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Measurements of trace elements in human blood and urine using atomic emission spectrometry

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Abstract: Arc-discharge atomic-emission spectrometry was used for determining trace amounts of metals in human blood and urine. Radiation spectra were recorded on an atomic-emission multichannel spectrometer AEMS. An optical multichannel analyzer (OMA) based on a photodiode array was used as the detector. The spectral resolution was characterized by the range 0.011 nm/diode and a spectral-line half-width no more than 0.033-0.044 nm. The samples were prepared for the measurements by the method of dry mineralization without acids. The obtained ash was mixed with a graphite powder in 3:7 mass ratio. For urine samples, the second variant of buffering a mixture of a graphite powder with potassium chloride (9.2 % of K) was also conducted. For determining the concentration of Pb, Cd, Cu and Zn in blood and the concentration of Co, Mn, Ni and Cu in urine three types of the calibrating mixtures containing, respectively, equal amounts of graphite powder and having predetermined concentrations of matrix elements (K, Na, Mg, Ca, Fe) were prepared. Lyphochek Urine Metals Control, Level 1 and Seronorm Trace Elements Urine was used as a reference material in determining trace amounts of elements in urine. Limits of detection were 0.13; 0.7 and 5.5 μg l^{-1} for Cd, Zn and Pb in blood and 1.2; 1.4; 1.5 and 1.9 μg l^{-1} for Ni, Mn, Co and Cu in urine respectively. The results obtained and the simplicity of preparation of samples for analysis, allow the conclusion that arc-discharge atomic-emission spectrometry can be used for determination of trace elements in human biological samples, forensic and clinical toxicology, screening investigations in ecologically adverse regions and workers in unhealthy industries.

Keywords: trace elements, blood, urine, limit of detection

1. Introduction

For some time arc discharge atomic emission spectrometry have been was among the main techniques used for quantitative chemical analysis of various biological objects and in clinical investigations [1,2]. In the variant with photo-graphic recording, the sensitivity of this technique is insufficient for reliable determination of the natural content of a number of elements, among them nickel, cobalt, chromium, barium, vanadium, in human urine and blood. The technique was not further developed and, according to the literature data, its application was stopped. This technique was replaced by the well-known techniques of inductively coupled plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry, electrothermal atomization atomic absorption spectrometry (ETAAS) or graphite furnace atomic absorption spectrometry (GFAAS), and inductively coupled plasma atomic emission spectrometry (ICP-AES) [3-11]. ETAAS is the most widely used technique for various biological situations, however it has poor multielement capability. Multielement analysis became possible with ICP-MS and ICP-AES techniques. Modern ICP-MS is the most sensitive technique and is now applied for direct determination of trace elements in blood and urine samples from a non occupationally exposed populations [12,13].

We have developed an atomic-emission multichannel spectrometer (AEMS) with recording of radiation spectra on an optical multichannel analyzer (OMA) based on a photodiode array. The use of an OMA as the detector allowed us to combine the advantages of the photographic and photoelectric recording of spectra - simultaneous recording of all the lines in a region of the radiation spectrum, automatic acquisition and processing of analytical signals with time resolution. The spectrometer has found application in determination of heavy metals in such samples as foodstuff and forages for animals at a level of toxic elements 0.1-1 mg kg^{-1}.

In the present paper we consider the results of simultaneous determination of the concentrations of metals in human blood and urine at a level 1-10 μg l^{-1} using of an AEMS. Reference concentrations for trace elements (Ni, Co, Mn, Cd) in human whole blood and urine are in the range 0.5 - 20 μg l^{-1} [12,13].

2. Experimental

2.1. Recording and Processing of Radiation Spectra

We used a photodiode array containing 3600 light-sensitive elements (channels) as the detector of radiation from a low temperature plasma. The signal from the photodiode array was processed on a computer. The spectral resolution was characterized by the range 0.011 nm/diode and a spectral line half-width of no more than 0.04 nm. The optical background was taken into account. Figure1 shows an example of taking into account the optical background for one of the of manganese lines.

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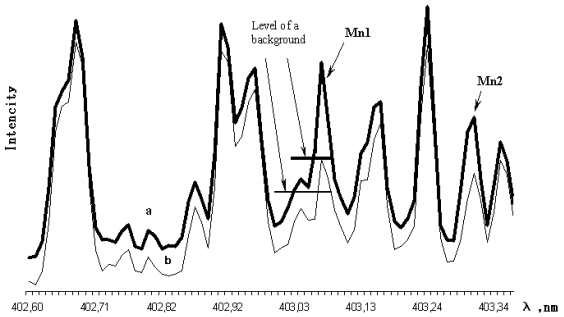

Figure 1. Region of the radiation spectrum in the range 402.75-403.4 nm of urine sample without additions (a) and with a 10 μg·l-1 addition of Mn (b); a signal from an analytical line was recorded in the range 403.071-403.082 nm, the background was taken into account in the ranges 402.939-403.027 nm and 403.137-403.236 nm.

The detection parameters were as follows: alternating current, current intensity 12 A, total time of radiation detection, no more than 64 s. The gap between the two graphite electrodes (upper and lower) was 3 mm. Radiation from the plasma region located at a distance of 0.5-0.9 mm above the edge in the lower graphite electrode into the cavity of which the sample studied was placed, was directed to the detector.

2.2. Graphite electrodes

The lower electrode with a hollow of diameter 2 mm at the ends and the upper cone electrode were made from graphite bars SE 6,0 (SRIECD, s. Electrougli). The mass of a sample placed in the hollow was about 10 mg.

Directly before the filling of the electrodes with a sample, they were burned in an alternating current arc. The burning parameters were as follows: current intensity, 16 A; time 24 s.

2.3. Subjects and Specimens

All human samples of urine (US0) and blood (BS0), used in the experiment, were taken from people who had not been connected with harmful manufacture. Standard samples of ions of metals analyzed of the Center of Research and Control of Water (St.-Peterburg) were added to a part of each sample (standard samples of urine US1 - US4, standard samples of blood BS1). Certified reference materials (SRM) used for method validation and quality control (QC) were Lyphochek Urine Metals Control, Level 1, Seronorm Trace Elements Urine and Seronorm Trace Elements Urine Blank.

2.4. Chemicals

Twice distilled water was used for dilution of dry urine control samples. All the reactants used for the preparation and calibration mixtures were chemically pure or extra pure.

For dilution of the ash of urine and blood samples we used a graphite powder, which corresponded to the reference samples Uw for urine and reference samples Bd for blood, or the mixture of a graphite powder with KCl (9,2 % of K) when the reference samples Uri were used for urine.

The matrices of the Uw and Uri calibration mixtures were made of KCl, KH_2PO_4, NaCl, $CaCO_3$ and MgO. The matrix of the Bd calibration mixtures included Fe_2O_3 in addition to the above-mentioned compounds. The Uw and Uri Matrices corresponded to composition of 16.9 % K, 17.3 % Na, 1.3 % Ca, 1.1 % Mg. The Bd matrix included 15.9 % K, 16.3 % Na, 1.2 % Ca, 1.0 % Mg, 4.0 % Fe. The calibration mixtures were prepared by grinding of 0.3 g of the matrix and 0.7 g of the buffer containing certain amounts of the elements analyzed.

2.5. Preparation and Samples Analysed

The blood and urine samples (1-2ml) were evaporated and charred in quartz cups and then were positioned into a muffel furnace heated to a temperature of 250 °C. Gradually (within 40 min) the temperature in the furnace was increased to 450 °C, the blood samples were mineralized within 60 minutes and the urine samples within 100 minutes.

Next the mineralized samples were cooled and weighed and they were mixed with buffers in 3:7 on mass ratio.

3. Result and Discussion

3.1. Spectral-kinetic characteristics and matrix control

The arc-discharge between the carbons electrodes, in the crater of one of which the sample to be analyzed is placed, represents a nonstationary process. Figure 2 shows the average kinetic curves of the intensity of different spectral components of the Uw calibration mixtures and of the prepared urine samples with close concentration of the elements analyzed. These curves illustrate the process of ingress of various elements into the plasma. It is seen that the kinetic curves of different elements found in the calibration mixtures and in the prepared samples are somewhat similar to each other (Fig. 2a and 2b). The egress of the elements analyzed is closest to the egress of the matrix element potassium (Fig. 2 c, d) and is terminated approximately within 30 s of the arc burning, while the egress of other matrix elements: calcium and magnesium, is more prolonged and lasts for 48 s. The background intensity corresponds to the plasma temperature: once the egress of the matrix elements cooling the plasma is terminated, its intensity increases sharply.

The Uri samples, as compared with the Uw samples, are characterized by a longer kinetic of intensity of both the matrix elements and the microelements studied. The time of complete egress of each element is longer by 5-7 s here. For Uri samples the time of attainment of a maximum background radiation corresponding to a higher plasma temperature in the absence of macroelements also increases accordingly. This is due to the large amount of potassium in the Uri samples. In the measurements with both types of calibration mixtures, for urine samples we used the same exposure time of 32 s, which covers not less than 90 % of the integral intensity of the radiation of each of the elements analyzed. The same exposure time was used for measuring zinc and copper in blood, and an exposure of 20 s was used for more volatile Cd and Pb.

In the case where there is a structured optical background (Fig. 1), the optimum conditions of recording of weak analytical lines is realized when the background intensities as well

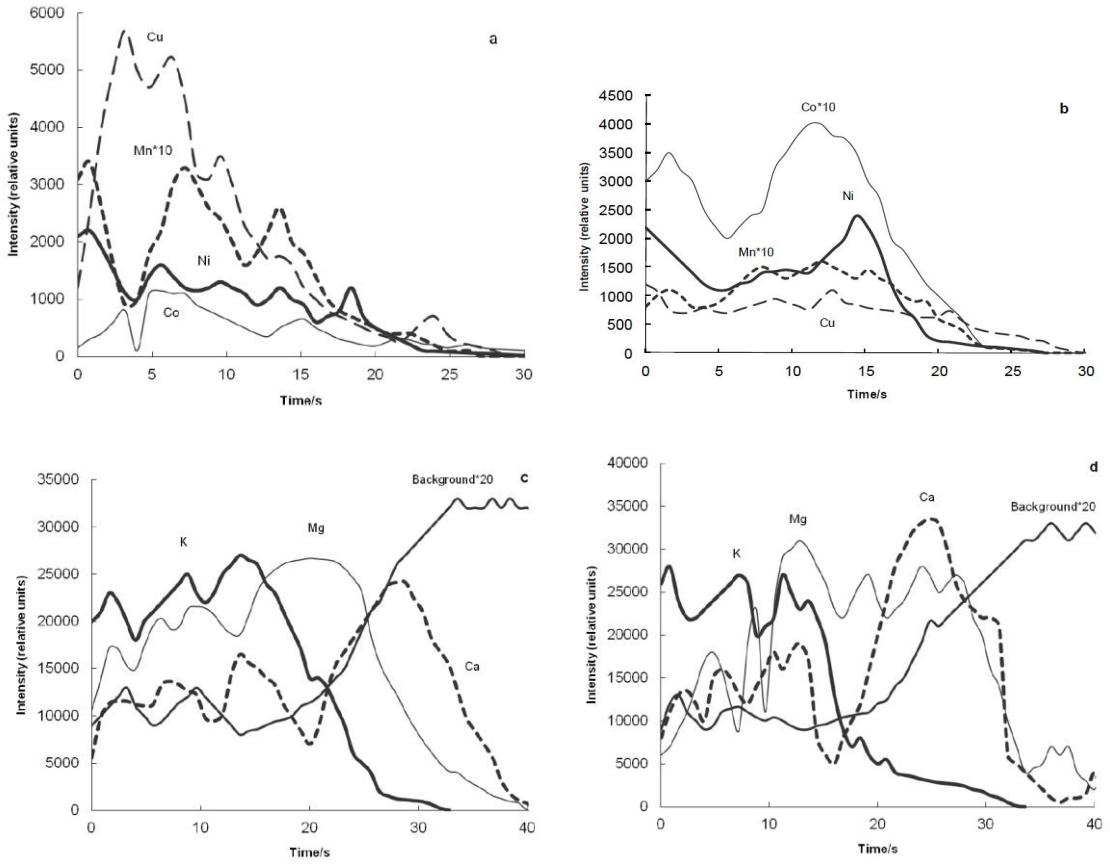

Figure. 2. Kinetic intensity curves averaged over four registrations: a, b) elements analyzed; c, d) matrix elements and optical background in the UW calibration mixtures and in the LUMC sample, respectively.

as the kinetic curves of egress of the elements analyzed of the calibration mixtures and the prepared samples coincide, which, first of all, can be provided by the same concentration of potassium - a matrix element with the lowest ionization potential. The data presented in Fig. 2 and in Tables 1 and 2 characterize, in our opinion, the conditions approximating fairly well to the conditions described above.

It is seen from Table 1 that the replacement of a part of the graphite powder by KCl when going from the Uw to the Uri calibration mixtures leads to a small decrease in the intensity of Mg and Ca lines. At the same time, the background radiation intensity practically does not decrease. As will be shown below in Tables 5 and 6, the absence of potassium in the buffer does not lead to the appearance of the matrix effect when the concentrations of the elements analyzed are determined.

3.2. Choice of lines and Detection limits

Choice of lines and Detection limits We used lines with a high intensity relative to the background as the analytical lines, which do not practically overlap with the atomic lines of other elements. Blood was analyzed with the use of the copper line 223.008 nm which was less intense than the lines 324.754 nm and 327.396 nm. The choice of these lines is explained by the fact that the concentration of copper in blood is fairly high and the concentration of Pb, Cd and Zn can be also measured in this case. The lines of Co, Cu and Ni were also simultaneously recorded when urine was analyzed. The element Mn was determined separately.

The limits of the detection were determined by the 3s-criterion for the signals from matrices of each of the calibration mixtures by no less than ten registrations (Tables 3 and 4). atomic or ionic lines.

We present examples of comparison of the detection limits (LOD) obtained by us with analogous characteristics of other methods, presented in recent publications. The LOD for manganese in urine is 2.3 times higher, than in the ETAAS method [4]. The LOD for copper and zinc in blood is, respectively, 2.5 and 86 times lower than in the ICP-AES method [5], and the LOD for cadmium is the same as in the ETAAS method [6].

The LOD obtained by us are low enough to be used in forensic and clinical toxicology, screening investigations in ecologically adverse regions and for sample from workers in unhealthy industries [14].

3.3. Influence of impurities

In the measurements of manganese, cobalt and lead concentrations close to the limits of detection, there no effect from impurities in the materials used, while for nickel and copper in urine and cadmium in blood a certain influence of the impurities on the results of the analysis, increasing the measurements error, was observed. The investigations using extra pure NaCl and KCl showed, that the impurities getting into

Table 1. Comparison of the radiation intensities ($I*10^{-5}$) of the matrix elements and the optical background in the Uw and Uri calibration mixtures and in the prepared US0-US4 and LUMC urine samples analyzed.

sample	$I_b \pm \sigma$ [a] 345.5 nm 32 s	$I_b \pm \sigma$ [a] 341.4 nm 32 s	$I_{Mg} \pm \sigma$ [a] 333.667 nm 48 s	$I_{Ca} \pm \sigma$ [a] 318.127 nm 48 s	$I_K \pm \sigma$ [a] 344.738 nm 40 s
Uw	0.95±0.05	1.22±0.07	0.70±0.04	0.61±0.02	0.48±0.02
US0-US4	0.94±0.05	1.23±0.06	0.86±0.04	0.86±0.04	0.49±0.02
LUMC	0.92±0.02	1.18±0.02	0.83±0.04	0.90±0.06	0.52±0.02
Uri	1.07±0.05	1.31±0.04	0.66±0.05	0.60±0.06	0.82±0.04
US0-US4[b]	0.94±0.04	1.14±0.02	0.85±0.02	0.82±0.04	0.85±0.04
LUMC[b]	0.90±0.02	1.14±0.04	0.80±0.04	0.87±0.06	0.86±0.02

[a] σ – corresponds to one standard deviation based on at least four separate analyses.
[b] Variant of buffering the mixture of graphite powder and KCl (9.2 % K).

Table 2. Comparison of the radiation intensities ($I*10^{-5}$) of the matrix elements and the optical background in the Bd calibration mixtures and in the prepared B0 and B1 blood samples analyzed.

sample	$I_b \pm \sigma$ [a] 228.9 nm 32 s	$I_{Fe} \pm \sigma$ [a] 322.207 nm 32 s	$I_{Mg} \pm \sigma$ [a] 333.667 nm 48 s	$I_{Ca} \pm \sigma$ [a] 318.127 nm 48 s	$I_K \pm \sigma$ [a] 344.738 nm 40 s
Bd	0.16±0.01	1.11±0.04	0.77±0.03	0.55±0.03	0.48±0.03
B0, B1	0.17±0.02	1.04±0.05	0.36±0.02	0.30±0.015	0.43±0.03

[a] σ – corresponds to one standard deviation based on at least four separate analyses.

Table 3. Line selection and LOD for urine.

Lines of element	Wavelength/ nm	LOD (Uw)/ μg l^{-1}	LOD (Uri)/ μg l^{-1}
Cu1	324.754	1.9	2.6
Cu2	327.396	2.5	3.5
Ni	341.476	1.2	2.1
Mn1	403.076	1.4	3.0
Mn2	403.307	2.0	5.0
Co	345.351	1.4	2.3
Co	345.351	1.4	2.3

Table 4. Line selection and LOD for blood.

Element	Wavelength/ nm	LOD/ μg l^{-1}
Cu	223.008	12
Pb	216.999	5.5
Cd	228.802	0.13
Zn	213.856	1.0

the plasma came from the graphite electrodes. This impurity signal is the same for the calibration mixtures and for the samples analyzed and is taken into account automatically in measurements.

3.4. Results of analysis

Two series of measurements of urine sample without additions (US0) and urine sample with additions of Standard Samples of solutions of cobalt, nickel, manganese and copper ions (US1-US4) as well as of a reference sample Lyphochek Urine Metals Control, Level 1 were carried out with each type of Uw and Uri calibration mixtures. Analogously, two series of measurements were carried out with a blood sample without additions and a blood sample with additions of Standard Samples of cadmium, copper, lead and zinc solutions. The average results are presented in Tables 5 and 6.

The results of the measurements of the cobalt, manganese, copper and nickel concentrations in the Lyphochek Urine Metals Control, Level sample correspond to the certified values. The concentration value 4.8 μg l-1 obtained by us with Uw calibration mixtures for cobalt differs insignificantly from the certified values 4.2 μg l-1 (ETAAS technique) and 4.6 μg l-1 (ICP-MS technique) because the range of measured values was 3.4-5.1 and 3.6-5.5 μg l-1, respectively, in the case of the Lyphochek certification.

The percentage measure of correctness for the samples with additions of Standard Samples of ions of solutions of the determined elements changed from 81 % (lead in blood) to 108 % (nickel in urine). This shows that there is no marked differences between the measurements with Uw and Uri calibration mixtures, the method of measurements with Uw calibration mixtures can be preferred only by lower limits of detection (Table 4).

Accuracy of the proposed technique was assessed on the basis of multielement analysis of three Certified Reference Materials. Results are presented in Table 7. For urine measured values for Co, Mn, Cu and Ni agreed well with recommended or certified values across a concentrations range of 2.3 - 40 mg l-1.

Among various spectrometric techniques ICP-MS provides the greatest sensitivity for trace element determination. The ICP-MS technique has an order of magnitude lower LOD than AEMS and improved precision at low concentration values and more appropriate for human biomonitoring of trace elements [12,13]. AEMS is relatively low cost, efficient and allows quick determination of metals in biological fluids for the purposes of forensic and clinical toxicology[14].

4. Conclusion

The technique of simultaneous multielement determination of Cu, Cd, Zn and Pb in blood and Ni, Mn, Co and Cu in urine with the use of an arc-discharge atomic emission spectrometer is desribed. It is believed that judged by the detection limits, the correspondence of the measured concentrations to the reference materials, and the simplicity of preparation

Table 5. Results of measurements of cobalt, nickel, manganese and copper in urine samples with the use of two types of calibration mixtures (Uw and Uri).

Element	Sample	Analysis of Lyphochek/ μg l^{-1}	Added/ μg l^{-1}	Found/ μg l^{-1}	Recovery/ (%)
Co	LUMC	4.6[a], 4.2[b]		4.78	4.43
	US0		0	1.22	<2[c]
	US1		2.00	3.06	2.99
	US2		4.00	5.07	4.91
	US3		6.00	7.34	7.40
	US4		8.00	9.03	8.82
Cu	LUMC	7.0[a], 10.3[b]		8.55	9.47
	US0		0	21.8	20.4
	US1		10.0	31.5	29.5
	US2		15.0	37.6	34.1
	US3		20.0	42.9	39.6
	US4		25.0	46.4	45.8
Mn	LUMC	< 3.5[a]		2.37	2.51
	US0		0	4.93	4.62
	US1		2.00	6.71	6.73
	US2		4.00	8.84	8.75
	US3		10.0	15.0	14.2
	US4		20.0	25.6	25.0
Ni	LUMC	<12[a]		3.84	4.81
	US0		0	<2[c]	<2[c]
	US1		5.00	5.66	5.47
	US2		10.0	10.9	10.1
	US3		15.0	17.2	16.4
	US4		20.0	19.2	19.6

Table 6. Results of measurements of the cadmium, copper, lead and zinc concentrations in whole blood samples.

Element	Sample	Added/ μg l^{-1}	Found/ μg l^{-1}	Recovery (%)
Cd	BS0	0	0.43	
	BS1	2.80	3.04	93
Cu	BS0	0	850	
	BS1	1500	2410	104
Pb	BS0	0	15.1	
	BS1	150	137	81
Zn	BS0	0	3600	
	BS1	7500	11200	101

Table 7. Certified reference values obtained by ETAAS and ICP-MS techniques and results of AEMS determination of the cobalt, nickel, manganese and copper concentrations in urine CRM samples.

Reference materials	Analitycal technique	Reference and measured concentrations μg l^{-1}			
		Co	Mn	Cu	Ni
Lyphochek Urine Metals Control, Level 1	ETAAS	4,6 ± 1,0	7,0 ± 1,8	<3,5	<12
	ICP-MS	4,2 ± 0,9	10,3 ± 2,5	–	–
	AEMS (this work)	4,8 ± 0,9	8,5 ± 1,7	2,4 ± 0,8	1,2 ± 0,4
Seronorm Trace Elements Urine	ETAAS	<0,2		13,0 ± 1,0	2,3 ± 0,7
	AEMS (this work)	<1,4		10,3 ± 0,9	2,5 ± 0,5
Seronorm Trace Elements Urine Blank	ETAAS	9,8 ± 1,0		12,9 ± 1,0	40 ± 1,6
	AEMS (this work)	9,0 ± 0,9		11,7 ± 1,0	44 ± 4

technique can be used for determination of trace elements in human biological samples, in forensic and clinical toxicology, and for screening investigations and mass examinations of population in ecologically adverse regions and of workers in unhealthy industries.

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