Minimization of phosphate interference in the direct determination of arsenic in urine by electrothermal atomic absorption spectrometry

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Abstract

A direct method for the determination of arsenic in urine by Zeeman atomic absorption spectrometry has been proposed by graphite furnace atomization with $(\text{NH}_4)_3\text{RhCl}_6$ + citric acid as a mixed matrix modifier. Different matrix modifiers including palladium, nickel and rhodium, were used for the elimination of phosphate interference, which was most troublesome in the arsenic determination in urine. Rhodium was preferred to palladium and nickel; in its presence the determination of arsenic in urine can tolerate a char temperature of 1600°C, a temperature high enough to drive off the urine matrix including calcium phosphate without losing arsenic. The citric acid in the furnace evolved active gases and carbon upon decomposition thus facilitating the creation of a favorable reducing environment for the formation of a Rh–As alloy or intermetallic compounds which stabilized arsenic to a high temperature. In the determination of 1 ng of arsenic, 1.0 mg ml⁻¹ phosphate in the form of calcium phosphate can be tolerated. Analytical results obtained for standard urine sample compared (>92% recovery) well with the certified value. The lowest concentration of arsenic that could be determined was 20 ng ml⁻¹ in the undiluted urine.

Keywords: Arsenic in urine; Atomic absorption spectrometry; Rhodium as matrix modifier; Zeeman background correction; Phosphate interference

1. Introduction

In biological samples arsenic is usually present in a matrix containing an excessive amount of phosphorus. For instance, mussel has a phosphorus content 10³-fold greater than that of arsenic and human urine is rich in phosphate. Phosphate buffers have been used as mobile phases in ion exchange and

liquid chromatography in the separation of arsenic species [1]. Therefore, the determination of arsenic in the presence of phosphate is of practical interest. However, the interference of phosphate on arsenic determination in the graphite furnace has been well documented [2–4]. According to Fernandez and Giddings [2], this interference was supposed to be due to the calcium phosphate in the sample which produced PO or P₂ during thermal decomposition and the interference was eliminated when Zeeman background correction was used. In contrast, other

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workers found that the PO band was effected by a magnetic field [5–7]. Ohlsson and Frech [8] were able to ascertain the presence of PO in the graphite tube during vaporization of a phosphate-containing matrix and showed that PO band absorption was effected by a magnetic field with the use of time-resolved high-resolution spectrographic measurements. For the elimination of phosphorus interference on arsenic determination, Bauslaugh et al. [3] found that the addition of Ni and Pt has a significant effect on the formation of gaseous phosphate decomposition products thereby decreasing the spectral interferences at As resonance lines. The interference is troublesome; according to Welz [4], in the presence of palladium phosphate at a concentration of 50 mg l^{-1} in the form of CaHPO_4 decreased the arsenic signal to about 75% of that obtained in the absence of phosphate. This is due most probably to a non-spectral interference, because there is no visible baseline distortion and the interference was also observed in an instrument with Zeeman background correction.

The problem of phosphate interference arsenic determination needs further study. The aim of the present work is to devise a method for the minimization of phosphate interference. This is achieved by using $(\text{NH}_4)_3\text{RhCl}_6$ +citric acid which was found to be capable of stabilizing arsenic in urine to a temperature as high as 1600°C , thus allowing the urine matrix to be eliminated. The method has been applied to the analysis of urine containing not less than 20 ng ml^{-1} arsenic.

2. Experimental

A Hitachi Z-8200 Polarized Zeeman Atomic Absorption Spectrometer equipped with a GA-3 graphite furnace was employed for all analyses. Acquisition and treatment of data were performed with an IBM compatible 80386 computer and Z-8200 windows-base application software. Data and absorbance were output though a Brother M-2724 printer. Pyrolytically coated graphite tubes (Beijing Research Institute of Materials and Technology, Ministry of Astronautics) were used. The internal gas flow was interrupted during the atomization cycle. Integrated absorbance was measured in all experiments.

Table 1

Furnace program for As in urine with $(\text{NH}_4)_3\text{RhCl}_6$ +citric acid

Stage no.	Stage	Temperature ($^\circ\text{C}$)		Ramp (s)	Hold (s)	Gas flow (ml min^{-1})
		Start	End			
1	Dry	50	140	10	30	200
2	Ash	200	1600	10	30	200
			1400 ^a			
3	Atom	2600	2600	0	10	0
4	Clean	2600	2600	0	4	200
5	Cool				5	200

^a For As in standard solution.

A high intensity arsenic hollow cathode lamp from the General Research Institute for Non-ferrous Metals, Beijing, China, operated at 6 mA, was used. The wavelength and slit width were set at 193.7 and 1.3 nm, respectively.

The furnace program is listed in Table 1.

2.1. Reagents

Doubly distilled water was used throughout.

An arsenic stock solution of 1 mg ml^{-1} was prepared by dissolving 0.1320 g of As_2O_3 in 50 ml of 0.5 M NaOH with warming. After cooling, 3 ml of 12 M HCl was added and diluted to 100 ml with water. Working solutions were prepared by serial dilution of the stock solution immediately before use.

A rhodium solution containing 2.5 mg ml^{-1} Rh was prepared by dissolving ammonium hexachlororhodate(III) $(\text{NH}_4)_3\text{RhCl}_6 \cdot 1.5\text{H}_2\text{O}$, extrapure, Tianjin) in water.

A palladium solution containing 2.5 mg ml^{-1} Pd was prepared by dissolving palladium sponge powder (Light, UK) in concentrated HNO_3 with a few drops of HCl and evaporating to near dryness. The palladium salt was dissolved in 0.1 M HNO_3 and diluted to volume.

A nickel solution containing 2.5 mg ml^{-1} nickel was prepared by dissolving $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Merck; Darmstadt, Germany, extrapure) in water.

Citric acid solution (Beijing Chemical Works, Analytical Reagent), 120 mg ml^{-1} , was prepared by dissolving the acid in water.

Ammonium dihydrogenphosphate solution (Beijing Chemical Works, Analytical Reagent), 10 mg ml^{-1} , was prepared by dissolving the salt in water.

Calcium nitrate solution, 10 mg $\text{Ca}^{2+} \text{ ml}^{-1}$, was obtained by dissolving calcium carbonate (standard material from National Research Center for Certified Reference Materials) in dilute nitric acid.

The phosphate and calcium solutions were mixed in a mole ratio of $\text{PO}_4^{3+} : \text{Ca}^{2+} = 1 : 0.6$ before use. The phosphate was added in the form of calcium phosphate.

All chemicals were of analytical reagent grade in which no arsenic was detected.

3. Results and discussion

3.1. Comparison of modifiers for the suppression of calcium phosphate interference

The most commonly used matrix modifier for stabilizing relatively volatile elements is palladium [9]. However, Welz et al. [4] found that in the presence of palladium loss of arsenic occurred even at a low phosphate concentration of 5 mg l^{-1} . Saeed and Thomassen [10] indicated that the addition of a large amount of nickel to a sample containing phosphate suppressed the non-spectral absorption signal. Nickel obviated the spectral interference by converting phosphate into free atoms. Bauslaugh et al. [3] found that a mixed matrix modifier of 0.1% Ni+2.5% Pt in 3 M HNO_3 effectively controlled the spectral interference found at the As resonance line. The amount of platinum added was excessive and the furnace will not tolerate the high acid concentration used [11]. Edgar and Lum [12] proposed the use of 5% nickel nitrate to eliminate the phosphate interference on arsenic in urine. This requires the addition of 100 μg of nickel when 10 μl of sample was injected into the furnace. The excessive amount of 100 μg of nickel added will contaminate the tube and graphite ring and will certainly shorten the lifetime of the graphite tube.

To compare the effect of different modifiers palladium, nickel and rhodium on the minimization of phosphate interference on arsenic determination, experiments were carried out using 25 μg of the modifiers. The recoveries of arsenic are depicted in Fig. 1. With nickel the recovery of arsenic decreases to 89% when 250 $\mu\text{g ml}^{-1}$ phosphate in the form of calcium phosphate is present. Palladium shows a

Fig. 1. Effect of phosphate on the recovery of 1.0 ng of As in the presence of 25 μg of different modifiers. (a). Rh as $(\text{NH}_4)_3\text{RhCl}_6$ and 1.2 mg of citric acid; (b) Pd; (c) Ni pyrolysis 1400°C, atomization 2600°C

similar effect, a recovery of 85% being found in the presence of the same amount of phosphate. The result is better than that reported by Welz et al. [4]. Rhodium gives more favorable results, 1000 $\mu\text{g ml}^{-1}$ phosphate can be tolerated when 25 μg of rhodium in the form of $(\text{NH}_4)_3\text{RhCl}_6$ and 1.2 mg of citric acid are added. The arsenic peak profile does not change much when 500 $\mu\text{g ml}^{-1}$ phosphate is introduced (Fig. 2(a)), but significant changes in arsenic peak profiles are observed with palladium or nickel (Fig. 2(b) and (c)). In the presence of phosphate, the peak profile for arsenic in palladium becomes much broader probably due to the stabilizing effect of phosphate on arsenic (Fig. 2(b)(2)). Arsenic with nickel in phosphate seems to give rise to two peaks, perhaps due to a more complicated mechanism of atomization such as formation of two different arsenic compounds prior to atomization.

Rhodium is therefore preferred to palladium or nickel in suppressing the phosphate interference. The rhodium compound used was ammonium hexachlororhodate(III) which is water soluble and convenient to prepare. In order to remove the chloride in the compound citric acid was added. The chloride present may cause the volatilization of AsCl_3 (bp 130°C) leading to the loss of arsenic signal. With the addition of citric acid the chloride will be replaced by citrate and HCl, which has a large dissociation energy of 427 kJ mol^{-1} and is stable in the high temperature environment, will be liberated [13]. Only 100 μg of citric acid is needed for 25 μg of Rh to obtain a steady

Fig. 2. Peak profiles for 1.0 ng of As in the presence of (a) Rh, (b) Pd, and (c) Ni. (1) without addition of phosphate; (2) with addition of 5 µg of phosphate in the form of calcium phosphate. Integrated absorbance for As: without phosphate (a) 0.2440 s, (b) 0.2355 s, (c) 0.2499 s; with phosphate (a) 0.2290 s, (b) 0.1738 s, (c) 0.2054 s. upper curve: background absorption; lower curve: absorption profile of As.

absorbance signal for 1 ng of arsenic (Fig. 3). An excess amount of 1.2 mg of citric acid was introduced into the furnace to ensure the elimination of interference from any chloride that might be present in the sample. The presence of the organic reagent has the further advantage of creating a more favorable reducing environment in the furnace. The organic ligand evolves active gases ($\text{CO} + \text{H}_2$) and produces active carbon [14], thus enhancing the reduction of arsenic and rhodium to the elements in highly dispersed forms and promoting the formation of a Rh–As alloy or intermetallic compounds. The pyrolysis curve for arsenic in the presence of ammonium hexachlororhodate and citric acid is shown in Fig. 4(a). It is interesting to find that arsenic can be stabilized up to a temperature of 1400°C. The higher pyrolysis temperature arsenic can tolerate, the less will be the interference effect. Subsequent experiments were carried out using

Fig. 3. Effect of citric acid on the integrated absorbance of 1.0 ng for As in the presence of 25 µg of Rh as $(\text{NH}_4)_3\text{RhCl}_6$ (pyrolysis 1400°C, atomization 2600°C).

Rh+citric acid as a mixed modifier for stabilizing arsenic and removing phosphate interference in human urine.

Fig. 4. Pyrolysis and atomization curves for 1.0 ng of As in the presence of 25 μg Rh as $(\text{NH}_4)_3\text{RhCl}_6$ and 1.2 mg of citric acid. Left branch: pyrolysis curves for (a) 1.0 ng of As and (b) 1.0 ng of As in urine diluted (1+4); atomization at 2600°C. Right branch: atomization curves for (a) 1.0 ng of As; pyrolysis at 1400°C; (b) 1.0 ng of As in urine (1+4), pyrolysis at 1600°C.

3.2. Analysis of urine

The direct determination of arsenic in urine was found to be virtually impossible with or without nickel matrix modification as summarized by previous workers [15,16]. In the presence of nickel the suppression was about 30% and 50% at 10- and 3-fold dilution, respectively. The detection limit was 50 ng ml^{-1} . Even with the addition of a large amount of nickel (5% nickel nitrate solution), the recovery was variable ranging from 48% to 80% despite using Zeeman background correction. Since a mixture of ammonium hexachlororhodate and citric acid was found to be efficient in overcoming the phosphate interference, it was expected that this modifier could be applied for the determination of arsenic in urine. The pyrolysis curve of arsenic in urine was constructed by setting the atomization temperature at 2600°C and the atomization curve was obtained by keeping the pyrolysis temperature at 1600°C, as preliminary experiments had shown that this temperature can be tolerated by arsenic in urine. The results are depicted in Fig. 4(b). The tolerated pyrolysis temperature in urine of 1600°C was higher than that in a standard solution probably due to the presence of phosphate in urine. When 5 μg of phosphate was added to a standard arsenic solution, the tolerated pyrolysis temperature increased from 1400°C to 1600°C. When atomization was carried

out in the temperature range 2400–2600°C, arsenic gave similar absorbance values, but the absorption peak was broader and less symmetrical when the temperature was kept below 2500°C. In urine analysis, 10 μl of freshly collected urine (diluted 1+4) was introduced into the furnace together with 10 μl each of 0.25% rhodium solution in the form of ammonium hexachlororhodate and 12% citric acid. The furnace program is listed in Table 1.

3.3. Analytical performance

The characteristic mass which gave an integrated absorbance of 0.0044 s was found to be 18 pg of As in urine diluted (1+4). An absolute detection limit (3σ) of 25 pg ($n=9$) was found in the urine (1+4) which resulted in a detection limit of 6.3 ng ml^{-1} in undiluted urine.

Recoveries for 50–100 $\mu\text{g ml}^{-1}$ As added to urine sample (1+4) were found in the range 92–96% with a standard deviation of 4%. The regression equation for the calibration curves for standard arsenic and arsenic in urine (1+4) were $y=0.2212x+0.0114$ and $y=0.2361x+0.00625$ with correlation coefficients of 0.9989 and 0.9999 ($n=6$), respectively, where x =analyte mass (ng) and y =integrated absorbance.

Results for real sample analysis are summarized in Table 2. The frozen urine GBW09102 is a Standard Reference Material certified by Beijing Medical School using various analytical techniques including flame atomic absorption spectrometry, inductively coupled plasma–mass spectrometry, neutron activation analysis and anodic stripping voltammetry. Samples 1–4 were freshly collected from volunteers in this laboratory. Samples 3 and 4 were obtained

Table 2
Determination of As in human urine

Sample	Concentration of As (ng ml^{-1})		Recovery of As (%) ^b
	Found ^a	Reference value	
Frozen urine			
GBW09102	28 $\pm$ 2	20 $\pm$ 6	93 $\pm$ 4
1	37 $\pm$ 7	—	92 $\pm$ 3
2	48 $\pm$ 2	—	96 $\pm$ 4
3	81 $\pm$ 11	—	—
4	106 $\pm$ 15	—	—

^a mean $\pm$ standard deviation ($n=5$).

^b mean $\pm$ standard deviation ($n=3$).

Fig. 5. profiles of (a) 0.18 ng of As in 20 μ l of urine (1+4) corresponding to 45 ng ml^{-1} in undiluted urine; integrated absorbance 0.0364 s; (b) a spiked of 1.0 ng of As in 20 μ l of urine (1+4); integrated absorbance 0.2305 s. pyrolysis 1600°C, atomization 2600°C; upper curve: background absorption lower curve: absorption profile of As.

from individuals not exposed to arsenic through occupation; the high levels of arsenic found probably originated from seafood. Since no detectable amount of As can be found in the reagents, the sensitivity can further be improved by successive double introduction of the urine sample (1+4) into the furnace, the first injection being followed by the second after drying and charring. Fig. 5 presents the absorption profiles for arsenic in a urine sample (1+4) with and without addition of 1.0 ng of As.

4. Conclusion

The proposed method has the advantage of specificity and speed of measurement and may serve as a monitor of recent arsenic exposure. Since no chemical pretreatment is required, the analyzed sample solution is free from contamination as acids

usually contain detectable amounts of arsenic. The technique can be used for the direct determination of trace arsenic in urine or other biological fluids containing arsenic levels not less than 20 ng ml^{-1} .

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