Determination of lead in microvolumes of blood after decomposition of the organic matrix

D. KALAVSKÁ

Department of Biology and Ecology of Man, Faculty of Natural Sciences, Comenius University, CS-842 15 Bratislava

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Modifications of decomposition of the organic matrix of blood have been studied with purpose to determine Pb in micro-bleedings (in volumes of 150 to 300 mm^3). To decompose organic compounds combination of wet and dry decomposition with addition of concentrated H_2SO_4 (100 to 200 mm^3) has been utilized at the temperature program of 150 °C for 2 h, 300 °C for 2 h, and 550 °C for 2 h. The determination of lead itself was carried out using anodic stripping analysis on a mercury working electrode after dissolution of ash of the sample in the mixture (100 mm^3) of HNO_3 and HCl (φ_r = 1 1) and dilution with water (2 cm^3). Concentrations of Pb^{2+} were calculated by linear regression using five additions of the standard Pb^{2+} solution.

Изучены модификации разложения органической матрицы крови с целью определения содержания Pb в микроколичествах крови (150—300 $мм^3$). Для разложения органических соединений использовалась комбинация мокрого и сухого способа разложения с добавлением концентрированной H_2SO_4 (100—200 $мм^3$) в температурном режиме 150 °C в течение 2 часов, 300 °C в течение 2 часов или 550 °C в течение 2 часов. Собственно определение свинца проводилось с использованием анализа анодного растворения на рабочем ртутном электроде после растворения золы образца в смеси (100 $мм^3$) HNO_3 и HCl (φ_r = 1 1) и разбавления водой (2 $см^3$). Концентрации Pb^{2+} были рассчитаны с помощью метода линейной регрессии, используя пять добавлений стандартного раствора Pb^{2+}

In connection with anthropogenic pollution of environment, much attention is being paid to determination of inorganic pollutants — heavy metals. With rapid growth of automobilism and increased production of lead, this metal has occurred in the components of environment in ever increasing amounts.

Lead is a natural microcomponent of soil, water, plants, and animals (the earth crust contains 1.6×10^{-3} mass % Pb) and consequently, it can be found also in foodstuffs. The daily intake by children should not exceed 150 μg Pb

because lead compounds belong to toxic substances with high degree of cumulation in living organisms. Therefore, determination of lead and other heavy metals in body liquids and organs is of interest to hygienists, ecologists, and analytical chemists alike.

On the basis of the content of lead in blood, urine, sweat, lymph, *etc.* it is possible to a great extent to evaluate the health of an individual already during his life. *Boeckx* [1] considered the lead content 400 $\mu g\,dm^{-3}$ in blood of children to be the upper limit. At higher contents anemia and various defects occur, at contents 600 to 700 $\mu g\,dm^{-3}$ encephalopathy is frequently encountered with and the lead content of 800 to 1000 $\mu g\,dm^{-3}$ is lethal.

These relevant facts led us to reexamination of methods used for determination of lead in blood, elaboration of a procedure for determination of lead in microvolumes of blood as well as to determination of the level of lead in blood of urban people, exposed to increased exhalations.

Works of ecological and hygienic nature, dealing with this problem, usually do not present analytical procedures and many chemical works describe the most important step, solubilization of the sample, only briefly. On the basis of our experience this step is the main source of errors leading to incorrect results. On the other hand, analytical works differ considerably in methods of decomposition of organic material. Some authors use mineralization with various mixtures of acids at different temperature and time relations in open Kjeldahl flasks or under reflux, others apply dry decomposition at different temperature programs. Of the Czechoslovak works the monograph [2] describes in most detail the methods for decomposition of the organic matrix of materials of animal origin. From this monograph and other current publications it follows that the methods of decomposition of the organic matrix have not been unified for any kind of animal analyte.

Experimental

Chemicals and solutions

All chemicals, including mercury in the working electrodes, were of Suprapur grade (Merck, Darmstadt). For preparation of the measured, auxiliary, and standard solutions as well as of the solutions for filling the reference electrode, thrice distilled water, purified on Spheron sorbents (Lachema, Brno) was used. After measurement of each sample the purity of water and vessel was checked on the basis of electrochemical records obtained at identical conditions. In each series of four samples a blank and a standard sample with known content of Pb were measured. The measurements were performed with tempered samples at 23 °C in laboratory without determination of the number of particles in the air, however, at strict hygienic regime and limited dustness.

Instruments and equipments

Blood samples were taken from finger-tips by 50 mm^3 glass micropipettes with capillary narrowing at both ends. Mineral acids were added with the same pipettes. Standard Pb^{2+} solutions were added with a 25 mm^3 automatic pipette (Eppendorf, GFR). Reproducibility of dosage was 26.13, 24.63, 25.75, 25.38, 25.00, 24.75, 26.30, 23.75, 27.30, and 28.30 mm^3

Wet decomposition was performed in 10 cm^3 conical flasks provided with a reflux and heated on a sand bath. Dry decomposition was carried out in a furnace with manual temperature regulation. Temperature was checked with a Pt—PtRh (6 %) thermocouple. The blood samples were decomposed in glass and silica tubes of 1.3 cm diameter provided with an untight glass plug.

Electrochemical measurements were performed on a Polarographic analyzer PA 3 (Laboratórní přístroje, Prague). A hanging mercury electrode, type P958B (Radiometer, Copenhagen) and a thermoelectrically regulated dropping mercury electrode of our own construction [3] served as working electrodes. The reference silver chloride electrode, the auxiliary platinum electrode, and the electrochemical vessel belonged to accessories of PA 3.

Concentrations were calculated by linear regression from five standard additions of the Pb^{2+} solution.

Analytical procedures

The blood samples (100—200 mm^3) were pipetted to flasks that contained porcelain boiling chips and concentrated $HClO_4$ (400—600 mm^3) was added. Mineralization was carried out under reflux for approximately 4 h to bleaching. After cooling the mixture was rinsed with highly pure water (2 cm^3) and transferred into the electrochemical vessel.

In alternative procedure the tubes containing blood samples (100—300 mm^3), concentrated H_2SO_4 (100—200 mm^3), and porcelain chips were heated in a furnace at 150 °C for 2 h, at 300 °C for 2 h, and at 550 °C for 2 h. The hot white ash was dissolved in the mixture of HNO_3 (50 mm^3) and HCl (50 mm^3) and diluted with water (2 cm^3). With regard to the dilution mentioned above, electrochemical determinations were performed at sensitivity 13, 14, deposition times 40 s and 80 s, and deposition potential −0.6 V The size of the mercury drop corresponded to 1 part on the micrometric screw (1 mg, measured in the air).

Results and discussion

The methods used so far for determination of heavy metals in body liquids and organs have treated 1—10 g of samples or several cm^3 of liquid [4]. After drying, decomposition of the organic material is carried out by mineralization with various mixtures of acids and hydrogen peroxide in open or covered vessels

and apparatuses. Combustion is provided mostly in covered flasks over 20 h at different temperature programs [2]. The determination itself has been carried out using DC and AC polarography and flame-absorption spectrometry. Recently, electrochemical stripping analysis and flameless technique of atomic absorption spectrometry have been used, the latter making possible, in some cases, to determine as much as 0.5 $\mu g\,dm^{-3}$ contents [5] without mineralization of the sample [6, 7].

High consumption of highly pure chemicals, long-lasting decomposition as well as high sensitivity of electrochemical stripping analysis (detection limit of Pb^{2+} determination 0.01 $\mu g\,dm^{-3}$ [5]) led us to determination of microamounts of lead in few drops of blood. Sampling of blood (100—300 mm^3) from finger-tips may be provided also by nonsanitary persons.

The blood samples were subjected to several modifications of wet decomposition with the mixture of HNO_3—H_2O_2—H_2SO_4 (φ_r = 1 1 : 0.1) [2], a mixture of $HClO_4$ and H_2O_2, the mixture of H_2SO_4—HNO_3—H_2O_2 (φ_r = 2 : 8 : 5) [8], and the mixture of $HClO_4$—H_2SO_4—HNO_3 (φ_r = 1 1 1), respectively. The most effective decompositions were achieved when using $HClO_4$ in three-fold or higher amounts with regard to the sample. Water contained in blood was sufficient to prevent explosion of $HClO_4$. Though the solutions obtained in all mineralizations were colourless and suitable for using atomic spectrometry, many of them were unsuitable for polarographic measurements, since they showed high signals in the region of potentials -0.6 to -0.1 V, overlapping the signal of Pb.

The most simple way of decomposition to inorganic compounds is the dry decomposition, combustion of the material. We have proved that though dry decomposition of microvolumes of blood is carried out at various rates of temperature rise, up to the final temperature of 550 °C, it is not lossless. In the range of 110—250 °C lead is carried along with considerable escape of tar vapours. The results obtained were the lower the faster was the smoke formed and the higher amounts of the sample were used (100—500 mm^3). These observations led us to combine the wet decomposition with the dry one. Then in the first stage lead is released from the organic matrix and the residue is removed by combustion. Escape of Pb was observed also in the case when using HNO_3.

When searching for the possibilities to prevent losses, the best results were obtained by addition of H_2SO_4 to blood in the ratio of 1 : 3 to 2 : 3 and decomposing for 2—3 h at 150 °C, for 2 h at 300 °C, and for 2 h at 550 °C. Some authors reject the use of H_2SO_4 for determination of Pb^{2+} because of formation of insoluble $PbSO_4$ ($K_s = 1.7 \times 10^{-8}$). At our conditions H_2SO_4 is advantageous for its relatively good thermal stability and mineralization ability. Lead(II) sulfate, formed during decomposition, makes possible to apply higher combustion temperatures as it decomposes only at 1000 °C and thus, escape of Pb

is prevented. At our conditions we can determine $c(Pb^{2+}) \approx 10^{-7}\,mol\,dm^{-3}$ when lead is present in the form of Pb^{2+} The results of determination of standard Pb^{2+} solution (Table 1) prove the correctness of determination. The data in Table 1 evidence that the relative standard deviation of the individual determinations did not exceed 10 %.

Table 1

Results of determination of lead contents in blood and standard solution with addition of blood

Mineralization	Blood : $HClO_4$ $\varphi_r = 2:3$	Blood : H_2SO_4 $\varphi_r = 3:2$ up to 550 °C				
Sample	*1*	*1*	*2*	*3*	*4*	S
$x_i/\mu g\,dm^{-3}$	309	273	90	138	68	96
	360	345	103	120	90	103
	331	372	90	110	103	95
	213	260	206	160	90	96
	350	224	206	190	108	106
		345	155		83	86
		379	172			
		276	155			
			223			
			152			
$\bar{x}/\mu g\,dm$	312.6	309.3	155.2	143.6	90.3	97
$s_{\bar{x}\%}/\%$	8.5	6.7	9.9	10.0	6.5	2.9
$e_{\bar{x}\%}/\%$						3.0

S — Standard Pb^{2+} solution with addition of blood after subtraction of average lead content in blood with real value $\xi = 100\,\mu g\,dm^{-3}$; x_i — results of the individual determination; $\bar{x}$ — arithmetic mean; $s_{\bar{x}\%}$ — relative standard deviation of the arithmetic mean; $e_{\bar{x}\%}$ — relative error of the arithmetic mean.

The results of the individual determinations (x_i) (Table 1) point not only to their dispersion or reproducibility of the procedure but also to the level of lead in blood of the donors, Bratislavians, that reflects also the level of total pollution. Samples of blood from 60 professionally not intoxicated donors of age 40 to 60 years, living and working in central and east parts of Bratislava, were treated. Table 1 presents the results of numerously repeated determinations (testing of the method) with samples taken from four selected typical representatives. The sample No. *1* is from a woman smoking moderately (in the case of heavy smokers the results are higher), the samples No. *2* and *3* are from nonsmokers (a man and a woman, respectively), and the sample No. *4* is from an 18 years old sportswoman.

The results presented indicate the global environmental pollution and intoxication of Bratislavians (excepting the western part of town) with lead. For

comparison, in paper [9] the upper limit of normal Pb level in blood in the U.S.A. is reported to be 400 μg dm^{-3} and the average level 250 μg dm^{-3} and in [10] the Pb content in blood of 80 % of the world-wide adult population is reported to be in the range of 110—300 μg dm^{-3} The works [9, 10] bring also that the lead content in blood of Scandinavians is lower than 100 μg dm^{-3}, while in industrial North Italy it is 240—350 μg dm^{-3} The lead content in blood of adult population in the medium-industrial West German town Jülich was determined to be in the range of 70—190 μg dm^{-3} [11].

The procedure suggested herein for determination of lead in blood is simple, economical, and provides sufficiently reproducible results. We recommend it to sanitary, ecological, and hygienic services. The procedure can be utilized for volumes of 100—600 mm^3 of blood with minimum 30 μg dm^{-3} lead content. In the case of blood plasma and other materials containing lower amounts of lead, it is necessary to prolong the deposition time and take higher amounts of samples.

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