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Direct Determination of Copper, Lead and Cadmium in the Whole Bovine Blood Using Thick Film Modified Graphite Electrodes.

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Abstract

The thick film modified graphite electrodes (TMGE) as working sensors are used for determination of heavy metals such as copper, lead and cadmium in the whole bovine blood. Due to the use of TMGE, a quick scan rate and registration of the voltammograms in the first derivative mode it is possible to analyze whole blood very quick and easily without deaeration of the solution, without the use of toxic mercury and its salts and without prior mineralization of the biological samples that contain the organic components. The accuracy of the developed technique is proved by recovery tests and by comparison with the results of analysis obtained by the independent method of ICP-AES (atomic emission spectrometry with excitation in inductively coupled plasma) and by comparison with the results of the anodic stripping voltammetric analysis of mineralized samples. The relative standard deviation (RSD) values obtained by the proposed technique of direct determination of heavy metals in whole bovine blood do not exceed 12 %.

Keywords - Bovine blood, Film graphite electrode, Heavy metals, Stripping voltammetry

INTRODUCTION

Providing the population with ecologically safe food is a top priority and urgent task of the modern agriculture [1-5]. The quality of agricultural products is largely determined by content of heavy metals in it including Cu, Pb, Cd, Zn [6]. The imbalance of trace elements in the animal body can have a negative impact not only on product quality but on the health of animals and, consequently, on the populations of cattle [7-11]. Therefore, it seems obvious and essential determination of heavy metals in different organs and tissues of farm animals [12].

At present the heavy metals are determined in almost all organs and tissues of farm animals [13-15]. But analysis of such animal biomaterials as liver [16-17], lungs [18], muscle tissue [19, 20] etc. is only possible after slaughter of the animal. For study of microelement status of the animal organism is more effective in vivo determination of heavy metals in animal body. This approach allow of watching the animal health, of controlling the content of metals in organism over time [21]. Besides such approach can be also applied to establish the source of heavy metals entry in organism and eliminate it. As in vivo markers to indicate the accumulation of heavy metals in farm animals, researchers use the analysis of bristle [22, 23], blood and its fractions [24, 25], ejaculate [24, 26-28]. However the most accessible and informative biological material for study the microelement status of cattle still remain blood.

According to the literature data the most popular methods used for analysis of biological objects including bovine blood is atomic absorption spectrometry [29-31] and atomic emission spectrometry. But these methods involve the use of bulky, expensive equipment, require operators of high qualification and eliminate the possibility of analyzing samples in field-conditions. Besides heavy metals is mostly determined after procedure of preliminary mineralization of the samples [32]. Mineralization is used to eliminate the interfering effect of the organic component of the blood matrix. But it is known that the sample preparation requires extra labor and can often be the source of systematic error of analysis results. Competitive in all aspects to the mentioned above methods for the study of the microelement status of cattle can be anodic stripping voltammetry method (ASV). It is characterized by high sensitivity, low detection limit, selectivity, universality, does not require expensive and complicated fixed equipment; there is the possibility of implementing it in field-conditions.

The most perspective working electrodes applied for analysis of biological liquids not requiring preliminary sample preparation are the electrodes insensible for interfering influence of biological matrix and dissolved oxygen. Such sensors can be thick film graphite electrodes (TGE) proposed in [33, 34]. Determinate ions are preliminary accumulated on the surface of TGE in the presence of mercury (II) salts with following registration of peak current of metals oxidation using quick scan rate. The thick film modified graphite electrodes (TMGE) made by screen printing method exclude using mercury and its toxic salts in analysis, make determination of copper, lead, cadmium and zinc in whole blood more simple and express [35, 36]. TMGE used in the quick scan rate mode has

proved to be indifferent to hindering influence of the organic components of the human blood matrix and dissolved oxygen. These approaches used TMGE as a working sensors were successfully applied for study of microelemental status of healthy people and patients with blood oncology diseases [37-39].

The aim of this work is to research the possibility of application of TMGE for direct determination of copper, lead and cadmium in presence of bovine blood matrix more rich with proteinaceous and lipid components in comparison with human blood.

2. MATERIALS AND METHODS

2.1. Apparatus

The linear-sweep voltammetric measurements were carried out with a voltammetric analyzer STA (ITM Co. Ltd., Tomsk, Russia) or IVA-5 (NPVP IVA Co. Ltd., Yekaterinburg, Russia). The first derivative mode was used. So the amplitude of derivative change (dI/dE) was used as the analytical signal.

A standard three electrode configuration and typical electrochemical cell filled with 0.5 mol L^{-1} HCl solution (10 mL) as an electrolyte were used in all measurements. Graphite or glassy carbon rod was used as the auxiliary electrode. The reference electrode was a silver-silver chloride electrode (Ag/AgCl) filled with saturated KCl solution. The thick film modified graphite electrode (Fig. 1.) was used as a working sensor (EcoBioTest Co. Ltd., Yekaterinburg, Russia).

The sensor surface was preliminary formed by standing the sensor in 0.5 mol L^{-1} HCl solution with applying a series of potentials -0.8 V, -1.0 V and -1.5 V for 60 s, 60 s and 300 s, respectively. Operability of modified sensor was remained during the working day. Vibrating stirring of the solution during the electrolysis was applied.

2.2. Chemicals

All reagents used were of analytical grade and were used without further purification. Only hydrochloric acid was further purified by distillation isopiestically at room temperature or by distillation without boiling. Twice distilled water (TDW) was used to prepare all solutions and clean glassware and electrodes. Copper (II), lead (II) and cadmium (II) standard solutions with concentration of 1000 μg L^{-1}, 500 μg $L^{-1}$and 500 μg L^{-1}, respectively were prepared from the primary certified standard solution (1000 mg L^{-1}) by serial dilution with 0.5 mol L^{-1} HCl solution. The solutions with concentration of 1000 and 500 μg L^{-1} were prepared every day.

2.3. Sample preservation and storage

The object of study was whole bovine blood and samples of blood after mineralization. Whole blood taken from the bulls in a volume of 5-10 mL was placed in a pre-prepared glass tube containing the appropriate amount of 5% EDTA (ethylenediamine tetra-acetic acid) solution as anticoagulant. Anticoagulant was put into the tube in amount of 0.04 mL per 1.0 mL of blood. Samples were thoroughly mixed and kept in freezer (-18^{0}C). This treatment stabilizes the blood for up to 30 days. For the analysis stabilized blood samples with a volume of 0.05 - 0.2 mL was put in the electrochemical cell containing electrolyte solution in the volume of 5 (10) mL. So the diluted sample was got. Dilution ratio (f) of the blood was calculated according to the formula:

$$f = \frac{V_{electrolyte} + V_{blood}}{V_{blood}} \quad (1),$$

where $V_{electrolyte}$ and V_{blood} – the volume (mL) of background electrolyte and an aliquot (mL) of blood introduced in the cell, respectively.

In order to obtain mineralized blood, the manner of decomposition under the action of microwave radiation was used. Blood decomposition was carried out in the mixture of nitric acid and hydrogen peroxide in the ratio of 4:1 in microwave oven MARS-5 at 180^{0}C.

3. RESULTS AND DISCUSSION

3.1. The choice of optimal electrolysis conditions

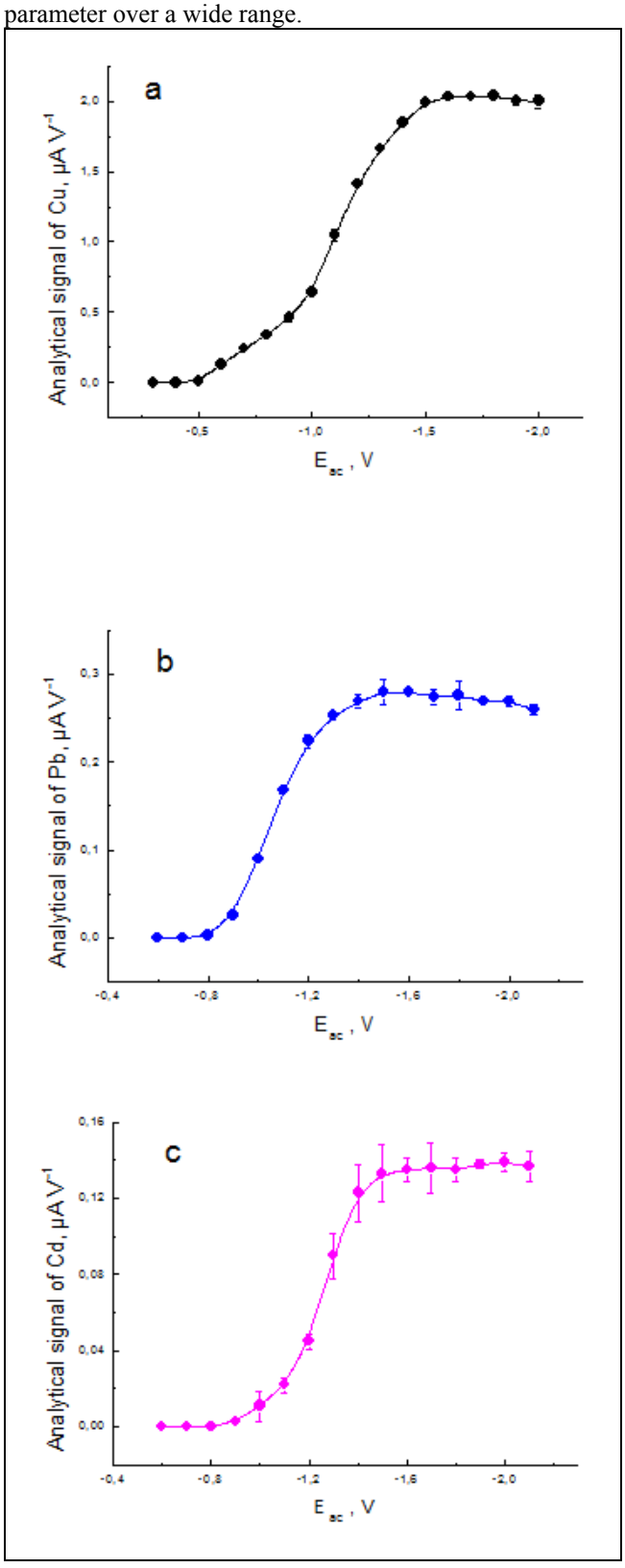
0.5 mol L^{-1} HCl solution is used as a background electrolyte for determination of copper, lead and cadmium as mentioned in [37].

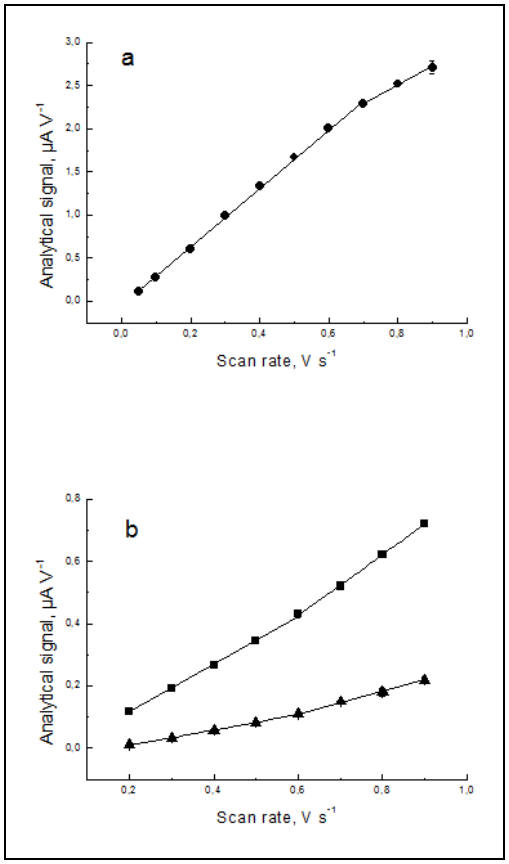
To select the accumulation potential (E_{ac}), the influence of the E_{ac} on the analytical signal (μA V^{-1}) of copper, lead and cadmium in 0.5 mol L^{-1} HCl solution containing 0.1 mL bovine blood are investigated. Figure 2b and 2c illustrates the pseudopolarograms obtained in case of cathodic precipitation of lead and cadmium, respectively. As you can see, plateau on it is observed in the range of accumulation potential (E_{ac}) -1.4 ÷ -2.1 V for lead and -1.5 ÷ -2.1 V for cadmium. In case of cathodic precipitation of copper the bend is observed on pseudopolarogram in the potential range of -0.7 ÷ -0.9 V (Fig. 2a). It looks like the first plateau is formed. The second plateau is observed in the potential range of -1.5 ÷ -2.0 V. Such electrochemical behavior of copper is similar to data presented in [37] and it is explained by complicated biochemical state of copper-containing proteins and enzymes. So the accumulation potentials more negative than -1.4 V are suitable for simultaneous accumulation of copper, lead and cadmium on thick film modified graphite electrode surface. But E_{ac} = -1.6 V is used in all further experiments because of the lower standard deviation of the analytical signals.

The dependences of analytical signal (μA V^{-1}) of copper, lead and cadmium on scan rate (υ) are presented on Figure 3. Figure 3a indicates that the relationship between the analytical signals of copper and scan rate is direct proportional up to 700 mV s^{-1}. It means that electrode process does not include diffusion delivery of electrochemically active material in the electrode reaction zone. In the other words, the metal dissolution occurs from either the solid phase or from very thin amalgam film which has a thickness comparable with a thickness of diffusion layer [41, 42]. Dependences of analytical signals (μA V^{-1}) of lead and cadmium on the scan rate are linearly increased (Fig 3b). But under υ > 600 mV s^{-1} the slope ratios of dI/dE = f (υ) are changed. It can be related with exchanging the kinetics of electrode process. So the scan rate is not more than 600 mV s^{-1} can be suitable for

simultaneous registration the analytical signal of copper, lead and cadmium.

Relationship between the analytical signals of determined analytes and accumulation time (t_{ac}) is directly proportional up to 180 s. So, it allows varying this parameter over a wide range.





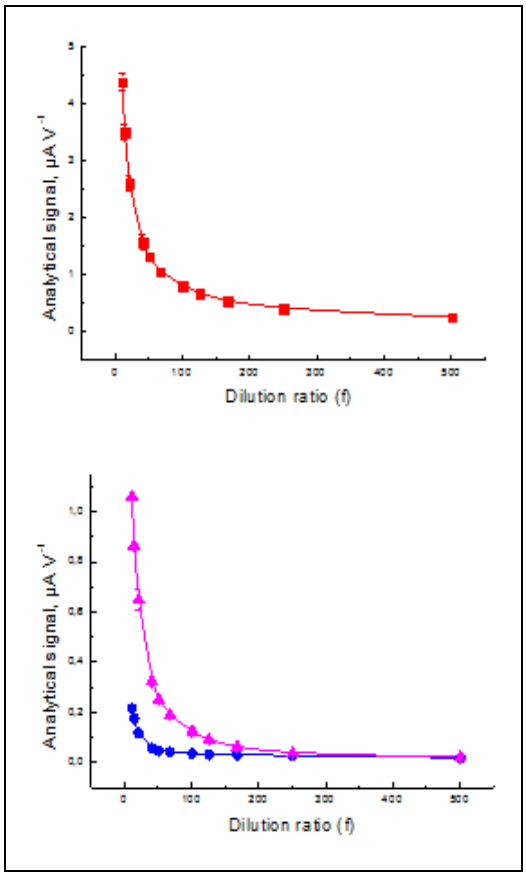


Table 1. Accuracy of copper, lead and cadmium determination in bovine blood

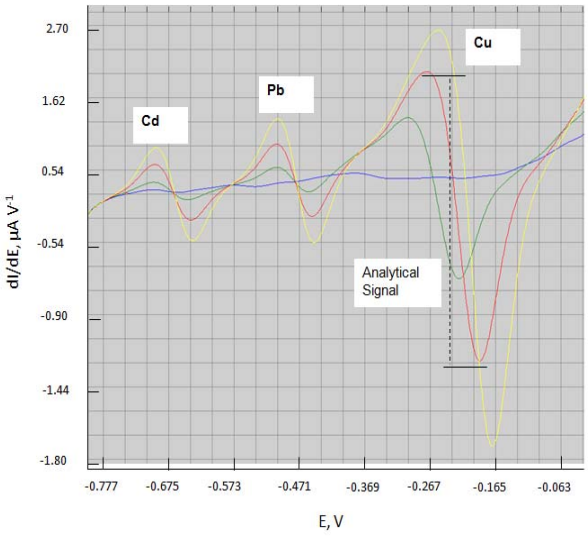
Sample	Breed	Analyte	C (μg·L^{-1})			Recovery (%)
			Detected in the sample	Spiked	Found	
1[a]	Semental	Cu	1180±70	1000	980±90	98
		Pb	107±5	250	253±15	101
		Cd	65±2	150	155±6	103
2[a]	Red Danish	Cu	1290±75	1000	990±95	99
		Pb	6.3±0.6	25	24.3±0.3	97
		Cd	<5	-	-	-
3[b]	Holstein	Cu	670±60	1000	960±100	96
		Pb	28±2	50	48±4	96
		Cd	6.0±0.5	25	25.5±0.5	102

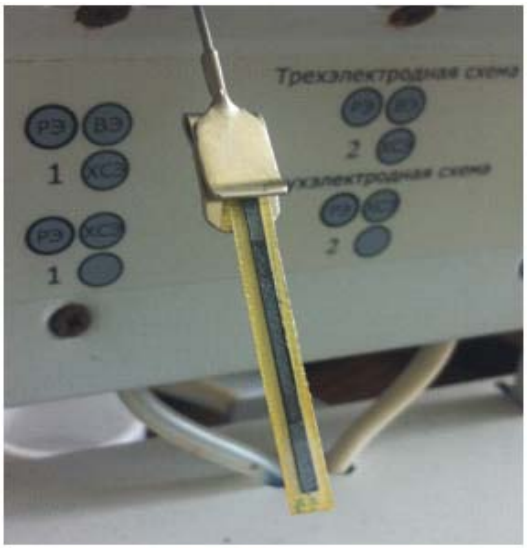
[a]Altay region, Barnaul city, "Barnaul breeding", [b]Kemerovo region, Vaganovo village, "Vaganovo breeding"

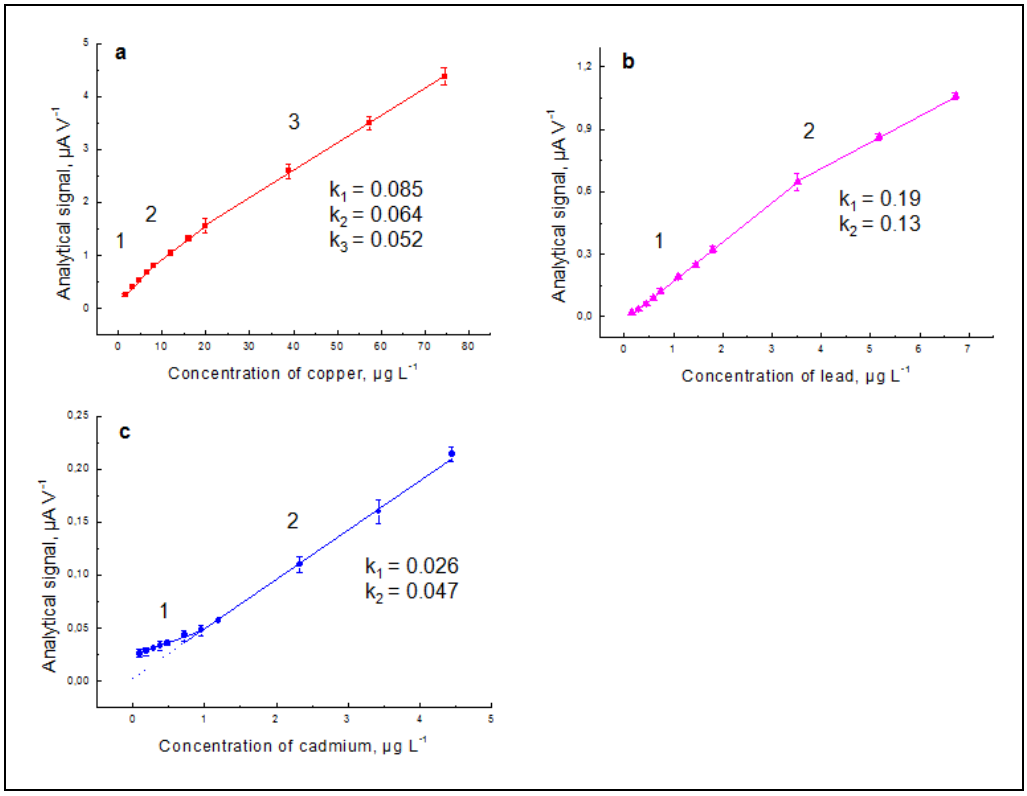
Table 2. The results of the whole bovine blood analysis obtained by three independent methods

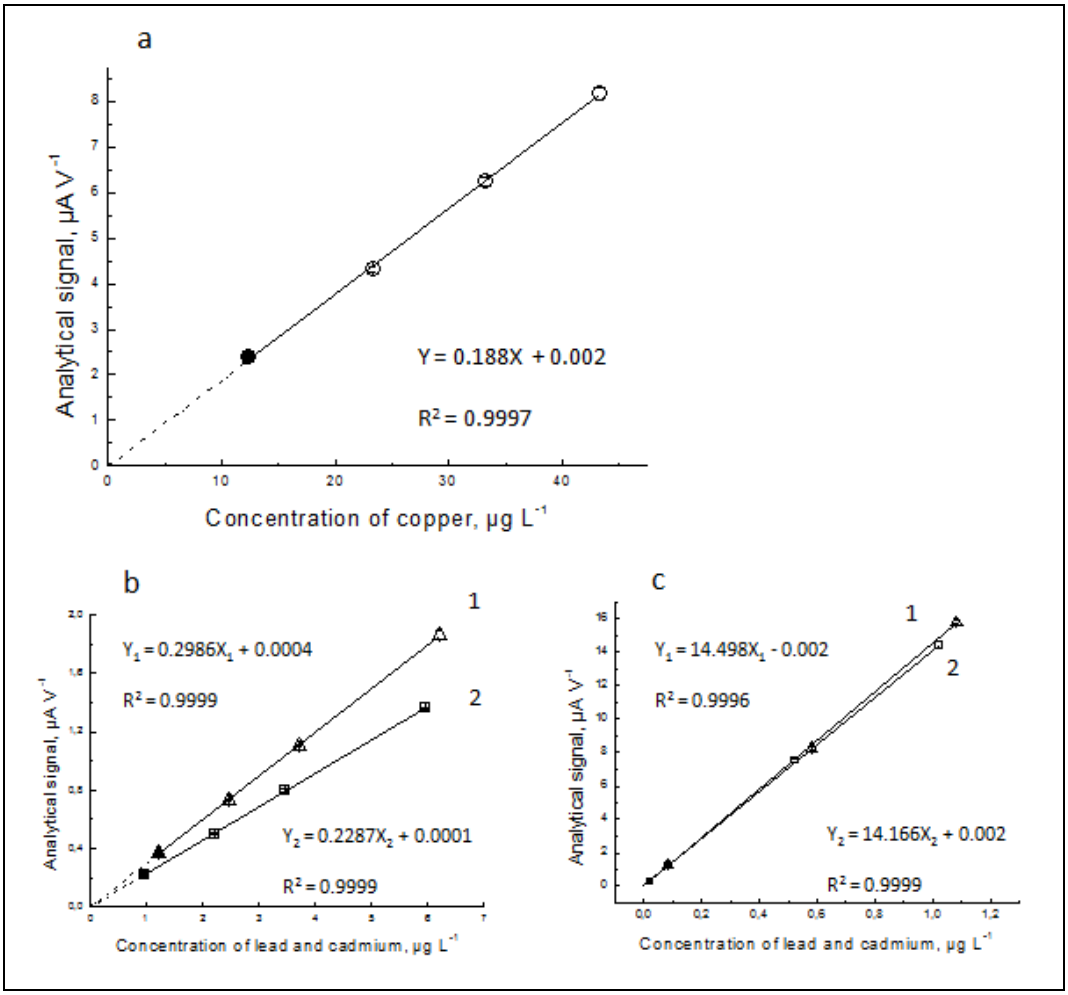
Sample number	Breed	C (Cu), μg L^{-1} (RSD, %)			C (Pb), μg L^{-1} (RSD, %)			C (Cd), μg L^{-1} (RSD, %)		
		I	II	III[a]	I	II	III*	I	II	III*
1	S	890 (3.9)	-	880 (9.1)	170 (5.9)	-	N/F, LOD: 400 μgL^{-1}	86 (7.0)	-	93 (14)
2	S	1180 (7.6)	1040 (9.6)	910 (22.0)	107 (5.6)	109 (6.4)		66 (3.0)	66 (4.5)	75 (16)
3	S	910 (9.9)	890 (13.5)	780 (12.8)	41 (4.9)	38 (13.1)		61 (3.3)	56 (8.9)	58 (6.9)
4	S	840 (5.9)	770 (10.4)	745 (10.7)	160 (5.6)	150 (8.0)		121 (2.5)	110 (9.1)	130 (11.5)
5	S	1180 (6.8)	1060 (9.4)	1010 (11.9)	73 (9.6)	72 (9.7)		65 (6.1)	60 (11.7)	60 (15)
6	S	900 (10)	970 (9.3)	980 (10.2)	15.6 (5.8)	14 (14.3)		N/F, LOQ: 5 μgL^{-1}	N/F	N/F, LOD: 20 μgL^{-1}
7	S	820 (12.2)	870 (13.8)	700 (17.1)	97 (5.1)	106 (9.4)				
8	Bl.-M.	1150 10.4)	1000 15.0)	910 (16.5)	14.0 (10.7)	15 (13.3)		8 (12.5)	7 (21.4)	
9	Bl.-M.	1250 (8.0)	1080 11.1)	1050 (17.1)	105 (5.7)	99 (12.1)		3.9 (12.8)	4.0 (15)	
10	Bl.-M.	920 (6.5)	930 (9.7)	900 (11.1)	40 (10)	45 (13.3)		N/F	N/F	
11	Bl.-M.	1080 (7.4)	940 (10.6)	1070 (9.3)	138 (5.1)	134 (11.2)		10 (10)	11 (13.6)	
12	Bl.-M.	1100 (10)	-	960 (14.6)	110 (9.1)	-		91 (9.9)	-	76 (15.8)
13	Bl.-M.	850 (12.9)	-	650 (23.0)	36 (11.1)	-		46 (8.7)	-	40 (20)
14	Bl.-M.	960 (10.4)	-	790 (15.2)	28 (10.7)	-		47 (10.6)	-	54 (16.7)
15	A	700 (8.6)	650 (13.8)	700 (14.3)	86 (8.1)	93 (8.6)		3.3 (9.1)	3.0 (16.7)	N/F LOD: 20 μgL^{-1}
16	R.D.	1200 (7.5)	1150 (7.8)	1080 (13.9)	26 (11.5)	30 (13.3)		N/F	N/F	
17	A	1200 (8.3)	1080 11.1)	1000 (20.0)	45 (8.9)	47 (10.6)		14 (14.3)	12 (16.7)	
18	R.D.	950 (10.5)	-	800 (15.0)	92 (5.4)	-		52 (3.8)	-	46 (15.2)

I - Direct ASV, II - ASV with mineralization, III - ICP-AES
S - Simmental, Bl.-M. - Black-Motley, A – Angler, R.D. - Red Danish
a – spectra were obtained under standard conditions as mentioned in [43]
N/F – not found






a
Analytical signal, μA V-1
Concentration of copper, μg L-1
k1 = 0.085
k2 = 0.064
k3 = 0.052
b
Analytical signal, μA V-1
Concentration of lead, μg L-1
k1 = 0.19
k2 = 0.13
c
Analytical signal, μA V-1
Concentration of cadmium, μg L-1
k1 = 0.026
k2 = 0.047


a
Analytical signal, μA V-1
Y = 0.188X + 0.002
R2 = 0.9997
Concentration of copper, μg L-1
b
Y1 = 0.2986X1 + 0.0004
R2 = 0.9999
Y2 = 0.2287X2 + 0.0001
R2 = 0.9999
Analytical signal, μA V-1
Concentration of lead and cadmium, μg L-1
c
Y1 = 14.498X1 - 0.002
R2 = 0.9996
Y2 = 14.166X2 + 0.002
R2 = 0.9999
Analytical signal, μA V-1
Concentration of lead and cadmium, μg L-1

3.2. Choosing the dilution ratio

The dependences of the analytical signal of copper, lead and cadmium on dilution ratio and on concentration of these ions are presented in Fig. 4 and Fig. 5, respectively. The analytical signals are exponentially reduced when increasing of dilution ratio (Fig. 4). The dilution ratio of the blood is changed depending on the aliquot of the sample introduced into the electrochemical cell. When V_{blood} = 0.02 ÷ 1.0 mL, dilution ratio is in the range from 501 to 11 according to the formula 1 (provided that $V_{electrolyte}$ = 10 mL). However the sensitivity of determination of copper, lead and cadmium is different in examined blood dilution range. The following low is observed: sensitivity coefficient in case of copper and lead determination in the bovine blood is decreased with increasing blood aliquot introduced in the electrochemical cell. Sensitivity coefficient decreasing can be explained by slowing down of process of metals accumulation on the electrode surface in the cathodic stage [33]. The dependence of the analytical signal of copper on concentration has a three linear concentration range with sensitivity coefficient (k) of 0.085, 0.064 and 0.052, respectively (Fig. 5a). In the first part of the graph the dependence dI/dE = f ($C_{Cu(II)}$) is directly proportional with the best sensitivity coefficient of 0.085. It corresponds to the blood dilution range of 501 ÷ 101. This range can be used as an optimal for determination of copper. The dependence dI/dE = f ($C_{Pb(II)}$) is directly proportional up to blood dilution factor of 21 (see the first part of the graph on Fig. 5b). Sensitivity coefficient for this range is equal to 0.19. So it is possible to vary the sample volume in the more wide range in the case of determination of lead in the bovine blood. The different situation is observed in case of cadmium determination. The dependence of analytical signal of cadmium on concentration has a two linear concentration range (Fig. 5c). Sensitivity coefficient in the first range is lower than in the second one. Since dilution ratio of 51 sensitivity of cadmium determination is higher. Sensitivity coefficient is equal to 0.047. It means that the smaller blood dilution is required for determination of cadmium in the bovine blood when its concentration <20 μg L^{-1}. The first dilution range (f = 501 ÷ 51) with sensitivity coefficient 0.026 can be used for bovine blood analysis if cadmium concentration is more than 20 μg L^{-1}.

So the optimal dilution ratio can be 101. It is suitable for simultaneous determination of copper, lead and cadmium in the bovine blood. But under the low cadmium concentration (<20 μg L^{-1}) in the blood sample it is necessary to determine the cadmium and lead separately from copper. In this case it is necessary to using smaller blood dilution since f=51and less or t_{ac} > 90 s.

3.3. Whole blood analysis and in-house validation of the developed procedure

Under chosen optimal conditions mentioned in the sections 3.1 and 3.2 calibration graphics for copper, lead and cadmium are straight linear and come from point of origin with correlation coefficient (R^2) above than 0.999 (Fig. 6). Linearity is observed up to a concentration of 45.0 $\mu g \cdot L^{-1}$ for copper using a 60 s accumulation time and the plot dI/dE = f($C_{Cu(II)}$) follow the relationship dI/dE = 0.188 $C_{Cu(II)}$ + 0.002. Fig 6b and 6c show the plot of analytical signals of lead and cadmium versus concentration obtained under accumulation time of 60 s and 120 s, respectively. The plots dI/dE = f($C_{Pb(II)}$) and dI/dE = f($C_{Cd(II)}$) demonstrate a good linearity in both cases and submit the following regression equations: dI/dE = 0.2986 $C_{Pb(II)}$ + 0.0004 and dI/dE = 0.2287 $C_{Cd(II)}$ + 0.0001 using t_{ac} = 60 s; dI/dE = 14.498 $C_{Pb(II)}$ - 0.002 and dI/dE = 14.166 $C_{Cd(II)}$ + 0.002 using t_{ac} = 120 s. The overall investigated concentration range is 0.08 to 6.5 $\mu g \cdot L^{-1}$ for lead and 0.02 to 6.0 $\mu g \cdot L^{-1}$ for cadmium. The data mentioned above proves the propriety of application of standard addition method for quantification of copper, lead and cadmium in the whole bovine blood by ASV. The concentration ranges considered for copper, lead and cadmium is absolutely enough for determination of these metals in the bovine blood. There is no need to investigate the linearity outside of these ranges.

The Fig. 7 illustrates the typical linear-sweep anodic stripping voltammetric responses of copper, lead and cadmium accumulated at thick film modified graphite electrode surface from 0.5 mol L^{-1} HCl (10 mL), containing 0.1 mL sample. In this case the sample is the whole bovine blood taken from one of the bulls of Black-Motley breed (Altay region, Barnaul city, “Barnaul breeding”). The found concentration of copper, lead and cadmium calculated on standard addition method equal to 1100, 120 and 88 μg L^{-1}, respectively, and they are in agreement with results obtained by ISP-AES method (see Table 2). The error of a single determination is less than 5 %

The values of limit of detection (LOD) calculated as $3SD_{blank}$ are 0.3, 0.09 and 0.24 $\mu g \cdot L^{-1}$ for copper, lead and cadmium, respectively. LOQ (limit of quantification) was determined in this work for lead and cadmium and the values of LOQ obtained is 2.0 and 5.0 $\mu g \cdot L^{-1}$, respectively. LOQ was estimated as a minimum concentration which is determined according to the proposed technique with relative standard deviation (RSD) 33%. The LOD and LOQ was obtained using a 60 s accumulation time for copper and 120 s accumulation time for lead and cadmium.

Bovine blood reference material with certified concentrations of copper, lead and cadmium was not available. Thus, accuracy of the developed technique is proved by recovery tests and by comparison with the results obtained by the independent methods. Table 1 shows the results of the accuracy test of the method proposed for bovine blood of three breeds – Semental, Red Danish and Holstein. Recoveries ranged from 96 to 103%, indicating that the proposed procedure is accurate. Comparative results of the copper, lead and cadmium determination obtained by direct ASV method, by ICP-AES method (atomic emission spectrometry with excitation in inductively coupled plasma) and by anodic stripping voltammetry method after sample mineralization are summarized in Table 2. As it is seen, the results of direct ASV analysis of whole bovine blood insignificant differ from the results obtained by independent methods of ISP-AES and by ASV method after acid mineralization of samples.

The precision of the method was evaluated in terms of within day repeatability (Re) and intermediate precision (IP). Re values of 5.8, 4.6 and 5.3 % and IP values of 7.3, 6.7 and 9.4 % were obtained in case of copper, lead and cadmium determination, respectively. The relative standard deviation (RSD) values obtained do not exceed 12 %.

4. CONCLUSIONS

Presented above data clearly indicate that in the chosen conditions direct and rapid analysis of whole bovine blood using anodic stripping voltammetry method is possible for the study of the microelement status of bovine animals. Proposed technique does not require preliminary sample decomposition, removal of dissolved oxygen from analyzed solution and the use of toxic mercury and its salts in the analysis. It is realized due to the use of thick film modified graphite electrodes, faster than normally used in voltammetry the scan rate and the registration voltammograms in the first derivative mode.

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