DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
42

DETERMINATION OF SOME IMPORTANT TRACE METAL IONS IN HUMAN BLOOD

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Dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Chemistry on April 28, 2004, by the Doctoral Committee of the Department of Chemistry, University of Tartu.

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Commencement: June 21, 2004 at 2 Jakobi St. Room 430

Publication of this dissertation is granted by University of Tartu

Tartu Ülikooli Kirjastus
www.tyk.ut.ee
Tellimus nr. 244

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I. **Jaanus Kruusma** and Lembit Nei*, Joanna L. Hardcastle and Richard G. Compton, ”Sonoelectroanalysis: Anodic Stripping Voltammetric Determination of Cadmium in Whole Human Blood”, *Electroanalysis*, Vol. 16 (2004), p. 399.

II. **Jaanus Kruusma**, Peter Tomčík, Craig E. Banks and Richard G. Compton*, “Sonoelectroanalysis in Acoustically Emulsified Media: Zinc and Cadmium” (*Electroanalysis*, accepted).

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V. **Jaanus Kruusma**, Craig E. Banks, Lembit Nei and Richard G. Compton*, “Electroanalytical Detection of Zinc in Whole Blood” *Analytica Chimica Acta*, Vol. 510 (2004), p. 85.

VI. **Jaanus Kruusma**, Craig E. Banks, and Richard G. Compton*, “Mercury Free Sono-Electroanalytical Detection of Lead in Human Blood Utilising Bismuth Film Modified Boron-Doped Diamond Electrodes” (*Analytical and Bioanalytical Chemistry*, accepted).

1. INTRODUCTION

We all, citizens of this industrial world, living in an environment different from the natural. Making our lives more and more comfortable we are forced up the production of the goods we want to have. Unfortunately we have no absolutely clean technologies in our service. Every production stage pollutes in its own way the environment in which we are living. Very often harmful trace metals are released and precipitated into surrounding [1]. There they will be introduced into biological chain ending very often inside us — in humans. The most common toxic heavy metal ions are Pb^{2+}, Cd^{2+}, Hg^{2+}, As^{3+} and Cu^{2+} causing different disease [2]. Therefore it is essential to monitor the situation in the environment. When a human has been exposed to some kind of harm, it is very critical to find quickly the substance causing poisoning. But critical is not only to identify this substance/compound but also to measure the amount (concentration) of this pollutant to find the best and reliable way for cure.

On the other hand sometimes the deficiency of some kind of trace metal ions can be a reason or indicator of some kind of disease [3]. Clinical features of deficiency include testicular atrophy, skeletal maturation and retardation of growth [4]. For health and environmental monitoring several parts of body are used. Commonly hair [5–9], sweat [10–12], urine [7, 9, 12–21], some tissue [22] and blood [12, 21, 23–31] or other body fluids [32, 33] are used as samples. Mainly AAS [34–41] or ICP-MS [42–46] instruments are used for analysis, which have a need for time consuming sample pretreatment. It must be mentioned that these analytical instruments are also expensive and not portable. Therefore we have interest for cheaper and more rapid analytical methods.

One of the cheapest ways to perform analyses of large variety of heavy metal ions is to use electrochemical methods [47]. The most sensitive are stripping voltammetric methods allowing the determination of these metals amount of down to 10^{-13}–10^{-15} mol/dm^3 on a routine basis [48]. The metals, which cations can be analysed by stripping analysis, are: Ag, As, Au, Ba, Bi, Cd, Co, Cs, Cu, Fe, Ga, Hg, In, K, Mg, Mn, Mo, Na, Ni, Pb, Pd, Pt, Rb, Sb, Se, Sn, Sr, Tc, Te, Ti, Tl, U, V and Zn [47, 48]. Cations of metals like Al, Cr, La, Mn, Th and Zr can be analysed via reduction of a suitable ligand [48]. From medical point of view interesting metals from this list include: Al, As, Bi, Cd, Cr, Co, Fe, Mn, Hg, Ni, Pb, Pt, Cu, Se, Sn, Tl and Zn [15]. They are sources or indicators of illness [15].

Unfortunately voltammetric analytical methods, when applied to analyses with a high organic content (i.e. body fluids) require sample pretreatment just as atomic absorption spectrometric or mass-spectrometric methods because of the likely passivation of the surface by surface-active compounds adsorbing on the electrode surface. To overcome this kind of problem the ultrasound has been taken into arsenal of electrochemistry [49–60]. The applied ultrasound provides

continues cleaning of the electrode surface and removal of passivating organic complexes during deposition step of these metals. The cavitational effects of ultrasound have been studied in detail [50, 55, 56, 58–64] and shown to facilitate the determination of low metal ions concentrations in highly passivating media via *in situ* cavitational cleaning of the electrode [49–51, 53, 59, 65]. In addition, acoustic streaming significantly increases the rate of mass transport resulting in faster deposition, higher sensitivity and reduced detection limits [50, 54, 57, 58]. Sonovoltammetric stripping analysis has been successfully utilised in the determination of copper ions in beer [59] and blood [31], lead ions in wine [65] and river sediments [66], as well as manganese ions in tea [67].

Traditionally such metals like Au, Pt, Hg and non-metallic conducting materials like glassy carbon are used as working electrode materials. Unfortunately all they have specific complications. Noble metals have a narrow region of ideal polarisability and they are not very sensitive. Glassy carbon has a wider region of ideal polarisability and better sensitivity, then noble metals, but much less, than the mercury-drop or Hg-film electrodes [47, 48, 68]. It seems that Hg-type electrodes should be the best ones, but they are not perfect also. The lack of mechanical robustness and environmental toxicity are limiting factors for the application of mercury in modern science and analytical tests. Therefore is it useful to explore and apply new and better working electrode materials. The most promising of these are boron doped diamond [66, 67, 69–72] and bismuth-coated electrodes [73–83].

The aim of this thesis is to demonstrate the versatility of anodic stripping voltammetry coupled with the ultrasound as a tool for the detection of some heavy metals ions (Cd^{2+}, Pb^{2+} and Zn^{2+}) in human blood. Analyses of these metal ions using voltammetric methods are highly advantageous due to the compact size of the equipment allowing potential portability and the relatively low cost compared to the spectroscopic analytical methods. Also the use of toxic mercury electrode is minimised. Our attention is turned to blood analysis while it is most common media for this kind of analyses giving detail overview due being in direct contact with almost intestinal organs. In blood metal cations like Cd^{2+} and Pb^{2+} are bound almost completely with red blood cells [15].

2. ABREVIATIONS USED

AAS	—	atomic absorption spectrometry
ASV	—	anodic stripping voltammetry
BDD	—	boron doped diamond
BDDE	—	boron doped diamond electrode
DP-ASV	—	differential pulse anodic stripping voltammetry
GC	—	glassy carbon
GCE	—	glassy carbon electrode
I	—	intensity of applied ultrasound
ICP-MS	—	inductively coupled plasma mass spectrometry
NCGCMFE	—	Nafion® coated glassy carbon mercury film electrode
RE	—	reference electrode
SCE	—	saturated calomel electrode
SW-ASV	—	square wave anodic stripping voltammetry
WE	—	working electrode

3. THEORETICAL BACKGROUND

3.1 Cavitation phenomenon

The application of ultrasound in chemistry is quite old — the first work was done in the 1930's and was connected with the lowering of the potential for electrochemical synthesis of gases down to the equilibrium potential [84]. Today sonication is applied in electroplating enhancing adhesion, giving a brighter finish and increasing the deposition current density. In organic chemistry ultrasound is used assisting electropolymerisation [84] and synthesis [85–87]. Ultrasonic agitation was applied also for high-rate controlled potential coulometry [85] and amperometry [60, 88], enhancement of the sensitivity of flow-cell electrochemical sensors, variation of the convection rate of the solution at GCE in hydrodynamic modulation voltammetry [84] and for different type of electron transfer rate measurements [89–92]. Compton et al [31–33, 55, 65, 93–96] have successfully shown that sonification can be used for reactivation of WE in highly passivating media.

Sonification can accelerate mass transport to WE mainly in two ways: 1) via acoustic streaming and 2) by micro jet formation after cavitational collapse of gas bubbles in the vicinity of the WE surface [50, 54, 60, 84, 87, 88, 97–100]. The studies of Marken et al [101] and Maisonhaute et al [102] showed that the acoustic streaming plays the most important role in mass transport giving value for steady state sono limiting current. Despite of this fact the cavitationaly collapsing gas bubbles have very important position in sonochemistry. These gas bubbles will preferentially form randomly where "cavitation nuclei" are present. Such nucleus can be surface inhomogenities like holes and cracks, bubbles remaining from previous cavitation events or impurities [103]. The formation of cavitating and expanding gas bubbles going to collapse at radius R_M presumes the presence of microbubbles filled with gas and vapour [84]. The collapse of an empty spherical cavity (bubble) with initial radius R_M in a liquid with density ρ under constant external pressure P_e was first studied in 1917 by Rayleigh [84]. Noltingk and Neppiras [84] modified his equation to a more general form. After neglecting the compressibility and viscosity of the liquid they described the instant bubble radius R as follows:

$$R\frac{d^2R}{dt^2}+\frac{3}{2}\left[\frac{dR}{dt}\right]^2=\frac{\Delta P}{\rho} \qquad (1)$$

In that equation ρ is density of the liquid while ΔP is difference between the external and pressure of liquid P_L and the internal pressure of gas and/or solvent vapour P_g inside cavitating bubble:

$$\Delta P = P_L - P_g \quad (2)$$

The pressure P_L itself is sum of the external ambient pressure P_e, the surface tension pressure P_ρ ($P_\rho = 2\rho / R$) due to the surface tension ρ, and the acoustic (ultrasonic) pressure P_{ac} ($P_{ac} = P_A \sin 2\pi ft$) where P_A ($P_A = (2\ \rho c I_0)^{1/2}$) is the ultrasonic pressure amplitude:

$$P_L = P_e + 2\ \rho / R + P_A \sin 2\pi ft \quad (3)$$

The f is the applied ultrasonic frequency, t is time, c is velocity of sound in a liquid being under study and I_0 is ultrasonic intensity.

If the P_A is larger than internal pressure inside the bubble:

$$P_A > P_e + 2\ \rho / R \quad (4)$$

a negative pressure is formed in the underpressure phase (the minimal P_L value is $P_e + (2\ \rho / R) - P_A$) and the bubble starts fast to grow. The underpressure will be changed sinusoidally to overpressure in compression phase and the expansion of the bubble ceases at the time t_M when the maximum radius R_M is obtained. This compression can lead to violent collapse of bubble [84] to very small microbubble as far as the sphericity of the bubble is not disturbed by some external influence. Consequently the gas compression is almost adiabatic, obtaining instantaneous temperatures up to 5000° K and pressures up to 500 atm [106]. Cum et al [107] found experimental evidences suggesting, that gas bubble radii in a real liquid follows a Gaussian distribution, when an external gas atmosphere is present.

This phenomenon, called transient cavitation is believed to affect chemical effects in homogeneous sonochemistry [84].

This collapse process differs when it occurs in the vicinity of solid material (i.e. at working electrode), this means that the spherical symmetry of the system will be lost. The resistance of the motion of the liquid from the boundary side results in the deformation of the bubble, and consequently to the formation of the liquid jet toward to the solid boundary (Fig. 1) [84].

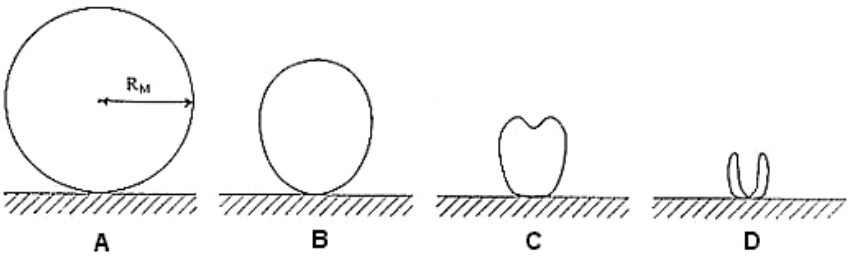

Figure 1. Asymmetric collapse of the oscillating bubbles at solid surface.

The existence of such jets was proved experimentally by Benjamin and Ellis [73] by a series of photographs. The gas compression in the bubble collapsing at the solid surface is much smaller and slower compared to the case of unperturbed collapse, while the bubble decomposes long before large compression. Therefore the influence of ultrasound on heterogeneous processes is mainly brought about by the effects of the jets of liquid on the solid surface properties and their contribution to mass transport [84] (see Fig.2).

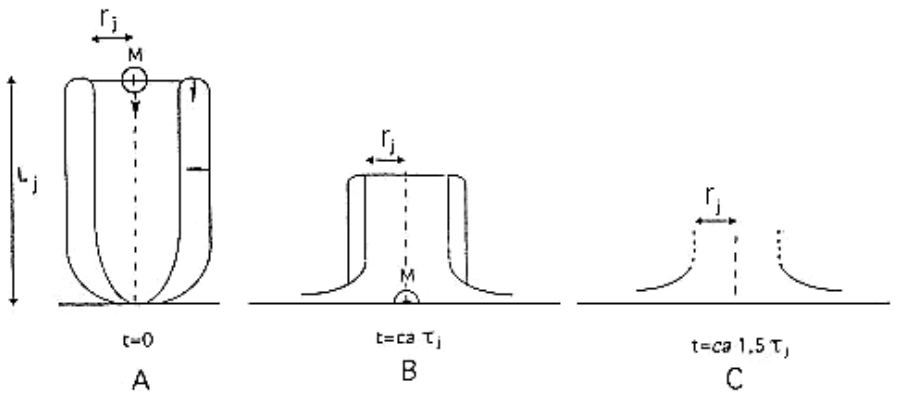

Figure 2. Expected bubble collapse jet history after the moment when it touches the surface [84].

When the jet is once formed the speed of its tip is high enough to produce an initially high impact pressure when it strikes solid boundary. This short duration pressure is called as "liquid hammer pressure". The pressure and duration for water are 2000 atm and 0.1 μs if $\Delta P = 10^5$ Pa [84]. This pressure is strong enough to make serious damage to working electrode as is noted in previous works [55, 108]. After collision this pressure decreases rapidly down to lower value (ca. 80 atm) called as the "stagnation pressure" [84].

When the bubble is not initially in contact with the wall a similar jet is formed, but its length and duration are shorter. The jet flow will be impeded by the bulk solution before it reaches the solid surface and therefore the effect of the jet on solid surface (WE) and the mass transport are lower [84].

3.2 Diffusion layer reduction. The enhancing effect of sonification on the electrochemical measurements

The numerous studies [54, 84, 92, 98, 101, 109–111] of the enhancing effect of the sonification to controlled potential electrochemical measurements have shown large increase of the limiting diffusion current of the electrochemical reaction compared to non agitated media. The limiting current obtained by sonification has specific "noisy" shape when the electrochemical process is occurring. Under more careful study of these voltammograms it is visible that it contains some kind of current jumps — peaks having different amplitude. In the absence of either ultrasound or electroactive compounds no current peaks were observed (Fig. 3.) [100].

Using microelectrodes it was showed that these current peaks have shape similar to these observed in chronoamperometry reflecting just the current decay due diffusion layer thickness expansion [102] (Fig. 4 [100]). According to Birkin and Silva-Martinez [100] the peak maximum corresponds to the minimal diffusion layer thickness (Fig. 5 [54]). Maisonhaute et al [102–105] observed on the platinum microelectrodes some decay of limiting current before the current spike. They explained this as a result of very fast collapse of gas bubble bringing new portion of electroactive spices to working electrode covered previously by the collapsed bubble.

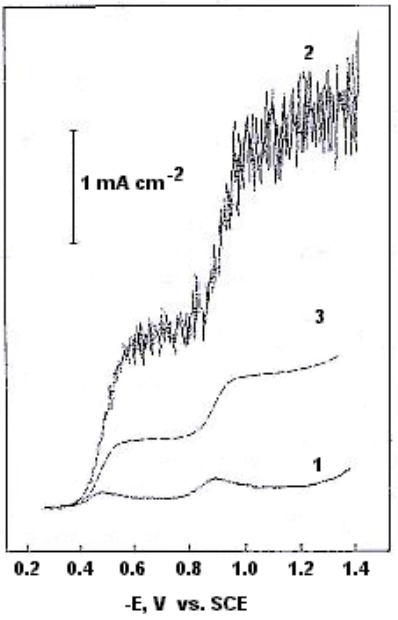

Figure 3. Voltammograms of 0.5 mM methylviologen (MV^{2+}) obtained at stationary Pt wire electrode: 1 – without sonification, 2 – with sonification (I = 5W/cm^2, v = 0.1 V/s, A = 16 mm^2), 3 – at Pt RDE (A=3.14 mm^2, ω = 80 rpm, v = 0.020 V/s).

The limiting current of an electrochemical reaction will be not completely damped by the bubble blocking the microelectrode indicating that some kind of thin layer in the range 45–75 nm will remain between electrodes surface and the bubble. This is due net force caused by the viscosity shear stress on the surface, resulting that the collapse can not occur if the bubble touches the electrode while fluid velocity on the surface is zero [104].

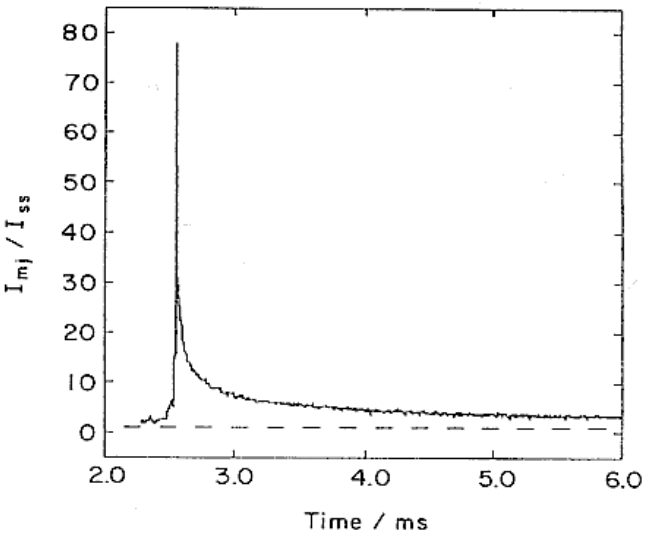

Figure 4. A figure presenting a single normalised current-time (micro jet current / steady state current) transient recorded at a 25 mm Au microelectrode exposed to ultrasound.

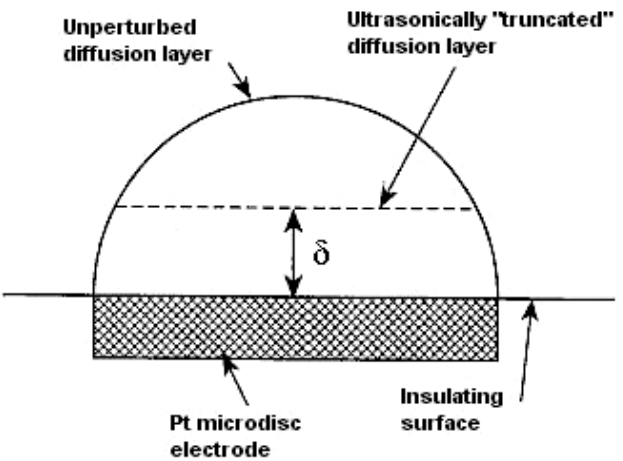

Figure 5. Supposed thinning of the diffusion layer caused by ultrasonically caused cavitation in solution.

Measuring diffusion limited currents I_{lim} the diffusion layer thicknesses can be calculated using simple and well known equation [50, 53, 59, 98, 101]:

$$I_{lim} = \frac{nFADC_b}{\delta} \tag{20}$$

were n is the number of electrons, F is Faraday constant, A is WE area, D is diffusion constant of electro active substance, C_b is bulk concentration of this electroactive compound and δ is diffusion layer thickness. The value of δ depends upon intensity of applied ultrasound and sono tip (ultrasound source) to WE distance due I_{lim} [59, 90, 91, 100]. Minimal values of δ are measured using "sonotrodes" being only few microns under "face-on" conditions [59, 101]. Applying high sono intensity (power) or very close electrode to horn distance in aqueous media, the diffusion layer thickness for "face-on" electrode geometry converges towards the value found for the sonotrode. This means that the sonotrode can be regarded as the "limiting case" of the "face-on" geometry [101].

Birkin and Silva-Martinez showed [92] also that sonification does not affect the rate of electron transfer, so that it can be used for determination of electron transfer rate constants in aqueous as well as in non-aqueous media [63, 91, 92, 100].

For any kind of voltammetric measurements it is needed to know the power emitted by sono source. Mechanic methods are not simple and precise due to erosion of sono probe tip. Margulis and Maltsev suggested to use calorimetric measurements for determination of emitted sonopower P [112]:

$$P = (dT / dt)\, C_p\, M \tag{21}$$

were (dT / dt) is temperature increase (gradient) per time unit, C_p is specific heat capacity and M is mass of used liquid. Since sono probes have different sizes it is better to report not the power of the emitting tip, but the power per area A_s of emitting tip i.e. the intensity of applied power I [113]:

$$I = P / A_s \tag{22}$$

Many authors [60, 84, 113] confirmed the validity of the method, which is commonly used now.

3.3 Additional factors affecting the cavitational collapse of bubbles and sono analyse

Analysing the influence of temperature on the cavitation process Mason et al [113] found that when systems temperature is increased, the ultrasonic power need to be increased to keep cavitational collapse time i.e. process intensity constant. Alternatively, if the ultrasonic power is fixed, then the lower is system temperature the greater will be the sonochemical effect.

Birkin and Silva-Martinez [100] have given some information about this phenomenon. According to their explanation the process of cavitation depends strongly upon the pressure differential acting the exterior and interior of the particular cavitation bubble. The energies of transient cavitation will be dependent upon vapour pressure of the solvent employed. A solvent with high vapour pressure will be expected to cavitate at low acoustic pressures but produces cavitation, which will have relatively low energy in comparison with cavitation in a liquid with low vapour pressure. Suslick et al [114–116] demonstrated the effect of solution vapour pressure on a chemical reaction known to be affected by irradiation with ultrasound. It was found that decreasing the vapour pressure of the solvent accelerated the reaction, so giving evidence for an increased sonochemical reaction rate with increased cavitation bubble collapse energy.

While ultrasound affects chemical reactions and enhances mass transfer to WE surface due cavitationing and collapsing bubbles, it is important to know circumstances affecting the efficiency of the formation of these bubbles. To have more reproducible results a bubbled gas has been used. Most effective for such processes are monoatomic gases like argon in comparison with diatomics like nitrogen. The polyatomics gases have very poor effect [113]. This is directly related to the ratio for specific heats of these gases and the highest ratios will give the greatest cavitational effects [113].

The influence of ultrasound frequency on this process is still not very well established and is mathematically not explained although many papers [59, 97, 107, 111, 113, 117–119] have been published. Despite of this some important conclusions can be made. The studies of Petrier and co-workers [117–119] have shown that if sono frequency grows, it produces more free radicals. Especially due to the destruction of water and dissolved oxygen molecules in aqueous media:

$$H_2O \rightarrow H^{\cdot} + HO^{\cdot} \quad (23)$$

$$O_2 \rightarrow O^{\cdot} + O^{\cdot} \quad (24)$$

These free radicals formed in collapsed bubble can recombine or escape to bulk solution to react with other molecules. Mainly the organic compounds are decomposed in the radical reactions. Petrier et al [119] found that sonosystem emitting frequency 500 kHz, produces 6.2 times more hydrogen peroxide, then

low frequency (20 kHz) system. Petrier et al have supposed that at least for halocarbons this destruction process has performed inside a cavitationing bubble leading mostly to formation of carbon dioxide and chloride ions [117].

3.4 Ultrasonic cleaning

As noted in introduction one advantage of ultrasound is the cleaning effect. Traditionally it was explained by micro jet formed in final stage of collapse of cavitating bubble close to electrodes surface [49–51, 53, 59, 65]. According to Maisonhaute et al [105] this process is more complex. As they found in previous studies [102, 104] the gas bubble will detach from the electrode surface to allow collapse occur. Therefore the micro jet if it is formed at all is weak [103, 104]. Shear stress involved during the collapse is suggested to be an important factor. The efficiency of the ultrasonic cleaning itself strongly depends upon the size of the adsorbed particle to be removed and on the electrode to horn distance. Another parameter to take into account may also be the cavitation frequency and the bubble sizes. While cavitating bubbles preferentially forms in surface holes or cracks the collapse and following cleaning can occur locally.

To characterise better the cleaning process a parameter like α ($\alpha = \sigma R_p / 1.5\ W_A$, where σ is the surface tangential stress, R_p is spherical particle radius to be moved from the electrode and W_A is the work of adhesion, which is the sum of the surface energy of the two contacting materials minus the interfacial energy) was introduced [103]. It is difficult to determine which value of α leads to an effective cleaning since other parameters like time and surface roughness also play an important role [103]. Also a non-spherical adsorbate has higher adsorption energy than spherical one and then needs higher stresses to be removed. Value of α decreases with the particle radius and reaches very low values for molecular adsorbates. It was observed that for example oxidised ascorbic acid was impossible to remove from platinum electrodes by ultrasound [103]. The use of shorter horn to electrode distances down to 1 mm did not improve desorption rate. When ultrasound effects are observed with small adsorbates, they should be attributed to a mass transport increase, which may enhance dissolution of the adsorbates, rather than to mechanical cavitation effects. As soon as α and then the size of adsorbed particles reaches some critical value (which depends on the adsorbate size and adsorption energy but also on the sonication power and time and on the electrode to horn distance) ultrasound may be very beneficial. In natural samples, a major drawback for electroanalysis is protein or surfactants adsorption. Since they are large molecules, they are readily removed by sonication, which is then beneficial both for cleaning purposes and increase of mass transport [103].

3.5 Sono electrodes

Three types of sono electrodes having different geometries have been used and described [50, 91, 101, 120] and are illustrated on figure 6 [101].

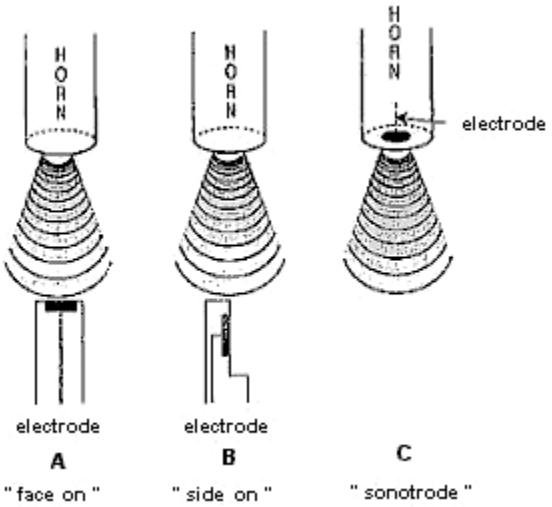

Figure 6. Three types of electrode geometries used in sonovoltammetric experiments.

The most conventional configuration of electrodes is “face on” geometry where WE is locating “face to face” with sono horn (Fig. 6A). Acoustic stream is trained directly to WE surface and the turbulent liquid flow is passing normally to the surface as with a rotating disk electrode. In this mode excellent solution mixing and mass transfer conditions are achieved. Also due ability to change sono tip to WE distance (typically 5 (3) – 40 mm [(31, 32), 89, 95, 120) and applied sono intensity (typically 0–300W/cm^2 [31, 60, 120]) it is the most flexible system for sono electrochemical studies and analyses. Approximately uniform diffusion layer with average thickness ca. 6–7 μm is calculated using Eq. 20. Common electrode materials like Au, Pt, GC, and Hg [31–33, 65, 94–96, 100] are used to prepare WE. Novel electrode materials are BDDE and Bi-coated GC or other carbon based electrodes. The BDDE is remarkable because of its very low background current, mechanical durability and wide region of ideal polarisation [121].

S configuration type is “side on” (Fig. 6B) with the WE placed perpendicular to the tip of ultrasonic horn [50]. Electrode to horn tip distance is measured from the centre of WE and mass transport conditions are similar to WE placed in flow cell [47, 48]. Characteristic for the “side on” placed electrode is this that the diffusion layer thickness is not uniform. The minimal thickness can be ca. 10–12 μm at the downstream edge of electrode.

The third type (Fig. 6C) of electrode is “sonotrode”. Characteristic to this is that WE is directly connected to sono horn which is supplying it with energy. The estimated diffusion layer is approximately uniformly thinned and has thickness much less than 1 μm [120]. Marken et al [101] found also that within experimental error the observed limiting current is almost constant, and therefore independent of the applied range of ultrasound intensities. So the minimal value for diffusion layer thickness can be 0.7 ± 0.1 μm. Pt-disk is used as WE and normally it is connected to and isolated from sono horn by an epoxy resin [50, 91, 121–124]. Due to non-stability of adhesives (or even in the case of strong cement) only relatively low sonopower can be used [123].

4. DETERMINATION OF SOME HEAVY METAL IONS IN HUMAN BLOOD

4.1 Determination of Cd^{2+} ions in human blood applying Nafion® coated glassy carbon mercury film electrode

Environmental contamination by cadmium ions often occurs: a) in areas surrounding zinc, copper and lead smelters; b) as a result of coal burning, as in the case for the other heavy metals; c) in the disposal via incineration of waste materials containing cadmium or cadmium ions [125]. Normal dietary intakes of cadmium ions are in the range of 0.21–0.42 mg per week, which are close to the WHO recommended maximum [126]. Cadmium ions are nephrotoxic and can cause renal dysfusion with proteinuria, the slow onset of which may not become apparent for some years [127]. Normal levels of cadmium ions in blood are under 5 μgL^{-1} [128], but acute toxic effects are observed when this level exceeds 50 μgL^{-1} [I]. The most common sources of chronic exposure, which elevate blood cadmium ions levels, are spray painting of organic based paints without the use of protective breathing apparatus, and smoking [129, 130].

As obvious from previous introduction very low concentration of Cd^{2+} ions in blood is present. Therefore a sensitive analytical method is needed. Preliminary experimental studies showed that Nafion® coated GCE does not have needed sensitivity and resolution (Fig. 7) and therefore it was returned to more traditional applications.

Wang et al and Esteban et al [47, 131] used Hg drop electrode for the determination of low concentrations of Cd^{2+} ions. The authors in [33, 53, 65, 95] successfully used *in-situ* as well *ex-situ* mercury coated working electrodes in surface active compounds containing media for determination of Cd^{2+} and Pb^{2+} ions applying optimised ultrasound intensity.

Unfortunately Marken et al and Agra-Gutiérrez et al [53, 58] observed that Hg droplets are unstable under intensive ultrasound field due ablation phenomenon of ultrasound. Agra-Gutiérrez et al [132] successfully resolved this problem covering the GC working electrode with thin Nafion® film. This film is strong enough being stabile under intensive ultrasound. It can decompose only if significant amount of Hg is collected onto WE surface or if hydrogen evolution is too intensive [132].

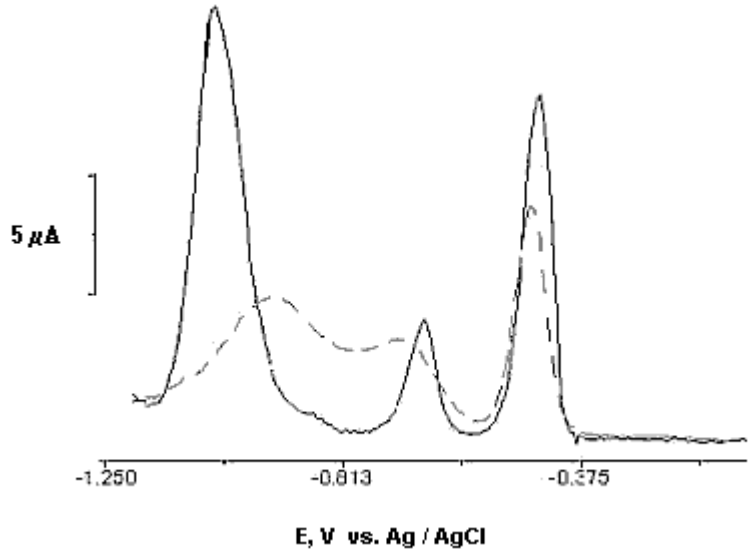

Figure 7. Sono DP-ASV of Zn^{2+}–, Cd^{2+}– and Pb^{2+} ions at the concentration $4.02 \cdot 10^{-6}$ M and $1.75 \cdot 10^{-4}$ M Zn^{2+} ions on NCGCE (scored line). The solid line indicates the situation, when $1.4 \cdot 10^{-4}$ M Hg^{2+} ions are present in the same solution. (Deposition time: 60 s, I = 27.2 W/cm^2, used reference electrode was Ag/AgCl in 3M KCl solution).

The thermostated (25.0±0.5°C) electrochemical cell used for voltammetric and sonovoltammetric experiments has been described previously [109]. The cell used throughout this work (figure 1 [I]) had three necks — for the counter electrode, the reference electrode and a pH electrode. All experiments were performed using a computer-controlled PGSTAT30 Autolab potentiostat (Eco-Chemie, Utrecht, Netherlands). Platinum gauze served as a counter electrode and Ag/AgCl electrode in 3 M KCl solution (Metrohm, Switzerland) as a reference electrode. The working electrode was prepared using as substrate a glassy carbon electrode with a diameter of 3 mm (Metrohm, Switzerland). The positioning of the working electrode in the cell was directly opposite the transducer sonic horn (equipped with 3 mm diameter titanium microtip emitting 20 kHz ultrasound with an intensity of 102.3 Wcm^{-2}) and located near the cell bottom with a transducer to working electrode distance of 5 mm. All reagents were used as received without any further treatment. All solutions were prepared using Milli-Q+ (Millipore) water of resistivity not less than 18 MΩ cm. All glassware, electrodes, the ultrasonic probe and the stainless steel cooling coil were soaked into diluted nitric acid (ca. 1 M) and thoroughly rinsed with purified water before use. The pH of the blood solution was measured using an E6121 pH-meter (Evikon, Estonia). The content of Cd^{2+}, Pb^{2+} and Zn^{2+} ions in used buffer/supporting electrolyte was electroanalytically measured using a hanging mercury drop electrode system. Obtained results were taken

into account in the selection of buffer/supporting electrolyte concentration for following electrochemical studies. (See reference [I] for additional details.)

For the analyse amount of 0.01 M acetic buffer (pH = 4.60) supporting electrolyte solution was 50 mL. The low concentration of buffer/supporting electrolyte solution was used to minimise solution contamination due impurity of used reagents. Human blood samples were analysed without any additional preparation except dilution with the buffer/supporting electrolyte solution. The best results were obtained at the 50 times dilution of the blood sample in buffer/supporting electrolyte solution. Less diluted blood samples (for example at 25 time's dilution) caused blocking of the electrode surface and after 3–4 potential sweeps the peaks corresponding to the cadmium oxidation disappeared. At the 100 times dilution of blood samples the cadmium oxidation peaks were not clearly expressed.

To visualise destructive effect of 20 kHz ultrasound we made some optical photos. First 1.0 ml of blood sample was taken to make 50 ml of 1:50 diluted blood in 0.01 M acetic buffer (pH = 4.60) solution. Some drops of this blood solution was taken and fixed between two glass plates and exposed under microscope system "Axiophot 2" (Zeiss, Germany). Healthy red blood cells were observed. One ordinary red cell was exposed and photographed (figure 8 A). Next we sonicated previously obtained solution for 30 s ($I = 67.2$ W/cm^2) using the same apparatus and electrochemical cell as for sono electrochemical studies. Healthy red blood cells were disrupted completely to little particles and no non-disrupted cell were found (figure 8 B). This disruption process should enhance the liberation process of trace metal ions.

Prior to obtaining the voltammetric measurements, all solutions were deoxygenated for at least 20 min using Ar gas (MESSER, purity 99.993%). After addition of Cd^{2+} standard solution, the samples were deoxygenated and mixed for a period of 180 s. Cadmium was preconcentrated i.e. deposited in the presence of ultrasound at a potential of –1.4 V vs. Ag/AgCl. The equilibration time between preconcentration and anodic stripping was 20 s. During the analytical oxidation step the potential was swept over the range –1.0 V to –0.6 V, thereafter the standby potential +0.15 V was applied. The differential pulse parameters used were: 5 mV step potential, 50 mV modulation amplitude, 0.01 s pulse modulation and 0.1 s interval times. The background concentration of cadmium ions in the supporting electrolyte solution, detected using the scheme described above was subtracted from the results obtained. In order to stabilise the working electrode four deposition — stripping (reduction-oxidation) cycles were carried out before recording the series of anodic stripping voltammograms for Cd^{2+} ions.

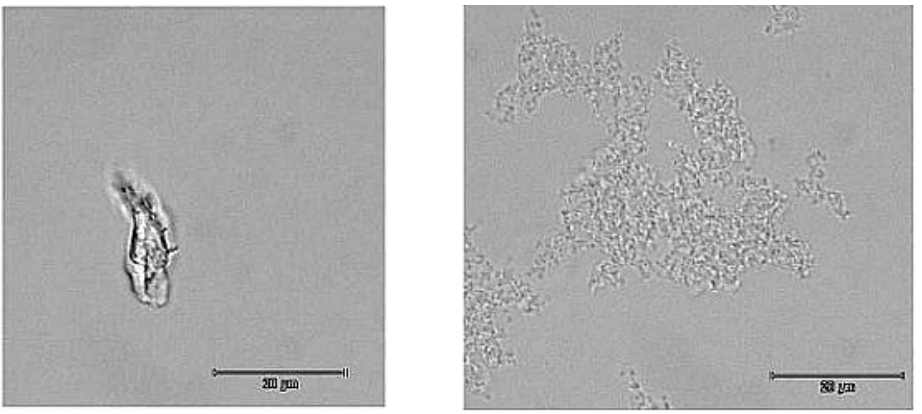

Figure 8. The disrupting effect of applied ultrasound. A – a red cell image in 1:50 diluted blood (0.01 M acetic buffer, pH = 4.60). B – the same blood solution after 30 s sonification (I = 67.2 W/cm^2). A digital microscope "Axiophot 2" (Zeiss, Germany) was used for making blood cells images. Scale unit on the images is 20 µm.

The anodic stripping peaks corresponding to the oxidation of cadmium deposited on the electrode occurred at — 0.74 V vs. Ag/AgCl. In the presence of 2% whole human blood, anodic stripping peaks corresponding to the oxidation of cadmium preconcentrated at the working electrode were followed at — 0.72 V vs. Ag/AgCl. Typical differential pulse voltammograms in the presence of ultrasound for the samples containing 2% blood are shown in figure 9. The calibration plot, showing the dependence of the cadmium oxidation peak height on cadmium ions concentration in the sample, obtained using microadditions of 1.0×10^{-6} M Cd^{2+} solution, was linear in the range 1.0×10^{-10} M to 4.0×10^{-9} M Cd^{2+} with excellent correlation of the experimental results (R^2=0.999). This demonstrates that sensitive quantitative measurements of cadmium ions content can be obtained (Fig. 10). At concentrations above 4.0×10^{-9} M the calibration curve became non-linear.

The value of the background concentration of cadmium ions in the supporting electrolyte $(5.3 \pm 0.4) \times 10^{-11}$ M (0.0060 ± 0.0004 µgL^{-1}) was subtracted from the total value obtained for the analysed sample. The standard microaddition method provided average cadmium content of 9.8 ± 0.6 nM (1.10 ± 0.07 µgL^{-1}) in whole human blood (background corrected). This result was close to the value of the reference analyse 11.8 ± 0.4 nM (1.30 ± 0.05 µgL^{-1}) obtained using atomic absorption spectroscopy, analytical method described in detail in [133]. The results of individual measurements can be seen in Table 1 [I].

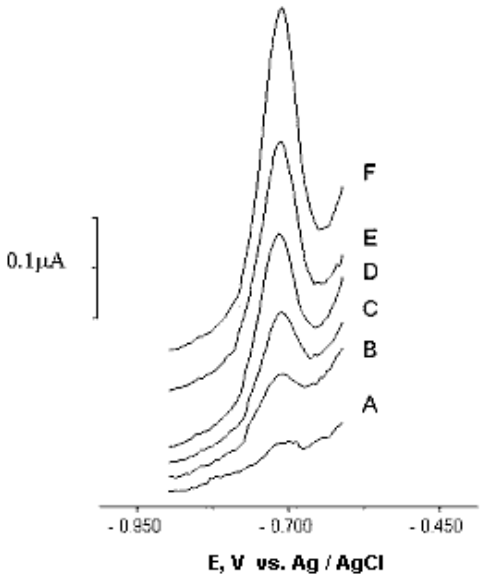

Figure 9. Differential pulse anodic stripping voltammogram of aqueous solutions prepared by mixing of 1 cm^3 human blood and 50 cm^3 0.01 M acetic buffer supporting electrolyte (pH = 4.6): A – sample, B – 10 μL, C – 20 μL, D – 55 μL, E – 80 μL and F – 130 μL of 1.0×10^{-6} M Cd^{2+} standard solution added. An Ag/AgCl (in 3 M KCl solution) reference electrode was used.

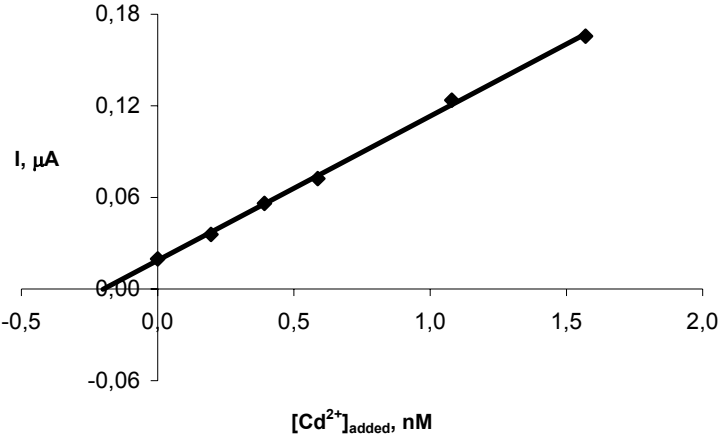

Figure 10. Standard additions plot of Cd^{2+} ions in the aqueous solutions prepared by mixing of 1 cm^3 human blood and 50 cm^3 0.01 M acetic buffer supporting electrolyte, $R^2 = 0.999$.

Table 1. Determination of cadmium ions in 2% human blood solution using sono DP-ASV and independent AAS analysis.

Test No	$[Cd^{2+}]$ / nM (DP-ASV)	Mean $[Cd^{2+}]$/ nM (DP-ASV)	RSD/ % (DP-ASV)	$[Cd^{2+}]$/ nM (AAS)	Mean $[Cd^{2+}]$/ nM (AAS)	RSD/% (AAS)
1	9.52			11.9		
2	9.25	9.81	6.2	11.4	11.8	3.1
3	10.7			12.1		

The cadmium ion concentrations in human blood of medical interest are above 10 μgL^{-1} (ca. 90 nM) [128] and the corresponding relative errors of the results obtained using sonovoltammetric stripping measurements were within ± 5%. The results obtained for the blood samples spiked up to level 10.0 μgL^{-1} (89.0 nM) Cd^{2+} can be seen in Table 2 [I].

Table 2. Determination of cadmium ions from spiked blood sample containing 10.0 μgL^{-1} (89.0 nM) Cd^{2+} using ASV.

Test No	$[Cd^{2+}]$ / nM	Mean $[Cd^{2+}]$ / nM	Recovery /%	SDV / nM	RSD /%
1	87.2				
2	92.5	87.8	99	4.2	4.8
3	83.6				

4.2 Determination of Zn^{2+} ions from blood by sono-double extraction method

Zinc ions are naturally present in blood at a high level (typically 10^{-5} M [134]) with deviations from normal used for the early diagnosis of certain illnesses [135]. In human beings, chronic metabolic disturbances may result from an excess or deficiency of zinc ions [136]; clinical features of deficiency include testicular atrophy, skeletal maturation and retardation of growth [4]. The measurement of red blood cell zinc ions concentration has been reported to be able to discriminate between hyperthyroid Grave's disease and transient thyrotoxicosis [137] while it may be a prognostic indicator of the sepsis syndrome in infancy [138].

Zinc ions in blood are usually analysed using variations of atomic absorption spectroscopy (AAS) [139, 140], such as graphite furnace AAS [141], microwave assisted mineralization and flow injection AAS [142], derivative microsampling flame AAS [143] or inductively coupled plasma mass spectrometry (ICP-MS) method [144, 145]. Szpunar et al [135] has pointed out

that although ICP-MS plays an important role in analytical laboratories, the determination of copper- and zinc ions can be plagued by several spectral interferences [135].

Uniquely Sun [146] has determined zinc ions in blood by digesting the sample by heating with nitric and perchloroic acids followed by analysing via differential stripping potentiometry.

Trying to find the best solution for zinc ions electroanalytical determination in blood it was studied at bare GC and BDD as well as Nafion® film coated GC working electrodes [V].

The most reliable results were obtained using sono-double extraction method using dithiazone as complexing agent [II, III, V]. Test of this analytical method was performed using artificial, toothpaste and shampoo samples [II].

All reagents were used as received without any further treatment. Shampoo and toothpaste were obtained from a local pharmacy. All subsequent solutions were made using deionised water of resistivity not less than 18.2 MΩ cm (Vivendi water systems, UK) (See [II] for additional details). A three electrode arrangement was used in the electrochemical cell with a glassy carbon (3 mm diameter, BAS, Indiana, USA) or boron-doped diamond (5 mm × 5mm, DeBeers Industrial Diamond Division, Ascot, UK) serving as the working electrodes, with a bright platinum wire used as the counter electrode. A saturated calomel reference electrode (SCE) (Radiometer, Copenhagen, Denmark) completed the circuit (following values of working electrode potentials are recalculated to Ag/AgCl (in 3 M KCl) reference electrode values for better comparing). The glassy carbon-working electrode was polished in between experiments using diamond-lapping compounds. The BDD was cleaned using 3 μM alumina polishing compound, then rinsed to remove any surface residue. Voltammetric measurements were carried out on a μ — Autolab (ECO-Chemie, Utrecht, Netherlands) potentiostat.

The ultrasonic horn used in this work was a model CV 26 (Sonics and Materials Inc. USA) with operating frequency of 20 kHz fitted with a 3 mm diameter titanium alloy microtip (Jencons, Leyton Buzzard, UK), with the intensity of the ultrasound being determined calorimetrically [95]. The working electrode was placed in a face-on arrangement to the ultrasonic horn [II].

The electrochemical cell was thermostated with a stainless steel cooling coil at a constant temperature of 221°C (±2°C). The volume of the extraction solution was sufficient for the horn to be immersed beyond the shoulder of stepped tip. This was essential to ensure an efficient mixing regime in the biphasic system. Solutions were de-aerated and mixed with nitrogen gas (99.99+%, BOC, Manchester, UK).

Square-wave anodic stripping voltammetry was explored using a –1.97 V vs. Ag/AgCl deposition potential for 120 s followed by a potential sweep in the potential range of –1.87 V to 0.03 V vs. Ag/AgCl, for increasing additions of zinc ions to pH = 6.7 phosphate buffer (amplitude 25 mV, frequency 50 Hz).

A linear response over the range 0.1–5.1 μM was observed with the limit of detection found to be 8.1×10^{-8} M, further additions resulted in the calibration plot "curving". Next, the deposition Zn^{2+} was investigated with the application of an (non-optimised) acoustic field during the deposition procedure. It was found in order to achieve a similar limit of detection, a much shorter 20 s deposition time was required at — 1.97 V vs. Ag/AgCl, with the application of the acoustic field (120 W cm^{-2}). Well-defined voltammograms were observed for a horn to electrode distance of 5 mm. Clearly, the silent deposition of BDDE has a lower limit of detection compared to glassy carbon, consistent with the low background currents, which is characteristic for this material [II], while applying ultrasound during the deposition procedure, the analytical sampling time may be decreased.

The double extraction procedure was investigated for the detection of zinc ions using the ligand dithiazone. 10.0 mL (5.0 mM) dithiazone in chloroform was insonated with 10.0 ml aqueous solution of zinc ions (pH = 10) containing 0.50 mL of 0.050 M sodium thiocyanate in ethanol, 10.0 mL (0.40 M) ammonium citrate and 10.0 mL (9 M) ammonia. The microemulsion was then left to equilibrate back into two phases (within minutes). The organic phase was separated and insonated with 1 M sulphuric acid for 60 s. This aqueous phase containing the zinc ions was then added to an electrochemically clean aqueous volume of 0.7 M phosphate buffer in which the electrochemistry was done. This involved an insonated deposition step of 60 s –1.97 vs. Ag/AgCl on either glassy carbon or boron doped diamond followed by silent stripping step in the potential range of –1.97 V to 0.03 V vs. Ag/AgCl. The extraction process using the dithiazone ligand was investigated for various ranges of zinc ions concentrations. These are summarised in Table 3 [II].

Table 3. Extraction results for the determination of zinc ions extracted from 40 mL of aqueous phases contained: 10.0 mL of zinc ions samples with different concentrations, 10.0 mL chloroform solution of [L] = 5.0×10^{-3} M, 10.0 mL (0.40 M) ammonium citrate, 0.5 mL 0.050 M sodium thiocyanate in ethanol, 10.0 mL (9 M) ammonia. For back-extraction to aqueous phase 10.0 mL of 1.0 M sulphuric acid water solution was used.

[L]:[Zn^{2+}]	Spiked Zn^{2+}/ M	Detected Zn^{2+}/ M	Extraction efficiency / %
5:1	1.00×10^{-3}	8.50×10^{-4}	85 ± 2
50:1	1.00×10^{-4}	9.60×10^{-5}	96 ± 2
500:1	1.00×10^{-5}	1.01×10^{-5}	101 ± 1

For the determining the amount of zinc ions in toothpaste 1.10 g of toothpaste was dissolved in 10.0 mL (0.40 M) ammonium citrate. This formed solution was then filtered to remove non-dissolved species deriving from toothpaste

additives. Finally 10.0 mL water, 0.50 mL of 0.050 M sodium thiocyanate in ethanol, and 10.0 mL (9 M) ammonia was added giving total volume 30.0 mL of sample solution. This solution was then insonated with 10.0 mL of 5.0 mM dithiazone in chloroform for 240 s. Two different phases were allowed to separate and the organic phase containing the bound ligand was removed and insonated with 1.0 M sulphuric acid for 240 s. 2.0 mL sample of the aqueous phase containing zinc ions was transferred into the sonoelectroanalytical cell and diluted with 18.0 mL water. Using square-wave anodic stripping voltammetry, the zinc ions content was quantified by using the standard addition technique, which involved applying a 20 s deposition at –1.97 V vs. Ag/AgCl (in 3M KCl) for the silent case, or a 5 s deposition step at –1.97 V vs. Ag/AgCl (in 3 M KCl) with the application of 120 W cm^{-2} ultrasound at a horn to electrode distance of 10 mm.

The corresponding voltammograms of standard additions of Zn^{2+} ions for a 5 s deposition at 1.97 V vs. Ag/AgCl using ultrasound is shown in figure 11, with the standard addition plot found to be linear (figure 12). The measured amount of zinc ions was 1.30×10^{-4} M (8.5 mg dm^{-3}), which is in close agreement with the manufactures reported value of 1.1×10^{-4} M (7.2 mg dm^{-3}). It was observed in the absence of ultrasound that the voltammetric signals were non-linear and unresponsive to increasing additions of zinc ions, however the application of an acoustic field produced quantifiable and reproducible signals.

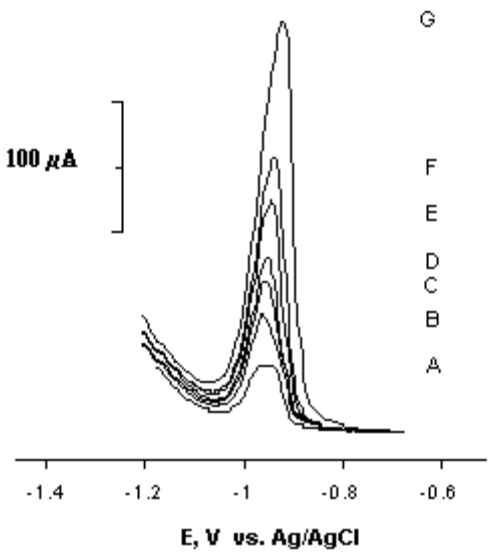

Figure 11. Voltammograms of zinc (II) ions measured in the toothpaste extract: A – sample, B – 0.5 μL, C – 5.5 μL, D – 15.5 μL, E – 25.5 μL, F – 35.5 μL and G – 65.5 μL of 0.1 M Zn^{2+} standard solution added. Deposition time 5 s under sonification horn to working electrode distance 10 mm and I = 120 W/cm^2 applying deposition potential – 1.97 V vs. Ag/AgCl (in 3 M KCl).

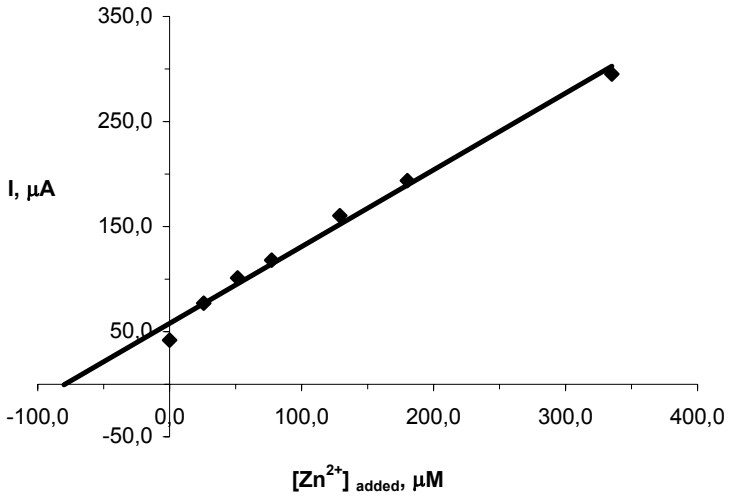

Figure 12. Standard additions plot of Zn^{2+} ions in the back extract of toothpaste sample in 0.5 M sulphuric acid supporting electrolyte, $R^2 = 0.990$.

For blood analysis same electroanalytical condition were used as noted above. The ultrasonic generator used was a model VCX 5000 (Sonics and Materials, USA) horn equipped with 3 mm diameter titanium microtip emitting 20 kHz ultrasound with an intensity of 49 Wcm^{-2} as determined calorimetrically which was used for emulsifying the aqueous and organic phases. The volume of the extraction solution was sufficient for the horn to be immersed beyond the shoulder of stepped tip; this is essential to ensure an efficient regime in the biphasic system.

The optimum deposition potential of zinc ions on boron-doped diamond working electrode was investigated and found again that a potential of –1.97 V vs. Ag/AgCl (in 3 M KCl) produced the optimum amount of deposition material (avoiding intensive hydrogen gas evolution). An optimised deposition potential of 300 s was found to produce a detection limit of 1.55×10^{-6} M with additions of increasing concentrations of zinc ions found to be linear over the range $0.2–1.3 \times 10^{-5}$ M.

100 μL of the sample was diluted with 9.9 mL of water, 10.0 mL (0.40 M) ammonia citrate, 500 μL of 0.050 M potassium thiocyanate (ethanolic solution) and 10.0 mL (9 M, pH = 10) ammonia solution. Finally, 10.0 mL (5.0 mM) dithiazone in chloroform was added and insonated for 240 s applying an intensity of 49 W cm^{-2}. Than, the lower chloroform phase was separated and insonated with an equal volume of 1 M sulphuric acid to decompose metal-ligand complex. The formed mixture was allowed to separate again, before the lower organic phase containing impurities was discarded, while the acidic aqueous phase (pH ca. 1) was allowed to analyse. The aqueous extraction

sample (10 mL) was diluted with an equal volume of pure water within which the electroanalysis was performed. Electrochemical anodic stripping voltammetric measurement was performed on bare boron doped diamond electrode having better sensitivity than ordinary GCE [II, V]. The volume of the initial blood sample was sufficient to provide accurate and low volume sampling (less hazardous), while increasing volume resulted in longer and poor separation during the extraction process.

The voltammetric response of zinc ions after sono double extraction of blood sample was studied in quiescent mode on BDDE using square-wave anodic stripping voltammetry (amplitude 25 mV, frequency 50 Hz) in 20.0 mL of solution containing 0.5 M sulphuric acid as supporting electrolyte. Using a deposition potential of –1.97 V vs. Ag/AgCl (in 3 M KCl) for 300 s, an equilibration time of 5 s and a potential sweep over the range –1.97 to –0.07 V vs. Ag/AgCl, a well-defined oxidation signal was observed at –0.97 V vs. Ag/AgCl (Fig. 13).

The average amount of zinc ions was found to be 1.99 (± 0.02) × 10^{-5} M (1.30 ± 0.01 mg dm^{-3}) (number of analysis, n = 3), which is in good agreement with the value of (1.74 ± 0.1) × 10^{-5} M (1.14 ± 0.07 mg dm^{-3}) (n = 3) obtained from independent AAS analysis. An example of standard addition plot is presented on figure 14.

Thus benefits of double extraction are clear. If these results are compared with conventional ASV, in which a glassy carbon electrode is used in diluted blood with an applied deposition time of 600 s –1.97 V vs. Ag/AgCl, a limit of detection of ca. 100 times higher was found than is required for the direct determination.

The rapid analysis (total time less than 60 minutes) and simple detection of the zinc ions in blood has been shown to be possible by applying the acoustically assisted double extraction technique, which requires only pinprick size volumes of blood. Direct sonoelectroanalysis requires much larger volumes of blood [31], while ASV analysis in diluted blood was unreliable [V].

This rapid technique is advantageous due to the low levels of target metal ions that can be extracted and consequently analysed. Contaminants present in the test solution will ‘prefer’ to remain in the initial aqueous phase, or will transfer to the extractive organic phase, but are unlikely to be doubly transferred into the clean final aqueous phase [147, II, III]. The application of ultrasound (as opposed to mechanical stirring) is advantageous in creating an efficient emulsification [148, 149], increasing the extraction rate and decreasing the analytical analysis time.

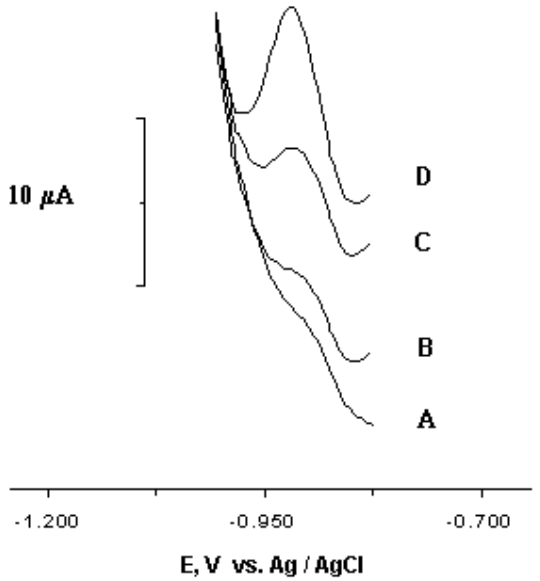

Figure 13. Square wave anodic stripping voltammograms of increasing additions of zinc ions over the concentration in 0.5 M sulphuric acid solution: A – sample, B – 10 μL, C – 20 μL and D – 30 μL of 1.0×10^{-4} M Zn^{2+} standard solution added. A deposition potential –1.97 V vs. Ag/AgCl (in 3 M KCl) was applied during 300 s in quiescent mode prior to the anodic stripping scan.

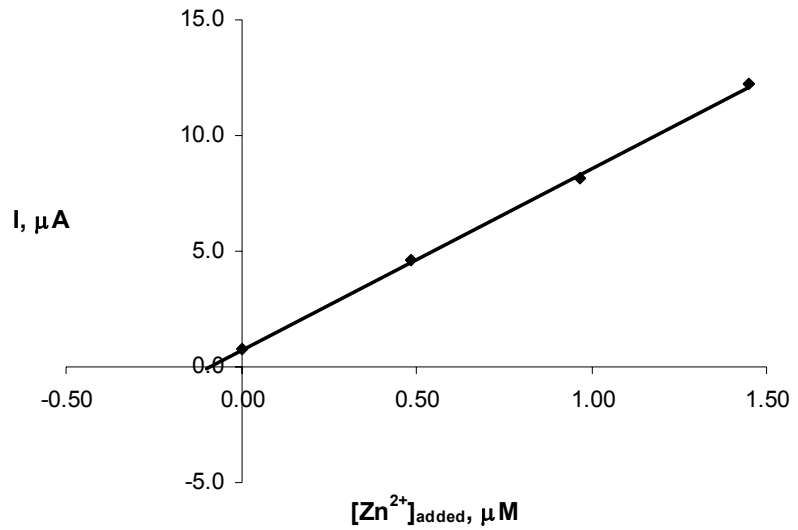

Figure 14. Standard additions plot of Zn^{2+} ions in the back extract of 100 μL blood sample in 0.5 M sulphuric acid supporting electrolyte, $R^2 = 0.999$.

4.3 Determination of Pb^{2+} ions in blood applying in-situ deposited Bi boron doped diamond electrode

The toxicity of lead ions is well documented [33] and is of particular concern due to the acute toxic effects experienced by children [150–152]. Normal lead ion levels in blood lie in the range from 0 to 2×10^{-7} M. [33] Jagner et al [150] and Hoyer and Florence [12] reported that the clinically significant levels of exposure to lead ions are in the micro-molar range.

Traditionally mercury film electrodes have been used in electroanalysis. For example Jagner et al [153] investigated potentiometric stripping analysis for the determination of lead ions in blood utilising a glassy carbon mercury film electrode. Subsequently, Hoyer and Florence used Nafion® coated mercury film electrodes for the determination of lead- and copper ions in clinical samples via anodic stripping voltammetry [12]. They demonstrated, that the only sample pre-treatment of the blood required was dilution with hydrochloric acid. Recently Mathieu and co-worker have reported potentiometric stripping analysis for lead ions in blood [154]. Nevertheless, the preparation of the Nafion® coating is time consuming, difficult to prepare and potentially irreproducible [V]. As noted in the introduction section, the toxic nature and risks associated with mercury have led to some restriction in its use with complete bans in some countries. One possible way to avoid the use of mercury is the use of new electrode substrates like BDD which has a low level background currents and an attractive wide range of ideal polarisability in aqueous media [67, 155–158]. For example, BDD has been used in the electroanalytical quantification of manganese ions in instant tea [67] and cytochrome C [158]. The sensitivity of these bare-electrodes can be increased applying sonication during the deposition period, permitting the detection of trace metal ions in otherwise passivating media where normal electroanalysis can fail [159].

Recently bismuth modified electrodes have attracted attention and have been described as “environmentally friendly” having a low toxicity compared to that of mercury [72, 73]. Wittstock and co workers [76] first described the use of a bismuth-modified platinum electrodes for glucose oxidation. Later, Wang has tested the bismuth film electrode and exploited its use in electroanalytical detection of trace metal ions [72–74, 79–81, 160, 161]. Bismuth-film carbon paste electrodes have been also been proposed [83, 162]. These can be generated *in-situ* by involving bismuth (III) ions, alternatively the bulk of the carbon paste is modified with bismuth (III) oxide which can be reduced to bismuth at the electrode surface [83, 162]. However, the limited number of published work on bismuth film electrodes deals with insights into the behaviour of bismuth film electrodes in ASV. To our knowledge Kefala and co-workers are the only group to have used bismuth for real sample analysis,

namely in the quantification of lead- and zinc ions in tap water and lead ions in human hair using a rotating bismuth film glassy carbon disc electrode [163].

Our target was to study the applicability of bismuth coated electrode [VI] for the determination of lead ions in blood instead of mercury coated ones. As first, we studied the stability of Bi coating on GCE. All reagents were used as received without any further purification [VI].

A three electrode electrochemical measurement system was used in the electrochemical cell with a glassy carbon disc (3 mm diameter, BAS, USA) serving as the working electrode, and a large area bright platinum wire used as the counter electrode. A saturated calomel reference (Radiometer, Copenhagen, Denmark) electrode completed the circuit (following values of the working electrode potentials are recalculated to Ag/AgCl (in 3 M KCl) reference electrode values for better comparing with other data). Voltammetric measurements were carried out on a μ-Autolab (Eco-Chemie) potentiostat. The glassy carbon working electrode was polished between experiments using diamond-lapping compounds [IV]. The ultrasonic generator used was a model VCX 5000 (Sonics and Materials, USA) horn equipped with 3 mm diameter titanium microtip emitting 25 kHz ultrasound where an amplitude of 10% was found to correspond to 57 Wcm^{-2} as determined calorimetrically [IV]. The electrochemical cell was thermostated with a stainless steel cooling coil at a constant temperature 22°C (±2°C). Solutions were de-aerated and mixed with nitrogen gas (99.99+%, BOC, Manchester, UK). AFM measurements were performed using a Digital instruments MultiMode SPM system operating in contact mode. A type 'J' scanner with x and y limits of 125 μm and a z limit of 6 μm was used with standard silicon nitride tip (type 'NP') (supplied by Digital Instruments) with a force constant of approximately 0.32 N m^{-1}. Scans were collected at a scan rate of 1 Hz [IV].

A polished glassy carbon working electrode was modified with bismuth by deposition from a 0.48 mM solution of bismuth (III) nitrate in 0.1 M sodium acetate buffer (pH = 5.2) at a potential of –1.17 V vs. Ag/AgCl for 360 s; this is similar to the procedure of Wang et al. The deposited bismuth was then electrolytically oxidised by sweeping the potential from –1.17 V to 0.43 V vs. Ag/AgCl. This plating — sweeping (reduction-oxidation) sequence was repeated until a consistent oxidation peak was achieved. Under these conditions well-defined oxidation (stripping) peak of bismuth was observed at –0.005 V vs. Ag/AgCl, which is in excellent agreement with that reported by Wang et al [72, 79]. From integration of the bismuth-oxidation peak, the mean thickness of the deposit was found to be ca. 75 nm. Then a bismuth modified glassy carbon electrode, newly prepared as described above, was placed into a 0.1 M sodium acetate solution (pH = 4.6) in a face-on arrangement under the ultrasound source (10 mm horn-to-electrode distance) and subjected to 60 s of insonation (I = 57 W cm^{-2}). Upon termination of the ultrasound, the bismuth deposit was swept in the potential range –1.17 V to 0.53 V vs. Ag/AgCl (under quiescent conditions), to see if any bismuth had been ablated from the applied ultrasound.

This was then repeated at longer insonation times. A plot of peak area i.e. charge of the bismuth oxidation peak plotted against increasing periods of ultrasound is shown in figure 15 [IV].

Experiments showed initial decrease in the bismuth oxidation signal (to ca. 21% of the original peak) after 15 s of applied ultrasound, but further insonation of the bismuth-modified electrode leads to no further change [IV]. Additional voltammetric tests of sensitivity with Zn- and Cd ions [IV] showed again the applicability of Bi coated electrodes in electroanalysis. As follows, the elaborated analytical method was tested on blood samples. All reagents needed for this test used as received without any further purification (see for details in [VI]). A three electrode system was used in the electrochemical cell with a boron-doped diamond electrode (3 mm diameter, available from Windsor Scientific Ltd, UK) serving as the working electrode, with a large area bright platinum wire used as the counter electrode. An Ag/AgCl (in 3 M KCl) reference electrode (Metrohm, Switzerland) electrode completed the circuit. Voltammetric measurements were carried out on a PGSTAT 30 Autolab potentiostat (Eco-Chemie, Utrecht, Netherlands). The BDD electrode was polished with alumina gel (0.05 micron, Buehler, USA) which was rinsed before use with ethanol.

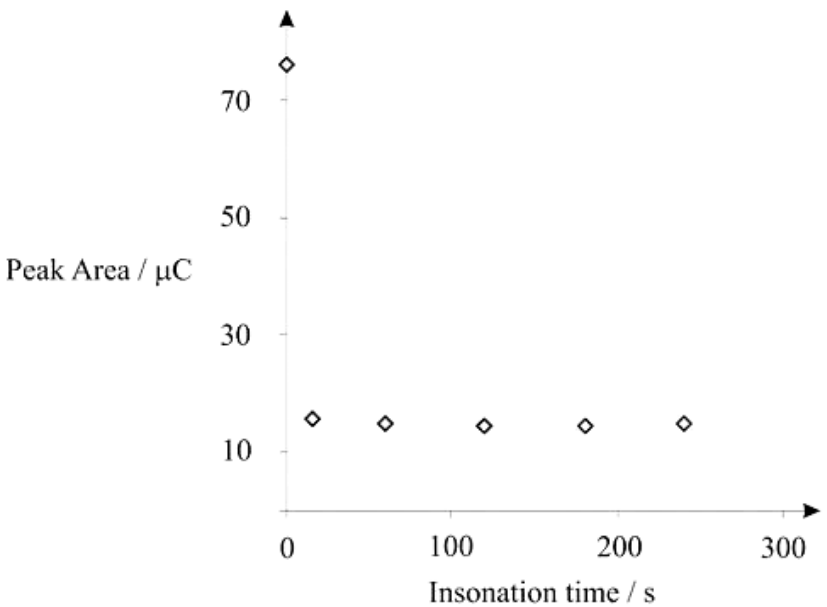

Figure 15. Stability of a bismuth film coated glassy carbon electrode monitored by the bismuth oxidation signal as a function of the duration of applied ultrasound to the bismuth film. The bismuth film was prepared using square-wave anodic stripping voltammetry from a 0.48 mM bismuth (III) solution in 0.1 M sodium acetate buffer (pH = 4.6) using a 360 s deposition at a potential of –1.17 V vs. Ag/AgCl. Applied ultrasonic intensity was 57 W/cm^2 at the electrode to horn distance 10 mm.

The used ultrasonic generator was a model VCX 5000 (Sonics and Materials, USA) horn equipped with 3 mm diameter titanium microtip emitting 20 kHz ultrasound with an amplitude of 20% found to correspond to an intensity of 24 W cm^{-2} as determined calorimetrically [VI]. Blood samples were prepared and supplied by Clinic of the University of Tartu. The blood samples were prepared as follows: 200 mL of blood sample was mixed with 100 mL of a solution containing 327 mg citric acid monohydrate, 2.63 g sodium citrate, 251 mg sodium dihydrogenphosphate dihydrate and 2.55 g D-glucose monohydrate in a total aqueous volume of 100 mL. The samples were centrifuged into two phases with the phase containing the erythrocytes used for analysis.

The concentrations of lead ions in blood were verified via independent AAS using the method of Muzyka et al [133]. Then knowing the starting concentration of lead ions in blood, the blood was easily and accurately spiked to the required levels.

A 0.1 M nitric acid (pH 0.9) solution containing 4.0×10^{-7} M (82 ppb) lead ions was first prepared. Low pH value was selected since Jagner with co-workers [153] and Florence with co-worker [12] have previously shown that lead ions are completely and instantly released from the blood matrix under these conditions. Square-wave anodic voltammetry at BDD electrode using a deposition potential of –1.4 V vs. Ag/AgCl for 120 s was explored. As can be seen in figure 16 wave A, a relatively small lead oxidation peak is observable at – 0.5 V vs. Ag/AgCl. Next, the SW-ASV was re-run with an addition of 1.2×10^{-6} M Bi^{3+} ions. As is shown in figure 16, wave B, the bismuth oxidation peak is clearly visible at 0.0 V vs. Ag/AgCl while the lead oxidation peak has considerable increased in magnitude, suggesting bismuth can be used to increase the sensitivity of lead ions detection. At this pH, good separation of the two oxidation peaks is visible.

A sample of blood was spiked with Pb^{2+} ions to give a final concentration of 5.00×10^{-6} M. This value in the undiluted blood sample corresponds to an exposed person, which is in the clinical range of interest [33]. The sample was then diluted by a factor of one part blood to 50 parts 0.1 M nitric acid (pH = 0.9) to give a final concentration of 1×10^{-7} M (21 ppb) of Pb^{2+} ions. Using SW-ASV via an insonated deposition time of 300 s, the magnitude of the lead oxidation peak was monitored as the deposition potential was changed. A sharp increase in the lead oxidation peak was observed between –1.0 V and –1.7 V vs. Ag/AgCl. After this potential the peak height slowly increased reaching a plateau at –2.7 V vs. Ag/AgCl. A large overpotential is required to achieve optimised conditions.

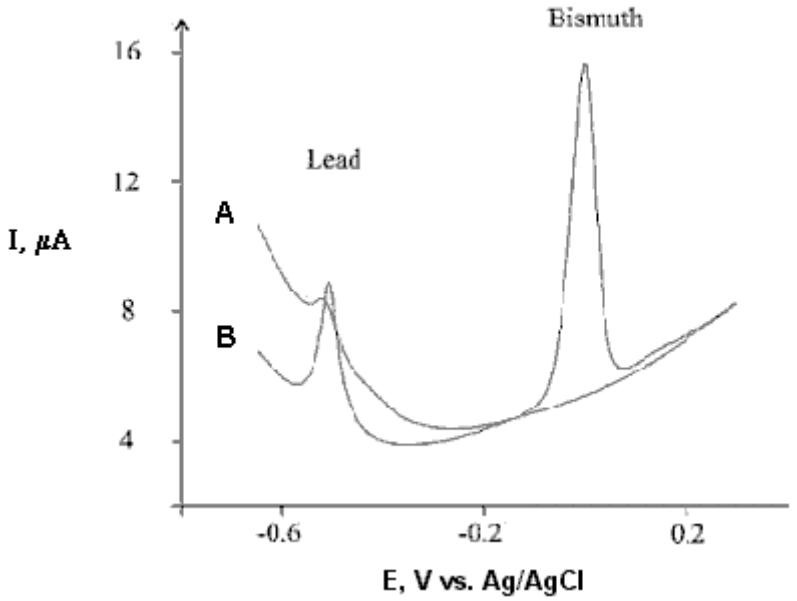

Figure 16. Square-wave anodic stripping voltammetry for 4.0×10^{-7} M lead ions (curve A) in a 0.1 M nitric acid solution, and then with 1.2×10^{-6} M bismuth (III) (curve B) at a boron-doped diamond electrode. Parameters: –1.4 V vs. Ag/AgCl deposition for 120 s, frequency 50 Hz, step potential 5 mV and amplitude 50 mV [VI].

It was noted that the mass transport effects of ultrasound removing gas formed at the electrode surface and any possible surface passivating species make the electroanalytical signal reproducible at this highly negative deposition potential. The ultrasound intensity and working electrode to sono source tip optimisation studies showed that an ultrasound intensity of 34.3 W cm^{-2} with a horn-to-electrode distance of 10 mm produced the most enhancement [VI].

Next, the horn-to-electrode distance and intensity in blood was investigated by monitoring the variation in peak height, as described above. It was found that an ultrasound intensity of 34.3 W cm^{-2} with a horn-to-electrode distance of 10 mm produced the most enhancement.

After optimisation of all needed technical parameters new clean blood solution was prepared as described above, with the response of increasing concentration of lead ions added to the solution was explored. Using SW-ASV with a 300 s accumulation period at – 2.7 V vs. Ag/AgCl and insonation (I = 34.3 W cm^{-2}), a linear response was observed over the range 4.3×10^{-7} (89 μg dm^{-3}) to 3.0×10^{-8} M (6.2 μg dm^{-3}) with a limit of detection found to correspond to 4.2×10^{-8} M (8.7 μg dm^{-3}) Pb^{2+}. Thus, the sensitivity seen in the blood solution under quiescent conditions has increased from 35.8 A M^{-1} (silent) to 411.9 A M^{-1} (insonated), which can be attributed to enhanced mass

transport effects removing gas formation at the electrode surface and allowing more negative deposition potentials to be used [VI].

Next sample of blood was spiked with Pb^{2+} ions to give a concentration of 5.00×10^{-6} M (1 ppm). This was diluted by a factor of one part blood to 50 parts 0.1 M nitric acid (pH = 0.9) to give a final concentration of 1.0×10^{-7} M (21 μg dm^{-3}) Pb^{2+} ions in measurement cell. Previous experimental tests showed that 1: 50 dilution is the minimum dilution to provide accurate quantifications. Using SW-ASV applying an insonated accumulation time of 300 s at –2.7 V vs. Ag/AgCl, standard additions were performed on the blood sample. Figure 17 shows typical voltammetric response from such addition to a blood sample and figure 18 appropriate standard solution addition plot. The average value of lead ions was found to be $(4.85 \pm 0.02) \times 10^{-6}$ M (1.005 ± 0.004 mg dm^{-3}) (n = 3), which is in excellent agreement with the spiked value of 5.00×10^{-6} M (1.03 mg dm^{-3}) Pb^{2+}.

The inherent sensitivity of the above protocol is useful for distinguishing between exposed (10^{-4}–10^{-6} M) and healthy persons (10^{-7}–10^{-8} M). This suggests the above method could be used for clinical testing of lead ions in other complex media such as urine and saliva.

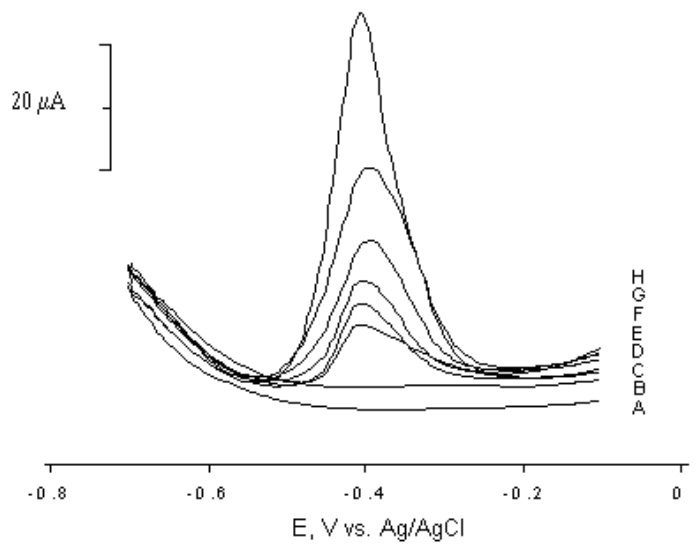

Figure 17. An example of standard additions of Pb^{2+} to a 1:50 diluted blood solution using SW-ASV with an insonated accumulation time of 300 s at — 2.7 V vs. Ag/AgCl: A – blank (1:50 diluted blood), B – after addition of 60 μl 4.8 mM Bi^{3+}, C – 25.1 μL (spikeing up to level 5.0×10^{-6} M level of Pb^{2+} in blood), D – 2.5 μL, E – 7.5 μL, F – 12.5 μL, G – 17.5 μL and H – 27.5 μL of 1.0×10^{-4} M Pb^{2+} standard solution added. An Ag/AgCl (in 3 M KCl solution) reference electrode was used.

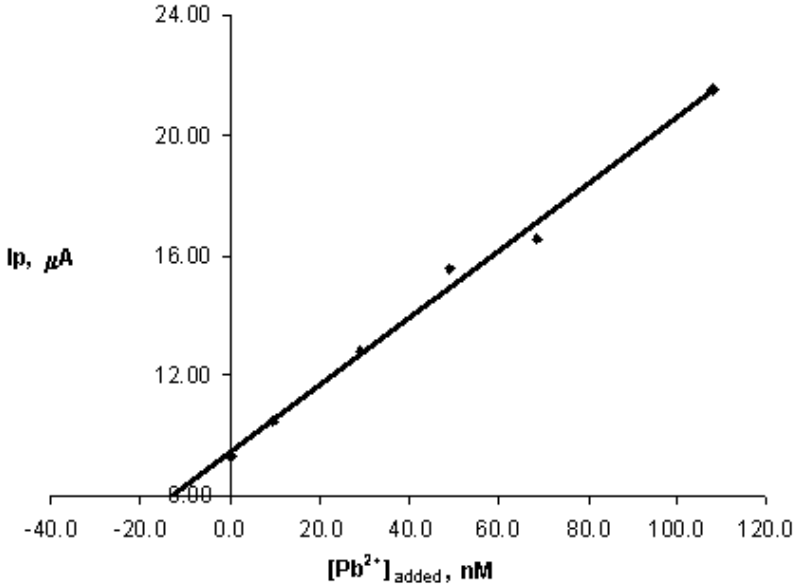

Figure 18. Standard additions plot of Pb^{2+} ions to the aqueous solutions of 1:50 diluted up to level 5.0×10^{-6} M spiked human blood in 0.01 M acetic buffer supporting electrolyte, $R^2 = 0.993$.

CONCLUSION

In the present work the determination of Cd^{2+}, Zn^{2+} and Pb^{2+} ions in human blood were studied employing sono voltammetric measurements.

Our attention was turned to blood analysis while it is most common media for this kind of analyses giving detail overview due being in direct contact with almost intestinal organs. In blood metal ions like Cd^{2+} and Pb^{2+} are bound almost completely with red blood cells [15].

The accurate determination of the concentration of cadmium ions in human blood [I] was demonstrated in an ultrasound enhanced electroanalytical system without the additional need for previous sample treatment, which complicates and lengthens the analytical protocol. The amount of cadmium ions detected in human blood using the methodology of differential pulse sono anodic stripping voltammetry on the *ex-situ* formed Nafion® coated glassy carbon mercury (thin) film electrode was compared with the results obtained using AAS. These results were in excellent agreement, showing the feasibility of the methodology proposed here. We conclude that sonoelectroanalysis of cadmium ions from human blood has the following advantages: minimal experimental procedure, elimination of sample pretreatment, possibility for portable apparatus and relatively inexpense compared with spectroscopic methods giving it the potential to become a powerful and useful method for medical and environmental purposes.

For the determination of zinc ions in media in which classical electroanalytical methods are not viable, the double extraction approach based on acoustic emulsification was used. Using the dithiazone as the ligand needed for Zn^{2+} ions extraction from the toothpaste and shampoo samples and the boron doped diamond working electrode in the final electroanalytical step, the measured concentration of zinc ions was found to be in close agreement with the value obtained from independent AAS analysis [II]. The above described and proved sono double extraction based approach for the determination of zinc ions in highly passivating media was next applied for blood analysis [III]. The amount of zinc ions found in the blood sample was again in close agreement with the value obtained from independent AAS analysis.

A mercury free electroanalytical detection of Pb^{2+} ions in whole blood was shown to be possible using an *in-situ* formed bismuth film modified boron-doped diamond electrode. A sample of blood was spiked with Pb^{2+} ions to give a final concentration of 5.00×10^{-6} M. This value in the undiluted blood sample corresponds to an exposed person, which is in the clinical range of interest [33]. The average measured value of lead ions was in excellent agreement with the spiked value of Pb^{2+} ions. Detection limits in the order of 10^{-8} M in a 1:50 diluted blood solution are obtained with excellent inter- and intra reproduci-

bility. This analytical protocol offers a rapid, sensitive, non-toxic method for the clinical sensing of lead (II) ions in human blood over existing techniques.

As summary it can be concluded that three different methods for the electrochemical analyse, employing ultrasound, of three main toxic trace metal ions in human blood was developed [I, III, VI]. The connection of ultrasound to the electroanalytical procedures improved the sensitivity as well as reduced the total analyse time of the studied toxic metal ions in such complex matrix like blood, toothpaste or shampoo [I, II, III, VI]. Also the continuous step for the avoiding of the use of mercury in electrochemical analysis was done applying bare as well bismuth coated BDDE for electroanalytical measurements [II, III, IV, VI]. These three different analytical methods of the determination of Cd^{2+}, Zn^{2+} and Pb^{2+} ions in blood can be good base for the continuous development of new series of electroanalytical methods for the determination of the other trace metal ions in blood being under medical interest.

REFERENCES

1. S. F. Zakrzewski, Principles of Environmental Toxicology. ACS Professional Reference Book, Am. Chem. Society, Washington, DC (1991) p. 150.
2. S. E. Manahan, Environmental Chemistry. 6th edition, Lewis Publishers, Boca Raton, Ann Arbor, London, Tokyo (1994) p. 667.
3. J. Szpunar, J. Bettmer, M. Robert, H. Chassaigne, K. Cammann, R. Lobinski and O. F. X. Donard. *Talanta,* 1997, 44, 1389.
4. Carl A. Burtis and Ashwood, E. R., eds., Tietz Fundamentals of Clinical Chemistry. 5th edition, W. B. Saunders, NY 2001.
5. B. Nowak and J. Chmielnicka, Ecotoxicology and Environmental Safety, Vol. 46 (2000), 265
6. B. Nowak, Science of The Total Environment, Vol. 209, Issue 1 (1998), p. 59.
7. E. Krušlin, C. M. Hodel and H. Schurgast, Toxicology Letters, Vol. 88, (1996), p. 84 .
8. W. Wasiak, W. Ciszewska and A. Ciszewski, Anal. Chim. Acta, Vol. 335, Issue 3 (1996), p. 201.
9. H. M. Anawar, J. Akai, K. M. G. Mostofa, S. Safiullah and S. M. Tareq, Environment International, Vol. 27, Issue 7 (2002), p. 597.
10. J. L. Stauber, T. M. Florence, Science of The Total Environment, Vol. 60 (1987), p. 263.
11. J. L. Stauber, T. M. Florence, Science of The Total Environment, Vol. 74 (1988), p. 235.
12. B. Hoyer, T. M. Florence, Anal. Chem. Vol. 59 (1987), p. 2839.
13. B. Hoyer, T. M. Florence, Anal. Chem. Vol. 59 (1987), p. 1608.
14. M. C. Agramunt, A. Domingo, J. L. Domingo and J. Corbella, Toxicology Letters, Vol. 146, Issue 1 (2003), p. 83.
15. P. Apostoli, Journal of Chromatography B, Vol. 778, Issues 1–2, 5 (2002), p. 63.
16. T. Schäfer, J. Heinrich, M. Wjst, C. Krause, H. Adam, J. Ring and H. E. Wichmann, Environmental Research, Vol. 81, Issue 2, August 1999, p. 151.
17. Valderi L. Dressler, Dirce Pozebon and Adilson J. Curtius, Spectrochimica Acta Part B: Atomic Spectroscopy, Vol. 53, Issue 11, (1998), p. 1527.
18. A. K. M. Kabzinski, Talanta, Volume 46, Issue 2 (1998), p. 335.
19. S. A. Taylor, I. D. Chivers, R. G. Price, M. Arce-Tomas, P. Milligan, I. Francini, R. Alinovi, S. Cavazzini, E. Bergamaschi, M. Vittori, A. Mutti, R. R. Lauwerys, A. M. Bernard, H. A. Roels, M. E. De Broe, Guy D. Nuyts, M. M. Elseviers, G. Hotter, I. Ramis, J. Rosello, E. Gelpi, H. Stolte, U. Eisenberger, Luder M. Fels, Environmental Research, Vol. 75, Issue 1 (1997), p. 23.
20. Håkan Jönsson, Thor-Axel Stenström, Jan Svensson and Annika Sundin, Water Science and Technology, Vol. 35, Issue 9 (1997), p. 145.
21. Bensryd, L. Rylander, B. Högstedt, P. Aprea, I. Bratt, C. Fåhraéus, A. Holmén, A. Karlsson, A. Nilsson, B.-L. Svensson A. Schütz, Y. Thomassen and S. Skerfving, The Sci. of Total Environ., Vol. 145, Issues 1–2, (1994), p. 81.
22. J. L. Hardcastle and R. G. Compton, Electroanalysis, Vol. 13 (2001), p. 89.
23. B. Xu, S. E. Chia, C. N. Ong, Biological Trace Element Research, Vol. 40, Issue 1 (1994), p. 49.

24. R. M. Tripathi, R. N. Khandekar, Radha Raghunath and U. C. Mishra, Atmospheric environment (1967), Vol. 23, Issue 4 (1989), p. 879.
25. S. E. Chia, C. N. Ong, L. H. Chua, L. M. Ho, S. K. Tay**,** Journal of Andrology, Vol. 21, Issue 1 (2000), p. 53.
26. B. Xu, S. E. Chia, M. Tsakok, C.N. Ong, Reproductive Toxicology (Elmsford, N.Y.), Vol. 7, Issue 6 (1993), p. 613.
27. P. Drbohlav, V. Bencko, J. Masata, J. Bendl, J. Rezácová, T. Zouhar, V. Cerný, E. Hálková, Ceska Gynekologie / Ceska Lekarska Spolecnost J. Ev. Purkyne, Vol. 63, Issue 4 (1998), p. 292.
28. L. M. Marco, E. Jimenez, E.A. Hernandez, A. Rojas, E. D. Greaves, Spectrochimica Acta Part B-Atomic Spectroscopy, Vol. 56 (11), Issue 30 (2001), p. 2195.
29. E. Lane, A. J. Holden, R. A. Coward, The Analyst, Vol. 124, Issue 3 (1999), p. 245.
30. R. Prakash, R. C. Srivastava and P. K. Seth, Electroanalysis, Vol. 14 (2002), p. 303.
31. J. L. Hardcastle, G. G. Murcott, R. G. Compton, Electroanalysis, Vol. 12 (2000), p. 559.
32. C. E. West, J. L. Hardcastle, R. G. Compton, Electroanalysis, Vol. 14 (2002), p. 1470.
33. J. L. Hardcastle, C. E. West, R. G. Compton, Analyst, Vol. 127 (2002), p. 1495.
34. W. J. Walsh, H. R. Isaacson, F. Rehman, A. Hall, Phys. & Behaviour, Vol. 62 (1997), p. 327.
35. R. M. Tripathi, R. Raghunath, S. Mahapatra, S. Sadasivan, Sci. Total Environ., Vol. 277 (2001), p. 161.
36. B. Xu, S. E. Chia, C. N. Ong, Biol. Trace Element Research, Vol. 40 (1994), p. 49.
37. S. Emanuele, L. Alessandra, P. Lucia, S. Maria Renata, F. Giovanni, Ecotoxicology and Environ. Safety, Vol. 55 (2003), p. 293,
38. R. M. Tripathi, S. Mahapatra, R. Raghunath, V. N. Sastry, T. M. Krishnamoorthy, Sci. Total Environ., Vol. 250 (2000), p. 43.
39. W. Y. Wong, G. Flik, P. M. W. Groenen, D. W. Swinkels, C. M. G. Thomas, J. H. J. Copius-Peereboom, H. M. W. M. Merkus, R. P. M. Steegers-Theunissen, Reproductive Toxicology, Vol. 15 (2001), p. 131.
40. P. J. Parsons, C. Geraghty, M. F. Verostek, Spectrochim. Acta, Part B, Vol. 56 (2001), p. 1593.
41. I. P. Hallen, L. Jorhem, B. J. Lagerkvist and A. Oskarsson, Sci. Total Environ., Vol. 166 (1995), p. 149.
42. J. M. Marchante-Gayón, C. S. Muñiz, J. I. G. Alonso, A. Sanz-Medel, Anal. Chim. Acta, Vol. 400 (1999), p. 307.
43. E. Bárány, I. A. Bergdahl, L.-E. Bratteby, T. Lundh, G. Samuelson, A. Schütz, S. Skerfving, A. Oskarsson, Sci. Total Environ., Vol. 286 (2002), p. 129.
44. E. Bárány, I. A. Bergdahl, L.-E. Bratteby, T. Lundh, G. Samuelson, A. Schütz, S. Skerfving, A. Oskarsson, Environ. Res., Section A, Vol. 89 (2002), p. 72.
45. E. Bárány, I. A. Bergdahl, L.-E. Bratteby, T. Lundh, G. Samuelson, A. Schütz, S. Skerfving, A. Oskarsson, Toxicology Letters, Vol. 134 (2002), p. 177.
46. F. Perucci, N. Violante, O. Senofonte, M. De Gregorio, A. Alimonti, S. Caroli, G. Forte, A. Cristaudo, Microchem. J., Vol. 76 (2004), p. 131
47. J. Wang, Stripping analysis. Principles, Instrumentation, and Applications, VCH Publishers, Inc. Deerfield Beach, Florida (1985), 160 p.

48. J. Wang, Analytical Electrochemistry, VCH Publishers, Inc. N.J. (1994), 198 p.
49. A.M. Oliveira Brett, C.M.A. Brett, F.-M. Matysik, S. Matysik, Ultrason. Sonochem., Vol. 4 (1997), p. 123.
50. R.G. Compton, J. C. Eklund, F. Marken, Electroanalysis, Vol. 9 (1997), p. 509.
51. F.-M. Matysik, S. Matysik, A.-M. Oliveira Brett, C. M. A. Brett, Anal. Chem., Vol. 69 (1997), p. 1651.
52. N. A. Madigan, T. J. Murphy, J. M. Fortune, C.R.S. Hagan, L. A. Coury, Anal. Chem., Vol. 67 (1995), p. 2781.
53. F. Marken, T. O. Rebbitt, J. Booth, R.G. Compton, Electroanalysis, Vol. 9 (1997), p. 19.
54. R.G. Compton, J. C. Eklund, S. D. Page, T. J. Mason, D. J. Walton, J. Appl. Electrochem., Vol. 26 (1996), p. 775.
55. R.G. Compton, J. C. Eklund, S. D. Page, G. H. W. Sanders, J. Booth, J. Phys. Chem., Vol. 98 (1994), p. 12410.
56. A. M. Oliveira Brett, F.- M. Matysik, Biochem. Bioenerg., Vol. 42 (1997), p. 111.
57. C. Agra-Gutiérrez, R. G. Compton, Electroanalysis, Vol. 10 (1998), p. 204.
58. C. Agra-Gutiérrez, R. G. Compton, Electroanalysis, Vol. 10 (1998), p. 603.
59. J. C. Ball and R. G. Compton, Electrochem., Vol. 67 (1999), p. 912.
60. A. J. Saterlay, R. G. Compton, Fresenius J. Anal. Chem., Vol. 367 (2000), p. 308.
61. C. Agra-Gutiérrez, J. L. Hardcastle, J. C. Ball, R. G. Compton, The Analyst, Vol. 124 (1999), p.1053.
62. Q. Hong, J. L. Hardcastle, R. A. J. McKeown, F. Marken, R. G. Compton, New J. Chem., 23 (1999), p. 845.
63. J. L. Hardcastle, J. C. Ball, Q. Hong, F. Marken, R.G. Compton, S. D. Bull, S. G. Davies, Ultrasonics. Sonochem., Vol. 7 (2000), p. 7.
64. L. Nei, F. Marken, Q. Hong, R.G. Compton, J. Electrochem. Soc., Vol. 144 (1997), p. 3019.
65. R. P. Akkermans, J. C. Ball, Q. Hong, F. Marken, R.G. Compton, Electrochim. Acta, 43 (1998), p. 3443.
66. A. J. Saterlay, C. Agra-Gutiérrez, F. Marken, R.G. Compton, Electroanalysis, Vol. 11 (1999), p. 1083.
67. A. J. Saterlay, J. S. Foord, R. G. Compton, Analyst, Vol. 124 (1999), p.1791.
68. A. J. Bard and L. R. Faulkner, Electrochemical Methods. Fundamentals and Applications, John Wiley & Sons, N.Y. Chichester, Brisbane, Toronto (1980).
69. C. Prado, S. J. Wilkins, F. Marken, R.G. Compton, Electroanalysis, Vol. 14 (2002), p. 262.
70. Y. C. Tsai, B. A. Coles, K. Holt, J. S. Foord, F. Marken, R.G. Compton, Electroanalysis, Vol. 13 (2001), p. 831.
71. R. G. Compton, J. S. Foord, F. Marken, Electroanalysis, Vol. 15 (2003), p. 1349.
72. J. Wang, J. Lu, S. B. Hocevar, P. A. M. Farias, B. Ogorevc, Anal. Chem., Vol. 72 (2000), p. 3218.
73. J. Wang, R. P. Deo, S. Thongngamdee, B. Ogorevc, Electroanalysis, Vol. 13 (2001), p. 1153.
74. J. Wang, J. Lu, Electrochem. Comm., Vol. 2 (2000), p. 390.
75. G.-U. Flechsig, O. Korbout, S. B. Hocevar, S. Thongngamdee, B. Ogorevc, P. Gründler, J. Wang, Electroanalysis, Vol. 14 (2002), p. 192.
76. G. Wittstock, A. Strübing, R. Szargan, G. Werner, J. of Electroanal. Chem., Vol. 444 (1998), p. 61.

77. S. B. Hocevar, J. Wang, R. P. Deo, B. Ogorevc, Electroanalysis, Vol. 14 (2002), p. 112.
78. J. Wang, J. Lu, Ülkü A. Kirgöz, S. B. Hocevar, B. Ogorevc, Anal. Chim. Acta, Vol. 434 (2001), p. 29.
79. J. Wang, Ülkü A. Kirgöz, J. Lu, Electrochem. Comm., Vol. 3 (2001), p. 703.
80. J. Wang, J. Lu, S. B. Hocevar, B. Ogorevc, Electroanalysis, Vol. 13 (2001), p. 13.
81. E. A. Hutton, B. Ogorevc, S. B. Hocevar, F. Weldon, M. R. Smyth, J. Wang, Electrochem. Comm., Vol. 3 (2001), p. 707.
82. K. Vytras, I. Svancara, R. Metelka, Electroanalysis, Vol. 14 (2002), p. 1359.
83. A. Królicka, R. Pauliukaite, I. Švancara, R. Metelka, A. Bobrowski, E. Norkus, K. Kalcher, K. Vytras, Electrochem. Comm., Vol. 4 (2002), 193.
84. J. Klima, C. Bernard, C. Degrand, J. of Electroanal. Chem., Vol. 399 (1995), p. 147.
85. J. D. Wadhawan, F. J. Del Campo, R. G. Compton, J. S. Foord, F. Marken, S. D. Bull, S. G. Davies, D. J. Walton, S. Ryley, J. Electroanal. Chem., Vol. 507 (2001), p. 135.
86. A. J. Fry, J. Touster, Electrochim. Acta, Vol. 42 (1997), p. 2057.
87. P. Cognet, A.-M. Wilhelm, H. Delmas, H. Aït Lyazidi, P.-L. Fabre, Ultrasonics Sonochem.,Vol. 7 (2000), p. 163.
88. M. J. Moorcroft, L. Nei, J. Davis, R. G. Compton, Anal. Letters, Vol. 33 (2000), p. 3127.
89. L. Nei, F. Marken, Q. Hong, R. G. Compton, J. Electrochem. Soc., Vol. 144 (1997), p. 3019.
90. A. M. O. Brett, F.-M. Matysik, Electrochim. Acta, Vol. 42 (1997), p. 945.
91. R. G. Compton, J. C. Eklund, F. Marken, T. O. Rebbitt, R. P. Akkermans, D. N. Waller, Electrochim. Acta, 42 (1997), p. 2919.
92. P. R. Birkin, S. Silva-Martinez, Anal. Chem. Vol. 69 (1997), p. 2055.
93. R. P. Akkermans, M. Wu, R. G. Compton, Electroanalysis Vol. 10 (1998), p. 814.
94. J. Davis, R. G. Compton, Anal. Chim. Acta Vol. 404 (2000), p. 241.
95. Y. C. Tsai, J. Davis, R. G. Compton, Fresenius J. Anal. Chem. Vol. 368 (2000), p. 415.
96. A. N. Blythe, R. P. Akkermans, R. G. Compton, Electroanalysis Vol. 12 (2000), p. 16.
97. J. C. Ball, R. G. Compton, J. Phys. Chem. B Vol. 102 (1998), p. 3967.
98. C. Agra-Gutierrez, J. C. Ball, R. G. Compton, J. Phys. Chem. B Vol. 102 (1998), p. 7028.
99. J. Klima, C. Bernard, C. Degrand, J. Electroanal. Chem. Vol. 367 (1994), p. 297.
100. P. R. Birkin, S. Silva-Martinez, J. Electroanal. Chem. Vol. 416 (1996), p. 127.
101. F. Marken, R. P. Akkermans, R. G. Compton, J. Electroanal. Chem., Vol. 415 (1996), p. 55.
102. E. Maisonhaute, P. C. White, R. G. Compton, J. Phys. Chem. B, Vol. 105 (2001), p. 12087.
103. E. Maisonhaute, C. Prado, P. C. White, R. G. Compton, Ultrasonics Sonochem., Vol. 9 (2002), p. 297.
104. E. Maisonhaute, B. A. Brookes, R. G. Compton, J. Phys. Chem. B, Vol. 106 (2002), p. 3166.
105. E. Maisonhaute, F. J. Del Campo, R. G. Compton, Ultrasonics Sonochem., Vol. 9 (2002), p. 275.
106. K. S. Suslick, Science Vol. 247 (1990), p. 1439.

107. G. Cum, G. Galli, R. Gallo, A. Spadaro, Ultrasonics, Vol. 30 (1992), p. 267
108. F. Marken, S. Kumbhat, G. H. W. Sanders, R. G. Compton, J. Electroanal Chem. Vol. 414 (1996), p. 95.
109. R. G. Compton, J. C. Eklund, S. D. Page, J. Phys. Chem., Vol. 99 (1995), p. 4211.
110. D. Shoup, A. Szabo, J. Electroanal. Chem., Vol. 160 (1984), p. 1.
111. C. R. S. Hagan, L. A. Coury Jr, Anal. Chem., Vol. 66 (1994), p. 399.
112. M. A. Margulis, A. N. Maltsev, Russ J. Phys. Chem., Vol. 43 (1969), p. 1055, (in Russian).
113. T. J. Mason, J. P. Lorimer and D. M. Bates, Ultrasonics, Vol. 30 (1992), p. 40.
114. E. B. Flint, K. S. Suslick, Science Vol. 253 (1991), p. 1397.
115. K. S. Suslick, D. A. Hammerton, R. E. Cline, J. Am. Chem. Soc., Vol. 108 (1986), p. 5641.
116. E. B. Flint, K. S. Suslick, J. Am. Chem. Soc., Vol. 111 (1989), p. 6987.
117. A. Francony, C. Pétrier, Ultrasonics Sonochem., Vol. 3 (1996), p. 77.
118. C. Petrier, A. Jeunet, J.-L. Luche, G. Reverdy, J. Am. Chem. Soc., Vol. 114 (1992), p.3148.
119. C. Petrier, B. David, S. Laguian, Chemosphere, Vol. 32 (1996), p. 1709.
120. J. Eklund, F. Marken, D. N. Waller, R. G. Compton, Electrochim. Acta, Vol. 41. (1996), p. 1541.
121. R. G. Compton, J. S. Foord, F. Marken, Electroanalysis, Vol. 15 (2003), p. 1349.
122. J. Reisse, H. Francois, J. Vandercammen, O. Fabre, A. K. Mesmaeker, C. Maerschalk, J.-L. Delplancke, Electrochim. Acta, Vol. 39 (1994), p. 37.
123. R. G. Compton, J. C. Eklund, f. Marken, D. N. Waller, Electrochim. Acta, Vol. 41 (1996), p. 315.
124. R. P. Akkermans, J. C. Ball, F. Marken, R. G. Compton, Electroanalysis, Vol. 10 (1998), p. 26.
125. C. Baird, Environmental Chemistry, 2nd edition, W. H. Freeman and Company, New York (2000), p. 408.
126. P. O'Neill, Environmental Chemistry, 2nd edition, Chapman & Hall, London (1994), p. 220.
127. Fundamentals of Clinical Chemistry, (Eds: C.A. Burtis, E.R. Ashwood), W.B. Saunders Co., PA. USA, (1996).
128. I. Pais, J.B. Jones, The Handbook of Trace Elements, St. Lucie Press, Boca Raton, Florida, (1997), p. 94.
129. M. Beacklund, N. L. Pedersen L. Bjorkman, M. Vahter, Environmental Research, Vol. 80 (1999), p. 222.
130. M. del'Omo, G. Muzi, R. Piccini, A. Gambelunghe, P. Morucci, T. Fiordi, M. Ambrogi, G. Abbritti, Science of the Total Environment, Vol. 226 (1999), p. 57.
131. M. Esteban, I. Ruisánchez, M. S. Larrechi, F. X. Rius, Anal. Chim. Acta, Vol. 268 (1992), p. 95.
132. C. Agra-Gutierrez, M. E. Suarez, R. G. Compton, Electroanalysis, Vol. 11 (1999), p.16.
133. V. Muzyka, S. Bogovski, A. Viitak, T. Veidebaum, Sci. Total Environment, Vol. 286 (2002), p. 73.
134. B. Xu, S. E. Chia and C. N. Ong. Bio. Trace Elem. Res., Vol. 40 (1994), p. 49.
135. J. Szpunar, J. Bettmer, M. Robert, H. Chassaigne, K. Cammann, R. Lobinski and O. F. X. Donard, Talanta, Vol. 44 (1997), p. 1389.

136. R. M. Tripathi, R. Raghunath, S. Mahapatra and S. Sadasivan, Sci. Total Environ., Vol. 277 (2001), p. 161.
137. N. Sayama, K. Yoshida, K. Mori, H. Fukazawa, H. Hori, M. Nakazato, J.-I. Tani, Y. Nakagawa and S. Ito, Endocr. J.(Tokyo), Vol. 45 (1998), p. 767.
138. G. Saner, S. U. Baysal, E. Unuvar and T. Ozden, J. Trace Elem. Experiment. Med., Vol. 13 (2000), p. 265.
139. Brockhaus. VDI-Berichte, Vol. 203 (1973), 20.
140. T. Maeda, M. Nakagawa, M. Kawakatsu and Y. Tanimoto, Shimadzu Hyoron, Vol. 37 (1980), p. 75.
141. M. Tuzen, Trace Elem. Electrolytes, Vol. 19 (2002), p. 202.
142. J. L. Burguera, M. Burguera and M. R. Brunetto, At. Spectrosc., Vol. 14 (1993), p. 90.
143. X. Ji and J. Ren. Analyst, Vol. 127 (2002), p. 416.
144. H. Vanhoe, C. Vandecasteele, J. Versieck and R. Dams, Anal. Chem., Vol. 61 (1989), p. 1851.
145. P. Schramel, J. Begerow and H. Emons, Anal. Hazard. Substan. Bio. Mat., Vol. 6 (1999), p. 1.
146. X. Sun, Zhonghua Yufang Yixue Zazhi, Vol. 27 (1993), p. 176.
147. J. L. Hardcastle and R. G. Compton, Electroanalysis, Vol. 14 (2002), p. 753.
148. C. E. Banks and R. G. Compton, CPPC, Vol. 4 (2003), p. 169.
149. C. E. Banks, N. V. Rees and R. G. Compton, J. Electroanal. Chem., Vol. 535 (2002), p. 41.
150. Y. von Schirnding, A. Mathee, M. Kibel, P. Robertson, N. Strauss and R. Blignaut, Environ. Res., Vol. 93 (2003), p. 259.
151. R. Albalak, R. H. McElroy, G. Noonan, S. Buchanan, R. L. Jones, W. D. Flanders, C. Gotway-Crawford, D. Kim, T. Dignam, W. R. Daley, J. Jarrett, E. Eduardo and M. A. McGeehin, Archives of Environ. Health, Vol. 58 (2003), p. 172
152. E. Sanna, A. Liguori, L. Palmas, M. R. Soro and G. Floris, Ecotoxicology and Environmental Safety, Vol. 55 (2003), p. 293.
153. D. Jagner, M. Josefon, S. Westerlund and K. Aren, Anal. Chem., Vol. 53 (1981), p. 1406.
154. H. Mathieu, F. Le Moigne, G. Panteix, P. Derache and E. Jouzier, Annales de Biologie Clinique, Vol. 61 (2003), p. 667.
155. J. Wang, Acc. Chem. Res., Vol. 35 (2002), p. 811.
156. C. E. Banks, M. E. Hyde, P. Tomcík, R. Jacobs and R. G. Compton, Talanta, Vol. 62 (2004), p. 279.
157. J. Xu, M. C. Granger, Q. Chen, J. W. Strojek, T. E. Lister and G. M. Swain. Anal. Chem., Vol. 69 (1997), p. 591A.
158. S. Haymond, G. T. Babcock and G. M. Swain. J. Am. Chem. Soc, Vol. 124 (2002), p. 10634.
159. C. E. Banks and R. G. Compton. Electroanalysis, Vol. 15 (2003), p. 329.
160. S. B. Hocevar, B. Ogorevc, J. Wang and B. Pihlar. Electroanalysis, Vol.14 (2002), p. 1707.
161. J. Wang, R. P. Deo, S. Thongngamdee and B. Ogorevc. Electroanalysis, Vol.13 (2001), p. 1153.
162. R. Pauliukaite, R. Metelka, I. Svancara, A. Krolicka, A. Bobrowski, K. Vytras, E. Norkus and K. Kalcher. Anal. Bioanal. Chem, Vol. 374 (2002), p. 1155.
163. G. Kefala, A. Economou, A. Voulgaropoulos and M. Sofoniou. Talanta, Vol. 61 (2003), p. 603.

SUMMARY IN ESTONIAN

Antud töös uuriti Cd^{2+}, Zn^{2+} ja Pb^{2+} ioonide sonovoltamperomeetrilist määramist inimverest, kuna meditsiinis on oluline teada lisaks haigust põhjustavale toksilisele ainele/ühendile ka ta kontsentratsiooni. Meie tähelepanu koondus vere uurimisele, kuna ta on kirjanduse andmete põhjal üks enamlevinumaid analüüsiobjekte andes ühtlasi nii meedikutele, kui ka analüütikutele üpris tõetruu pildi inimorganismis toimuvast. Enamlevinud uurimismeetodid eeldavad tänapäeval aatom absorbtsioonspektromeetrite või induktiivpaar plasma massspektromeetrite kasutamist raskmetallide analüüside tegemisel. Püüdsime luua ja rakendada voltamperomeetrilisi analüüsimeetodeid, sest antud aparatuur on odavam ja kaasaskantavam oma väiksemate mõõtmete tõttu. Lisaks sellele oli eesmärgiks lühendada proovi ettevalmistusaega või vältida seda hoopis rakendades proovi eelöötlemiseks ja tööelektroodi pinna aktiivsena hoidmiseks ultraheli.

Esimesena, rakendasime seda meetodit kaadmiumi-ioonide määramisel inimverest ning kasutasime eelnevalt elavhõbedaga kaetud Nafion® klaassüsinik elektroodi. Analüüsiks kasutasime eelnevalt keemiliselt töötlemata 2% vere erütrotsüütide (punaliblede) lahust. Kirjandusviite [15] põhjal võib väita, et Cd^{2+} ja Pb^{2+} ioonid ongi just vere erütrotsüütides. Väljatöötatud anaüüsimeetodiga määratud vere kaadmiumi-ioonide sisaldust võrreldi aatom absorbtsioonspektromeetrilisel (AAS) analüüsil saadud tulemustega, mis olid omavahel väga heas kooskõlas.

Tsingi ioonide voltamperomeetriliseks määramiseks rakendasime sono topeltekstraktsioonilist proovi eelpuhastust kasutades ligandina ditisooni kloroformilahust. Tagasiekstraheerimisel saadud veefaasi analüüsiti voltamperomeetriliselt boori lisandiga dopeeritud teemantelektroodil, mis omas kõrgemat tundlikust tsingi-ioonide suhtes, kui klaassüsinik elektrood eelkõige tänu oma madalamatele foonvoolu väärtustele [II, III]. Nenede mõõtmiste tulemusena määratud tsingi ioonide kontsentratsioonid langesid väga hästi kokku sõltumatul (AAS) analüüsil saadud tulemustega [III].

Plii(II) ioonide voltamperomeetriliseks määramiseks oli vaja veelgi suurendada tööelektroodi tundlikust, kuna Pb^{2+} ioonide sisaldus inimveres on ca. 100–1000 väiksem, kui Zn^{2+} ioonide oma, vältides seejuures elavõbeda kasutamist, kuna ta kasutamist püütakse paljudaes maailma riikides piirata või hoopis keelustada. Seepärast otsustasime kasutada vismutiga kaetud tööelektroodide, kuna vismutit peetakse "keskkonnasõbralikumaks" võrreldes elavhõbedaga [59, 60]. Meie [VI] kasutasime pidevalt taasmoodustuva vismutiga kaetud boori lisandiga dopeeritud teemantelektroodi. Analüüsimeetodi väljatöötamiseks ja testimiseks lisasime Pb^{2+} ioone vereproovile just nii palju, et saavutada lahjendamata inimveres kontsentratsioon 5.0 μM, mis on meditsiiniliselt kriitiliseks piiriks normaalse ja kõrgendatud plii (II) ioonide sisalduse vahel

[33]. Väljatöötatud analüüsimeetodi kontrollimisel saavutati hea kokkulangevus analüüsil määratud ning eelnevalt lisatud Pb^{2+} ioonide kontsentratsiooni vahel [VI].

Kokkuvõtteks võib tõdeda, et antud töös on väljatöötatud kolm erinevat meetodit kolme olulisima toksilise raskmetalli määramiseks verest [I, III, VI]. Samuti on edukalt tõestatud ultraheli kasulikkust elektrokeemilisel analüüsil [I, II, III, IV, V, VI] ja astutud järgmine samm elavhõbeda elektrokeemilises analüüsis vältimise suunas kasutades tema asemel piisavalt hea analüütilise tundlikusega katmata ja vismutiga kaetud boori lisandiga dopeeritud teemant-elektroodi [IV, VI]. Eelpoolnimetatud kolm väljatöötatud Cd^{2+}, Zn^{2+} ja Pb^{2+} ioonide verest määramise elektroanalüütilist määramismeetodit võivad olla uute meditsiiniliselt oluliste veres sisalduvate raskmetallide elektroanalüütiliste määramismeetodite aluseks.

ACKNOWLEDGEMENTS

I would like thank Mrs A. Viitak (Technical University of Tallinn) for conducting the blood AAS measurements, Prof. Lembit Nei (Estonian Agricultural University Environmental Protection Institute) for idea of this work and scientific advises, Dr Ivo Kolts and Mr. Agu Raudheiding (Tartu University) for making blood images. Special thanks to my official supervisors Prof. Enn Lust and docent Heldur Keis and the greatest one to my supervisor Prof. Richard Guy Compton and advisor Craig E. Banks (Physical and Theoretical Chemistry Laboratory, Oxford University, UK) thank whose intensive support I could make as these studies as to write my publications.

As last I would like to thank my parents and sister for their moral support.

Many-many thanks to all who supported me in my PhD studies.

I owe thanks also to the Wellcome Trust for their funding program (Grant 062759) resolving my financial needs at least for this time.

PUBLICATIONS

Jaanus Kruusma and Lembit Nei*, Joanna L. Hardcastle and Richard G. Compton, ”Sonoelectroanalysis: Anodic Stripping Voltammetric Determination of Cadmium in Whole Human Blood”, *Electroanalysis*, Vol. 16 (2004), p. 399.

Jaanus Kruusma, Peter Tomčík, Craig E. Banks and Richard G. Compton*, “Sonoelectroanalysis in Acoustically Emulsified Media: Zinc and Cadmium” (*Electroanalysis*, accepted).

Jaanus Kruusma, Craig E. Banks, Enn Lust, Heldur Keis, Lembit Nei and Richard G. Compton*, “Electroanalytical Determination of Zinc in Human Blood Facilitated by Acoustically Assisted Double Extraction”, (*Analytica Chimica Acta*, accepted)

Craig E. Banks, **Jaanus Kruusma**, Michael E. Hyde, Abdollah Salimi, Richard G. Compton*, “Sonoelectroanalysis: Investigation of Bismuth Film Modified Glassy Carbon Electrodes”, (*Analytical and Bioanalytical Chemistry*, Vol. 379 (2004), p. 277.

Jaanus Kruusma, Craig E. Banks, Lembit Nei and Richard G. Compton*, “Electroanalytical Detection of Zinc in Whole Blood” *Analytica Chimica Acta*, Vol. 510 (2004), p. 85.

Jaanus Kruusma, Craig E. Banks, and Richard G. Compton*, “Mercury Free Sono-Electroanalytical Detection of Lead in Human Blood Utilising Bismuth Film Modified Boron-Doped Diamond Electrodes” (*Analytical and Bioanalytical Chemistry*, accepted).

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3. H. Keis, J. Pullat, J. Kruusma. Polarography and stripping voltammetry of lead-polycarboxylate complexes on mercury drop and rotating disc electrodes, Abstracts of 51st Annual ISE Meeting, Warsaw, 2000, s-2, 803.
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6. J. Kruusma, L. Nei, J.L. Hardcastle, R.G. Compton, E. Lust, H. Keis. Sonoelectroanalysis: Anodic Stripping Voltammetric Determination of Cadmium in Whole Blood, *Electroanalysis*, 16 (2004), p. 399.
7. Jaanus Kruusma, Craig E. Banks, Enn Lust, Heldur Keis, Lembit Nei and Richard G. Compton*. Electroanalytical Determination of Zinc in Human Blood Facilitated by Acoustically Assisted Double Extraction, *Analytica Chimica Acta*, Vol. 510 (2004), p. 85.
8. Craig E. Banks, Jaanus Kruusma, Michael E. Hyde, Abdollah Salimi, Richard G. Compton*, "Sonoelectroanalysis: Investigation of Bismuth Film Modified Glassy Carbon Electrodes", *Analytical and Bioanalytical Chemistry*, Vol. 379 (2004), p. 277.
9. J. Kruusma, P. Tomčík, C. E. Banks and R. G. Compton*, Sono-electroanalysis in Acoustically Emulsified Media: Zinc and Cadmium, (*Electroanalysis*, accepted).
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2. H. Keis, J. Kruusma, and J. Pullat, Polarography and stripping voltammetry of lead-polycarboxylate complexes on dropping mercury and rotating disc electrodes, *Proc. Estonian Acad. Sci. Chem.*, Vol. 49 (2000), 3, p.156.

3. H. Keis, J. Pullat, J. Kruusma. Polarography and stripping voltammetry of lead-polycarboxylate complexes on mercury drop and rotating disc electrodes, Abstracts of 51st Annual ISE Meeting, Warsaw, 2000, s-2, 803.
4. J. Kruusma, L. Nei, J.L. Hardcastle, R.G. Compton, E. Lust, H. Keis. Determination of Cadmium Ions Concentration in Human Blood, Abstracts of 28th Estonian Chemistry Days, 2002, p.70.
5. Jaanus Kruusma and Lembit Nei*. Sonoelectroanalysis: Anodic Stripping Voltammetric Determination of Cadmium, Zinc and Lead in Human Blood, Abstracts of Second Triennial International Conference on Electroanalytical Chemistry and Allied Topics (ELAC-2004). Goas (India)
6. J. Kruusma, L. Nei, J.L. Hardcastle, R.G. Compton, E. Lust, H. Keis. Sonoelectroanalysis: Anodic Stripping Voltammetric Determination of Cadmium in Whole Blood, *Electroanalysis*, 16 (2004), p. 399.
7. Jaanus Kruusma, Craig E. Banks, Enn Lust, Heldur Keis, Lembit Nei and Richard G. Compton*. Electroanalytical Determination of Zinc in Human Blood Facilitated by Acoustically Assisted Double Extraction, *Analytica Chimica Acta*, Vol. 510 (2004), p. 85.
8. Craig E. Banks, Jaanus Kruusma, Michael E. Hyde, Abdollah Salimi, Richard G. Compton*, "Sonoelectroanalysis: Investigation of Bismuth Film Modified Glassy Carbon Electrodes", *Analytical and Bioanalytical Chemistry*, Vol. 379 (2004), p. 277.
9. J. Kruusma, P. Tomčík, C. E. Banks and R. G. Compton*, Sono-electroanalysis in Acoustically Emulsified Media: Zinc and Cadmium, (*Electroanalysis*, accepted).
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