Simultaneous Determination of Lead and Cadmium in Biological Samples by Multichannel Graphite Furnace Atomic Absorption Spectrometry

SHOJI IMAI, HIROSHI ISHIKURA, TOSHIYUKI TANAKA,^a KENGO SAITO^b
and YASUHISA HAYASHI

Department of Chemistry, Joetsu University of Education, Joetsu, Niigata 943, Japan,

Department of General Education, Tottori University College of Medical Care

Technology,^a Yonago, Tottori 683, Japan and Nissei Sangyo S I Center,^b

Sakamachi, Shinjuku, Tokyo 160, Japan

(Received April 10, 1991)

A simple and rapid method was investigated for the determination of $\mu\text{g/l}$ levels of lead and cadmium in biological samples, by multichannel graphite furnace atomic absorption spectrometry using ammonium dihydrogen phosphate as a matrix modifier. Bovine whole blood and human serum were selected as biological samples for application. The method has a detection limit of $0.36 \mu\text{g/l}$ for lead and $0.026 \mu\text{g/l}$ for cadmium. Concentration of lead $788 \pm 14 \mu\text{g/l}$ and cadmium $13.4 \pm 0.1 \mu\text{g/l}$ in a certified reference material, bovine blood, was in good agreement with the certified value (lead $772 \pm 11 \mu\text{g/l}$; cadmium $12.4 \pm 0.5 \mu\text{g/l}$), respectively. This method was applied to ten sera of normal subject and levels of lead ($3.3 \pm 1.0 \mu\text{g/l}$) and cadmium ($0.28 \pm 0.11 \mu\text{g/l}$) obtained showed reasonable values.

Keywords — graphite furnace atomic absorption spectrometry; lead; cadmium; ammonium dihydrogen phosphate; blood; serum; simultaneous determination

Introduction

Simultaneous determination by multichannel graphite furnace atomic absorption spectrometry is an effective method for the determination and monitoring of toxic metals because of lower detection limit, less sample mass and less working time. Human exposure to lead and cadmium, whose toxicity are well known, has been assessed through biological monitoring. However, in a biological sample often only a small quantity is available and troublesome pretreatment is needed because of a large amount of matrix. Although a biological sample contains a large quantity of matrices, concentrations of lead and cadmium are very low. For example, human serum contains $3700 \pm 200 \text{ mg/l}$ of chloride ion and 780 mg/l of total sulfur,¹⁾ the level of lead in

a normal subject is variously reported as $5.4 \pm 1.6 \mu\text{g/l}$, $14.5 \mu\text{g/l}$ and $22.3 \pm 15.3 \mu\text{g/l}$ and the level of cadmium is variously reported as $0.50 \pm 0.24 \mu\text{g/l}$ and $25 \pm 5 \mu\text{g/l}$.²⁾ It is well known that determination of lead and cadmium is interfered from chloride ion because of vaporization loss of lead or cadmium chlorides in the thermal pretreatment stage. To avoid this interference, some matrix modifiers such as ammonium dihydrogen phosphate,³⁾ palladium nitrate,⁴⁾ and chromium nitrate⁵⁾ were proposed. The purpose of this work is investigation of a simple and rapid method free from the interference to determine $\mu\text{g/l}$ levels of lead and cadmium in biological samples without troublesome pretreatment using ammonium dihydrogen phosphate as a matrix modifier.

Experimental

Two graphite furnace atomic absorption spectrometers (Hitachi Model Z-8000 and Z-9000) were used. An uncoated graphite tube (standard type) was also used. The sample was injected by an automatic sampler and absorption data were automatically printed out and displayed.

Commercially available stock solution of lead and cadmium was used (Wake Pure Chemical Industry, Ltd.). An aliquot of this solution was diluted with water to a suitable concentration before use. Chromium nitrate was reagent grade by Kanto Chemical Co., Ltd. Other reagents were also of reagent grade (Wako Pure Chemical Industry, Ltd.). A biological sample was diluted with a diluent (1 m/V% Triton X-100, 0.1 mol/l nitric acid, matrix modifier, and an aliquot of lead). The water was purified by a Milli-Q system after distillation. Sera from normal subjects were obtained from Tottori University Hospital and kept frozen in plastic vials until use. Bovine blood (No. 196) which was a certified reference material ($772 \pm 11 \mu\text{g/l}$ for lead and $12.4 \pm 0.5 \mu\text{g/l}$ for cadmium) was prepared by the Commission of the European Community.

Recommended procedure for determination of lead and cadmium is as follows: Optimum instrumental conditions for simultaneous determination of lead and cadmium are shown in Table I. Biological sample (bovine blood or human serum) was diluted 5 times by the diluent containing 1 m/V% $\text{NH}_4\text{H}_2\text{PO}_4$ as a matrix modifier. Twenty

microliters of the diluted sample was injected into the graphite furnace by automatic sampler.

Results and Discussion

Several matrix modifiers were examined to determine the amount of lead and cadmium in serum by single channel atomic absorption spectrometry. Optimization of the ashing conditions for lead and cadmium was first performed using a serum sample. Ammonium dihydrogen phosphate (1 m/V%), $\text{Cr}(\text{NO}_3)_3$ (2 gCr/l) and $\text{Pd}(\text{NO}_3)_2$ (1 gPd/l) were employed as matrix modifiers. The effect of increasing ashing temperature on peak area was examined under the following atomizer conditions: drying, 120°C, ramp time 30 s; ashing, various temperatures, ramp time 20 s and hold time 10 s; atomizing, 2000°C without $\text{Pd}(\text{NO}_3)_2$ (3000°C), hold time 5 s; cleaning 3000°C, hold time 3 s. Reproducibility of peak area was higher than that of peak height. A loss of signal for lead was observed above 600°C without the modifier, 1200°C with $\text{NH}_4\text{H}_2\text{PO}_4$, 800°C with $\text{Cr}(\text{NO}_3)_3$ and 1500°C with $\text{Pd}(\text{NO}_3)_2$, and for cadmium, a loss of signal was observed above 400°C, 1100°C, 500°C and 1200°C, respectively. $\text{NH}_4\text{H}_2\text{PO}_4$ or $\text{Pd}(\text{NO}_3)_2$ is favorable as a modifier because it allows higher ashing temperature. The ashing temperature was optimized at 1000°C for both $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{Pd}(\text{NO}_3)_2$. The peak area of both elements was kept constant for 60 s of ashing hold time when $\text{NH}_4\text{H}_2\text{PO}_4$ or

TABLE I. Standard Instrumental Conditions for Simultaneous Determination of Lead and Cadmium

Temperature program		Temperature (°C)		Time (s)	Inner gas flow (ml/min)
Step		start	stop		
1 Drying		65	70	30	200
2 Drying		70	100	30	200
3 Ashing		100	800	20	200
4 Ashing		800	800	10	200
5 Atomizing		2000	2000	5	30
6 Cleaning		2700	2700	3	200
Wavelength	Lead 283.3nm,	Cadmium 228.8nm			
Lamp current	Lead 7.5mA,	Cadmium 7.5mA			
Time constant	0.1s				

$\text{Pd}(\text{NO}_3)_2$ was used. In order to simultaneously determine lead and cadmium, common heating programs and a common matrix modifier are needed. The atomization condition was optimized: 2000°C with 5 s for $\text{NH}_4\text{H}_2\text{PO}_4$ and 3000°C with 5 s for $\text{Pd}(\text{NO}_3)_2$. Using the optimized atomizer conditions, the detection limit of lead and cadmium was 0.46 $\mu\text{g/l}$ and 0.019 $\mu\text{g/l}$ with $\text{NH}_4\text{H}_2\text{PO}_4$ and 0.52 $\mu\text{g/l}$ and 0.044 $\mu\text{g/l}$ with $\text{Pd}(\text{NO}_3)_2$, respectively. The detection limit indicates the concentration for $\bar{x}_b + 3S_b$, where $\bar{x}_b$ is a mean value of blank and S_b is a standard deviation of x_b . $\text{NH}_4\text{H}_2\text{PO}_4$ was selected as a modifier for simultaneous determination of lead and cadmium because of lower detection limit and high ashing temperature.

Simultaneous determination of lead and cadmium in serum was performed using the modifier of 1 m/V% $\text{NH}_4\text{H}_2\text{PO}_4$ after the optimization of atomizer conditions because of the instrument's characteristics. Reproducibility in peak height was higher than that in peak area. Two steps for drying condition were used because of bumping of the serum sample: ramp time, 30 s from 65 to 70°C

Fig. 1. Effect of Increasing Ashing Temperature on the Peak Height Signals of Simultaneous Measurement for Lead (12 $\mu\text{g/l}$) and Cadmium (1.2 $\mu\text{g/l}$) in 5 Times Diluted Human Serum with 0.1 mol/l Nitric Acid, 1 m/V% Triton X-100 and 1 m/V% $\text{NH}_4\text{H}_2\text{PO}_4$

1: lead, 2: cadmium. Apparatus, Hitachi model Z-9000.

and 30 s from 70 to 100°C. The effect of increasing ashing temperature on peak height of lead and cadmium in serum sample under the standard atomizer conditions is shown in Fig. 1. A loss of peak height was observed above 1000°C for lead and 800°C for cadmium. The atomizing condition was optimized at 2000°C for 5 s using the optical temperature controller. The relative standard deviation of peak height was 1.6% for lead and 2.4% for cadmium at an ashing temperature of 800°C. The detection and determination limits were 0.36 and 0.44 $\mu\text{g/l}$ for lead and 0.026 and 0.032 $\mu\text{g/l}$ for cadmium, respectively. The determination limit indicates a concentration for $\bar{x}_b + 10S_b$. The reported values of the detection limit in plasma are 2 $\mu\text{g/l}$ lead⁶⁾ and 0.063 $\mu\text{g/l}$ cadmium.⁷⁾

Bovine blood (No.196), a certified reference material prepared by the Commission of the European Community, was analyzed to confirm the accuracy of the proposed method by the standard addition method and the results are shown in Table II. The concentration of lead and cadmium obtained is in agreement with the certified values. The proposed method was applied to the determination of lead and cadmium in ten sera of normal subjects. A curve equation obtained by the standard addition of lead and cadmium was used as a calibration curve and the results are shown in Table III. A mean concentration of 3.3 ± 1.0 $\mu\text{g/l}$ was observed for lead and 0.28 ± 0.11 $\mu\text{g/l}$ for cadmium. Lead level of serum or plasma in a normal subject is variously reported as 5.4 ± 1.6 $\mu\text{g/l}$, 14.5 $\mu\text{g/l}$ and 22.3 ± 15.3 $\mu\text{g/l}$ and cadmium level is variously reported

TABLE II. Concentration of Lead and Cadmium in Bovine Blood^{a)}

	Pb ($\mu\text{g/l}$)	Cd ($\mu\text{g/l}$)
Singlechannel method	758 ± 10	11.7 ± 0.9
Multichannel method	788 ± 14	13.4 ± 0.1
Certified value	772 ± 11	12.4 ± 0.5

a) Bovine blood (No. 196) prepared by the Commission of the European Community.

TABLE III. Determination^{a)} of Lead and Cadmium in Human Serum

No.	Pb ($\mu\text{g/l}$)	Cd ($\mu\text{g/l}$)	No.	Pb ($\mu\text{g/l}$)	Cd ($\mu\text{g/l}$)
1	2.4	0.24	6	4.4	0.30
2	2.7	0.29	7	3.8	0.39
3	2.8	trace ^{b)}	8	2.5	0.24
4	4.9	0.39	9	2.8	0.52
5	4.4	trace	10	trace	trace

Mean $\pm$ standard deviation: Pb $3.3 \pm 1.0 \mu\text{g/l}$, Cd $0.28 \pm 0.11 \mu\text{g/l}$.

a) 3 determinations.

b) detection limit < trace < determination limit.

as $0.50 \pm 0.24 \mu\text{g/l}$ and $25 \pm 5 \mu\text{g/l}$.²⁾ The mean concentrations of lead and cadmium determined in this work thus seem reasonable.

In conclusion, simultaneous determination of $\mu\text{g/l}$ levels of lead and cadmium in a

biological sample are feasible by multichannel graphite furnace atomic absorption spectrometry using $\text{NH}_4\text{H}_2\text{PO}_4$ as a matrix modifier.

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