Determination of Zinc in Whole Blood and Its Fractions by Means of Stripping Voltammetry Using Modified Thick-Film Carbon-Containing Electrodes

N. F. ZAKHARCHUK[1], O. I. SUDAEVA[1], S. YU. SARAEVA[2], L. I. KOLYADINA[2] and KH. Z. BRAININA[2]

[1]*Nikolaev Institute of Inorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, Pr. Akademika Lavrentyeva 3, Novosibirsk 630090 (Russia)*

E-mail: nzak@che.nsk.su

[2]*Ural State University of Economics, Ul. 8-go Marta 62, Yekaterinburg 620219 (Russia)*

E-mail: baz@usue.ru

(Received December 12, 2003)

Abstract

A rapid method to analyze the whole blood and its fractions for zinc content involving stripping voltammetry with the use of the modified thick-film carbon-containing electrodes is proposed. The method allows one to make the analysis free from metal mercury and its soluble salts, preliminary deproteinization of samples, and also to decrease the sample necessary for analysis to 5–50 μl. The measurable range of zinc concentration in blood and in its fractions is 100–20 000 μg/l.

INTRODUCTION

As long ago as in 1869, John Roulin showed that zinc is one of the most important essential microelements necessary for the harmonious growth of a living organism [1]. By present, it has been reliably established that zinc is a part of the genetic apparatus of a cell and is incorporated in more than 300 metalloenzymes. Thus, one molecule of a widespread enzyme carbonic anhydrase which exhibits especially high activity in cells and tissues participating in breathing processes, contains about 260 amino acids and one ion Zn^{2+} tightly bound with them. It is zinc that renders this enzyme tremendous catalytic activity. The first-order rate constant of the reaction $CO_2 + H_2O \rightarrow H^+ + HCO_3^-$ [3] which is very important for any living organisms is equal to $0.03\ s^{-1}$ without catalysts reaches $10^6\ s^{-1}$ in the presence of carbonic anhydrase [3]. Zinc plays an important part in the immune response [4], in nucleic exchange, transcription, stabilization of nucleic acids, proteins and especially the components of biological membranes, in providing the cells with energy, in ensuring the stability to stress [5]. In addition, zinc is an essential microelement necessary for the development of brain. The importance of zinc for the formation of higher psychical functions is due to its general biological properties, the presence of proteins bound with zinc in brain; these proteins provide structural and functional maturing of brain and zinc-containing mediators that participate in the formation of memory [1]. This is a powerful factor of antioxidant protection [6, 7] playing an important part in the synthesis of insulin. Lack of zinc is especially dangerous [8, 9]; its deficiency in an organism causes a number of grave diseases, such as chronic diseases of lungs and bronchi, anemia, degradation of immunity, changes in appetite, growth and pubescence inhibition [10], sterility, parakeratosis with baldness, poor growth of

the hair and nails, rash on face and extremities, skin ulcer [8, 11], mental confusion, lagging in psychic development, bad memory, apathy, depression, loss of the sense of smell and gustatory sense [12], overweight. As a rule, deficiency of this microelement is accompanied by depression of the formation of antibodies, a decrease in the number of lymphocytes, causes impairment of gastrointestinal tract and kidneys [13]. The lack of zinc is especially dangerous for expectant mothers because it causes severe impairment of the health of newborn children: low mass, indices of immaturity, prematurity [14]. Zinc in excess (more than 153 mmol/l) is often observed in the patients suffering from pancreatic diabetes [13]. It is not surprising that at present we survive a sharp increase in the attention to more thorough investigation of the biological role of this element, its exchange with other microelements, their participation in pre-disease, aetiology and pathogenesis of human diseases and corresponding approaches to their diagnostics, prophylactics and treatment [14–26]. Investigations of this kind require reliable analysis procedures.

One of these methods is stripping voltammetry (SV) [27, 28]. Thanks to unique thick-film modified carbon-containing electrodes [29, 30], direct analysis of blood and its fractions has become possible, without labour-intensive, rather complicated sample preparation procedures [31] which are an additional source of errors in the analysis of biological samples. Discussion of the applicability of other methods for determination of elements in biological samples would take too much space in the present paper. Because of this, we will only note that almost all the analytical methods make way for stripping voltammetry in the direct analysis of biological samples. Moreover, the existing voltammetric procedures of the analysis of biological samples for the presence of metal microimpurities [16, 32–35] in the majority of cases require preliminary mineralization of blood or extraction of microelements before analysis [36]. Stripping voltammetry, which is less sensitive to the effect of sample matrix composition, with the working electrode in the form of glass carbon disc modified with mercury in the *in situ* mode was used by the authors of [37, 38] to investigate the level of cadmium, lead, copper and zinc in blood serum of smokers and the level of lead in whole blood of school children.

The goal of the present work was to study the possibility to use modified thick-film electrodes for SV analyses of the whole blood and its fractions for zinc content without preliminary destruction of the organic components of the samples.

EXPERIMENTAL

Apparatus and electrodes

An automatic software-programmable voltammetric IVA-5 analyzer (JSC NPVP IVA, Yekaterinburg, Russia) with an IBM PC computer (OS Windows 95/98) was used.

The inverse voltammetric curves (voltammograms) of the metals under determination were recorded in the differential mode of the direct current SV with linear scan of potential. The analytical signal was the amplitude of changes in the (dI/dE) derivative (below it is designated as R – response). A three-electrode electrochemical cell 5 ml in volume was used. Oxidizers dissolved in the electrolyte (oxygen) were not removed. The auxiliary electrode was graphite or glass carbon rod, the reference electrode was silver chloride electrode (Ag/AgCl) filled with the saturated KCl solution, or a track composed of silver-containing paste (JSC ELMA-Pasty, Zelenograd, Russia) applied on a polymeric substrate by means of screen printing. The working electrodes (sensors) were thick-film modified carbon-containing electrodes (TMCE) (JSC NPVP IVA, Yekaterinburg). Preliminary formation of the working surface of the electrode was carried out by means of its stepwise polarization for several potential values in 0.5 M HCl solution. The work capacity of such an electrode is conserved, as a rule, during the whole working day. The solution was mixed with a magnetic mixer during electrolysis. All the experiments except those considered below were carried out under the following conditions: accumulation potential $E_{acc} = -1.4$ V, accumulation duration $t_{acc} = 30$ s, potential scan

rate v = 1.0 V/s. In order to estimate the correctness of the results of analysis of whole blood for zinc content, the samples were analyzed using independent physicochemical methods: atomic absorption spectrometry (AAS) and atomic emission chemical spectral analysis (AECSA) after acidic mineralization of the sample under analysis, and also by means of SV after acidic decomposition in the Hach set-up (USA).

Reagents

Reagents of ultrapure grade were used in the work. Tridistilled water was used to prepare solutions and to wash the equipment. Hydrochloric acid was additionally purified by the isopiestic method. Solutions containing 1.0, 0.5 and 0.2 mg/l of zinc (II) ions were prepared from the State Standard samples of zinc ions with the concentration of 1 g/l by consecutive dilution with 0.005 M HCl solution. The solutions with the concentration of 1 mg/l were prepared once a week, 0.5 mg/l every three days, and 0.2 mg/l every day.

Samples and their preparation

Venous blood in the amount of 1 to 5 ml was placed in a test tube containing anticoagulant (5 % solution of EDTA or 4 % solution of glugicir, 0.04 and 0.25 ml per one milliliter of blood). Blood with anticoagulant will be referred to as the blood sample. The degree of dilution of blood in the samples $f_1 = (V_{ant} + V_{bl})/V_{bl}$, where V_{ant} and V_{bl} are the volumes of anticoagulant and blood, was 1.04 and 1.25 for EDTA and glugicir, respectively. The samples were thoroughly mixed and stored in a refrigerator at a temperature of 4–5 °C or in a cooler (−18 °C). Unlike for [39], under these conditions of blood stabilization, we did not observe any effect of the types of containers or of temperature on the results of zinc determination during 30 days and more. The degree of blood dilution in the cell f was calculated according to the equation $f = f_1[(V_{el} + V_{sam})/V_{sam}]$, where V_{el} and V_{sam} are aliquots (ml) of the electrolyte and the blood sample, f_1 in the initial degree of blood dilution.

In order to obtain serum, venous blood was placed in a test tube without adding any anticoagulant, heated to 37 °C and settled (or centrifuged) with the rotation frequency of 1500 min^{-1}, The filtrate was used for analysis. In order to obtain plasma, blood with anticoagulant was heated to 37 °C and centrifuged with the rotation frequency of 1500 min^{-1}; the filtrate was analyzed in this case, too. In order to obtain the erythrocyte mass, after centrifuging of blood with anticoagulant and separation of plasma, the precipitate was several times washed with the physiological solution till the washing solution became completely colourless. All the erythrocytes were collected with the volumetric pipette, their volume was measured, they were transferred into another test tube and twice diluted with the physiological solution. The contents of the test tube was centrifuged with the rotation frequency of 6000 min^{-1}; the filtrate was analyzed.

RESULTS AND DISCUSSION

Choosing the supporting electrolyte

Determination of zinc by means of stripping voltammetry in various samples [27, 28, 40] including biological ones after preliminary mineralization of sample [16, 32–35, 41, 42] is usually carried out in solutions with pH 5 ± 0.5. This is most often the acetate buffer, a mixture of sodium (potassium) chloride and acetate, or sodium (potassium) chloride. In order to eliminate the effect of copper on the height of zinc signals, a gallium salt is added into the solution. For whole blood, the indicated electrolytes are physiological solutions; no hemolysis of blood occurs in them, the solution remains gently pink; the analytical signal of zinc is absent from the voltammograms. In addition, blood samples to be analyzed always contain anticoagulants (usually glugicir or EDTA); their effect on the signal of zinc is not studied yet. The same is true for gallium; its action under the indicated conditions is unknown.

The data characterizing the effect of the composition of supporting electrolyte on the

TABLE 1

Effect of the composition of supporting electrolyte on the results of analysis of whole blood for zinc content ($n = 8$, $P = 0.95$)

Blood sample	Anticoagulant	Composition of the supporting electrolyte	SV		Independent method	
			$\overline{C}$, μg/l	S_r	$\overline{C}$, μg/l	S_r
Blood No. 1	Glugicir	0.1 M NaCl + 0.003 M HCl	5650	0.07	5600[a]	0.12
		0.04 M NaCl + 0.003 M HCl	5600	0.04		
		0.04 M NaCl + 0.003 M HCl + 800 μg/l Ga(III)	11 250	0.05		
		0.04 M NaCl + 0.02 M HCl	5500	0.04		
		0.04 M NaCl + 0.02 M HCl + 800 μg/l Ga(III)	7550	0.05		
	EDTA	0.04 M NaCl + 0.003 M HCl	No signal			
		0.04 M NaCl + 0.003 M HCl + 1200 μg/l Ga(III)	6500	0.09		
		0.04 M NaCl + 0.02 M HCl	5650	0.04		
		0.04 M NaCl + 0.02 M HCl + 800 μg/l Ga(III)	6600	0.05		
Blood No. 2	Glugicir	0.04 M NaCl + 0.02 M HCl	9200	0.04	9300[b]	0.15
	EDTA		9300	0.05		
Blood No. 3	Glugicir	0.04 M NaCl + 0.003 M HCl + 600 μg/l Ga(III)	9800	0.03	5300[c]	0.09
	EDTA	0.04 M NaCl + 0.02 M HCl	5350	0.06		
			5400	0.05		

[a]Atomic absorption spectroscopy.

[b]Atomic emission chemical spectral analysis

[c]Stripping voltammetry after mineralization of the sample.

results of analyses of whole blood are generalized in Table 1. One can see that the analysis of blood stabilized with EDTA is impossible with weakly acidic electrolytes. In order to detect the signal of zinc, it is necessary to add rather large amount of gallium ions into the solution. However, the result of analysis turns out to be overstated in this case. In weakly acidic solutions in the presence of gallium, even more overstated results were obtained when analyzing the samples of blood stabilized with glugicir. The effect of acidity, concentrations of chlorides, anticoagulants and gallium was further examined both using the model solutions and using blood samples analyzed preliminarily with the help of independent methods. The microelement content of them is shown below in the corresponding Tables.

The voltammograms of zinc and the dependence of the signal of zinc on the concentration of hydrochloric acid in the solutions containing sodium chloride and blood stabilized with EDTA or glugicir are shown in Fig. 1. One can see that the signal height is substantially dependent on HCl concentration; the character of this dependence is determined by the nature of anticoagulant. In the case of EDTA, with low concentration of the acid, the signal does not appear at all; the likely reason may be the formation of a strong complex with zinc [43]. Since the goal of the present work is to choose the conditions of zinc determination in blood samples independently of the coagulant used, as a rule, further investigations involved the solutions containing HCl not less than 0.02 M.

The effect of sodium chloride concentration on the height of zinc signals is shown in Fig. 2. One can see that an increase in NaCl concentration causes a decrease in the signal of zinc; however, with NaCl concentration

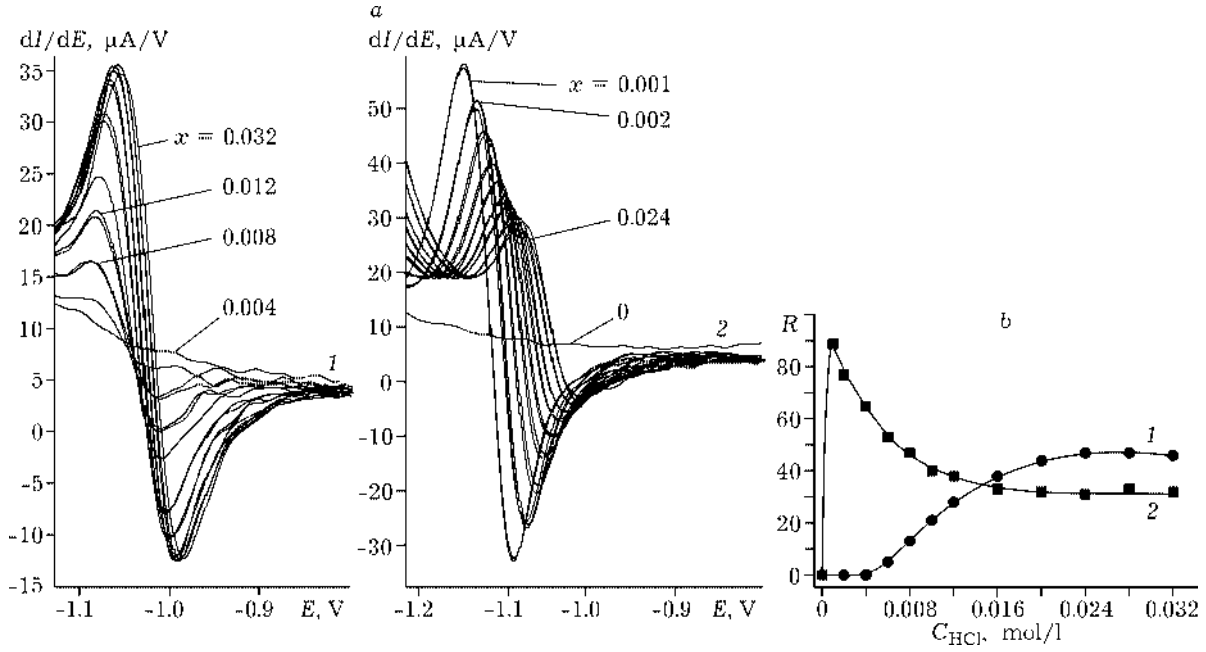

Fig. 1. Differential stripping voltammetric curves (*a*) and dependence of the maximal analytical signal of zinc *R* on HCl concentration (*b*) in 0.04 M NaCl + *x*M HCl solution containing blood stabilized with EDTA (*1*) or glugicir (*2*). Blood sample No. 3, f = 250.

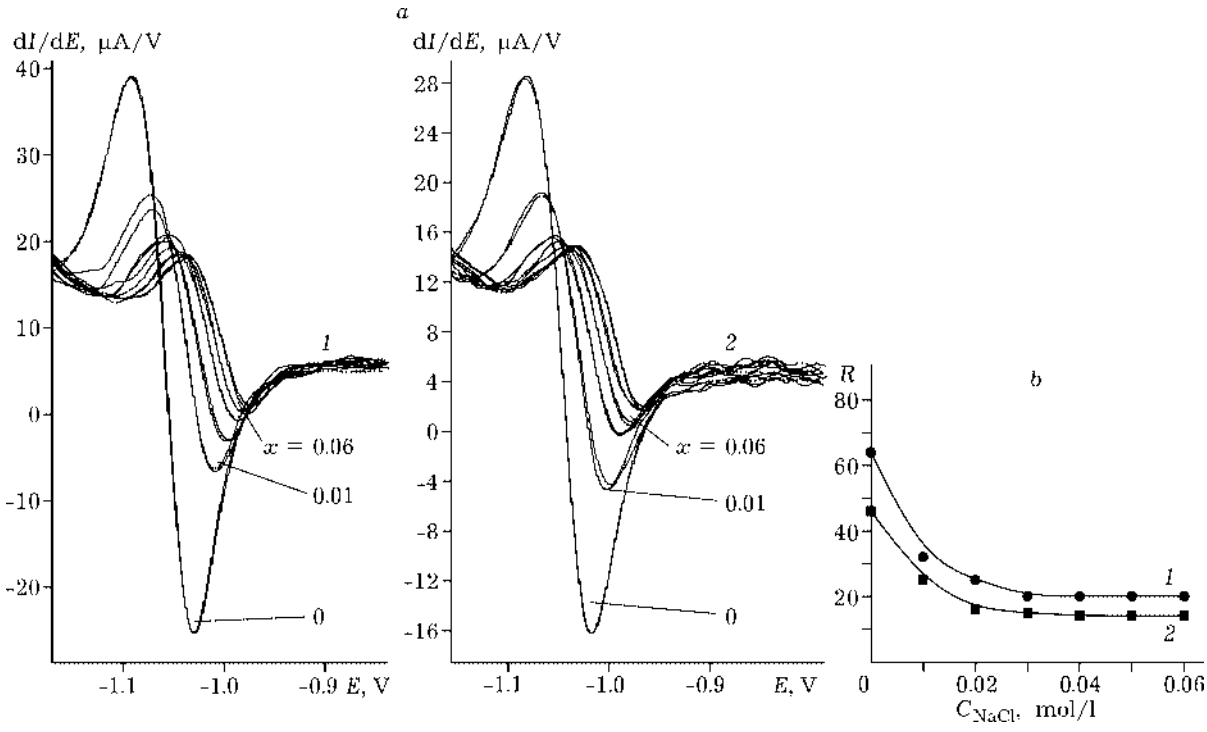

Fig. 2. Differential stripping voltammetric curves (*a*) and the dependence of the maximal analytical signal of zinc R on NaCl concentration (*b*) in *x*M NaCl + 0.02 M HCl solution containing blood stabilized with EDTA (*1*) and glugicir (*2*). Blood sample No. 3, f = 250.

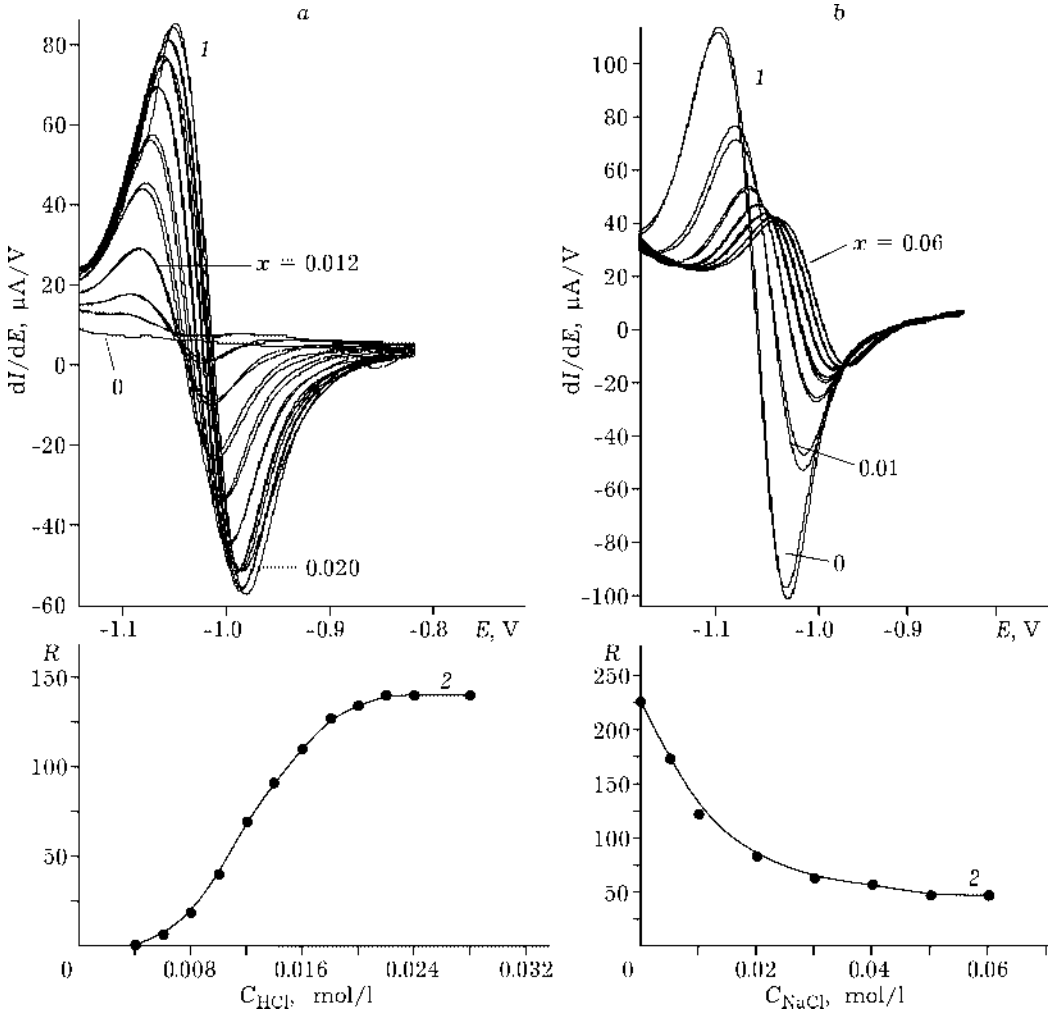

Fig. 3. Differential stripping voltammetric curves (*1*) and the dependence of the maximal analytical signal of zinc R (*2*) on the concentrations of HCl (*a*) and NaCl (*b*) in the solutions: 0.04 M NaCl (*a*) and xM NaCl + 0.02 M HCl (*b*) containing erythrocyte mass of blood stabilized with EDTA. Blood sample No. 3, f = 250.

above 0.02 M this dependence disappears. Further, the supporting solutions with NaCl concentration 0.04–0.08 mol/l were used. This allowed us to level the effect of dilution degree of blood containing NaCl with respect to NaCl concentration and to obtain stable results.

One can see in Figs. 1–3 that the $R = f(C_{HCl})$ and $R = f(C_{NaCl})$ dependences in the solutions containing blood or erythrocyte mass are of similar character. The same is observed in the investigation of plasma and serum.

Choosing the accumulation potential and the potential scan rate

The dependences of the analytical signal R (in μA/V) on the potential of zinc accumulation (a) and on potential scan rate (b) are shown in Fig. 4. One can see that at the background of the solution containing 0.02 M HCl + 0.04 M NaCl the $R = f(E_{acc})$ dependence has a rather narrow border of potential −1.3...−1.4 V, where a plateau is observed. For the accumulation potential more negative than −1.4 V, a decrease in the analytical signal is observed. For a potential more positive than −1.3 V, the analytical signal is unstable since it is on the kinetic part of the so-called pseudo-polarogram. The signal of zinc obtained by the analysis of blood increases linearly with an increase in the potential scanning rate u up to 1.2 V/s.

So, the value of zinc accumulation potential involved in the previous and subsequent experiments, which is equal to −1.4 V, can be

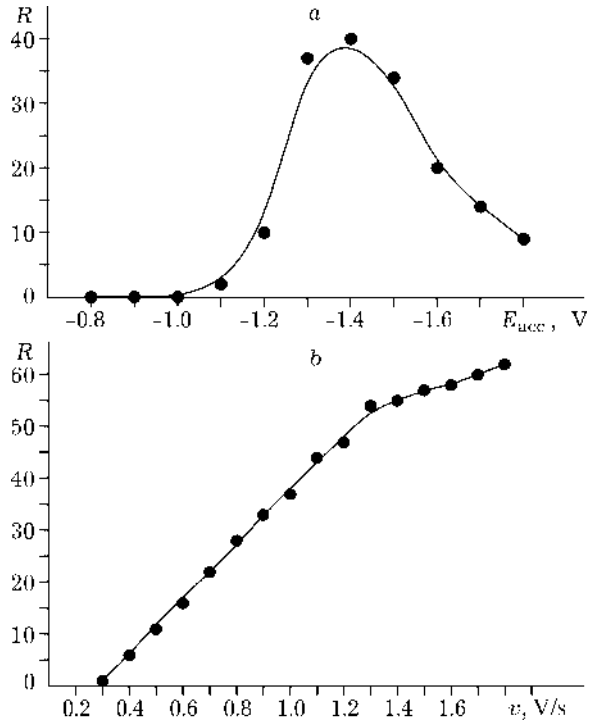

Fig. 4. Dependences of the analytical signal of zinc R on accumulation potential (*a*) and potential scan rate (*b*) in 0.04 M NaCl + 0.02 M HCl solution containing blood stabilized with EDTA. Blood sample No. 1, f = 250.

considered to be optimal. The potential scanning rate of 1 V/s allows obtaining a clearly expressed zinc signal.

The effect of copper and gallium

Binding of zinc into an intermetallic compound with copper, in the opinion of the authors of [40, 44], causes underestimation of zinc determination results. In order to diminish the systematic error of analysis, gallium ions are introduced into the solution to be analyzed; their concentration is much higher than that of Cu(II) ions (till the signal of gallium oxidation appears). According to the generalized data [45–47], copper and zinc are present in human blood in concentrations of 0.93–1.01 and 6.54–7.00 mg/l. With these concentration ranges, a negative effect of copper on zinc determination results should be observed; the introduction of Ga(III) into solution during analysis seems reasonable. An additional argument in favour of the introduction of gallium (III) into the solution is the possibility of zinc (II) displacement from the complexes with EDTA as a result of formation of a stronger complex of EDTA with Ga(III) [43].

The voltammograms recorded within the potential range from −1.1 to +0.05 V are shown in Fig. 5, *a*. The signals of zinc, gallium, lead and copper are seen. The dependences of the signals of zinc and copper on the concentration of zinc (II) ions in model solutions with glugicir, either containing gallium ions or not, are shown in Fig. 5, *b*. One can see that the calibration plot for zinc starts from the point of origin only in the absence of gallium ions in solution (curve *1*). The dependence of zinc signal on the concentration of zinc in the solution in the absence of Ga(III) is directly proportional in spite of the fact that some increase in the intensity of copper signal is observed with an increase in the concentration of zinc ions. The calibration plot for zinc in the presence of gallium (curve *2*) is parallel to the first plot. However, it does not pass through the point of origin; it is likely that this may cause overestimation of the analysis results. Similar dependences were also obtained in the case of analysis of the model solutions with EDTA containing gallium ions and not containing them.

Since the introduction of copper ions into the solution under the chosen conditions (acidity, the presence of complex-forming anticoagulants) has almost no effect on the calibration plot for zinc, the use of gallium may be not necessary under these conditions. The correctness of the analysis results in this case confirmed by the data of analysis blood using independent methods: AAS and AECSA, and SV after chemical mineralization of the sample (Table 1).

As an example, the results of the analysis of the model solution and blood stabilized with EDTA are shown in Fig. 6. The same figure illustrates the possibility of multiple use of the once-formed electrode. The results obtained when analyzing blood sample No. 1 stabilized with EDTA or glugicir (Fig. 7) almost coincide with the data of analysis carried out using the independent methods.

So, the electrolyte containing 0.04–0.1 M NaCl and 0.02–0.03 M HCl can be considered

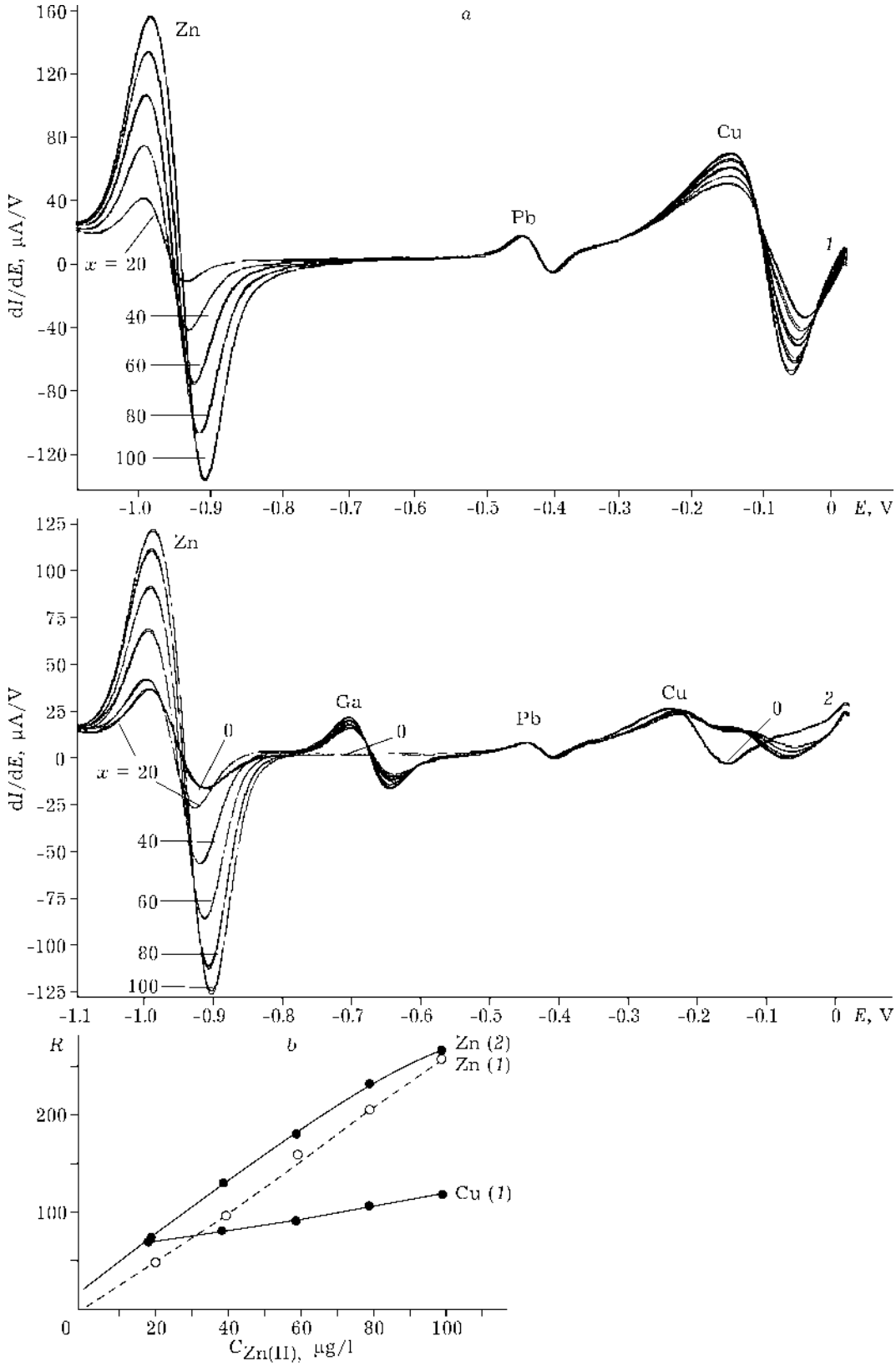

Fig. 5. Differential stripping voltammetric curves (*a*) and dependences of the analytical signals of zinc and copper on the concentration of zinc ions (*b*) in model solutions: *1* – 0.02 M HCl + 0.04 M NaCl + 2.5 10^{-3} % glugicir + 10 μg/l Cu(II) + 2 μg/l Pb(II) + *x* μg/l Zn(II) solution; the same + 300 μg/l Ga(III). Voltammetric curve 0 (experiment *2*) was obtained without adding gallium ions.

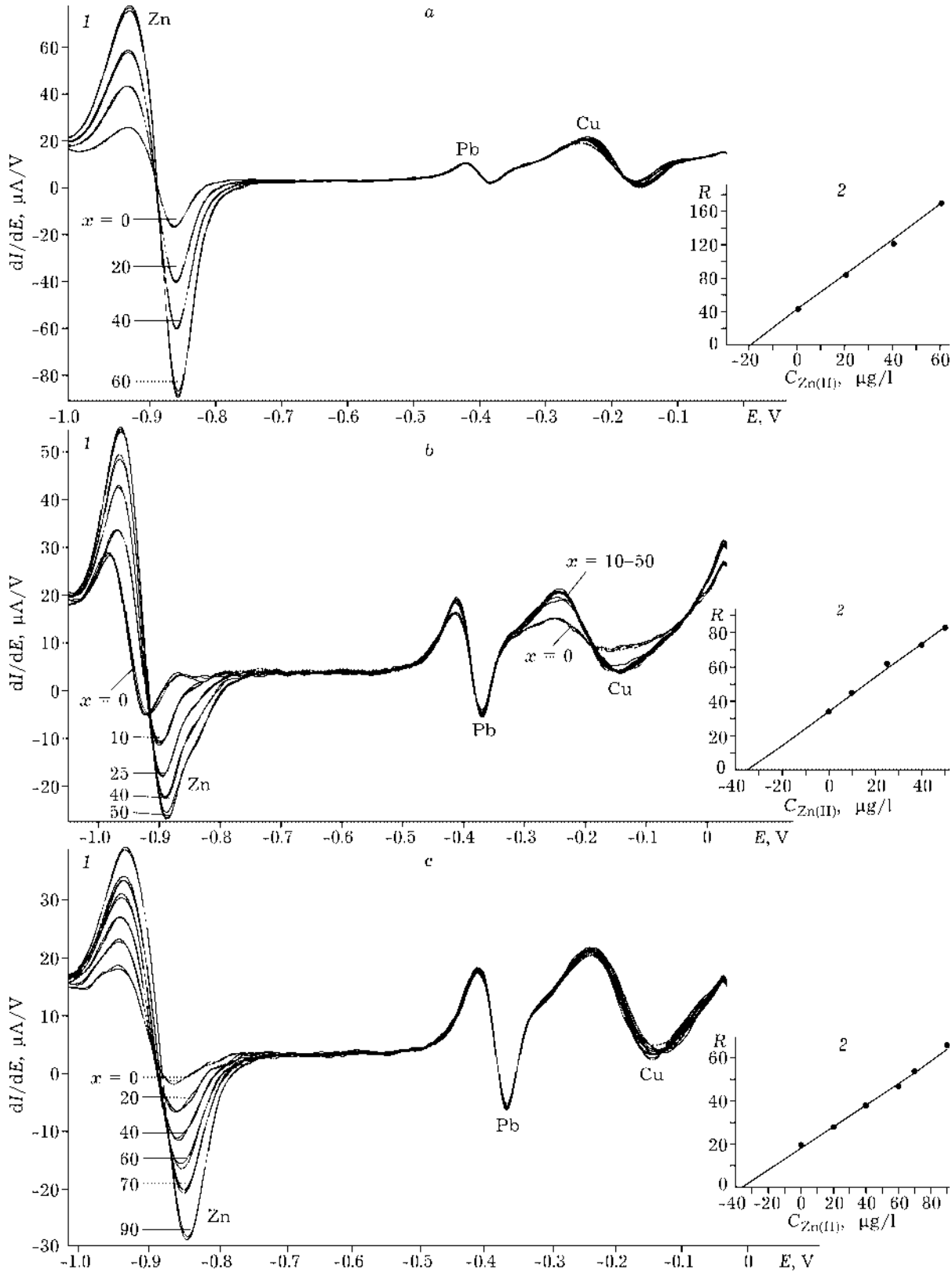

Fig. 6. Differential stripping voltammetric curves (*1*) and the dependence of the analytical signal of zinc R on the concentration of its ions (*2*) in 0.02 M HCl + 0.04 M NaCl + x μg/l Zn(II) solution containing the model sample (*a*) and whole blood stabilized with EDTA (*b*, *c*): *a* – composition: 5000 μg/l Zn(II), 1000 μg/l Cu(II), 150 μg/l Pb(II) and 0.2 % EDTA; f = 250; *b*, *c* – blood sample No. 3, f = 260 (*b* – freshly formed electrode, *c* – after 5 experiments of blood sample analysis).

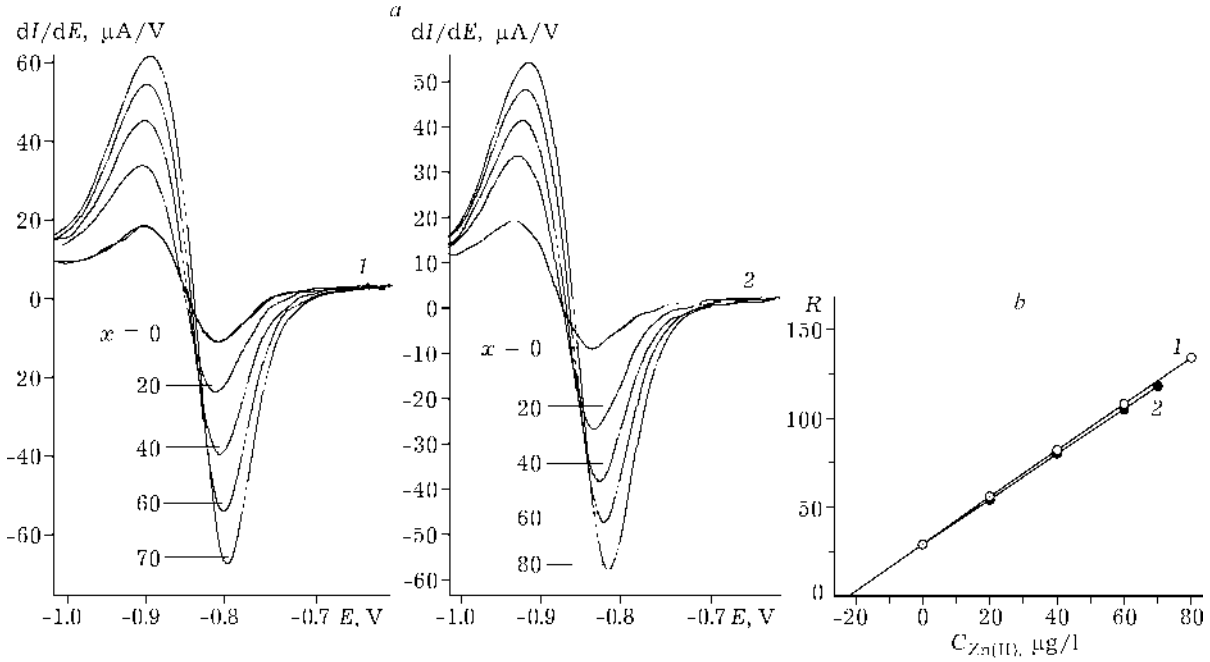

Fig. 7. Differential stripping voltammetric curves (*a*) and dependence of the analytical signal of zinc *R* on the concentration of its ions (*b*) in the solution: 0.04 M NaCl + 0.02 M HCl + *x* μg/l Zn(II) + blood stabilized with EDTA (*1*) or glugicir (*2*). Blood sample No. 3, $f = 250$.

as a universal one and used for the analysis of the erythrocyte mass, serum, plasma and blood stabilized with EDTA or glugicir. The introduction of gallium ions into the solution is unreasonable, because this causes overestimation of the results of analyses.

Table 2 characterizes the correctness of the results of analysis of blood and its fractions. The estimation of correctness was carried out in agreement with the recommendation MI 2336–95 (algorithm 8) of the State System of Provision of Unity of Measurements (Yekaterinburg, 1998). We calculated the error check standard; the results of check were considered to be satisfactory if the condition $K_{exp} \le K_{calc}$ was fulfilled. All the data reported here are in rather good agreement with the expected values. The detected concentration of

TABLE 2

Estimation of the correctness of results of the SV analysis of whole blood stabilized with EDTA, and blood fractions for zinc content using the method of standard additives in combination with the dilution method

Sample	Test sample ($f_1 = 1.04$), μg/l		Diluted test sample ($f_1 = 2.08$), μg/l		Diluted test sample with Zn(II) added, μg/l		C_{ad}, μg/l	Found ($C_z - C_y$), μg/l		Error check standard K	
	C_x	S_x	C_y	S_y	C_z	S_z		C_{ad}	S_{ad}	K_{exp}	K_{calc}
Plasma of Blood No. 1	2320	120	1130	90	2610	150	1500	1480	110	80	178
The same, serum	1190	78	570	60	1150	58	600	590	65	70	96
Blood No. 4	8100	400	3900	230	7000	190	3000	3100	210	200	419
The same, plasma	3350	170	1700	110	3250	210	1500	1530	170	100	245
The same, erythrocyte mass	14 650	850	7300	360	13 200	460	6000	5900	420	150	866
Serum of blood No. 4	1250	90	610	50	1150	60	500	540	40	10	100

Note. $K_{exp} = |C_z + C_y - C_x - C_d|$, $K_{calc} = 0.84\sqrt{(S_x)^2 + (S_y)^2 + (S_z)^2}$.

TABLE 3

Comparative determination of Zn(II) content in test samples of blood from patients, carried out by means of stripping voltammetry (SV) and using independent methods, μg/l

Patient, disease	Anticoagulant	SV	Independent method
A, practically healthy	EDTA	7000 ± 380 (3)	6800[a] (1)
			7900[b] (1)
	Glugicir	7870 ± 150 (3)	7000 ± 540[a] (2)
B, practically healthy	EDTA	8470 ± 150 (2)	8620 ± 210[a] (2)
C, eczema	Glugicir:		
	before medical treatment	3850 ± 120 (4)	3700 ± 170 (3)
	after medical treatment	6700 ± 170 (4)	6430 + 320[b] (3)
D, practically healthy	EDTA	9408 (1)	9150 ± 180[a] (2)
F, acute leucemia	»	6000 ± 840 (4)	5640[a] (1)
G, leucogranulomatosis	»	6500 ± 400 (3)	6860[a] (1)

[a] AAS.

[b] AECSA.

an additive was close to the introduced one in all the cases.

The results of examination of a number of patients, both almost healthy ones and those suffering from definite pathologies, are listed in Table 3. In this case we again observe a good agreement between the results of analyses carried out using the proposed method and using the conventional independent methods.

One can see in Table 4 giving an estimation of the systematic deviation between the results of SV analyses and the analyses performed with independent methods that the deviation is insignificant ($t_{exp} < t_{tab}$) and the results of analyses performed using different methods are close to each other ($F_{exp} < F_{tab}$).

CONCLUSIONS

The reported data provide a convincing evidence that under the chosen conditions it is possible to carry out the SV analysis of plasma, erythrocyte mass, serum and whole blood for

TABLE 4

Estimation of the systematic deviation of results of SV analysis and the data obtained by independent methods for the analysis of blood stabilized with EDTA, and its fractions for zinc content (n = 10 for SV, n = 3 for independent methods, P = 0.95)

Sample	SV		Independent method		F criterion (F_{exp})	t criterion (t_{exp})
	$\bar{C}$, μg/l	S	$\bar{C}$, μg/l	S		
Blood No. 3	5400	270	5300[a]	500	3.43	0.33
The same, erythrocyte mass	12 100	730	11 250[a]	1050	2.07	1.31
Blood No. 5	7100	550	7300[b]	650	1.40	0.48
The same, plasma	2800	170	2700[c]	270	2.52	0.61
Erythrocyte mass of blood No. 5	13 750	1100	14 100[b]	1450	1.74	0.39
The same, serum	1250	65	1300[c]	110	2.86	0.75

Note. F_{tab} = 3.71, t_{tab} = 2.20.

[a, b, c] SV after mineralization of blood, AECSA and AAS, respectively.

the concentration of zinc (II); this method does not require preliminary decomposition and concentrating. The results obtained using the proposed method and the existing ones (atomic absorption spectroscopy, atomic emission chemical spectral analysis) are in good agreement with each other. The use of thin-film modified carbon-containing electrodes, fast potential scan and the differential regime of curve recording allowed us to make the analysis procedure free from toxic mercury and its soluble salts and to eliminate oxygen removal from the solution to be analyzed. Small volume of the blood sample (5–50 μl) required for the analysis is one more advantage for examination of the population.

Acknowledgements

The authors gratefully thank MSTC for the financial support of Projects 342-C and 2132 within which the present investigation was carried out; the authors also thank T. A. Chanysheva, N. F. Beizel and N. P. Zaksas for participation in the investigation of the microelemental composition of blood and its fractions with the help of independent methods.

REFERENCES

1 A. P. Avtsyn, A. A. Zhavoronkova, M. A. Rish, L. S. Strochkova, Mikroelementozy cheloveka, Meditsina, Moscow, 1991, 272 p.
2 V. I. Slesarev, Osnovy khimii zhivogo, Khimizdat, St. Petersburg, 2001, 782 p.
3 Yu. A. Ershov, T. V. Pletneva, Mekhanizmy toksicheskogo deystviya neorganicheskikh soyedineniy. Biokhimiya krovi, Meditsina, Moscow, 1989, 485 p.
4 A. S. Prasad, *Mol. Cell Biochem.*, 188 (1998) 63.
5 A. H. Ringwooda and G. Dinovob, *Marine Environ. Res.*, 42, 1–4, June–October (1996) 53.
6 J. M. Hempe, R. J. Cousins, *Proc. Natl. Acad. Sci. USA*, 88 (1991) 9671
7 P. May, G. Berthon (Eds.), in: Handbook of Metal-Ligand Interaction in Biological Fluids: Bioinorganical Chemistry, Marcel Dekker, New York, 1995, pp. 1184–1194.
8 B. Y. Juergen, D. Kruse-Jarres, *Amer. Clin. Lab.*, April (2001) 17.
9 V. M. Karlinskiy, Tsinkdefitsitnye sostoyaniya, Medical Sciences Doctoral Dissertation, Moscow, 1980, 47 p.
10 L. R. Nozdryukhina, Biologicheskaya rol mikroelementov v organizme zhivotnykh i cheloveka, Nauka, Moscow, 1977, 184 p.
11 E. A. Wilkinson, C. I. Hawke, *Arch. Dermatolol.*, 134 (1998) 1556.
12 A. S. Prasad, *Nutrition*, 11 (1995) 93.
13 N. U. Tits, Klinicheskaya otsenka laboratornykh testov, Meditsina, Moscow, 1986, 480 p.
14 L. A. Shaplygina, T. I. Legonkova, T. Yu. Moiseeva, *Russ. Med. Zh.*, 10 (2002) 730.
15 J. O. Odland, E. Nieboer, N. Romanova *et al.*, *Int. J. Circumpolar Health*, 58, Jan. (1999) 4.
16 R. Raghunath, R. M. Tripathi, A. Vinod Kumar *et al.*, *Environ. Res. Sect.*, A80 (1999) 215.
17 H. T. Delves, *Clinic Endocrinol. Metab.*, 14 (1985) 725.
18 I. Bureau, R. A. Anderson, J. Arnaud *et al.*, *J. Trace Elem. Med. Biol.*, 16 (2002) 9.
19 A. Frkovir, B. Medugorac and Alibir-Juretir, *Sci. Total Environ.*, 192, 2, Dec. (1996) 207.
20 S. H. Lee, J. W. Huang, K. Y. Hung *et al.*, *Artif. Organs*, 24 (2000) 841.
21 W. Windisch, *Anal. and Bioanal. Chem.*, 372 (2002) 240.
22 R. Stanwell-Smith, S. G. Thompson, A. P. Haines *et al.*, *Fertil. Steril.*, 40 (1983) 670.
23 K. Osman, A. Akesson, M. Berglund *et al.*, *Clin. Biochem.*, 33 (2000) 131.
24 S. Telisman, J. Jurasovic, A. Pizent, P. Clitkovic, *Environ. Res.*, 87, 2 (2001) 57.
25 P. Saltman, *Ann. Int. Med.*, 98, 5 Pt 2 (1983) 823.
26 B. Benes, V. Spevackova, J. Smid *et al.*, *Cent. Eur. J. Public Health*, 8 (2000) 117.
27 Kh. Z. Brainina, Inversionnaya voltamperometriya tverdykh faz, Khimiya, Moscow, 1972, 102 p.
28 Kh. Z. Brainina, Stripping Voltammetry in Chemical Analisys, Haisted Press, New York *etc.*, 1974, p. 222.
29 Kh. Z. Brainina, A. V. Ivanova, N. A. Malakhova, *Anal. Chim. Acta*, 349 (1997) 85.
30 Kh. Z. Brainina, N. Yu. Ztozhko, N. A. Malakhova, A. V. Ivanova, *Mikrosistemnaya Tekhnika*, 2 (2002) 10.
31 N. F. Zakharchuk, S. Yu. Saraeva, L. I. Kolyadina *et al.*, *Khimiya v interesakh ustoichivogo razvitiya*, 11 (2003) 725.
32 A. Izquierdo, M. D. Luquede Castro and M. Valcarcel, *Electroanalysis*, 6 (1994) 764.
33 M. A. Moreno, C. Marin, F. Vinagre, P. Ostapczuk, *Sci. Total Environ.*, 229 (1999) 209.
34 R. N. Khandekar, R. Raghunath, U. C. Mishra, *Ibid.*, 66 (1987) 185.
35 J. L. Stauber, T. M Florence, *Ibid.*, 74 (1988) 235.
36 E. E. Tekutskaya, L. I. Sofiina, L. V. Bender, N. P. Onishchenko, *Gigiena i Sanitariya*, 4 (1999) 72.
37 F. Marcheggiani, F. Stella, S. Battistelli *et al.*, *Bull. Soc. Ital. Biol. Sper.*, 66, 10 (1990) 921.
38 D. Jagner, L. Renman, Y. Wang, *Electroanalysis*, 6 (1994) 285.
39 J. C. Meranger, B. R. Hollebone, G. A. Blanchette, *J. Anal. Toxicol.*, 5 (1981) 33.
40 C. L. da Silva, J. C Masini, *Fresenius J. Anal. Chem.*, 367, 3 (2000) 284.
41 C. W. Huang, *Biol. Trace Elem. Res.*, 38 (1999) 233.
42 C. J. Horng, *Analyst*, 121, 10 (1996) 1511.
43 G. Swarzbach, G. Flashka, Kompleksonometricheskoye titrovaniye, Khimiya, Moscow, 1970, 140 p.
44 E. Ya Neiman, L. G. Petrova, V. I. Ignatov, G. M. Dolgopolova, *Anal. Chim. Acta*, 113 (1980) 277.
45 H. J. M. Bowen, Environmental Chemistry of Elements, Acad. Press, London, 1979.
46 J. W. Emsley, The Elements, Clarendon Press, Oxford, 1993.
47 In: Chelovek. Mediko-biologicheskiye dannye, Meditsina, Moscow, 1977, pp. 304, 330.