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Determination of arsenic in biological fluids by electrothermal atomic absorption spectrometry

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A procedure for the determination of the total content of arsenic in urine, serum and blood by electrothermal atomic absorption spectrometry (ETAAS) is described. Zeeman correction is used to compensate the high background signals. The samples are diluted (1 + 1 for urine and 1 + 3 for both serum and blood samples) in a medium containing 0.1% w/v Triton X-100 before being introduced directly into the furnace. A solution containing 15% w/v hydrogen peroxide, 0.65% w/v nitric acid and 0.5% w/v nickel is also introduced into the atomizer by means of a separate injection. Calibration is carried out against aqueous standards for blood and serum samples and using the standard additions method for urine samples. The detection limit is 20 pg (2 ng ml^{-1}). The reliability of the procedure is checked by analyzing three certified reference materials and by recovery studies.

Introduction

The main route of arsenic uptake is perorally together with food and beverages, by inhalation in factories and smelters, and during the spraying of insecticides. Arsenic is bound to proteins in serum and to the globin part of hemoglobin in erythrocytes; it is stored first in the liver, kidney, spleen and lung. Normal levels in body fluids and tissues are influenced by food to a greater extent than the environment. The daily intake of arsenic with food is estimated as 0.05–0.1 mg, while the total body content of arsenic may be 0.3–4.0 mg. Blood levels of unexposed persons have been reported in the range 0.5–40 μg l^{-1}. Normal urine levels are generally lower than 10 μg l^{-1} although they may increase up to 80 μg l^{-1} reaching, after the consumption of seafood, as much as 300 μg l^{-1}.[1] Although it has not been proved that arsenic is an essential element for humans, it is probably so.[2]

Electrothermal atomic absorption spectrometry (ETAAS) is widely used for trace and ultratrace metal analyses in biological samples because it offers excellent detection limits with minimum sample consumption. Most methods for total arsenic determination require a complete decomposition of all arsenic compounds present in the sample.[3] This can be achieved by a number of decomposition procedures, with wet ashing being the most frequently used. However, this procedure is time-consuming and prone to contamination or analyte loss. An alternative is the direct analysis of the biological fluid without the previous mineralization step. In any case, matrix modification is necessary to allow increased pyrolysis temperatures, and thus to decrease the non-specific absorption signals originating from the matrix. Nickel salts[4–6] have been widely used as matrix modifiers for arsenic determination, although other chemicals have also been suggested for the same purpose.[7–11] In addition, the presence of iron and phosphate in biological matrices causes deuterium arc background correction systems to overcompensate at arsenic resonance lines such as 193.7 and 197.2 nm and so Zeeman-based background correction is commonly recommended.[9,10,12]

Despite the use of this correction procedure, problems in arsenic determination caused by high background levels and the build-up of carbonaceous residues inside the atomizer still remain, drawbacks which can be avoided by including an oxygen or air–pyrolysis step in the heating cycle.[13,14] However, this option is not possible for all the commercial electrothermal atomizers and involves a risk of decreasing the useful lifetime of the expensive pyrolytic material. Recently, it has been demonstrated that the addition of both hydrogen peroxide and nitric acid to samples of a high organic content can also alleviate these problems; an additional advantage being that no damage is produced in the graphite atomizers.[15–17] This is a simple way of producing an oxidizing environment inside the atomizer and effective *in situ* mineralization of the sample.

In this study, a procedure based on such a simple strategy for the determination of total arsenic in human urine, serum and blood is discussed. The samples are simply diluted in a medium containing Triton X-100 and directly introduced into the electrothermal atomizer. A second aliquot containing hydrogen peroxide, nitric acid and a nickel salt is then injected, which allows reduced background signals to be obtained.

Experimental

Apparatus

An ATI-Unicam (Unicam Atomic Absorption, Cambridge, UK) 939QZ atomic absorption spectrometer equipped with a GF90 electrothermal atomizer and a FS90 Plus autosampler were used. Zeeman correction was used to obtain the background-corrected integrated absorbance, which acted as the analytical signal. Pyrolytic platforms (reference 9423 393 95191) were obtained from ATI-Unicam. Argon was used as the inert gas, the flow rate being 300 ml min^{-1} during all the stages except atomization, when the flow was stopped. Measurements were performed, with a spectral bandwidth of 1.0 nm at 193.7 nm using a hollow cathode lamp (Photron PTY. LTD., Australia) operated at 8 mA.

Reagents

All the chemicals used were of the highest purity available and all the glassware and plasticware was nitric acid-washed and rinsed with ultrapure water. High quality water, obtained using

a Milli-Q system (Millipore, Milford, M.A.), was used exclusively. Arsenic stock standard solution (1000 μg ml^{-1}) was obtained from Panreac (Barcelona, Spain) and diluted as necessary to obtain working standards. High quality concentrated (65% w/v) nitric acid (Merck, Darmstadt, Germany), 30% w/v hydrogen peroxide (Fluka, Buchs, Switzerland), Triton X-100 (Merck) and nickel nitrate hexahydrate (Fluka) were used. Other chemicals used, ammonium dihydrogen phosphate, lanthanum nitrate hexahydrate, sodium chloride, potassium sulphate and urea, were obtained from Fluka. Palladium(II) nitrate was obtained from Sigma (St. Louis, USA). In a previous stage, all these chemicals were examined and found to be free of arsenic.

Samples

Urine samples from volunteers were collected directly into acid-washed high-density polyethylene containers and analyzed as soon as possible after collection. A sample of serum was provided by the Medicine Faculty of our University and maintained at –20 °C in a cleaned plastic tube with a colourless stopper. Freeze-dried urine reference samples, SRM 2670 (normal level and elevated level) were supplied by The National Institute of Standards & Technology (NIST), USA. Samples were supplied in freeze-dried form and were reconstituted by adding 20 ml of distilled water to each bottle. A whole blood reference sample, Seronorm 404108, was supplied by Seronorm (Norway). The blood sample was supplied in a lyophilized form and was reconstituted by dissolving the total content of a vial in 5 ml of distilled water.

Procedure

The addition of nitric acid or nickel to blood or serum samples produced protein precipitation and so, these samples could not be diluted with the solution containing the nitric acid or the matrix modifier. For this reason, two successive aliquots of 10 μl, separated by a drying step, were injected using the autosampler in the dry last method, as previously described.[18] The first injection consisted of the diluted sample (dilution 1 + 1 for urine and 1 + 3 for both serum and blood samples) containing 0.1% w/v Triton X-100. The second aliquot injected was a solution containing 15, 0.65 and 0.5% w/v of hydrogen peroxide, nitric acid and nickel, respectively. This modifier solution represented the addition of 50 μg nickel to the graphite tube. Between both injection steps, the capillary tip of the autosampler was washed using a solution containing 0.1% w/v Triton X-100 and 0.65% w/v nitric acid. The heating program given in Table 1 was run and the Zeeman background-corrected peak areas due to the analytes obtained. Calibration was performed against aqueous standards for the determination of arsenic in serum or blood samples, and using the standard additions method for the determination of arsenic in urine samples.

Table 1 Furnace heating program

Step	Temperature/°C	Ramp/°C s^{-1}	Hold/s
Dry[a]	200	35	30
Calcination	1600	20	15
Atomization[b]	2500	0	4
Cleaning	2650	0	3

[a] The step was run twice. First the aliquot of sample was dried, then the furnace cooled and the modifying solution injected. [b] The flow of argon was stopped during the atomization step.

Results and discussion

To improve sample reproducibility and to decrease the problems associated with the accumulation of carbonaceous residues and high background signals, the samples were diluted (dilution 1 + 1 for urine and 1 + 3 for both serum and blood samples), and 0.1% w/v Triton X-100 was incorporated in the solutions before they were introduced into the atomizer.

Halls demonstrated that, in many instances, the heating programs can be simplified by replacing the conventional drying and pyrolysis stages by a modified drying stage.[19,20] This so-called fast-program methodology, which considerably shortens the duration of the heating cycle, was tried first, but the results indicated that it is unsuitable in the particular case considered here due to the high background signals. Consequently, a conventional heating cycle was used.

The optimal drying temperature and the holding time to be used in the drying step were studied so that the samples were completely dry before calcination. The optimal values were 200 °C with a 35 °C s^{-1} ramp to avoid sputtering and a 30 s holding time. Because it was necessary to perform two successive injections, a drying step was carried out after the first injection and the heating program was stopped. After injection of the second aliquot the total program was run. The pyrolysis temperature was increased in steps of 200 °C in the range 400–2200 °C using aqueous solutions of arsenic as well as urine and serum samples in the presence of the nickel salt as the chemical modifier. Analyte losses appeared above 1600 °C for all the samples assayed and consequently this temperature was selected (Fig. 1).

The atomization temperature was varied from 2000 °C to 2600 °C, using samples of aqueous arsenic (65 ng ml^{-1}), 1 + 1 diluted urine (SRM 2670 urine elevated level, 245 ng ml^{-1}) and 1 + 3 diluted serum spiked with arsenic (75 ng ml^{-1}), while the pyrolysis temperature was maintained at 1600 °C. As can be seen in Fig. 1, in all cases the analyte signal increased with the temperature, 2500 °C being finally selected. Very narrow atomization peaks leading to a reduced linear response range were obtained when using higher temperatures. A cleaning stage was also included to avoid cross-contamination. It should be taken into account that the values for the temperatures quoted here are those programmed on the electrothermal atomizer and, consequently, they are indicative rather than absolute. The exact temperatures reached inside the atomizer depend on the particular instrument. These temperatures therefore must be checked when using another spectrometer.

The concentrations of hydrogen peroxide and nitric acid were optimized for both maximum analytical signal and minimum

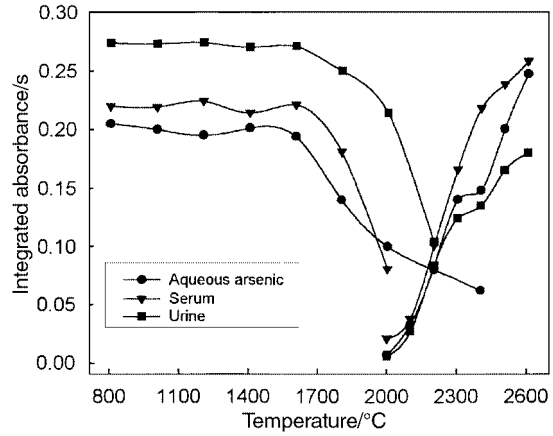

Fig. 1 Variation of the analytical signal with calcination and atomization temperatures, in the presence of 0.5% w/v nickel as chemical modifier, for an aqueous standard (65 ng ml^{-1}), a 1 + 1 diluted urine sample and 1 + 3 diluted serum spiked with arsenic (75 ng ml^{-1}). Nitric acid and hydrogen peroxide concentrations were 0.65 and 15% w/v, respectively.

background level and using 0.5% w/v nickel as the matrix modifier. Fig. 2 shows the results obtained for urine and aqueous standards of arsenic when the nitric acid concentration was varied in the 0–5% w/v range. A 0.65% w/v nitric acid concentration, which corresponds to 1% v/v concentrated acid, was chosen. Higher acid concentrations are not recommended because no advantages as regards the background level or atomization peak shape were obtained, and the useful lifetime of the pyrolytic material is shortened. The concentration of hydrogen peroxide was varied in the 0–20% w/v range. The higher the oxidant concentration, the lower the background signal without change in the analytical signal. For concentrations above 10% w/v the accumulation of carbonaceous residues clearly decreased and so 15% was selected as optimal. The pyrolysis temperature was studied again using this concentration of oxidant and the value of 1600 °C was confirmed as optimum. This is a simple way of producing an oxidizing environment inside the atomizer and an effective *in situ* mineralization of the sample. It should be emphasized that the presence of the oxidant did not produce premature deterioration of the pyrolytic atomizer.

All the above experiments were carried out in the presence of a nickel salt as the chemical modifier because the volatility of arsenic requires thermal stabilizers. Presumably, nickel forms a thermally stable arsenide that prevents volatilization of arsenic until concomitant compounds have been removed during the thermal pretreatment step.[4] The optimal nickel concentration to be used was studied experimentally using first aqueous standards and then the biological fluids mentioned. It was verified that, by using 0.5% w/v nickel, the pyrolysis temperature could be raised up to 1600 °C. When higher concentrations of nickel were used, the sensitivity considerably decreased. Fig. 3 shows that using this concentration in the modifier, which corresponds to the introduction into the atomizer of 50 μg nickel, the analytical signal was stable. Fig. 4 shows an arsenic atomization profile obtained under the recommended conditions. It should be noted that under the optimized experimental conditions, the atomization profiles obtained from the biological samples studied presented low background signals.

The matrix effects were studied by comparing the slopes of aqueous calibration graphs and standard additions graphs. Each graph was constructed with five points and each point was repeated three times. The slopes for aqueous standards and standard additions obtained with urine samples, even when submitted to several degrees of dilution, were quite different, indicating a matrix effect (Table 2). This means that, in this case at least, direct calibration against aqueous standards is unsuitable, the standard additions method being necessary. However, as can be seen in Table 2, for the cases of blood and serum, direct calibration is possible.

The standard additions method is a well-established and widely used procedure but it is tedious in practice. For this reason, a number of experiments were carried out in an attempt to overcome the matrix effect in the determination of arsenic in urine and thus to render the simplest and most direct calibration against aqueous standards feasible. The procedure based on matched standards was first tried by adding some of the main compounds present in the urine samples to the standards. No changes in the slopes of the calibration graphs were observed when 0.1% sodium chloride or 2.5% urea (amounts found normally in urine samples) were incorporated in the standards. The addition of 0.25% w/v potassium sulfate led to such high

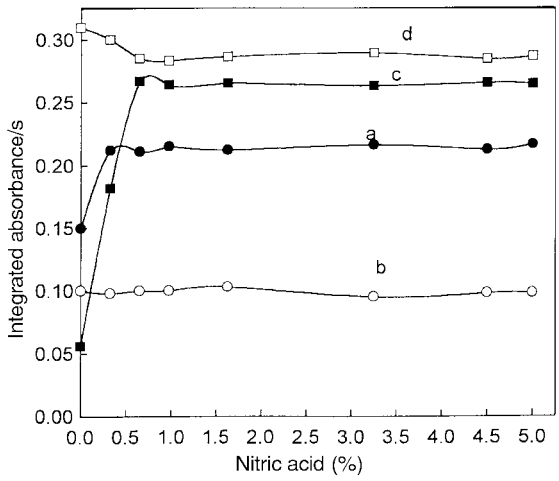

Fig. 2 Effect of the nitric acid concentration: (a) and (b) analytical signal and background, respectively, obtained from aqueous arsenic (65 ng ml^{-1}); (c) and (d) analytical signal and background, respectively, obtained from a urine sample. Hydrogen peroxide concentration was 15% w/v.

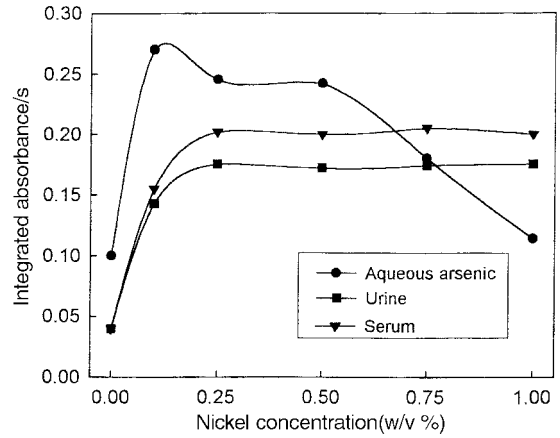

Fig. 3 Effect of nickel concentration on the analytical signal obtained from aqueous arsenic (75 ng ml^{-1}), urine and serum. Nitric acid and hydrogen peroxide concentrations were 0.65 and 15% w/v, respectively.

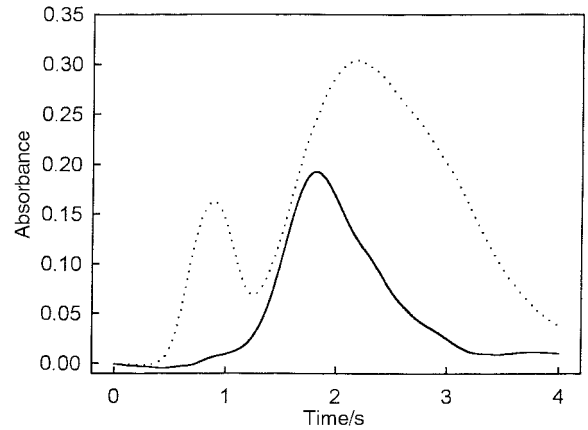

Fig. 4 Profile obtained for the atomization of a 1+3 diluted serum spiked with aqueous arsenic (65 ng ml^{-1}), in the presence of 0.5% w/v nickel . The broken line shows the background signal. Nitric acid and hydrogen peroxide concentrations were 0.65 and 15% w/v, respectively.

Table 2 Slopes of aqueous calibration and standard additions calibration graphs for atomization of arsenic

Sample	Slopes/ml ng^{-1} (mean ±*s*, *n* = 3)
Aqueous standard	0.00303 ± 0.00002
Undiluted urine	0.00102 ± 0.00007
1 + 1 diluted urine	0.00110 ± 0.00005
1 + 3 diluted urine	0.00141 ± 0.00004
1 + 1 serum	0.00292 ± 0.00008
1 + 3 serum	0.00301 ± 0.00006
1 + 1 blood	0.00286 ± 0.00009
1 + 3 blood	0.00293 ± 0.00006

Table 3 Effect of phosphate concentration on the slope of calibration graphs

Phosphate concentration/μg ml^{-1}	0	217	982	4000	10 000
Slope/ml ng^{-1}	0.0030	0.0019	0.0016	0.0008	0.0003

Table 4 Results and recovery study

Sample	As content/ ng ml^{-1}	As added/ ng ml^{-1}	Recovery (%)
Urine 1	ND	20.0	102.1
Urine 2	32.4	20.0	98.4
Urine 3	62.2	20.0	99.0
Serum	ND	20.0	101.5

Table 5 Results for standard reference materials[a]

	Content/ng ml^{-1}	
Sample	Found	Certified
Seronorm 404108 Whole blood	9.49 ± 1.2	(10)[a]
SRM 2670 Urine normal level	57.8 ± 4.3	(60)[a]
SRM 2670 Urine elevated level	487.3 ± 21.1	480 ± 100

[a] Values in brackets are not certified, and are given for information only.

background levels that the analytical signal was distorted. In agreement with the recent report of Ni *et al.*,[10] the presence of potassium monohydrogen phosphate considerably affected the slope of the calibration graph. As can be seen in Table 3 the higher the phosphate concentration the lower the slope of the calibration graph. In addition, significant changes in the arsenic atomization profiles were observed in the presence of phosphate, the peak profile becoming much broader[10] and the time for the appearance of the maximum decreasing. The deleterious effect of phosphate was additionally verified by obtaining standard additions graphs from ten different urine samples, the slopes ranging from 0.00043 to 0.0016 ml ng^{-1} due to the different phosphate concentrations.

Neither the addition of 0.5 or 1.0% w/v lanthanum nitrate to the urine samples or the replacement of the nickel salt by a palladium salt eliminated the interference of phosphate. This agrees with the report of Welz *et al.*,[21] who found that, in the presence of palladium, arsenic was lost even at a low phosphate concentration of 5 mg l^{-1}. No advantages were found when magnesium nitrate was used together with nickel or palladium. All the attempts to overcome the interference by modifying the heating cycle also failed, so that, for urine samples, the standard additions method proved essential.

The calibration graph was linear up to 125 ng ml^{-1} of arsenic. The detection limit calculated for ten successive injections of the blank and using the 3σ criterium (three times the standard deviation of the blank) was 2 ng ml^{-1} (20 pg). The characteristic mass, defined as that amount causing a measured signal of 0.0044 per second when using peak area, was 15 pg.

The repeatability was calculated using the relative standard deviation of ten successive injections. The RSD values were ±2.5% for an aqueous standard (20 ng ml^{-1}), ±3.1% for a urine sample (1 + 1 dilution), ±4.3% for blood (1 + 3 dilution) and ±4.0% for serum (1 + 3 dilution).

Table 4 summarizes the results obtained for serum and several samples of urine using the discussed procedure, as well as the results of a recovery study. It must be mentioned that the arsenic content is within a wide range, from not detectable values up to 62 ng ml^{-1}. This variability in the content of arsenic agrees with the values found in the literature. The average recovery (n = 10) was 100.2% (range 98.4–102.1%). The reliability of the method was checked by using three certified reference materials. As can be seen (Table 5), a good agreement between the reference values and the results was obtained.

Conclusion

Arsenic determination in biological matrices such as urine, serum or blood may be carried out by direct injection of the samples into the atomizer, provided they are submitted to a simple dilution stage. The addition of hydrogen peroxide, nitric acid and a nickel salt to the samples, together with the use of conventional furnace programs considerably reduces the appearance of carbonaceous residues inside the atomizer. The use of the Zeeman-based correction is mandatory to obtain reliable atomization profiles. The advantage of the procedure is its simplicity, because no pre-treatment is necessary, and so the risk of contamination is reduced.

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