

# Direct Measurements of Cadmium, Lead and Copper in Whole Blood Using the Stripping Voltammetric Method with Modified Thick-Film Graphite Electrodes

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## Abstract

The existing methods for measurement of Cu(II), Pb(II) and Cd(II) in whole blood need expensive complicated equipment, require preliminary destruction of the blood matrix, and generally cannot provide the necessary detection limit. We have reported the use of the stripping voltammetric method with modified thick-film electrodes for the analysis of whole blood and its fractions. The said electrodes, a quick potential sweep rate, and recording of differential voltammograms excluded extremely toxic mercury and its soluble salts from analysis, made the operations of the solution deaeration and chemical and electrochemical sample pretreatment unnecessary, and allowed decreasing the volume of test samples to 25–400  $\mu\text{l}$ .

## INTRODUCTION

The vigorous industrial development and, hence, the global problem of the technogenic environmental pollution forces out purely natural forms of pathological states of all living creatures. For example, cardiovascular diseases, which were of low occurrence in the 17th and 18th centuries, are the main cause of death in highly industrialized countries today [1]. Modern researchers think [2, 3] that an unbalance of elements in the organism is most intimately related to malfunctions of the cardiovascular system [2], impairment of immunity, diseases of the skin, hair and nails, allergoses (including bronchial asthma), hypertension, scoliosis, osteoporosis, osteochondrosis, diseases connected with the formation of stones in the liver and in the kidneys, blood diseases, anemia, chronic gastritis and

colitis, retardation of growth and development of children [3].

Versatile methods have been available for the determination of microelements in clinical materials [4–13]. Each of them has its advantages and drawbacks and can be used to solve a particular clinical problem. However, relatively low sensitivity of, *e. g.*, X-ray fluorescent [4] and photocolometric [5] methods requires preliminary accumulation of the majority of analytes. Studies of the microelemental status of the organism by means of the highly sensitive neutron activation analysis [6] are limited by laborious procedures and a large cost of the analysis. Inductively coupled plasma mass spectrometry [7–9] is available only to large research centres because of very expensive equipment. Atomic absorption spectroscopy (AAS) takes the lead today in the analysis of biological specimens [5, 10–15]. AAS can

be used directly for determination of elements in some biological fluids with the concentration of not less than 500  $\mu\text{g/l}$  (Cu, Zn, Fe) if samples are diluted with an appropriate buffer solution [11, 13]. Elements, the concentrations of which are lower, can be measured using electrothermal atomization. For example, Pb [14] and Cd [15] were measured directly in urine. It should be noted that the composition of samples has a considerable effect on analysis results. It is for this reason probably that AAS methods of direct analysis of whole blood are so rare. Most methods deal with the analysis of serum [5, 11]. The elimination of the adverse effect of the matrix [16] by mineralization represents the most complex and laborious task in the development of particular methods for measuring microelements in real materials [17]. Also, AAS equipment is rather expensive and is almost unavailable to small clinical laboratories. The examination of the microelemental status of a living organism in the medical practice requires primarily the availability of accessible inexpensive equipment. The method should provide an express analysis, which is very important for urgent diagnosis of diseases, and a very simple analysis procedure without time-consuming laborious preparation of samples. It should be extremely sensitive and capable of analyzing microdoses of test materials (for example, blood, all its fractions, the cerebrospinal fluid, and internal tissues) not only at toxic levels, but also at the level of essential, *i. e.* vital, concentrations. The stripping voltammetric method [18] may prove to be comprehensively competitive in the analysis of the microelemental status of the organism. It is distinguished for high sensitivity, low detection limit, selectivity, versatility, and simplicity of both the analysis procedure and processing of analysis results. This method often allows simultaneous determination of several metals including Zn, Cd, Tl, Bi and Cu [19, 20]. It does not require expensive complicated equipment and can be implemented as on-line variants. In recent years the SVA method has been used on a progressively wider scale in analytical control of the environment. The scope of clinical test samples has been expanded [21–29]. SVA equipment uses small quantities of test materials,

such as 50  $\mu\text{l}$  of blood [21]. The SVA analysis of biological fluids encounters two problems. The first problem is due to oxygen dissolved in test samples, which decreases the signal-to-noise ratio. It is difficult to remove oxygen from these fluids with an inert gas flow, because in most cases this procedure is accompanied by superfluous foaming and splashing [22]. The second problem involves, as in other methods of analysis, the interference of albumen components of samples, *e. g.*, proteins and amino acids. Adsorption-desorption of surface-active proteins at the working electrode decreases or completely disguises responses of metals [24, 25]. For these reasons, most researchers think it is necessary to mineralize the matrix of samples before the SVA analysis [21, 26–31]. Diverse methods are used for sample mineralization, but all of them are laborious, incur errors caused by contaminants in reagents (a “blank” test), and involve high risk of accidental contamination of samples. The latter factors are especially harmful for the determination of cadmium, because its concentration in biological materials is low. Instead of acid mineralization of samples for measurements of lead in whole blood, researchers [32] proposed to use reagents (mainly acid solutions of chromium and calcium salts) capable of replacing some ions of heavy metals in metal-containing biological molecules. It was found however [29] that these reagents cannot eliminate completely the effect of dissolved oxygen and the matrix on the electrochemical behaviour of lead. Despite this fact, the method has found its advocates [33]. When acidified samples were filtered through microcellulose instead of mineralization, lead was determined in whole blood at a level of 40 to 300  $\mu\text{g/l}$  with an accuracy of 13.5 % [22]. The method of stripping potentiometry, which is less sensitive to the adverse effect of oxygen and organic compounds, was used successfully for direct measurements of lead [34–37] and, also, of lead and cadmium [38, 39] in whole blood. The volume of blood samples was reduced to 2.5  $\mu\text{l}$  [37] thanks to refinement of the experimental technique. In our opinion, the question of the validity of samples less than 5  $\mu\text{l}$  in volume is still open. Studies concerned with working electrodes, which are in-

different to the interference of both the matrix and dissolved oxygen, may prove to be most promising for the analysis of biological fluids without sample pretreatment. Using a special polymer membrane to protect the working surface of a glassy carbon electrode, B. Hoyer and M. Florence [40] measured lead concentrations in whole blood immediately after acidification of samples. However, deproteinization was required before copper measurements. Thick-film graphite electrodes (TGE's) [41] operating at a fast potential sweep rate proved to be little sensitive to the effect of dissolved oxygen [42] and the blood matrix [43]. Therefore, it was possible to propose a method for the determination of not only lead, but also copper and cadmium in whole blood omitting a special treatment of samples. Analyte ions and mercury were pre-concentrated on the TGE surface and then the oxidation current was recorded at a linear potential sweep under the integral or differential regime (the latter eliminated the adverse effect of oxygen completely). However, toxic soluble salts of mercury (II) had to be added to the electrolyte during the analysis. This operation presents a considerable drawback.

According to the literature data [10, 11, 44–46], the concentration of copper in blood is 630 to 1550  $\mu\text{g/l}$ . The information about the concentration of lead in blood is contradictory. The normal concentrations of 220 to 300  $\mu\text{g/l}$  [11, 46] and 70 to 190  $\mu\text{g/l}$  [28] have been reported. Similarly to lead, reported normal concentrations of cadmium in blood are conflicting: 0.3 to 2.0 [28], 3 to 4 [10], and 27  $\mu\text{g/l}$  [46].

The objective of this study was to:

- eliminate mercury and toxic mercury salts from the analysis;
- examine the electrochemical behavior of copper, lead and cadmium in the presence of surface-active proteins in blood;
- simplify the analysis procedure;
- decrease minimum measurable concentrations of lead and cadmium in whole blood and reduce the measurement error of the three elements;
- make the method more rapid.

## EXPERIMENTAL

### *Equipment and electrodes*

Automatic program-controlled voltammetric analyzers of IVA-3AK and IVA-5 types (IVA Co. Ltd., Ekaterinburg, Russia) and an "Autolab" analyzer (Eco Chemie, Netherlands) were used. Anodic voltammograms of analyte metals were recorded under the standard integral and differential regimes of DC stripping voltammetry with a linear potential sweep. The maximum current ( $I_{\text{max}}$ ) and the variation amplitude of the derivative  $dI/dU$  ( $R$  – response) served as the response in the first and second cases, respectively. A 5-ml three-electrode electrochemical cell was used. Oxidants (oxygen), which were dissolved in the electrolyte, were not removed. A graphite or glassy-carbon rod served as the auxiliary electrode. The reference electrode was a silver/silver chloride (Ag/AgCl) electrode filled with a saturated KCl solution or a strip of a silver-containing paste, which was screen-printed on a polymer substrate. A modified thick-film graphite electrode (MTGE) or a modified thick-film carbon-containing electrode (MTCE) (IVA Co. Ltd., Ekaterinburg, Russia) served as the working sensors. The indicator electrodes were prepared by the screen printing method using graphite-containing (IVA Co. Ltd., Ekaterinburg, Russia) and carbon-containing (Metech, USA) pastes. The reference electrode was made of a silver-containing paste (ELMA-Pastes, Zelenograd, Russia). The surface of the working electrodes was modified by consecutive application of modifying solutions and their drying in air. The sensor working surface was formed before the analysis by five-fold cycling of the potential between  $-0.05$  and  $-1.2$  V at a rate  $v = 0.45$  V/s. The potential sweep was stopped at  $-1.2$  V for 60 s after each cycle and the electrode was held at a potential of  $-0.05$  V for 30–60 s in 0.5 M HCl. Generally, the electrode remained serviceable throughout the working day. A magnetic stirrer agitated the solution during electrolysis.

### *Reagents*

The solutions were prepared and the devices were washed using tridistilled water. Rea-

gents of the "os. ch." (extra pure) grade were used. Hydrochloric acid was purified additionally by the isopiestic method. Solutions containing 1.0, 0.5 and 0.2 mg/l of copper (II), lead (II) and cadmium (II) ions were prepared from State standard samples of the corresponding ions having the concentration of 1 g/l by consecutive dilution with 0.5 M HCl. Solutions of 1 mg/l were prepared once a week, 0.5 mg/l every three days, and 0.2 mg/l daily.

### Samples and their preparation

The objects of study were whole blood and hemolysate. Venous blood in the amount of 5 (10) ml was placed in a test tube containing 0.2 (0.4) ml of a 5 % EDTA solution or 1.25 (2.50) ml of a 4 % glucicir solution as the anticoagulant. Thus, the initial degree of dilution of the test blood samples was 1.04 and 1.25, respectively ( $f_1 = (V_{\text{ant}} + V_{\text{bl}})/V_{\text{bl}}$ , where  $V_{\text{ant}}$  and  $V_{\text{bl}}$  denote the volume of the antioxidant and blood). In some cases, the CPDA blood stabilizer (USA) was used as the anticoagulant and the blood samples were diluted 1.14 times. The test samples were stirred thoroughly and were placed in a cooler at a temperature of  $-18^\circ\text{C}$ . This treatment stabilized the blood samples for a term of up to 30 days or more. Blood (0.025–0.100 ml) was taken from a finger directly into a pipette wetted with an anticoagulant and was placed immediately into the electrolytic cell. To prepare hemolysate, blood and an anticoagulant were centrifuged for 15 min at 6000 rpm (erythrocytes burst at this speed and their colorless membranes only settle to the bottom; as distinct from the plasma, this liquid fraction of blood includes contents of erythrocytes). The data in Table 1 and the figures were obtained for the blood sample No. 1, which was analyzed by the methods of atomic emission chemical spectral analysis with excitation of the spectrum in a DC arc after mineralization of the sample (Cu, Pb) and atomic absorption spectroscopy in a graphite cuvette after mineralization of the sample (Cd). The concentration of Cu(II), Pb(II) and Cd(II) ions in the sample was  $(1386 \pm 180)$ ,  $(295 \pm 35)$  and  $(20.5 \pm 4.2)$   $\mu\text{g/l}$ , respectively. The model sample was a 0.01 M HCl solution containing

25  $\mu\text{g/l}$  Cd(II), 250  $\mu\text{g/l}$  Pb(II), and 1000  $\mu\text{g/l}$  Cu(II). Blood samples having a certain degree of dilution were prepared by mixing corresponding volumes of 0.5 M HCl and the stabilized blood samples directly in the electrochemical cell. The total dilution of the blood samples,  $f$ , was calculated from the formula  $f = f_1[(V_{\text{el}} + V_{\text{bl}})/V_{\text{bl}}]$ , where  $V_{\text{el}}$  and  $V_{\text{bl}}$  denote aliquots (ml) of the electrolyte and blood respectively and  $f_1$  is the initial degree of the blood dilution.

### RESULTS AND DISCUSSION

Figures 1 and 2 present stripping integral (see Fig. 1) and differential (see Fig. 2) voltammograms, which were recorded after electrodeposition of copper, lead and cadmium from a 0.5 M HCl solution containing an aliquot of the model solution (see Fig. 1, a) and whole blood (see Fig. 1, b and c, Fig. 2). From these figures it is seen that the shape of the curves, which were recorded in solutions in the absence of blood and those containing different quantities of blood, change little. Response characteristics are given in Table 1.  $C_{\text{Me(II)}}$  in the cell were calculated as the ratio between  $C_{\text{Me(II)}}$  in blood (results of independent analysis methods) and  $f$ . Thus, blood has little effect on the oxidation potentials of lead and cadmium and causes a slight shift of the copper oxidation potential to the positive side. However, blood has a passivation effect, which shows up as the decrease in the response as compared to the model solution. The sensitivity coefficient diminishes by 20–40 % (see Table 1). If one considers the effect of the blood matrix on determination of the concentration of microelements as was observed in [23–25] and in this study, he should take into account at least three possible factors:

- 1) the change of the electrode process kinetics as a result of adsorption of surfactants present in blood [18, 47–49];

- 2) an incomplete release of ions of the analyte metals from their complexes or enzymatic and protein elements of blood during its dilution with the electrolyte [11, 52];

- 3) the retardation of diffusion of the analyte ions to the electrode surface by a layer of

TABLE 1  
Composition of the test solution and characteristics of responses of cadmium, lead and copper ( $P = 0.95, n = 3$ )

Composition and concentration of analyte ions in solution	Response potential ( $E_{el} \pm \delta$ ), V			Response value ( $R \pm \delta$ ), mA			Sensitivity coefficient $R/C_{Me(II)}$ , $\mu A / \mu g$		
	Cd	Pb	Cu	Cd	Pb	Cu	Cd	Pb	Cu
4.8 ml of 0.5 M HCl + 0.2 ml of model solution 1.0, 10.0 and 40.0 mg/l Cd(II), Pb(II) and Cu(II)	$0.65 \pm 0.05$	$-0.45 \pm 0.05$	$-0.26 \pm 0.06$	$0.63 \pm 0.07$	$3.5 \pm 0.30$	$7.75 \pm 0.47$	0.63	0.35	0.19
4.8 ml of 0.5 M HCl + 0.2 ml of blood stabilized with EDTA 0.9, 11.3 and 53.3 mg/l Cd(II), Pb(II) and Cu(II)	$-0.65 \pm 0.05$	$-0.46 \pm 0.05$	$-0.22 \pm 0.06$	$0.50 \pm 0.05$	$2.8 \pm 0.18$	$6.5 \pm 0.42$	0.56	0.25	0.12
4.8 ml of 0.5 M HCl + 0.2 ml of blood stabilized with glucicir 0.7, 9.4 and 44.3 mg/l Cd(II), Pb(II) and Cu(II)	$-0.67 \pm 0.05$	$-0.47 \pm 0.05$	$-0.21 \pm 0.06$	$0.37 \pm 0.03$	$2.3 \pm 0.16$	$5.4 \pm 0.34$	0.53	0.25	0.12

adsorbed organic molecules at the stage of accumulation [49].

These factors may show up as the shift of discharge and ionization potentials of the metals, a change in the shape of voltammograms, an expansion of the oxidation potential interval of the metals, and a drop of the maximum current, *i. e.* attenuation of the response. Unfortunately, a rigorous mathematical theo-

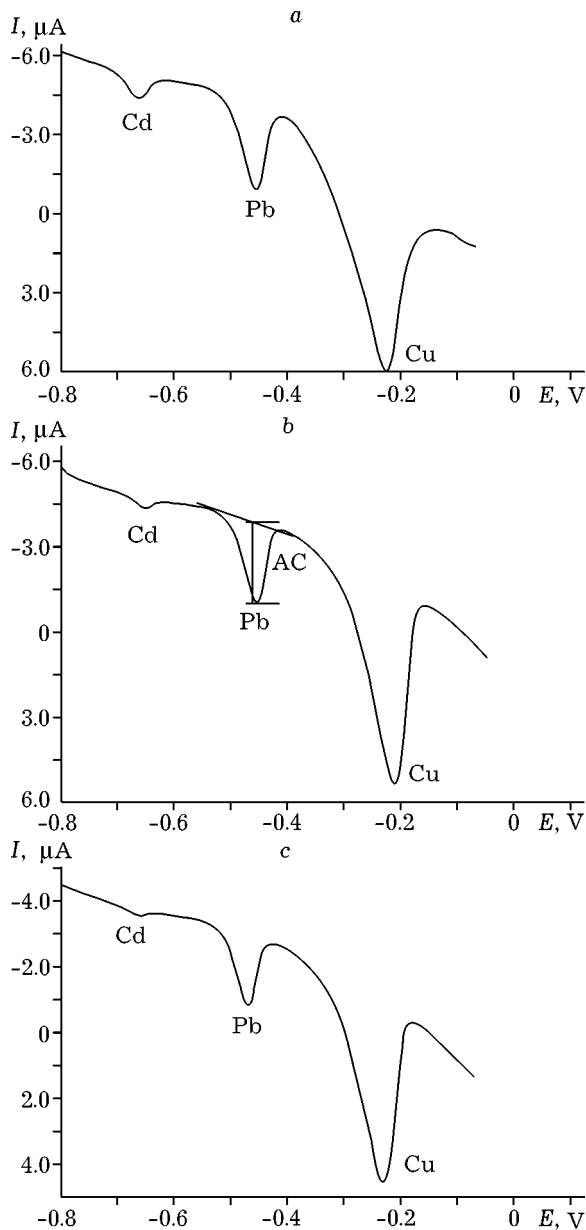


Fig. 1. Integral voltammograms of oxidation of Cu, Pb and Cd accumulated at  $E_{el} = -1.2$  V and  $t_{el} = 30$  s from a 0.5 M HCl solution containing (a) a model sample ( $f = 25$ ), (b) whole blood stabilized with EDTA ( $f = 26$ ), and (c) whole blood stabilized with glucicir ( $f = 31.25$ ).  $v = 0.3$  V/s.

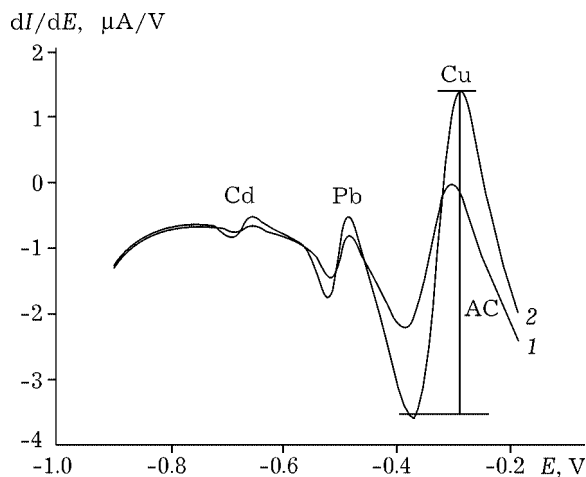


Fig. 2. Differential voltammograms of oxidation of Cu, Pb and Cd accumulated at  $E_{el} = -1.2$  V and  $t_{el} = 30$  s from a 0.5 M HCl solution containing whole blood stabilized with EDTA ( $v = 0.3$  V/s): 1 -  $f = 26$ ; 2 -  $f = 13$ . For the solution compositions refer to the text.

ry, which would provide a quantitative description of the effect of the said factors on measured parameters, has been unavailable.

It was shown earlier [50] that an ensemble of mercury microdroplets appears on the surface of a modified electrode during its electrochemical pretreatment. Since the metal is deposited at a lower overvoltage across mercury than across graphite and the crystallization overvoltage is absent in the first case, this electrode can be assumed as the one approaching an ensemble of microelectrodes. Therefore, the electroactive substance is transported to the electrode surface both through semi-finite and radial diffusion. Qualitative estimates can be made from the dependencies of the maximum stripping current or response value ( $I_{max}$  or  $R$ ) and its potential ( $E_{max}$ ) on the potential sweep rate ( $v$ ) and the log potential sweep rate ( $E_{max} - \lg v$  and  $I_{max}(R) - v$ , respectively), which were obtained by solving the problem of the metal discharge-ionization on the surface of solid [47, 48] and thin-film electrodes [51]. It follows from the cited studies that the slope ratio of the  $E_{max} - \lg v$  curve increases when the reversible process is replaced by the irreversible one. The directly proportional behavior of the  $R - v$  dependence suggests that the electrode process does not include the diffusion transport of the electroactive substance to the electrode reaction zone, *i. e.* the dis-

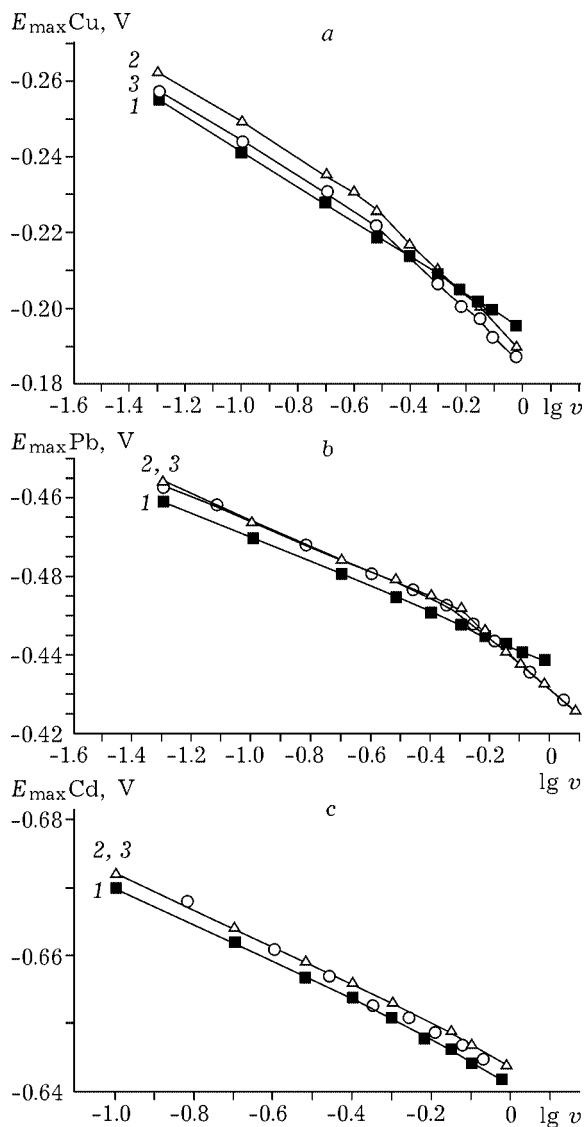


Fig. 3. Dependencies of the potential of maximums of copper (a), lead (b) and cadmium (c) responses on the logarithmic variation rate of the potential ( $v$ ). The dependencies were recorded in a 0.5 M HCl solution containing (1) a model sample ( $f = 25$ ), (2) whole blood stabilized with EDTA ( $f = 25$ ), and (3) whole blood stabilized with glucicir ( $f = 31.25$ ). Experimental conditions:  $E_{el} = -1.2$  V,  $t_{el} = 60$  s.

solved metal either represents a solid phase or resides in the amalgam film, the thickness of which is comparable with that of the diffusion layer. In the case of the diffusion transport, the response value is proportional to  $v^{1/2}$ .

Figure 3 presents  $E_{max} - \lg v$  dependencies, which were recorded during ionization of Cu, Pb and Cd against the background of the model solution (curve 1) and in the presence of blood (curves 2 and 3). Each point of

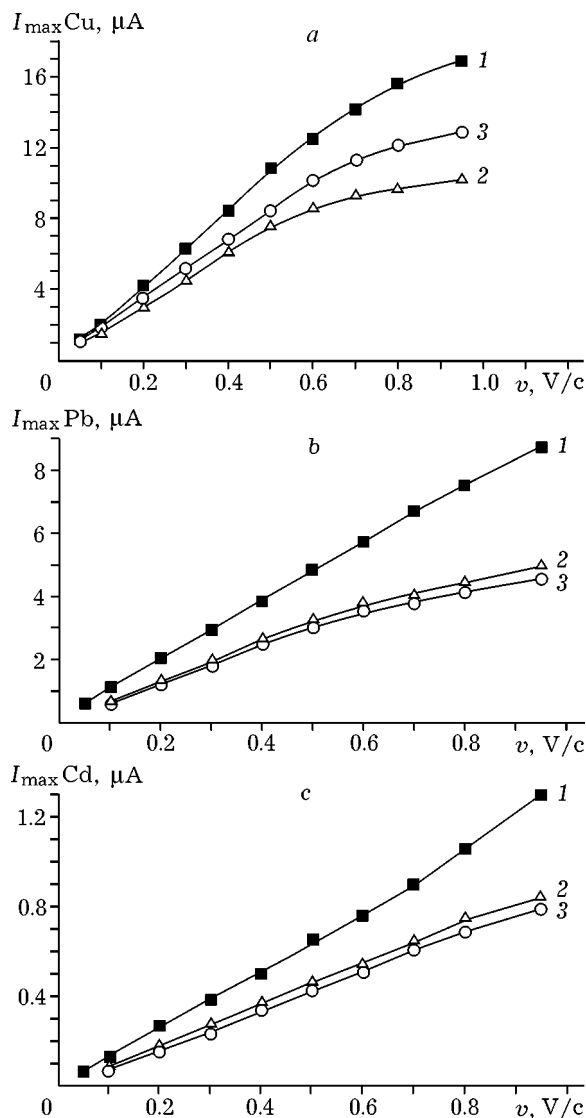


Fig. 4. Dependencies of copper (a), lead (b) and cadmium (c) responses on the potential sweep rate ( $v$ ). The dependences were recorded in a 0.5 M HCl solution containing (1) a model sample ( $f = 25$ ), (2) whole blood stabilized with EDTA ( $f = 25$ ), and (3) whole blood stabilized with glucicir ( $f = 31.25$ ). Experimental conditions:  $E_{\text{el}} = -1.2$  V,  $t_{\text{el}} = 60$  s.

the curves in this figure and all the subsequent curves is an arithmetic mean of 3 to 4 parallel measurements. One can see in Fig. 3 that the slopes of these dependencies do not change when blood is added to the solution if the potential sweep rate is not over 0.30 and 0.50 V/s ( $\lg v \leq -0.6$ ) during ionization of copper and lead, respectively, and over the whole interval of the potential sweep rates during ionization of cadmium. This is an indication that the electrode process kinetics remains unchanged over the

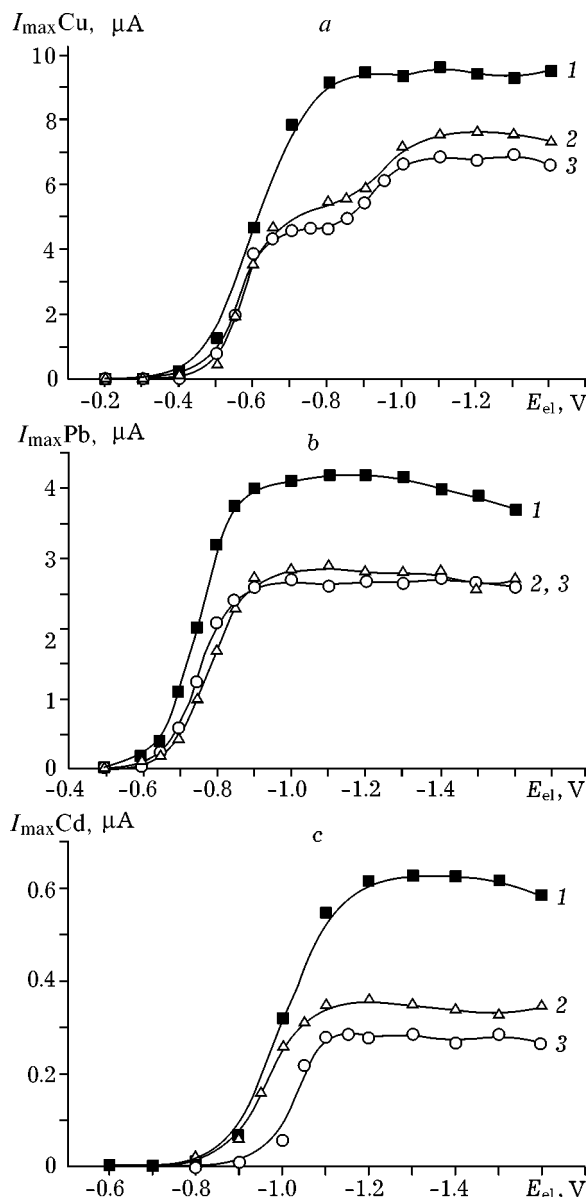


Fig. 5. Dependencies of copper (a), lead (b) and cadmium (c) responses on the electrolysis potential in a 0.5 M HCl solution containing (1) a model sample ( $f = 25$ ), (2) whole blood stabilized with EDTA ( $f = 25$ ), and (3) whole blood stabilized with glucicir ( $f = 31.25$ ). Experimental conditions:  $t_{\text{el}} = 60$  s,  $v = 0.3$  V/s.

given intervals of the potential sweep rates when blood is added to the solution. The increase in the slope ratio with growing potential sweep rate suggests that the limiting stage of the process is the electron transport.

The response value is directly proportional to the potential sweep rate  $v$  (Fig. 4). This means that the metals are dissolved from either a solid phase or a very thin film, the thickness of which is comparable with the thickness of

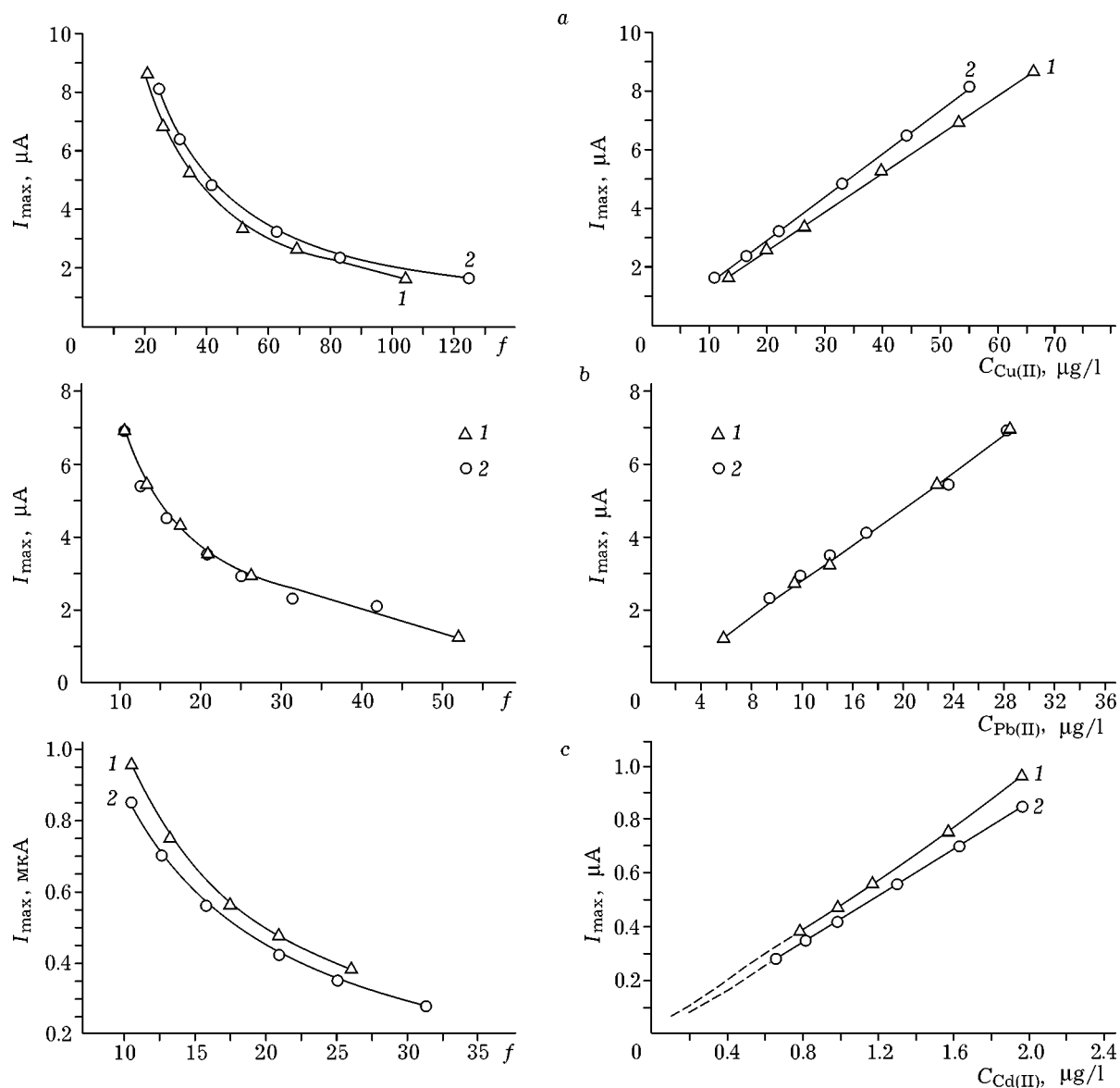


Fig. 6. Intensity of copper (a), lead (b) and cadmium (c) responses *versus* the degree of blood dilution ( $f$ ) and concentrations of the analyte ions in a solution comprising  $(5 - n)$  ml 0.5 M HCl +  $n$  ml blood: (1) blood stabilized with EDTA; (2) blood stabilized with glucicir. Experimental conditions:  $E_{\text{el}} = -1.2$  V,  $t_{\text{el}} = 60$  s,  $v = 0.3$  V/s.

the diffusion layer [47, 48, 51]. The variation of the slope ratio at  $v > 0.3-0.5$  V/s in the presence of blood is due probably to the change in the electrode process kinetics. This supposition is also confirmed by the fact that the slope ratio of  $R - v$  changes at potential sweep rates, at which we observe the variation of the slope ratios of the  $E_{\max} - \lg v$  curves shown in Fig. 3. The responses (the sensitivity coefficients) diminish probably due to the retardation of the metal accumulation on the electrode surface at the cathodic stage.

The latter conclusion is supported by the data in Fig. 5, which presents dependencies of the maximum metal oxidation currents on the accumulation potential. These dependencies were recorded in solutions without blood (curves 1) and with blood stabilized by EDTA (curves 2) and glucicir (curves 3). The limiting currents are higher in the first case than in the second and third cases. The deposition start potentials of lead and copper shift little upon addition of blood to the solution. The shift is much more pronounced for cadmium in



glugicir-stabilized blood. These results are an indication that diffusion of metal ions to the electrode surface is retarded at the accumulation stage. The deceleration shows up as the decrease in the sensitivity coefficient. The shift of the start potential of cadmium accumulation from blood to the negative region of potentials may be caused by complex formation.

The main requirement imposed on the response is that it should be related to the concentration of the analyte in the solution by a unique functional dependence (preferably a directly proportional one) and have, if possible, a maximum value. Therefore, it is reasonable to choose the following analysis conditions:

– oxidation currents of copper and lead are recorded at a potential sweep rate of not over 0.3 and 0.5 V/s, respectively;

– lead and cadmium are accumulated at potentials more negative than  $-0.9$  and  $-1.1$  V, respectively.

A more complicated situation arises during cathodic deposition of copper (see Fig. 5, *a*). This is due probably to a complex physical-biochemical state of copper-containing proteins and enzymes [52]. The  $R - E_{el}$  curve (curves 2 and 3 in Fig. 5, *a*) exhibits two plateaus: one plateau is located between  $-0.6$  and  $-0.9$  V, while the other plateau arises after  $-1.0$  V. In this case, it is absolutely clear that the analysis result will depend on the chosen potential of the electrode at the stage of cathodic deposition. One more important issue concerns calibration curves. Figure 6 presents dependencies of responses on the degree of blood dilution and the concentration of metal ions in solutions. The concentration of metal ions was changed by adding different aliquots of blood to the test solutions. One can see in Fig. 6 that the response value changes directly with the concentration of analyte ions in the solution at all the degrees of blood dilution ( $f = 8.6$  to 125). Therefore, different volumes of test samples can be analyzed. This finding is especially important for determination of cadmium, since its normal concentration in blood has not been established reliably yet, but can prove to be extremely low. If so, test samples should be diluted as little as possible. The applicability of the method of standard additions to calcu-

lation of concentrations of analyte elements in blood is proved by the calibration curves shown in Fig. 7. The behavior of standard additions of copper ions was studied in blood diluted with a supporting electrolyte to  $f = 35$  and 104 (see Fig. 7, *a* and *b*, respectively). The first points in the curves are concentrations of copper ions, which are due to the blood aliquot (here it is equal to  $1386/35$  and  $1386/104 = 39.5$  and  $13.3$   $\mu\text{g/l}$ , respectively). It can be seen from this figure that at a potential of  $-1.2$  V (curve 1), which is chosen for deposition of copper, the response value is directly proportional to the concentration of copper ions in the solutions containing an aliquot of the test sample and a standard addition of Cu(II). Moreover, standard additions do not change the slope of the calibration curve and the  $R/C_{\text{Cu(II)}}$  ratio remains equal to 0.12 (see Table 1 and the curve 1 in Fig. 6, *a*). Analogous calibration curves were obtained for lead and cadmium. When  $E_{el} =$

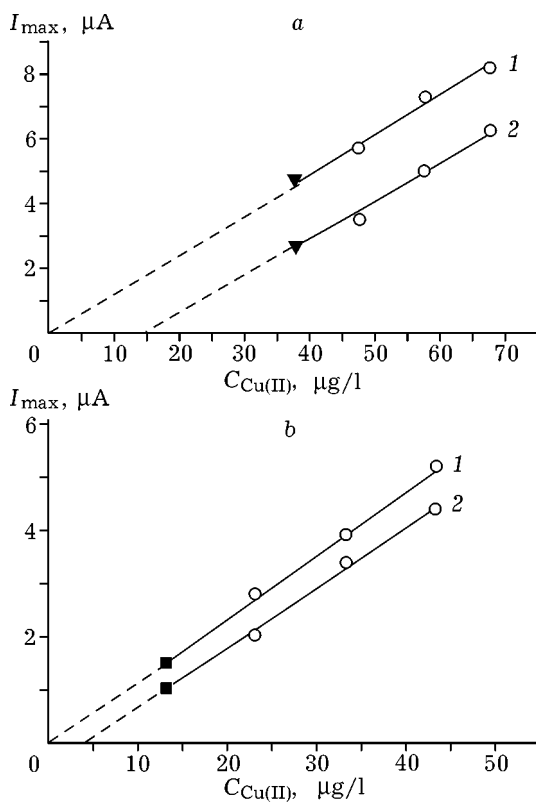


Fig. 7. Calibration curves for copper, which were obtained in blood diluted with a 0.5 M HCl solution to  $f = 25$  (a) and 104 (b) at the electrolysis potential of  $-1.2$  (1) and  $-0.9$  V(2). The points (▲) and (■) denote blood samples diluted to  $f = 35$  (▲) and 104 (■); (o) ditto + 10, 20, and 30  $\text{mg/l}$  Cu (II) added in sequence. Experimental conditions:  $t_{el} = 60$  s,  $v = 0.3$  V/s.

TABLE 2  
Comparison of results of analysis of blood samples obtained by different methods

Blood sample	Found, $X_{av}$ ( $\mu\text{g/l}$ ), $S_r$				Independent methods						
	SVA method				Independent methods						
	Whole blood				After chemical decomposition						
	Cu	Pb	Cd		Cu	Pb	Cd		Cu	Pb	Cd
1 (v)	1341, 0.09 (5)	312, 0.07 (5)	21.3, 0.12 (5)	—	—	—	—	1386, 0.13 (3) *	295, 0.12 (3)*	20.5, 0.12 (3)**	—
2 (v)	754, 0.08 (5)	62, 0.13 (5)	12.5, 0.10 (5)	59, 0.15 (5)	787, 0.11 (5)	—	10.9, 0.15 (5)	778, 0.10 (3)*	57, 0.13 (3)**	11.7, 0.13 (3)**	—
3 (v)	945, 0.7 (5)	123, 0.8 (5)	1.6, 0.13 (5)	132, 0.13 (5)	929, 0.15 (5)	—	1.4, 0.20 (3)	963, 0.12 (3)*	115, 0.13 (3)**	Not determined**	—
4 (v)	987, 0.07 (5)	121, 0.09 (5)	7.1, 0.14 (5)	—	—	—	—	1210, 0.18 (3)**	150, 0.15 (3)**	8.0, 0.15 (3)**	—
5 (v)	1155, 0.08 (11)	41, 0.12 (10)	6.5, 0.08 (10)	—	—	—	—	1090, 0.15 (5)**	45, 0.19 (5)**	5.8, 0.16 (5)**	—
6 (v)	920, 0.09 (5)	69, 0.09 (5)	1.1, 0.12 (5)	72, 0.11 (5)	898, 0.11 (5)	—	1.25, 0.24 (5)	—	—	—	—
7 (v)	884, 0.08 (5)	75, 0.09 (5)	0.5, 0.20 (5)	80, 0.10 (5)	905, 0.09 (5)	—	0.54, 0.19 (5)	—	—	—	—
8 (v)	1091, 0.06 (5)	245, 0.08 (5)	27.5, 0.09 (5)	—	—	—	—	1156, 0.11 (5) ***	254, 0.14 (5) **	26, 0.15 (5)**	—
9 (v)	989, 0.08 (5)	257, 0.09 (5)	8.7, 0.13 (5)	250, 0.12 (5)	982, 0.11 (5)	—	8.4, 0.14 (5)	—	—	—	—
10 (v)	—	—	—	223, 0.11 (5)	1028, 0.09 (5)	—	22.5, 0.11 (5)	969, 0.09 (5)*	238, 0.18 (5)*	20.4, 0.13 (5)*	—
11 (v)	1268, 0.08 (5)	29, 0.10 (5)	0.7, 0.14 (5)	—	—	—	0.64, 0.31 (5)	1215, 0.12 (5)*	35, 0.20 (5)**	Not determined**	—
11 (h)	1183, 0.08 (4)	33, 0.12 (4)	0.65, 0.15 (4)	—	—	—	—	—	—	—	—
11 (a)	1480, 0.11 (3)	21, 0.16 (3)	<0.3 (3)	—	—	—	—	—	—	—	—
12 (v)	983, 0.07 (5)	31, 0.07 (6)	0.8, 0.25 (6)	34.3, 0.13 (5)	1048, 0.15 (6)	—	—	—	—	—	—

Notes. 1. (v) venous blood; (a) arterial blood; (h) hemolysate. 2. The numbers of measurements are given in parentheses.

\*Atomic emission chemical spectral analysis with excitation of the spectrum in a DC arc after digestion of samples.

\*\*Atomic absorption spectroscopy in a graphite cuvette after digestion of samples.

\*\*\*Atomic emission spectral analysis with excitation of the spectrum in a double-jet arc plasmatron (without decomposition of samples, drying to the powder-like state at 80–90 °C).

-0.9 V (an optimal potential for accumulation of copper during analysis of most materials), only part of copper-containing molecules of the matrix probably participate in the reaction. In this case, the copper concentration is largely underestimated. The  $R - C_{\text{Cu(II)}}$  calibration curve (curve 2 in Fig. 7) cuts off a section on the concentration axis, which corresponds to the concentration of unreleased copper. One may see from the Figure that the line 2 intersects the abscissa at  $C_{\text{Cu(II)}} = 14.6$  (see Fig. 7, a) and 4.9 (see Fig. 7, b)  $\mu\text{g/l}$ . This means that copper ions, whose concentration in whole blood was 510  $\mu\text{g/l}$  ( $14.6 \times 35$  or  $4.9 \times 104$ ), did not participate in the cathodic process. Therefore, blood copper can be measured correctly only if electrolysis is realized at potentials more negative than -0.9 V. If  $C_{\text{Cu(II)}}/C_{\text{Pb(II)}}$  and  $C_{\text{Cu(II)}}/C_{\text{Cd(II)}}$  in blood are not over 20 and 200, respectively, all the three elements can be measured simultaneously after they are accumulated at  $E_{\text{el}} = -1.2$  V (these voltammograms are exemplified in Figs. 1 and 2). In this case, zinc does not affect copper measurements. The time required for analysis of one sample in two parallel measurements does not exceed 20–30 min. If  $C_{\text{Cu(II)}}/C_{\text{Pb(II)}} > 20$  and  $C_{\text{Cu(II)}}/C_{\text{Cd(II)}} > 200$  in blood, a much larger aliquot of the sample or a longer electrolysis time (120–180 s) is required for determination of lead and cadmium. In this case, copper can be measured more correctly separately after the solution is electrolyzed at  $E_{\text{el}} = -1.0 \dots -1.1$  V.

Results of the SV analysis of whole blood, which were obtained using the method of standard additions, and of mineralized blood only slightly differ from those obtained by independent methods (Table 2). Direct SVA measurements ( $S_r$  values in Table 2 are italicized) provide a much better accuracy than SVA measurements after acid decomposition of samples or independent methods. Despite the fact that the sensitivity coefficient of responses diminishes in the presence of whole blood as compared to its counterpart in the model solution, the method of standard additions ensures correct results. The conditions which were proposed above for the analysis of venous blood are quite acceptable for the measurements of

copper, lead and cadmium in hemolysate (see Table 2, rows 11(v) and 11(h)). To ascertain the origin of considerable differences between results of analysis of venous and arterial blood, it is necessary to assay additionally blood taken from several groups of patients and to use Wilcoxon's criterion for evaluation of the degree of discrepancy. Probably, microelemental compositions of venous and arterial blood differ and the observed discrepancies (by Student's criterion) are due just to this difference. We are unaware of any publications dealing with the study of simultaneous concentrations of copper, lead and cadmium in venous and arterial blood.

## CONCLUSION

The reported data convincingly prove that whole blood can be analyzed by the SVA method under the chosen conditions without preliminary operations of decomposition and accumulation. The results agree fairly well both with the results obtained by the SVA method after chemical decomposition of blood and those obtained by traditional methods of atomic emission chemical spectral analysis with excitation of the spectrum in a DC arc after mineralization of samples, atomic absorption spectroscopy in a graphite cuvette after mineralization of samples, and atomic emission spectral analysis with excitation of the spectrum in a double-jet arc plasmatron with mineralization of samples. The use of modified thick-film carbon-containing electrodes, the application of a faster potential sweep rate (as compared to the rates commonly used in voltammetry), and recording of curves in the differential regime allowed eliminating toxic mercury and its soluble salts from the analysis process. Also, oxygen need not be removed from test solutions.

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## REFERENCES

- 1 R. Masironi, *Bull. World. Health Org.*, 40 (1970) 103.
- 2 Proc. Int. Symp. on Atherosclerosis Problems in Stockholm, June 26–29, 2000.
- 3 Compensation-Adaptive Processes: Fundamental and Clinical Aspects: Abstracts, All-Russian Scientific and Practical Conf., Novosibirsk, 2002 (in Russian).
- 4 G. I. Poluyanov, M. Yu. Mironenko, V. F. Volkov, N. F. Losev, *Zavod. lab.*, 56, 9 (1990) 44.
- 5 N. U. Tits, Clinical Evaluation of Laboratory Tests, Meditsina, Moscow, 1986 (in Russian).
- 6 L. Xillei, Yu. Xiamei, H. Yaan, T. Gorecki, *Anal. Chim. Acta*, 211, 6 (1988) 231.
- 7 V. Bencko, B. Benes, M. Cikrt, *Arch. Toxicol.*, 36 (1976) 159.
- 8 J.-P. Buchet, R. Lauwerys, *Ibid.*, 57 (1985) 125.
- 9 M. R. Cornelis, *Ann. Clin. Lab. Sci.*, 26 (1996) 252.
- 10 L. R. Nozdryukhina, Biological Role of Microelements in Human and Animal Organisms, Nauka, Moscow, 1977, p. 184 (in Russian).
- 11 W. J. Price, Analytical Atomic Absorption Spectrometry, Pye Unicam Ltd, Cambridge, Heyden & Son Ltd, London *etc.*, 1972, 355 p.
- 12 K. S. Subramanian, *Sci. Total Environ.*, 89 (1989) 237.
- 13 N. K. Belskiy, L. P. Nozdryukhina, I. V. Kravchuk, N. V. Grebnykov, in: Biological Role of Microelements and Their Application in Agriculture and Medicine: Abstracts, VIII All-Union Conf., Ivano-Frankovsk, vol. 2, 1978 (in Russian).
- 14 D. J. Holges, *Analyst*, 10, 2 (1977) 1210.
- 15 F. C. Wrigth, J. C. Riner, *At. Abs. Newsletter*, 14, 8 (1975).
- 16 A. D. Olson, W. B. Hamlin, *Ibid.*, 3, 1 (1968).
- 17 N. K. Belskiy, in: Biological Role of Microelements, Nauka, Moscow, 1983, p. 206 (in Russian).
- 18 Kh. Z. Brainina, E. Ya. Neiman, Solid-Phase Reactions in Electroanalytical Chemistry, Khimiya, Moscow, 1982, p. 51 (in Russian).
- 19 W. R. Matson, R. M. Griffin, G. B. Schreiber, Trace Substances in Environmental Health, in: D. D. Hemphill (Ed.), Columbia, Missouri, 1971, p. 396.
- 20 J. Šinko, S. Gomišek, *Mikrochim. Acta*, 1 (1972) 163.
- 21 B. Searle, Wing Chan and B. Davidow, *Clin. Chem.*, 19 (1973) 76.
- 22 B. J. Feldman, J. D. Osterloh, B. H. Hata and A. D. Alessandro, *Anal. Chem.*, 66 (1994) 1983.
- 23 P. L. Brezonik, P. A. Brauner, W. Stumm, *Water Res.*, 10 (1976) 605.
- 24 A. M. Bond and J. B. Reust, *Anal. Chim. Acta*, 162 (1984) 389.
- 25 G. E. Batley and T. M. Florence, *J. Electroanal. Chem.*, 72 (1976) 121.
- 26 G. E. Batley and V. J. Farrar, *Anal. Chim. Acta*, 99 (1978) 283.
- 27 R. Copeland, R. A. Oseryoung and R. K. Skoerboe, *Ibid.*, 46 (1974) 2093.
- 28 P. Valenta, H. Rützel, H. W. Nürnberg and M. Stoeppler, *Fresenius' Z. Anal. Chem.*, 285 (1977) 25.
- 29 M. Oehme and W. Lund, *Ibid.*, 298 (1979) 260.
- 30 Joseph Wang, *J. Electroanal. Chem.*, 139 (1982) 225.
- 31 J. Golinawski, P. Valenta, M. Stoeppler and H. W. Nürnberg, *Talanta*, 26 (1979) 649.
- 32 G. Morrell and G. Ghridhar, *Clin. Chem.*, 22 (1976) 221.
- 33 M. Stoeppler, C. Mohl, P. Ostapczuk *et al.*, *Fresenius' J. Anal. Chem.*, 317 (1984) 486.
- 34 D. Jagner, L. Renman and Yudong Wang, *Electroanalysis*, 6 (1994) 285.
- 35 D. Jagner and Yudong Wang, *Ibid.*, 7 (1995) 614.
- 36 P. Ostapczuk, *Clin. Chem.*, 38 (1992) 1995.
- 37 D. Jagner, Feng Ma and Yudong Wang, *Electroanalysis*, 6, 10 (1996) 952.
- 38 L. Almestrand, D. Jagner and L. Renman, *Anal. Chim. Acta*, 193 (1987) 71.
- 39 D. Jagner, M. Josefson, S. Westlund and K. Aren, *Ibid.*, 53 (1981) 1406.
- 40 B. Hoyer and M. Florence, *Ibid.*, 59 (1987) 2839.
- 41 Kh. Brainina, N. A. Malakhova, A. Ivanova, Solid Carbon Containing Stripping Voltammetric Sensors, NATO Series Volume, 1997.
- 42 Kh. Z. Brainina and A. M. Bond, *Anal. Chem.*, 67, 5 (1995) 2586.
- 43 Kh. Brainina, H. Schafer, A. Ivanova and R. Khanina, *Anal. Chim. Acta*, 330 (1996) 175.
- 44 D. R. Williams, *The Metal of Life*, London *etc.*, 1971, 236 p.
- 45 Therapeutics Handbook of Washington University, in: A. Walon (Ed.), Praktika, Moscow, 1995 (in Russian).
- 46 Methods of Examination in Occupational Pathology, in: O. G. Arkhipova (Ed.), Biokhim, Meditsina, Moscow, 1988 (in Russian).
- 47 Kh. Z. Brainina, Solid-Phase Stripping Voltammetry, Khimiya, Moscow, 1972, 102 p. (in Russian)
- 48 Kh. Z. Brainina, Stripping Voltammetry in Chemical Analysis, Haisted Press, New York *etc.*, 1974, p. 222.
- 49 Kh. Brainina, R. Khanina, L. Roitman, *Anal. Lett.*, 18, 2 (1985) 117.
- 50 N. F. Zakharchuk, S. Yu. Saraeva, N. S. Borisova, Kh. Z. Brainina, *Electroanalysis*, 11, 9 (1999) 614.
- 51 W. T. De Vries, *J. Electroanal. Chem.*, 9 (1965) 448.
- 52 M. N. Hughes, *The Inorganic Chemistry of Biological Processes*, 2nd ed., John Wiley & Sons, Chichester *etc.*, 1979, 416 p.