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Rapid quantitative determination of metals in blood and liver by FAAS

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A simple method for determination of different metals (K, Na, Ca, Mg, Cr, Mn, Cu, Zn, and Pb) by flame atomic absorption spectrometry (FAAS) in human blood from patients infected with *hepatitis C* has been suggested. Negligible correlations between the concentrations of macro- and microelements in blood samples and their distribution in different patients were found. According to FAAS analysis data, Co, Ni and Cd were not present in the blood samples infected with *hepatitis C* or their concentrations were below the detection limits. The FAAS method was also applied for determination of Cu and Zn in liver samples. The results demonstrated that the analytical procedures could be successfully applied for a rapid and accurate determination of metals in different biological samples.

Key words: metal determination, flame atomic absorption spectrometry, blood analysis, liver analysis

INTRODUCTION

The physiological importance of microelements and macroelements in human organism, especially in blood cells, blood plasma or blood serum, liver and other parts of the body has been shown by many publications. For example, different metals present in the blood can form different complexes with amino acids, fatty acids, albumin, glucose, fibrinogen, salicylate, cholesterol and many other organic compounds and biomolecules which are the main constituents of blood plasma [1–3]. Depending on the concentration of metals in the parts of the body, different metal–ligand equilibria could be established in the system. Consequently, these changes could cause changes in global bioprocesses, or different clinical symptoms and metabolic stresses in a human organism could occur. Also, the variation of microelemts in body fluids such as blood, plasma and saliva could be informative of the genetic health [4].

Changes of selected macro- and microelements in blood plasma depending on age were observed by Lasar et al. [5]. Moreover, significant changes of calcium (from 2.49 to 4.96 mmol/l), magnesium (from 0.86 to 1.53 mmol/l), zinc (from 39.6 to 62.1 μmol/l) and iron (from 25.74 to 9.91 μmol/l) concentrations in the blood of females during different biological periods were determined. Different conditions in preventing the development of changes in blood plasma concentrations of microelements that occur during prolonged restriction of muscular activity have been evaluated [6]. The difference between the hyperhydrated and hypokinetic volunteers was found to be significant with respect to concentrations of microelements in their blood plasma.

At low concentrations, microelements play an important role in metabolism and biological processes as enzyme activators, stabilizers, functional components of proteins, etc. Above trace levels, however, these elements have other roles. It has been stated that for all trace elements that are considered to be essential there exists a fairly narrow "concentration window" between the essential and toxic levels. The toxic doses of microelements and their compounds can lead to serious health problems [7–9].

So, the biological importance of microelements requires their fast and accurate determination in different parts of human body. For determination of microelements, time-consuming extraction–atomic–emission [10], turbidimetric [11] and spectrophotometric methods [12] have been suggested. Flame atomic absorption spectrometry (FAAS) is one of the most

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extensively used techniques for determining various elements with a significant precision and accuracy. This analytical technique is remarkable for its selectivity, speed and fairly low operational cost [13–15]. However, in some cases numerous difficulties arise in determining traces of heavy metals in body fluid samples due to the insufficient sensitivity or matrix interferences. Thus, a preconcentration and/or separation step is necessary [16, 17].

The aim of the present study was to develop and propose a direct determination of micro- and macroelements in blood and liver without the preconcentration of metal ions prior to their FAAS determination. This paper also reports the application of the proposed method for determination of metals in samples from patients infected with *hepatitis C*.

EXPERIMENTAL

The content of metals in the blood from patients infected by *hepatitis C* and liver samples was determined by the flame atomic absorption spectroscopic method (FAAS, Hitachi 170–50). The instrumental parameters were adjusted according to the manufacturer's recommendations. The following conditions for metal determination by the FAAS method were used: (i) absorption line (766.5 nm (K), 589.0 nm (Na), 422.7 nm (Ca), 285.2 nm (Mg), 357.9 nm (Cr), 279.5 nm (Mn), 240.7 nm (Co), 232.0 nm (Ni), 324.8 nm (Cu), 213.8 nm (Zn), 228.8 nm (Cd), and 288.3 nm (Pb)); (ii) electric current of the lamp (15 mA (K, Ca, Cr, Co), and 10 mA (Na, Mg, Mn, Ni, Cu, Zn, Cd, Pb)); (iii) flame (propane-butane (K, Na, Mn, Co, Cu, Cd, Pb), and acetylene (Ca, Mg, Cr, Ni, Zn)); (iv) gas pressure ($9.81 \cdot 10^7$ Pa (K, Co, Cu, Cd), $1.47 \cdot 10^6$ Pa (Na), $2.94 \cdot 10^6$ Pa (Ca, Cr, Mn), $2.45 \cdot 10^6$ Pa (Mg, Zn), $3.43 \cdot 10^6$ Pa (Ni), and $7.35 \cdot 10^7$ Pa (Pb)). The pressure of air ($1.47 \cdot 10^7$ Pa) was the same during FAAS determination of all elements.

Double-distilled water and analytical-grade reagents were used for the preparation of stock standard solutions of metals, which were used to obtain calibration solutions by dilution. The calibration graphs for metals were found to be linear within the concentration ranges from 0.05 to 0.60 μg ml^{-1} for K, from 0.02 to 0.30 μg ml^{-1} for Na, from 0.5 to 8.0 μg ml^{-1} for Ca, from 0.05 to 0.4 μg ml^{-1} for Mg, from 0.25 to 6.0 μg ml^{-1} for Cr, from 0.1 to 4.0 μg ml^{-1} for Mn, from 0.2 to 2.0 μg ml^{-1} for Co, from 0.25 to 4.0 μg ml^{-1} for Ni, from 0.05 to 3.0 μg ml^{-1} for Cu, from 0.05 to 0.7 μg ml^{-1} for Zn, from 0.025 to 0.4 μg ml^{-1} for Cd, and from 0.1 to 4.0 μg ml^{-1} for Pb, with the detection limits of 0.02 μg ml^{-1} for K, 0.01 μg ml^{-1} for Na, 0.2 μg ml^{-1} for Ca, 0.02 μg ml^{-1} for Mg, 0.1 μg ml^{-1} for Cr, 0.05 μg ml^{-1} for Mn, 0.06 μg ml^{-1} for Co, 0.1 μg ml^{-1} for Ni, 0.02 μg ml^{-1} for Cu, 0.02 μg ml^{-1} for Zn, 0.01 μg ml^{-1} for Cd, and 0.04 μg ml^{-1} for Pb.

For the determination of metals in blood and liver samples, the specimens were burnt in the ordinary furnace at 800 °C. The obtained residuals were dissolved in 10 ml of nitric acid (1:1), transferred into a 25-ml volumetric flask and diluted with double-distilled water.

RESULTS AND DISCUSSION

The blood samples were taken from nine volunteer patients infected with *hepatitis C*. In order to avoid any psychological stress or undesired reaction the reader could have after reading this article, we do not elaborate here the severity and peculiarities of *hepatitis C* infection or how critical is the condition of each separate patient. The content of 12 metals in the blood specimens were found to be higher than the detection limits. Therefore, the determination of metals was performed directly without any preconcentration. Figure 1 shows the results of determination of two metals (K and Na) the concentrations of which in the blood are usually higher to compare with other elements. Evidently. The content of sodium was higher than of potassium in all samples. However, the levels of these metals did not vary much in the blood of different patients. A somewhat higher level of sodium was found in the blood sample from the 7th patient (3.3 mg/g). In the other samples the level of Na change varied from 2.3 to 2.9 mg/g. A similar situation was observed also for the distribution of potassium in the blood samples from different patients. The lowest level of potassium was determined in the blood sample from the 5th patient (1.3 mg/g). In the other samples the amount of K ranged from 1.7 to 2.1 mg/g.

The results regarding the distribution of two earth alkaline metals (Ca and Mg) in the blood samples from *hepatitis C* patients are shown in Fig. 2. One can see that the content of calcium slightly prevails over the content of magnesium in most of the sam-

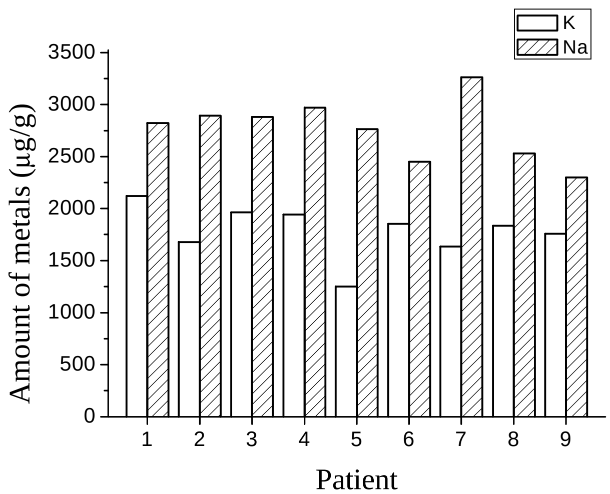

Fig. 1. Distribution of K and Na in blood samples from different patients

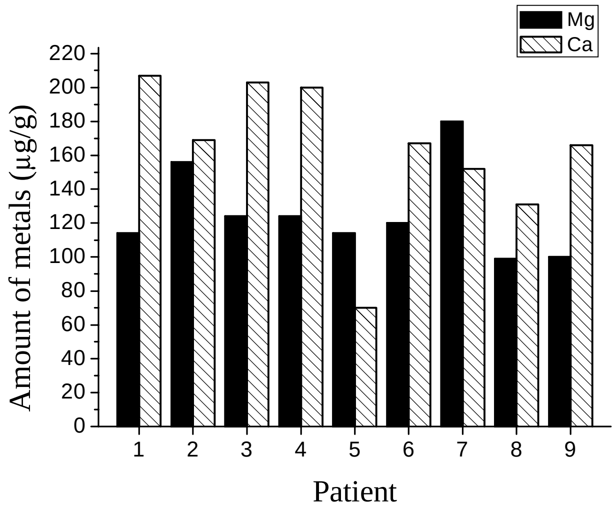

Fig. 2. Distribution of Ca and Mg in blood samples from different patients

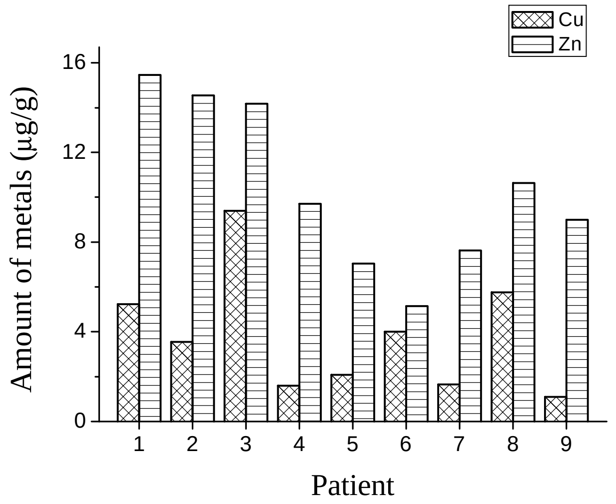

Fig. 3. Distribution of Cu and Zn in blood samples from different patients

ples, except the blood sample from the 7th patient. In this sample, the level of magnesium determined by FAAS was higher than of calcium. Moreover, like the level of sodium, the highest level of magnesium (0.18 mg/g) was found also in the same sample from the 7th patient. In the other samples the content of Mg varied from 0.10 to 0.15 mg/g. Surprisingly, the lowest level of calcium was found in the blood sample from the 5th patient (0.07 mg/g), whereas the interval of variation of calcium concentration in the other samples was 0.13–0.21 mg/g. Thus, the level of calcium in the sample from the 5th patient was 2–3 times lower than in the other blood samples. The low concentrations of K^+, Mg^{2+} and Ca^{2+} in the blood sample from the 5th patient were probably caused by the illness of the patient, which could influence the dehydration and osmosis processes and consequently promote the progress of disease.

The distribution of six transition elements from the first series (chromium, manganese, cobalt, nickel, copper and zinc) was also checked in the same samples. Interestingly, according to FAAS analysis data Co and Ni were not present in the *hepatitis C* patient blood samples or their concentrations were below the detection limits. The concentrations of very important two biological elements, Cu and Zn, in the blood samples were found to be rather comparable (Fig. 3). The concentration of zinc varied from 5.1 to 15.5 μg/g and of copper from 1.1 to 9.4 μg/g. In general, the blood samples contained more Zn than Cu. On the other hand, the level of zinc in the sample from the 6th patient (5.1 μg/g) was lower than that of copper in the sample from the 8th (5.8 μg/g) or 3rd (9.4 μg/g) patient. However, for each patient separately, the content of zinc was higher than that of copper in all samples. It is interesting to mention that we did not find any traces of cadmium none of the nine blood samples. On the other hand, Cd has bio-behaviour and properties very similar to those of Zn.

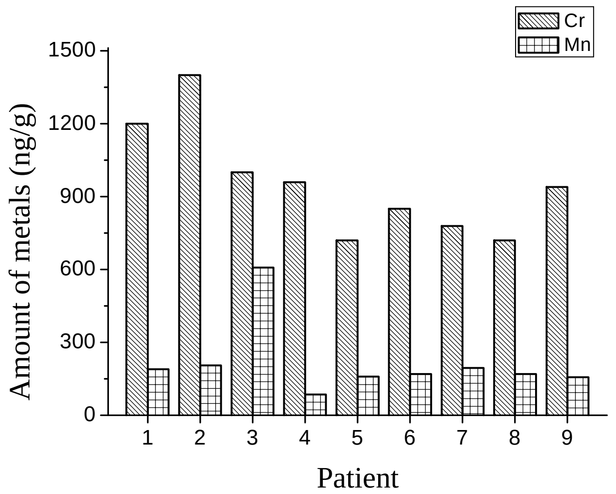

Fig. 4. Distribution of Cr and Mn in blood samples from different patients

Table. **Cu and Zn levels in liver samples from two volunteer patients**

Patient	Copper found by FAAS method (μg/g)	Zinc found by FAAS method (μg/g)
1	558.8	544.0
2	19.6	20.3

The distribution of two other transition elements from the first series, chromium and manganese, is presented by a diagram in Fig. 4. The concentrations of these two elements are significantly lower in the blood samples than the concentrations of copper and zinc. Moreover, the chromium content (0.72–1.4 μg/g) found in the specimens was higher in comparison with manganese content (0.086–0.609 μg/g) in all cases. An exceptionally low level of manganese (0.086 μg/g) was determined in the sample from the 4th patient. From the results presented in Figs. 3 and 4 we can conclude that the concentrations of all heavy elements (Cr, Mn, Cu and Zn) were highest in the blood samples from the first three patients (Nos. 1, 2 and 3) infected with *hepatitis C*. The other six samples contained relatively smaller concentrations of chromium, manganese, copper and zinc. Of course, it is difficult to state after analysis of blood only from nine patients that the concentration of these elements in the blood could be a signal of the seriousness and depth of the disease. However, the initial observations show such a tendency.

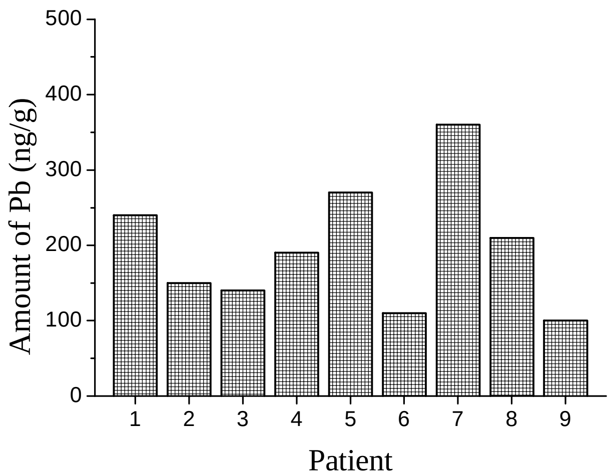

Fig. 5. Distribution of Pb in blood samples from different patients

The concentration of one more microelement (lead) was also checked in the blood samples (Fig. 5). The amount of Pb varied from 0.1 to 0.36 μg/g, and distributed almost evenly in all samples. The variations of lead concentration in blood and infection with *hepatitis C* seem to be not connected to each other. On the other hand, this conclusion should be supported by analysis of much more blood specimens from patients non-infected and infected with *hepatitis C*.

Direct determination of two microelements (Cu and Zn) in liver samples by the FAAS method was also performed. An exceptionally small amount of liver from biopsy was taken for different analytical purposes. Therefore, only two microelements were possible to select for quantitative determination. Copper and zinc are very important for the functioning of all organs of living organisms [6, 18–20], including the liver [21–23]. The content of these two metals in liver samples was determined by flame atomic absorption spectrometry. Two liver specimens from different volunteer patients were chosen for the analysis. Again, only because of the ethical considerations we do not elaborate whether the samples belong to seriously sick or more or less healthy patients. The results of Cu and Zn determination in liver samples are presented in Table. One liver sample contained an about 20–30 times higher level of these two microelements. Besides, the concentration of Cu prevailed in one liver sample, and the concentration of Zn predominated in another one. The results showed a possible variation of copper and zinc concentrations in differently affected liver. However, medical conclusions could be made only after a careful and systematic investigation of numerous patients. Nevertheless, in-depth characterization of the biological influence of microelements on the function of liver could be done using the results from rapid and accurate FAAS determination of the concentrations of metals in these samples.

CONCLUSIONS

The content of 12 metals in the blood samples from nine volunteer patients infected with *hepatitis C* was checked by flame atomic absorption spectrometry (FAAS). The determination of metals was performed directly without any preconcentration. The concentrations of nine elements (K, Na, Ca, Mg, Cr, Mn, Cu, Zn, and Pb) were found to be higher than the detection limits. However, no traces of Co, Ni, and Cd were found in either of the nine blood samples. The lower concentrations of K^+, Mg^{2+} and Ca^{2+} were detected only in the blood sample from one patient, whereas incidental distribution of Na^+ or Pb^{2+} was observed in all patients. The concentrations of transition metals (Cr, Mn, Cu and Zn) were highest in the blood samples from the first three patients infected with *hepatitis C*. Also, direct determination of two microelements (Cu and Zn) in liver samples by the FAAS method was performed. In conclusion, the proposed method is characterized by a low analytical cost, fairly easy operation, simplicity and a short time required for analysis. Thus, the proposed analytical methodology offers a simple and sensitive way for routine application in the analysis of biological samples.

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GREITAS KIEKYBINIS METALØ NUSTATYMAS KRAUJYJE BEI KEPENYSE FAAS METODU

S a n t r a u k a

FAAS metodas buvo taikomas ávairiø metalø (K, Na, Ca, Mg, Cr, Mn, Cu, Zn ir Pb) kiekiams þmoniø, infekuotø hepatito C virusu, kraujyje nustatyti. Tarp makroelementø ir mikroelementø kiekiø kraujo pavyzdþiuose ir jø pasiskirstymo skirtinguose ligoniuose pastebëtos neþymios priklausomybës. Be to, buvo nustatyta, kad þmoniø, infekuotø hepatito C virusu, kraujyje Co, Ni ir Cd nëra arba ðiø metalø koncentracijos yra þemesnës uþ jø aptikimo ribas. Taip pat FAAS metodas buvo taikomas greitai nustatyti varį ir cinkà ligoniø kepenø biopsiniuose ëminiuose. Gauti rezultatai leidþia padaryti iðvadà, kad FAAS metodas gali bûti sëkmingai taikomas greitai ir tiksliai nustatant metalus ávairiuose biologiniuose pavyzdþiuose.