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Zeeman graphite furnace atomic absorption spectrophotometric determination of eight life elements in human blood samples

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Abstract—A direct and simple method for the clinic muti-element determination of human blood sample by Zeeman graphite furnace atomic absorption spectrophotometry is reported. No pretreatment of the sample is necessary other than simple dilution of the sample with aqueous solution of Triton X–100. The detection limits of the elements investigated fall in the range of 0.1– 4.4 μ g/L with a relative standard deviation ranging from 1.6 for zinc to 3.73% for lead. Micro liters of samples are sufficient for the determination of each element.

Keywords: multi-element; micro-sized; human blood; Zeeman AAS.

It is difficult to directly analyze trace elements in biological fluids with conventional methods due to their extremely low concentration in blood sample (Cd, Cr, Mn) (Versieck, 1978); loss at ashing temperatures (Se, Zn); ubiquitous nature of elements (Mn, Zn) (Küpulainen, 1980) which would increase the likelihood of severe contamination in sampling and sample pretreatment, if they are not carried out with great care (Kümpulainen, 1980; Cui, 1987). The present work reports a direct method for the determination of Cr, Cd, Cu Zn, Se, Mn, Pb and Mg utilizing Zeeman effect graphite furnace atomic absorption spectrophotometry (ZGFAAS). No pretreatment is required other than simple dilution of sample with 0.2% aqueous solution of Triton X-100. The matrix modifiers which have undergone strict purification processes to avoid further contamination are added to eliminate severe interferences only when they cannot effectively be corrected by Zeeman effect. Blood sample with a maximum size of 50 μl is sufficient for the ultratrace analysis of the eight life elements investigated. The procedure proposed here can be directly used for the clinically diagnostic purposes.

EXPERIMENTAL

Instrumentation and reagents

A Perkin-Elmer AAS unit model Zeeman 5000 has been used. It was equipped with HGA-500 graphite furnace and programmer, PE As-40 autosampler and model 3600 data station. Hollow cathode lamps of Cu, Zn Mg, Cr, Cd, Mn and electrodeless discharge lamps of Pb and Se from PE were used for the rediation sources.

Chemicals used consisted of nitric acid (Superpure 65% Alfa 87920), SeO_2 (Reagent-grade Merck 9915), Triton X-100 (KOSO Chem. Co.), and other analytical reagents such as CuO, CdO, MgO, ZnO, $MnSO_4 \cdot H_2O$, $K_2Cr_2O_7$, $MgNO_3 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$ and NH_4NO_3 which were obtained as home-made products. The NH_4NO_3 was undergone further purification by recrystallization for three times at our laboratory. Deionized water undergone distillation twice with quartz apparatus was used for the experiment.

Sampling and preparation of blood and serum samples

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Vein-blood was sampled with a disposal polyvinyl syringe and stored in a capped centrifugal quartz tube. Serum was prepared by centrifugation of the whole blood sample after standing for 1 hr at 36°C and stored in polyvinyl capped bottle. The serum and heparinized whole blood samples were kept at 4°C for use, and the samples were not allowed to be kept longer than 24 h before they were diluted with triton X-100 and followed by Zeeman AAS measurement. L'vov platforms from Perkin-Elmer were used in the subsequent determination of the life elements including Zn, Cr, Mg, Pb, Cd and Se, Moreover, Zr-coated and pyrocoated tubes were used for Mn and Cu due to their particular behavior in the vaporization from the graphite surface. Great care has been thoroughly taken against any possible contamination and the whole part of the research section has been dust-proof.

RESULTS AND DISCUSSION

Results obtained in this study of the direct determination of eight life elements are summarized in Table 1, which indicates the normal values averaged from 60 healthy volunteers with comparison to that obtained from patients of cardiomyopathy (myocaridal infarction) of 31 subjects. The sensitivity, accuracy, detection limits and some other data can also be found in the table. Table 2 shows the microsize of a sample for each element ranging from 5 μl to 50 μl and the corresponding linearity of the detections.

Important considerations for the accuracy and reliability have drawn the attention of the authors. The first is in making successful measurement at ppb or subppb levels without interference from the complex matrix. The second is in avoiding contamination in the collection and pretreatment of blood or serum samples.

Table 1 Sensitivity, accuracy, detection limits and blood levels

Element	Pb	Mn	Cr	Cd	Cu	Zn	Mg	Se
Relative Stand. deviation, %	3.73	2.70	3.54	3.14	2.76	1.60	1.88	≤ 0
Detection limits, 2 μg/L	0.76	0.10	0.15	0.07	2.72	4.40	0.62	≤ 0
Characteristic mass, pg/0.0044 AS	37	2.82	9.24	0.98*	8.35	6.57	12.5	43.2
Recovery, %	100–102	90–91	92–103	93–100	99–102	97–99	97–103	98–99
Normal Value, $n = 60$	122.7 (μg/L)	4.07 (μg/L)	5.61 (μg/L)	1.52 (μg/L)	0.96 (mg/L)	1.13 (mg/L)	4.19 (mg%)	78.12 (μ/L)
Patient	148.6	9.07	14.92	1.25	1.19	1.26	3.06	63.05

*The characteristic mass for which is pg/0.0044 A

Table 2 Sample volume required for trace element analysis

Element	Sample volume	Times of dilution	Matrix modifier in diluent
Pb	10 μl whole blood	11	0.1 mol/L NH_4NO_3 0.1% $Mg(NO_3)_2$ in 0.2% w/v Triton X-100
Cu Zn	5μl serum	21–31	same as above
Cr Mn Cd	150 μl serum	2	0.2% Triton X-100
Se	50 μl serum	3	0.5 g Fe/L 1.15g Cu/L in 0.2% Triton X-100
Mg	5 μl serum	505	0.1 mol/L NH_4NO_3 in 0.2% Triton X-100

Elimination of interferences

Due to severe interferences from both organic and inorganic components in serum and blood matrix, procedures to eliminate such interferences (Lewis, 1985) become critical. In addition to the utilization of L'vov platform and Zeeman effect background correction, matrix modifiers have carefully been used for the determination of each element (Table 2). Chlorides were known to interfere seriously the determination of Pb and Cu (Bertenshaw, 1981; L'vov, 1981; Slavin, 1980), the addition of ammonium nitrate to the samples will minimize the interferences from chlorides due to the formation of volatile ammonium chloride. Serum samples with less than 2 ppb manganese which was encountered in most cases, can not simply be determined with L'vov platform due to the severe interferences from the elements co-existing in samples. In view of some possible hindrances in the vaporization of manganese in serum matrix from graphite surface by reaction with the surface (Fritzsche, 1979), we used Zr-coated tube instead of L'vov platform and recorded the signals by peak area mode achieving a direct determination of serum Mn successfully. The atomization of Cd from the platform often presented signals with several maxima which was also reported by some auther. This effect, according to L'vov macrokinetic theory (L'vov, 1981), is possibly associated with the mechanism for vaporization of the analyte from the two different regions (the K–and QK–regions). Ideal furnace conditions for eliminating chemical interferences are only given when samples are atomized into a thermal equilibrium environment, as under such conditions recombination of free atoms to molecules or loss of free atoms will be effectively avoided. On the basis of such consideration, we have circumvented this effect by applying a multi-stage heating program at drying and ashing steps with a long ramping time together with the applying of L'vov platform (Table 3) so as to remove as much matrix and chemical interferences as possible with a negligible loss of the analyte elements leading to a marked improvement in the accuracy and sensitivity.

Hydride-forming technique has usually been used for the analysis of traces of selenium in biological samples. It, however, can not be adopted for our direct procedure. Moreover, selenium was well known susceptible to loss in wet digestion or even possibly escaping during the drying step from the furnace in AAS techniques (Verlinden, 1981), and this is the important reason why in this paper a method for direct determination of serum Se has been designed instead of adopting the hydride technique to avoid both contamination and loss. By the use of graphite furnace AAS technique, Welz (Lewis, 1984) suggested to use Cu and Mg $(NO_3)_2$ as the matrix modifiers for selenium determination in an attempt to elevate the ashing temperature. We, however, added Fe instead of Cu as the matrix modifier to obtain an ashing temperature as high as 1000°C without appreciable loss of the analyte when L'vov platform was used with a desirable linearity 0-200 ppb.

Addition of aqueous solution of 0.2% Triton X-100 to serum or blood samples seems important as the reagent may prevent the furnace and platform from carbon deposition onto graphite surface during the ashing and atomization steps (Lewis, 1984).

Protection against contamination

It was well-known that the ubiquitous nature of some elements such as zinc and magnesium often causes severe contamination in the electrothermal atomic adsorption spectrophotometric determination. Steps against contamination have been taken as follows throughout the entire study:

The whole section of the research laboratory has been dustproof.

Critical reagents of ultrapurity have been used.

Modification in the procedure for the pretreatment of samples by simply diluting with appropriate solution instead of the wet digestion or solvent extraction.

Laboratory utensils of polyvinyl, polypropylene and quartz including the disposal tips of the Eppendorf micropipets were used in collecting, handling and measuring of the samples after a severe treatment by soaking them in 10% nitric acid for at least 72 hr before transferring the implements to another capped container of nitric acid for the second soaking. They were

thoroughly rinsed with water of high pirity immediately before use.

CONCLUSION

Method developed in this paper requires only micro-sized blood and serum sample for direct and simultaneous determinations of the same diluted sample for Cu and Zn, and for Mn, Cr and Cd. Under the conditions (Table 3 and Table 4) described in the present work, the ratio of curve slope of standard additions to that of standard solution is closely to be unity for Cu, Zn, Mn, Cr and Mg which allows a rapid determination and a practical procedure for clinical purposes.

Table 3 Furnace temperature programs

Setp		Element								
		Cu	Mn	Zn	Cr	Mg	Pb	Se	Cd	
Drying	Temperature, °C	110	110	110	110	110	110	110	80	130
	Ramp time, s	10	10	10	10	10	10	10	10	10
	Hold time, s	20	35	15	20	20	20	40	60	40
Ashing	Temperature, °C	800	1100	700	1100	1200	700	1000	300	450
	Ramp time, s	10	20	15	20	10	10	20	10	5
	Hold time, s	20	20	15	20	20	20	20	40	30
Atomizing	Temperature, °C	2550	2400	2100	2400	1700	2200	2100	1400	
	Ramp time, s	0	0	0	0	0	0	0	0	
	Hold time, s	5	2	3	4	3	3	3	1	
	Read	-1	-1	-1	-1	-1	-1	-1	-1	
	REC	-5	-3	-2	-3	-3	-3	-3	-3	
	Internal Ar flow, ml/min	20	0	200	0	300	0	0	0	
Cleaning	Temperature, °C	2600	2600	2500	2600	2500	2500	2600	2600	
	Ramp time, s	1	1	1	1	1	1	1	1	
	Hold time, s	3	3	2	3	3	3	3	3	

The internal argon flow rate for drying and ashing steps was kept at 300 ml/ min

Table 4 Instrument parameters

Element	Wavelength	Slitwidth	Current	Type of tube	Linearity	Mode of Calibration
Cu	324.8 nm	L 0.7	6 mA	Pyrocoated	0-100ppb	Working curve
Mn	279.5 nm	L 0.2	10 mA	Zr-coated	0-6ppb	Working curve
Zn	213.9 nm	L 0.7	5 mA	Platform	0-100ppb	Working curve
Cr	357.9 nm	L 0.7	25 mA	Platform	0-40ppb	Working curve
Mg	285.2 nm	L 0.7	12 mA	Platform	0-120ppb	Working curve
Pb	283.3 nm	L 0.7	10 w	Platform	0-150ppb	Standard additions
Se	196 nm	L 0.2	6 w	Platform	0-200ppb	Standard additions
Cd	228.8 nm	L 0.7	4 mA	Platform	0-4ppb	Standard additions

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