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DETERMINATION OF MANGANESE IN BLOOD SERUM BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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Abstract: A method for determination of manganese in blood serum by electrothermal atomic absorption spectrometry was developed. The preparation of the samples was done by deproteinization with different acids: trichloroacetic, sulfosalicic, perchloric and phosphomolibdenic, and some salts: sodium volframate, ammonium molibdate and uranyl acetate. The procedure was chequed by method of standard addition. The optimal instrumental conditions were established by extensive testing and they are: Dry: 100 °C, 30 s; Char: 700 °C, 60 s; Atomize: 2200 °C, 5 s. The values obtained from analyses of manganese in sera of 20 normal subjects, using this method, were between 4.2–9.3 $\mu g \cdot dm^{-3}$ (main value 7.3 $\mu g \cdot dm^{-3}$). The relative standard deviation of the method is from 1.63 to 4.14% for the range of 1 to 10 $\mu g \cdot dm^{-3}$ manganese in the serum. The limit of detection for this method is 0.1 $\mu g \cdot dm^{-3}$.

Introduction

Manganese is an essential element, but manganese deficiency has not been found in the general population. Excessive exposure through inhalation has been shown to cause effects on the lumps. Increased incidences of pneumonia and bronchitis have been found in population living near manganese-emitting industry. Excessive absorption in the lungs will cause accumulation in the brain, so manganese causes an irreversible brain disease [1]. From this, it can be seen that the determination of manganese is important in monitoring occupational exposure to this metal.

Most researchers have employed either neutron activation analysis or atomic absorption spectrometry. Usually, methods involving electrothermal atomic absorption spectrometry have given, higher value for normal levels than the work based on neutron activation analysis. The normal concentrations of manganese in human serum are reported to be 0.6–24 $\mu g \cdot cm^3$ either obtained by neutron activation analysis [2–4] or electrothermal atomic absorption spectrometry (ETAAS) [5–11].

The aim in the work described here was to suggest simple method for determination of manganese in serum by deprotenization with various reagents and by using ETAAS.

Experimental

Instrumentation

A Perkin-Elmer Model 703 atomic absorption spectrophotometer equipped with deuterium background corrector, an HGA-400 graphite furnace, and a Model 056 strip-chart recorder was used. A manganese cathode lamp was applied throughout the course of analysis.

Operation conditions for manganese determination were established by extensive testing and are given in Table I. Fig. 1 depicts the effect of charring temperature on the absorbance of the manganese in serum sample with addition of 100 ng Mn in 1 cm^3. The atomizing temperature of 2200 °C was found to produce acceptable absorbance (Fig. 2) and the 5-s time period was long enough to allow complete return of signal to baseline value.

Table I

Optimal instrumental conditions for manganese determination by electrothermal atomic absorption spectrometry

Wavelength		279.5 nm
Spectral slit		0.2 nm
Calibration mode		Peak height
Lamp current		20 mA
	Dry	
Temperature		100 °C
Time		30 s
Ramp time		2 s
	Char	
Temperature		700 °C
Time		60 s
Ramp time		1 s
	Atomize	
Temperature		2200 °C
Time		5 s
Ramp time		0 s
	Clean	
Temperature		2700 °C
Time		3 s
Internal gas		Argon

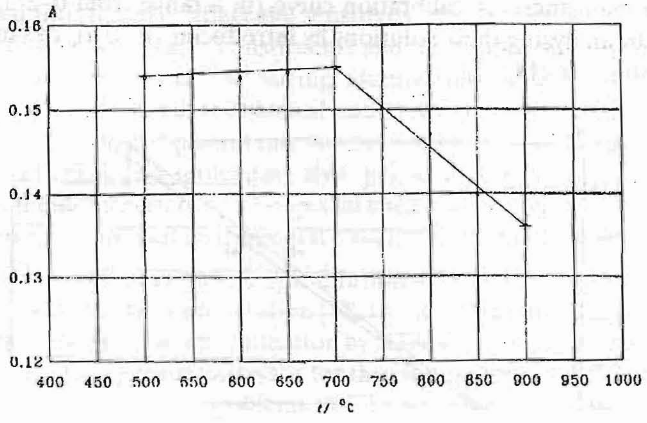

Fig. 1 – Determination of charring temperature

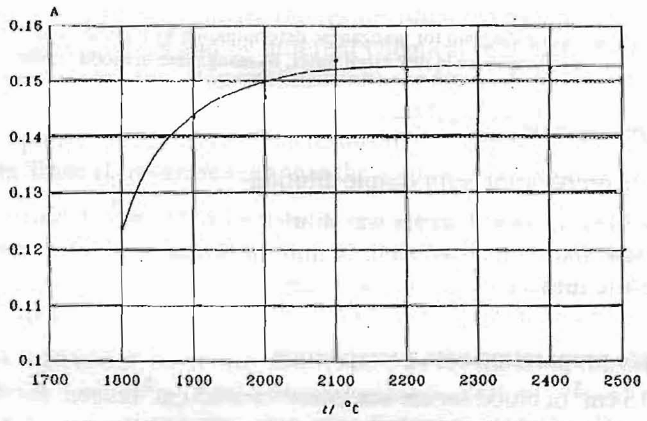

Fig. 2 – Determination of atomizing temperature

Reagents and samples

All reagents and standards were of analytical grade. Stock solution with concentration of 1000 mg$\cdot$dm^{-3} was prepared by dissolving of appropriate manganese concentrated solution. The other diluted solutions were prepared from this solution. Working standards containing 0, 10, 20, 50, 100 and 200 μg$\cdot$dm^{-3} manganese in blood serum were prepared from a stock blood serum sample obtained by venepuncture from healthy adults occupationally not exposed to manganese. A calibration curve (in a range from 0–2 ng) was constructed by analyzing these solutions by introducing of 20 μL of samples into a graphite furnace (Fig. 3).

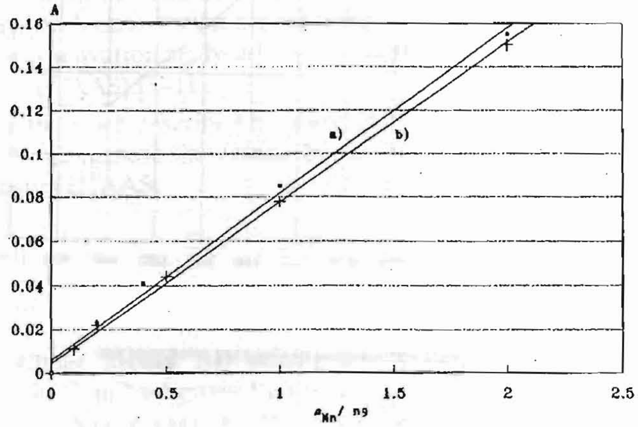

Fig. 3 – Calibration diagram for manganese determination by ETAAS (standard addition) a) manganese in deionized water, b) manganese in blood serum

Sample preparation

a) Sample preparation with simple dilution

0.5 cm^3 of blood serum was diluted with 0.5 cm^3 of deionized water in plastic tube and then mixed well. 20 mm^3 of this mixture were introduced into the graphite furnace.

b) Sample preparation with precipitation

0.5 cm^3 of blood serum was mixed with 0.5 cm^3 reagent for deproteinization. As a reagent it is possible to use: 2% sodium volframate, 1% ammonium molibdate, 2% sulphosalycilic acid, 0.31 mol·dm^{-3} trichloroacetic acid, 1 mol·dm^{-3} perchloric acid, 1.6% uranyl acetate or 4 % phosphomolibdenic acid. 20 mm^3 of the supernatant were introduced into the graphite furnace.

Results and discussion

Different type of interferences (physical, chemical and spectral) affect the determination of metals in serum by electrothermal atomic absorption spectrometry [12]. Physical interferences can effect the accuracy and precision of dispensing micro volumes of sample solution into the graphite tube and degree to which these solutions will spread along and diffuse into the tube wall.

Chemical interferences affect the sensitivity of these determinations with enhancement by the organic constituents and the suppression by inorganic constituents of matrix present during atomization. These interferences will depend from the adequate chemical and thermal pretreatment of the sample prior to atomization. Spectral interferences are expressed by light scattering of source radiation and molecular absorption. It is possible to eliminate, or reduce all these interferences by special chemical pretreatment of the samples and by using optimal instrumental conditions for manganese determination.

Most authors suggest simple dilution with destilled water (1+1) or with Triton x 100 for sample preparation [11, 13, 14, 15] or direct introducing [9, 10] of serum in manganese determination by ETAAS. In this case, it is most important to use a background corrector for the eliminations of different interferences. One of the frequent problems in ETAAS manganese determination is to complete ashing of the samples introduced into a graphite furnace. For this reason, it is very convenient to perform a deproteinization of the samples for releasing the metal from serum proteins. Some authors use trichloracetic acid [16, 17] or mineral acids [18, 19].

In our case, we compare the results obtained by simple dilution preparation of sera samples and by different chemical treatment by different solutions, especially for deproteinization: sodium volframate, ammonium molibdate, sulfosalicic acid, trichloracetic acid, perchloric acid, uranyl acetate and phosphomolibdenic acid. The results of the standard addition method are shown in Table II. It can be seen that the results are very satisfactory.

All results are obtained by using a deuterium background corrector with optimal instrumental condition given in Table I. Charring temperature is found to be 700°C for a longer time of 60 s. As it can be seen from the Table II this temperature is sufficient for complete ashing of the samples.

A calibration curve was constructed by results from the serum samples with standard addition of manganese standard solution. The calibration curve was constructed for samples containing from 0 to 2 ng Mn. The relative standard deviation for samples with concentration of 0.2 to 4 $\mu g \cdot dm^{-3}$ is from 1.63 to 4.14%. Applying the 3 SD detection limit rule a detection limit on order of 0.1 $\mu g \cdot dm^{-3}$.

Our results of main values of normal manganese concentration in serum are between 4 and 9 $\mu g \cdot dm^{-3}$ with mean value of 7.3 $\mu g \cdot dm^{-3}$. This concentration is higher than most of the results given in the literature, Table III. The manganese concentration in serum has been a most controversial topic in trace metal analysis. As it can be seen in Table III, there is great variation in the mean values, namely from 0.54 to 24 $\mu g \cdot dm^{-3}$. Some authors pay attention to a possibility of contamination in the procedure for sample collection. Versiek et al. [35, 36] found that stainless steel needles can introduce considerable contamination when used to collect biological samples. This contamination is a reason for obtaining higher results.

Table II

Results of the manganese determination in the samples with simple dilution and precipitation obtained by standard addition

Sample preparation	$Mn_{added}/\mu g \cdot dm^{-3}$	$Mn_{found}/\mu g \cdot dm^{-3}$	Recovery %
SIMPLE DILUTION	5.0	5.0	100.0
	10.0	9.8	98.0
	20.0	19.9	99.5
PRECIPITATION WITH:			
a) 2 % Sodium volframate	5.0	5.0	100.0
	10.0	10.2	102.0
	20.0	20.1	100.5
b) 1% Ammonium molibdate			
	5.0	4.8	96.0
	10.0	9.8	98.0
	20.0	19.9	99.5
c) 2% Sulfosalycilic acid			
	5.0	4.9	98.0
	10.0	10.1	101.0
	20.0	20.0	100.0
d) 5% Trichloroacetic acid			
	5.0	4.9	98.0
	10.0	10.1	101.0
	20.0	19.9	99.5
e) 10% Perchloric acid			
	5.0	4.9	98.0
	10.0	10.2	102.0
	20.0	20.2	101.0
f) 1.6% Uracil acetate			
	5.0	4.9	98.0
	10.0	10.2	102.0
	20.0	20.1	100.5
g) 4% Phosphomolibdenic acid			
	5.0	5.0	100.0
	10.0	10.2	102.0
	20.0	20.0	100.0

Table III

Reported normal manganese concentration in serum

Authors and year	Analytical method	Mean $\mu g \cdot dm^{-3}$	s.d. $\mu g \cdot dm^{-3}$	Range $\mu g \cdot dm^{-3}$	Number of subjects	Ref.
Uchida and Vallee, 1986	ETAAS	0.52	0.02	–	–	13
Damsgaard et al., 1973	NAA	0.54	0.16	0.36–0.78	11	20
Versieck et al., 1974	NAA	0.57	0.13	0.38–1.04	46	21
Halls and Fell, 1981	ETAAS	0.58	0.04	0.36–0.96	–	11
Cotzias et al., 1966	NAA	0.59	0.18	–	14	2
D'Amico and Klawans, 1976	ETAAS	1.02	0.19	0.74–1.25	19	9
Pleban and Pearson, 1979	ETAAS	1.82	–	0.94–2.92	–	10
Grafflage et al., 1974	ETAAS	1.94	–	0.50–7.87	50	6
Papavasiliu and Cotzias, 1961	AAS	2.50	–	2.05–2.97	16	22
Banta and Markesberg, 1977	ETAAS	–	–	2.1–3.1	–	23
Favier et al., 1982	ETAAS	1.17	–	–	–	24
Sirisena et al., 1986	ETAAS, NAA	1.47	0.5	–	109	25
Tsai et al., 1986	ETAAS	4.2	0.2	0.3–11.2	36	26
Olehy et al., 1966	NAA	4.3	0.5	–	12	27
Sullivan et al., 1966	NAA	6	4	–	37	28
Bayer, 1986	ETAAS	8.43	–	–	–	14
Wei et al., 1982	ETAAS	8.87	–	4.0–19.3	27	29
Muzzarelli and Roccetti, 1975	ETAAS	9.0	4.3	5 –16	9	8
Bowen, 1956	NAA	9.7	4.0	–	–	30
Alt and Massman, 1976	ETAAS	–	–	10–20	–	31
Hwang et al., 1972	ETAAS	11.0	–	–	–	32
Kanabrocki et al., 1967	NAA	13	–	11–14	–	3
Bek et al., 1974	ETAAS	13.4	–	6.5–18	–	5
Ross and Gonzales, 1974	ETAAS	21.8	–	8.5–40.5	–	7
Salvadeo et al., 1979	ETAAS	23.0	5.0	–	12	33
Mahoney et al., 1969	AAS	24	7	12–38	40	34
This work	ETAAS	7.3	–	4.2–9.3	20	–

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Резиме

ОПРЕДЕЛУВАЊЕ НА МАНГАН ВО СЕРУМ ОД КРВ СО ЕЛЕКТРОТЕРМИЧКА АТОМСКА АПСОРПЦИОНА СПЕКТРОМЕТРИЈА

Трајче Стафилов и Весна Ризова

Предложен е метод за определување на манган во серум од крв со помош на електротермичката атомска апсорпциона спектрометрија. Подготвувањето на пробите се врши со претходна депротеинизација со различни киселини: трихлороцетна, сулфосалицилна, перхлорна и фосфомолибденска, и со различни соли: натриум волфрамат, амониум молибдат и уранил ацетат. Постапката беше проверена со методот на стандардни адиции. Оптималните инструментални услови за определувањето на манганот со графитната печка се: сушење: 100 °C и 30 s; жарење: 700 °C и 60 s; атомизација: 2200 °C и 5 s. Со примена на овој метод е извршено определување на концентрацијата на манганот во серумот од крвта на 20 здрави пациенти, која се движи од 4,2 до 9,3 $\mu g \cdot dm^{-3}$ (со средна вредност од 7,3 $\mu g \cdot dm^{-3}$). Стандардната девијација се движи од 1,63 до 4,14% за опсег на концентрации на манганот од 1 до 10 $\mu g \cdot dm^{-3}$. Долната граница на детекција на манганот со овој метод изнесува 0,1 $\mu g \cdot dm^{-3}$.