Notes

Effect of Nickel Nitrate on Graphite-Furnace Atomic Absorption Spectrometry for Indium in Infant Formula and Breast Milk

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The determination of indium involves many difficulties because of the formation of volatile oxides due to reduction by the graphite surface and halides due to coexisting metallic halides in graphite-furnace atomic absorption spectrometry (GFAAS).¹⁻⁶ In order to overcome these difficulties, thermally stable carbide-forming elements have been proposed as a graphite surface-modification reagent, and many inorganic compounds have been proposed as chemical modifiers for this purpose. In our previous work, it was reported that a surface modification with W and Zr by the one-drop coating method greatly increases the sensitivity for indium.^{2,7} Palladium has been used as a matrix modifier for indium due to the formation of a palladium-indium intermetallic compound in the ashing stage.⁸⁻¹² Matsusaki reported that various nitrates, such as calcium, copper, nickel and aluminum nitrates, act as sensitivity-enhancement reagents for indium, and, especially, that a mixed solution of nickel, aluminum and ethylenediaminetetraacetic acid diammonium acts as a matrix modifier for indium in various chlorides.¹³ Yasuda and co-workers reported that the atomization of indium in nickel-indium intermetallic compounds was either delayed or shifted to a slightly higher temperature.¹²

Indium is an important metal; for example, it is used in soldering lead wires to germanium transistors, intermetallic semiconductor, sleeve-type bearing, glass-sealing alloys and dental alloys.¹⁴ Since the potential toxicity of indium to the liver, kidney and bone marrow has been suggested, environmental pollution and exposure will become serious concerns regarding its increasing use.¹⁵⁻¹⁷ However, there have only been a few studies concerning the determination of indium in biological samples by GFAAS with a complex pretreatment, such as solvent extraction and digestion.¹⁸⁻²⁰

The aim of this work was to study the effect of nickel

nitrate as a matrix modifier on the rapid determination of $\mu\text{g dm}^{-3}$ levels of indium in milk sample, such as infant formula and breast milk, which are an important diet for growing children, babies and infants, including complex matrix by GFAAS without a sample pretreatment.

Experimental

A Hitachi Model Z-8000 flame and graphite-furnace atomic absorption spectrometer equipped with a Zeeman-effect background corrector, an optical temperature-controller system (Hitachi Model 180-0341) and an automatic data processor were used. Ten microliters of a sample solution were injected by an automatic sampler. An Oki if-800 Model 50 personal computer was used to record the absorbance signal profiles at 20-ms intervals. The output data from the optical temperature controller were acquired at 4-ms intervals by the computer and stored on a diskette. Temperature data were calibrated using a Chino Model IR-AH1S radiation thermometer. The thermometer was calibrated with a Pt-Rh thermocouple. A pyrolytic graphite (PG) furnace (Lot No. P93101) was used.

A commercially available stock solution of indium was used (Kanto Chemical Co., Inc., Lot No. 708s9062). An aliquot of this solution was properly diluted before use. Nickel(II) nitrate hexahydrate was of high-purity reagent grade (99.95%) by Kanto Chemical Co., Inc. (Lot No. 506E1394). A commercially available stock solution of Ti, W and Zr and hafnium oxychloride octahydrate (99.99%+, Aldrich Chemical Co.) was used for a PG furnace treatment. Other reagents were also of reagent grade. Four brands of powdered infant formulae on sale were used. Twice-concentrated infant formula samples were prepared by dissolving double the amount of the powdered formula in hot water according to a diet recipe. (The recipe for diet: sample No. 1, 5.2 g/40 ml; No. 2, 13 g/100 ml; No. 3, 14 g/100 ml; No. 4, 14 g/60 ml) Breast milk was collected at Tottori University

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Table 1 Standard operation conditions

Atomizer operation Step			Temperature/°C	Time/s		Ar/ml m ⁻¹
				Ramp	Hold	
1	Injection	sample	120	30	0	200
2	Dry					
3	Cooling					
4	Injection	matrix modifier	120	30	0	200
5	Dry					
6	Ashing		1300	20	20	200
7	Atomizing ^a		2700	0	3	30
8	Cleaning		2800	0	3	200
Wavelength 325.6 nm			Bandwidth 1.3 nm		Time constant 0.02 s	

a. Optical temperature controller was used.

Hospital from eight healthy mothers. The breast milk was sampled by lactating the mothers directly into acid-washed polystyrene vials, and was kept frozen (-20°C) until use. A sample was diluted with a diluent of Triton X-100 (Wako Pure Chemical Industry, Ltd.). The water was purified by a Milli-Q Plus system after deionization and distillation.

The recommended procedure for the determination of indium is as follows: The standard operation conditions are given in Table 1. The twice-concentrated infant formula or breast milk (400 μl) was diluted by the diluents of 4w/v% Triton X-100 (200 μl) and an indium solution (or purified water: 200 μl). Ten microliters of the diluted sample were injected into the furnace by an autosampler and dried (step 1). After cooling, ten microliters of nickel nitrate solution (5000 mg dm^{-3} of nickel in 1 mol dm^{-3} HNO_3) as a matrix modifier were injected into the furnace by the autosampler, and the optimum atomization cycle was carried out. Calibration curves were obtained by the standard addition of 0, 50, 100, 150, 200 $\mu\text{g dm}^{-3}$ of indium into the sample.

Results and Discussion

The surface modification of a graphite furnace by a carbide-forming element, such as W and Zr, can lead to a great sensitivity enhancement with major changes in the atomization characteristics of indium. Hafnium, Ti, W and Zr were examined as a surface-modification reagent for a PG furnace. In the present work, prior to the injection of a sample solution, the PG furnace was treated simply with a solution of 500 mg dm^{-3} of metal by the injection of 40 μl of the solution into the furnace and heating according to the standard operation conditions given in Table 1. When the treatment process was repeated, the enhancement in absorbance was saturated after more than five treatments. The peak height and integrated absorbance of indium (100 $\mu\text{g dm}^{-3}$ in 0.1 mol dm^{-3} HNO_3) in the Hf, Ti, W and Zr-treated PG furnaces were increased by a factor of 23, 25, 29, and 22 and 15, 14, 19 and 14 compared to those which resulted in the bare PG furnace, respectively. When the treatment was repeated ten times, the W-treated PG furnace gave

Fig. 1 Comparison of the absorbance for 2 ng of indium in an infant formula using matrix modifiers (5000 mg dm^{-3} of metal) with a W-treated PG furnace. ○, peak height; ●, integrated absorbance.

stable signals for at least 200 and 100 firings for the indium standard solution and the infant formula sample, respectively. Hereafter, the W-treated PG furnace was used.

Figure 1 shows the peak height and integrated absorbance for 2 ng of indium in the presence of the infant formula according to the recommended procedure by the use of magnesium nitrate, aluminum nitrate, chromium nitrate and nickel nitrate and palladium nitrate-magnesium nitrate solutions including 5000 mg dm^{-3} of metal as a matrix modifier. In the absence of a matrix modifier, no signal was observed because of interferences by the infant formula matrix. Although no increase in the absorbance was found for the magnesium and chromium solutions, for the aluminum, nickel and palladium-magnesium solutions an increase in the absorbance was found. The enhancement effect for the peak height by the nickel solution was more remarkable than that of the palladium-magnesium and aluminum solutions, and for the integrated absorbance that was similar level to the palladium-magnesium solution and more remarkable than the aluminum solution. In the present work, nickel nitrate was used as a matrix

Fig. 2 Absorption profiles for 2 ng of indium with varying the concentration of nickel nitrate. Infant formula: A, absence; B, presence. Concentration of nickel in mg dm^{-3} : a, 0; b, 1000; c, 2000; d, 5000. 1% Triton X-100 was contained. Temperature profile: e.

modifier.

Figure 2 shows the effect of the nickel concentration on the atomic-absorption signal for 2 ng of indium in the presence and absence of the infant formula with the temperature profiles according to the recommended procedure with varying the concentration of nickel, in which the time axis is labeled in such a way that the zero time has elapsed before the atomization stage of the heating cycle. In the absence of the infant formula the integrated absorbance was independent of the nickel concentration above 1000 mg dm^{-3} , although a high-temperature shift of the signal was observed, which could be elucidated by the formation of a nickel-indium inter-metallic compound.¹² For the infant formula matrix, however, the absorbance was increased with increasing the nickel concentration. At a nickel concentration of 5000 mg dm^{-3} , the atomic-absorption signal in the presence of the infant formula was in agreement with that in the absence of the infant formula. Thereafter, a nickel concentration of 5000 mg dm^{-3} was used. The matrix-modifier additive method was examined. When the matrix modifier was injected after the injection of $10 \mu\text{l}$ of an infant formula sample spiked $50 \mu\text{g dm}^{-3}$ of indium (0.5 ng of indium) into the furnace without the sample dry step (step No. 2) and the atomic absorption signal was observed according to the atomization cycle after step No. 5 under the standard operation conditions, the relative standard deviation ($n=5$) was 3.7 and 3.7% for the peak height and integrated absorbance, respectively. When the matrix modifier was injected after drying $10 \mu\text{l}$ of an infant formula sample according to the recommended procedure, the relative standard deviations were 2.0 and 2.0% for the peak height and integrated absorbance, respectively. The average value of the peak height and integrated absorbance for the internal mixing method with the sample dry was in

Fig. 3 Effect of the ashing temperature for 2 ng of indium in the presence ($\blacktriangle, \bullet$) and absence ($\triangle, \circ$) of infant formula. $\triangle, \blacktriangle$: peak height; $\circ, \bullet$: integrated absorbance.

agreement with that without the sample dry, respectively. The poor relative standard deviation observed by the mixing method without the sample dry may have been due to the coagulation of proteins by the matrix-modifier solution.

Figure 3 shows the ashing-temperature effect for $200 \mu\text{g dm}^{-3}$ of indium (2 ng) in the presence and absence of the infant formula according to the recommended procedure with varying the ashing temperature. In the presence of the infant formula, although the peak height was not independent of the ashing temperature, the integrated absorbance was independent of that in the range $900\text{--}1600^\circ\text{C}$. A loss of peak height and integrated absorbance was observed above 1500 and 1600°C , respectively. In the absence of the infant formula, the peak height and integrated absorbance were independent of the ashing temperature over the range $900\text{--}1700$ and $900\text{--}1700^\circ\text{C}$, respectively. Above 1700°C a loss of the peak height and integrated absorbance was observed. The relative standard deviations ($n=5$) observed in the range $900\text{--}1700^\circ\text{C}$ are summarized in Table 2. The minimum relative standard deviation was observed at an ashing temperature of 1300°C . Thus, the optimum ashing temperature was 1300°C . The peak height and integrated absorbance for the infant formula sample were kept constant for 30 s of hold time during ashing (step 6 in Table 1) according to the standard temperature condition (Table 1).

A calibration curve for the integrated absorbance was obtained in the infant formula and breast milk by standard addition according to the recommended procedure. The calculated detection limit based on the validity of the sample ($3\sigma_s$) is 2.0 and $2.5 \mu\text{g dm}^{-3}$ (20 and 25 pg in $10 \mu\text{l}$) in the infant formula and breast milk, respectively. The detection limit obtained at a similar level to that ($2 \mu\text{g dm}^{-3}$) by Handy and co-workers for a plasma digested by microwave with $\text{HNO}_3\text{--H}_2\text{SO}_4$, and then treated with hydrogen peroxide for the complete oxidation of the organic matrix with the hafnium-magnesium matrix modifier has been reported (reviewed

Table 2 Relative standard deviation for 2 ng of indium at various ashing temperatures

Ashing temperature/°C	Relative standard deviation, %			
	Presence of infant formula		Absence of infant formula	
	Integrated absorbance	Peak height	Integrated absorbance	Peak height
900	0.3	1.5	2.2	0.8
1000	0.6	1.7	1.8	1.8
1100	1.2	1.1	1.4	1.9
1200	1.6	2.2	1.9	3.2
1300	0.2	0.7	0.2	0.2
1400	0.9	1.5	1.6	2.5
1500	1.0	2.4	2.3	2.4
1600	1.9	3.0	2.9	1.3

as ref. 18). The relative standard deviation ($n=5$) for $50 \mu\text{g dm}^{-3}$ of indium (0.5 ng) was 2.0 and 2.0% in the presence of the infant formula and breast milk, respectively. A recovery test of indium was carried out in four infant formula and the breast milk samples from eight mothers by standard addition. Table 3 gives the sensitivity, based on pg per 0.0044 abs s, and recovery as determined from the slope by the calibration curve, respectively. The average value of the sensitivity observed in the infant formula and breast milk was in agreement with that observed in the absence of the milk matrix (73 ± 2 pg per 0.0044 abs s) within the experimental errors. An average value of recovery of 97.2 ± 1.7 and $95.4 \pm 6.7\%$ was observed for the infant formula and breast milk, respectively. The relative standard deviation for breast milk is larger than that for infant formula, which may be due to the effect of varying the matrix concentration of breast milk. When $20 \mu\text{l}$ of both the matrix modifier and infant formula sample were used with a longer ramp time for drying (60 s) and with an Ar gas stop in the atomizing step (0 ml/m of Ar), the calibration curve for the integrated absorbance was obtained in the concentration range of 0– $20 \mu\text{g dm}^{-3}$ of indium in infant formula and breast milk samples. The detection limit ($3\sigma_s$) of 0.5 and $0.5 \mu\text{g dm}^{-3}$ (10 pg in $20 \mu\text{l}$) and the sensitivity of 60 ± 2 and 60 ± 2 pg per 0.0044 abs s were observed with relative standard deviations of 2.6 and 2.8% for $5 \mu\text{g dm}^{-3}$ of indium (0.1 ng) for infant formula and breast milk samples, respectively. Thus, it can be proposed that the proposed method is favorable for determinations at the $\mu\text{g dm}^{-3}$ level of indium in a complex matrix, such as milk, without any pretreatment.

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Table 3 Sensitivity and recovery for indium added to the infant formula and breast milk

No.	Sensitivity (pg per 0.0044 abs s)	Recovery, %
Infant formula 1	75	98.1
2	76	96.8
3	73	99.2
4	77	94.6
average	75 ± 2	97.2 ± 1.7
Breast milk ^a 1	71	91.4
2	78	91.7
3	77	88.5
4	73	103
5	69	109
6	79	94.5
7	85	88.9
8	81	95.9
average	77 ± 5	95.4 ± 6.7

a. Average value for breast milk from eight mothers.

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