO_3/HClO_4/H_2O_2$= 3:1:2, however the $HClO_4$ was discarded as it is a very powerful oxidant and has the potential to become dangerous when used on large quantities of organic matter. The NaCl was added to the supporting electrolyte because the stripping peak of mercury in chloride electrolyte was proven to be 6 times higher than in the absence of chloride. The procedure was tested by determinations (by standard addition method) carried out in certified hair samples and the regression coefficient of the addition was R = 0.999.

Table I (given overleaf) summarizes the type of samples in which anodic stripping voltammetry was performed for the determination of various heavy metals and the analytical performances of the methods reviewed before.

Conclusions

Though ASV is a cost-effective, precise and accurate method especially in the detection of heavy metals at trace amounts it is somewhat difficult to apply it to the great variety of types of biological samples, of which we have mentioned but a few. Also it is often, if not always necessary for biological matrices to be simplified before being submitted for analysis. Sample preparation is often the most time consuming step in this type of determination as for the few minutes that are spent on the actual measurement sometimes hours have to pass before the biological sample is brought to an ASV compatible solution.

Table I: *Analytical performance of anodic stripping voltammetry tests used for detection of heavy metals*

Analyte	Working electrode	Electro-chemical technique	Sample	LOD (ppb)	Data linearity range (ppb)	Slope (µA/ppb) unless stated otherwise	Ref.
Pb^{2+}	NCBFE	DPASV	Blood AOP pretreatment 1:40 dilution	0.13	1.7-27.9	0.148	[6]
Cd^{2+}				0.09	1.3-20.5	0.182	
Zn^{2+}				0.97	3.5-79.6	0.562	
Pb^{2+}	Disposable carbon microarray (plated ex situ Hg)	SWASV	Blood on EDTA 1:20 dilution 1.2M HCl	5	0-50	0.03	[15]
			Urine 1:2 dilution 1M HC ultrafiltered	0.44	0-50	0.017	
Pb^{2+}	W-Hg film glassy carbon electrode	SWASV (FIA)	Blood 1:10 dilution 1M HCl ultrafiltered	0.46	0-20	0.035	[16]
			Saliva 1:2 dilution 1M HCl ultrafiltered	1	0-10	0.463	
Pb^{2+}	Glassy carbon BiFE	DPASV	Blood digested in HNO_3	0.7	2-100	0.15	[18]
			Urine digested in HNO_3				
Hg^{2+}	Gold rotating electrode	SW-SASV	Urine Digested in $HNO_3/HClO_4$ 1:10 analyte dilution	0.8 for 5000 rpm	0.08-80	0.059 ± 0.005 $AV^{-1}/[Hg^{2+}]$*	[19]

Table I: (continued)

Analyte	Working electrode	Electrochemical method	Sample	LOD (ppb)	Data linearity range (ppb)	Slope (µA/ppb) unless stated otherwise	Ref.
Cd^{2+}	W-screen printed graphite (plated Bi in situ)	SWASV	"artificial saliva" 0.01M HCl	4.3	20-90	0.054	[21]
Pb^{2+}	SPCNTE (Plated Bi in situ)	SWASV (SIA)	Herbs Digested in $HNO_3/HClO_4$ 3:2 5g digested then dissolved in 5mL 1M HCl	0.2	2-100	0.388 (2-18 ppb) 0.668 (20-100)	[22]
Cd^{2+}				0.8	2-100	0.586 (2-18ppb) 0.799 (20-100)	
Zn^{2+}				11	12-100	0.175 (2-18ppb) 0.243 (20-100)	
Pb^{2+}	SPCE Modified with Hg film Screen printed gold	SWASV	Fish tissue Digested in H_2O_2 and HCl carb column purified 5g dry muscle/20 mL solution	0.3	1-1000	0.31	[23]
Cd^{2+}				1		0.10	
Cu^{2+}				0.5		0.25	
Hg^{2+}				0.9		0.04	
Hg^{2+}	Gold rotating electrode	SW-SASV	Human hair Digested in HNO_3 and H_2O_2 0.2g in 25mL solution	0.384	6.7-46.7	1	[24]

Legend: *analytical signal was not the peak height but a sum of the absolute values of slopes at the two inflexion points at the peak base. Then, instead of A / concentration, we have a formula** when adding the electrode area as 0.00661cm^2, the deposition time 180s, and the velocity 5000 rpm.

**) $$\frac{(6\pm0.6)A}{V\,cm^2\,[Hg2+]s\,rpm^{\frac{1}{2}}}$$

Also, certain methods of preparation, such as mineralization by the acid digestion, have significant tendencies to cause the sample contamination and the corresponding controlling steps must be implemented in order to reveal the unwanted contamination and/or analyte loss in the event that it occurs.

Finally, while electrochemical techniques have great potential for miniaturization, portability and a possibility to be employed in on-site analysis, the cited cases of the sample preparation still require a laboratory.

References

1. A.W. Bott: "Voltammetric Determination of Trace Concentrations of Metals in the Environment" *Current Separations,* **14** (1995) 24-29
2. E.A. McGaw, G.M. Swain: "A comparison of boron-doped diamond thin-film and Hg-coated glassy carbon electrodes for anodic stripping voltammetric determination of heavy metal ions in aqueous media" *AnayticaChimicaActa*, **575** (2006), 180-189
3. P. Sonthalia, E. McGaw, Y. Show, G.M. Swain: "Metal ion analysis in contaminated water samples using anodic stripping voltammetry and a nanocrystalline diamond thin-film electrode" *AnayticaChimicaActa*, **522** (2004), 35-44
4. S. Legeai, K. Soropogui, M. Cretinon, O. Vittori, A.H. De Oliveira, F. Barbier, M.F. Grenier-Loustalot: "Economic bismuth-film microsensor for anodic stripping analysis of trace heavy metals using differential pulse voltammetry" *Analytical and Bioanaytical. Chemistry*, **383** (2005), 839-847
5. S.M. Roda, R.D. Greenland, R.L. Bornacheln, P.B. Hammond: "Voltammetry Procedure Modified for Improved Accuracy of Blood Lead Analysis" *Clinical Chemistry*, **34** (1988), 563-567
6. B. Liu, L. Lu, M. Wang, Y. Zi: "A Study of Nafion-Coated Bismuth-Film Electrode for the Determination of Zinc, Lead, and Cadmium in Blood Samples" *Electroanalysis*, **21** (2008) 2363–2369
7. A.K. Rowden: "Pathophysiology and Etiology of Lead Toxicity "; http:// emedicine.medscape. com/article/2060369-overview
8. K. Kuboyama, N. Sasaki, Y. Nakagome, M. Kataoka: "Wet Digestion". *Analytical Chemistry,* **360** (2005), 184-191
9. http://www.copperinfo.co.uk/health/downloads/pub-34-copper-and-human-health.pdf

10. B. Searle, W. Chan, B. Davidow: "Determination of Lead in Blood and Urine by Anodic Striping Voltammetry" *Clinical Chemistry vol.19,* (1973) 76-80
11. A.J. Garcıá-Fernández, J.A. Sánchez-Garcıá, P. Jiménez-Montalbán, A. Luna: "Lead and cadmium in wild birds in southeastern Spain", *Environ ToxicolChem,* **14** (1995) 2049-2058
12. E. Martínez-López, A.R. Sousa, P. María-Mojica, P. Gómez-Ramírez, L. Guilhermino, A.J. García-Fernández: "Blood *d*-ALAD, lead and cadmium concentrations in spur-thighed tortoises (Testudograeca) from Southeastern Spain and Northern Africa" *Ecotoxicology,* **19** (2010) 670–677
13. P. Gómez-Ramírez, E. Martínez-López, P. María-Mojica, M. León-Ortega, A.J. García-Fernández: "Blood lead levels and d-ALAD inhibition in nestlings of Eurasian Eagle Owl (Bubo bubo) to assess lead exposure associated to an abandoned mining area" *Ecotoxicology,* **20** (2011) 131–138
14. B.J. Feldman, A. D'Alessandro, J.D. Osterloh, B.H. Hata: "Electrochemical Determination of Low Blood Lead Concentrations with a Disposable Carbon Microarray Electrode "*Clinical Chemistry*, **41** (1995) 557-563
15. W. Yantasee, Y. Lin, K. Hongsirikarn, G.E. Fryxell, R. Addleman, C. Timchalk: "Electrochemical Sensors for the Detection of Lead and Other Toxic Heavy Metals: The Next Generation of Personal Exposure Biomonitors" *Environ Health Perspectives*, **115** (2007) 1683–1690.
16. D.I. Bannon, J.J. Chisolm Jr.: "Anodic Stripping Voltammetry Compared with Graphite Furnace Atomic Absorption Spectrophotometry for Blood Lead", *Clinical Chemistry,* **47**(2001) 1703-1704
17. http://www.cdc.gov/nceh/lead/publications/1997/pdf/c1.pdf
18. N.V. Hop, D.V. Khanh, C.D. Kinh, H.T. Tin, T.V. Nghi: "Bismuth Film Electrode for Striping Voltammetric Determination of Blood Lead and Preliminary Assessment of Blood Lead Level in the Residents at Canh Duong Village, ThuaThien Hue Province", *Journal of Chemistry,* **45** (2007) 311 - 317
19. Y. Bonfil, M. Brand, E. Kirowa-Eisner: "Trace determination of mercury by anodic stripping voltammetry at the rotating gold electrode", *AnalyticaChimicaActa,* **424** (2000) 65–76
20. Š. Mesaroš, H. Hofbauerová, D. Bustin: " Determination of Trace Lead in Human Urine Using Hanging Mercury Drop Semimicroelectrode Influence of Matrix Effect and Its Elimination", *Chemical Papers,* **46** (1992) 98-101

21. M. Khairy, R.O. Kadara, D.K. Kampouris, C.E. Banks: "In situ bismuth film modified screen printed electrodes for the bio-monitoring of cadmium in oral (saliva) fluid", *Analytical Methods,* **2** (2010) 645–649
22. U. Injang, P. Noyrod, W. Siangproh, W. Dungchaia, S. Motomizu, O. Chailapakul: "Determination of trace heavy metals in herbs by sequential injection analysis-anodic stripping voltammetry using screen-printed carbon nanotubes electrodes", *AnalyticaChimicaActa,* **668** (2010) 54–60
23. V. Meucci, S. Laschi, M. Minunni, C. Pretti, L. Intorre, G. Soldani, M. Mascini: An optimized digestion method coupled to electrochemical sensor for the determination of Cd, Cu, Pb and Hg in fish by square wave anodic stripping voltammetry", *Talanta,* **77** (2009) 1143–1148
24. A. Gaston, P.E. Marinho: "Determination of Mercury in Hair by Square-Wave Anodic Stripping Voltammetry at a Rotating Gold Disk Electrode After Microwave Digestion ", *Biological Trace Element Research,* **103** (2005) 261-276