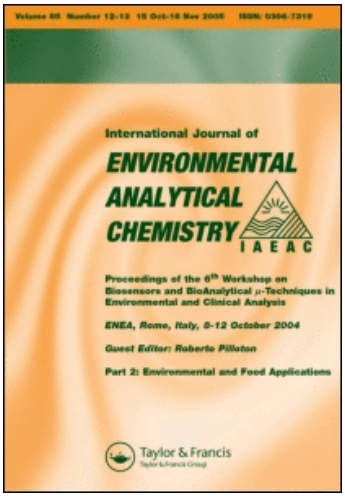

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ATOMIC FLUORESCENCE DETERMINATION OF SELENIUM USING HYDRIDE GENERATION TECHNIQUE

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Hydride generation followed by atomic fluorescence spectrometry, a simple and very sensitive technique, has been evaluated for selenium analysis in different environmental and biological samples. Research is focused on the interfering effects of several selected anions, cations, and acids on the selenium determination and the sample preparation procedures. Besides some transition metals such as Ni^{2+}, Co^{2+}, and Cu^{2+}, HNO_3 acid, the digestion medium used for sample preparation, shows strong interference. In order to reduce this interfering effect, matrix match or standard addition is recommended. This technique is validated by analyzing a number of standard reference materials. Results for selenium analysis in some biological samples are also presented.

Keywords: Atomic fluorescence spectrometry; selenium; environmental and biological samples; hydride generation and interferences

INTRODUCTION

From the perspective of human and environmental health, selenium is an important element because of its dual role as an essential nutrient at low concentrations, and a toxin when in excess.[1–5] For example, it is contained in the enzyme glutathione peroxidase (GSHPx), which affords cells protection against oxidative damage. Selenium compounds have also been reported to have anticarcinogenic activity and to prevent heavy metal effects. A selenium deficiency in man may also result in cardiomyopathy.[4] On the other hand, excessive intake of selenium can lead to toxic responses including "alkali disease" and "blind staggers".[6] The nutritionally required level is very close to the toxic concentration, and the

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narrow margin between the two opposite effects requires accurate and precise knowledge of selenium content in environmental and biological samples.

Analytical techniques for selenium analysis have recently been reviewed.[7,8] Although fluorimetric methods based on the reaction with a fluorophore have been reported,[8,9] they are labor intensive and prone to analytical errors. The element-specific detection systems, such as hydride generation-atomic absorption spectrometry (HG-AAS) and hydride generation inductively coupled plasma mass spectrometry (ICP-MS), are the techniques of current choice for selenium analysis from the selectivity point of view.[7,8,10] However, an on-line pre-concentration of the hydrides using a cryogenic trap, or off-line sample pre-concentration before analysis is often required because of the limited sensitivity provided by these techniques.[11] For example, although the ICP-MS technique exhibits very good analytical performance for ultratrace determination of a number of elements because of its high sensitivity, accuracy and multielement capability, the high ionization potential of selenium (9.8 eV) causes poor selenium ionization efficiency in the plasma (about 30%), resulting in a relatively low sensitivity for selenium determination.[11]

Atomic fluorescence spectrometry (AFS) is a very sensitive and selective method for the determination of a number of environmentally important elements. The advantages of AFS over AAS, in terms of sensitivity, linear range and spectral interferences, have been demonstrated some time ago both theoretically [12,13] and experimentally [14,15]. The first AFS determination of selenium was reported by Dagnall et al. [16] using a dispersive spectrometer equipped with an air-propane flame giving a detection limit of 0.25 μg ml^{-1}. Although dramatic improvements in detection limit were obtained since then [17], it was only recently that HG-AFS became a promising tool for the analysis of hydride forming elements, including selenium. This is mainly due to the availability of the commercial AFS instrument. Corns et al. recently described the design of the commercial AFS instrument and its performance in the determination of some hydride forming elements.[18] A miniature argon-hydrogen diffusion flame was used as the atomizer and the analyte elements were introduced as their gaseous hydride from a hydride generator. The hydrogen for the flame was chemically generated as a by-product of the sodium tetrahydroborate reduction. Excitation was achieved using a boosted-discharge hollow cathode lamp. The detection limit of 0.05 μg Se l^{-1} based on 3σ was reported under optimized conditions.

This paper presents a study on the performance of this relatively compact HG-AFS instrument in selenium analysis. The research was focused on the interferences caused by a variety of anions and cations generally present in the environmental and biological samples. Analytical steps, such as sample digestions, which potentially affect the performance of the technique, will be evaluated. The

validation of this analytical procedure was carried out by analyzing different standard reference materials (SRMs). Application of this technique to the analysis of some biological samples is also presented.

EXPERIMENTAL

Apparatus

A PSA 10.055 Millennium Excalibur System (P S Analytical Ltd, Kent, England) was used for selenium analysis. This system consists of an automated continuous flow hydride generation system for producing covalent gaseous hydrides. Two peristaltic pumps are used to deliver reductant and acidified sample, while a gas-liquid separator is used to separate the gaseous products and carry them to an atomic reservoir where subsequent determination can take place. A hygroscopic membrane dryer tube (Perma Pure Products, Monmouth Airport, Farmingdale, NJ, USA) is used to remove moisture. The AFS detector consists of a boosted discharge hollow cathode lamp (BDCHL) as an excitation source, a hydrogen diffusion flame as an atom cell, a collection of lenses to collect and focus useful radiation, a filter to achieve wavelength isolation and reduction of flame emissions, and a solar blind photo-multiplier tube.[19] The filter used has a peak wavelength at 200 nm and bandpass of ± 10 nm at half-width, which covers the three most important atomic fluorescence lines of selenium atoms (196.02, 203.98, and 206.28 nm). Although autosampler can be used for this system, samples were manually changed for this study.

Reagents and standards

Selenium standard (1000 mg l^{-1} atomic absorption standard) was purchased from Fisher Scientific (Pittsburgh, PA) and used without further purification. Sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) were purchased from Aldrich (Milwaukee, WI). Trace metal grade hydrochloric acid (HCl) and nitric acid (HNO_3) (Fisher) were used throughout this study. All interfering ions were prepared by dissolving quantitative amounts of the corresponding salts with 3 N HCl. The salts used were as follows: KCl, $MgCl_2$, $CaCl_2$, $SrCl_2{\cdot}6H_2O$, $BaCl_2{\cdot}2H_2O$, $FeCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $ZnCl_2$, $AlCl_3$, $NaNO_2$, H_3PO_4, and H_2SO_4. These chemicals were purchased either from Fisher or from Aldrich. The reductant, 0.7 % m/v sodium tetrahydroborate ($NaBH_4$) (Aldrich) in 0.1 mol l^{-1}sodium hydroxide (NaOH), was prepared

freshly each day. Distilled deionized water (DDW) was prepared using a Barnstead Fistream II Glass Still System (Barnstead Thermolyne Corp., Dubuque, Iowa) and was used in all standard and sample preparations. Ultrapure argon and nitrogen gases were supplied Air Products (Allentown, PA). Standard Reference Materials SRM1640 (natural fresh water), SRM 1643d (simulating fresh water), and SRM 1646a (estuarine sediment) were purchased from National Institute of Standards & Technology (NIST, Gaithersburg, MD, USA). Dorm-2 was obtained from the National Research Council of Canada (Ottawa, Ontario, Canada). Real water samples were collected from a small pond located at Florida International University, Miami, Florida. Fish (*Gambusia sp.*) and aquatic invertebrates were collected from the Florida Everglades. Urine samples were obtained from three volunteers in our laboratory.

Procedures

Standard working solution preparation

One ml of the 1000 mg l^{-1} selenium standard was transferred to a 250 ml glass beaker, diluted with 100 ml of 6N HCl, and digested at about 100 °C for 15 minutes using a heating plate. The digested solution was then transferred to a 200 ml volumetric flask and diluted to the mark with distilled water. The final acid concentration was 3 N and the final selenium concentration was 5.0 mg l^{-1}. This was transferred to a polyethylene bottle and used to prepare all calibration standards. The 5.0 mg l^{-1} selenium solution can be used for at least on month. When preparing daily calibration standards, serial dilution was made from 5.0 mg l^{-1} solution in 100 ml volumetric flask with 3N HCl.

Sample preparation

For water sample analysis, 25 ml of the water sample was transferred to a 150 ml glass beaker and 25 ml concentrated HCl (appxi. 12 N) were added. The sample was digested at 100 °C for 15 minutes and then diluted to 100 ml with distilled water. For biological material, 0.1 g of sample was placed in a 125 ml Erlenmeyer flask followed by addition of 10 ml of concentrated HNO_3. The sample was digested for 60 minutes at 100 °C using a heating plate. Next, 2 ml of H_2O_2 were slowly added (0.5 ml each time) followed by further heating for 20 minutes. In order to reduce the nitric acid concentration and to ensure that all Se(VI) had been converted to Se(IV) for hydride generation, the second digestion step was carried out by adding 50 ml 6 N HCl to the sample and heating for 60 minutes at 100 °C. The digested sample was then diluted to 100 ml with DDW water.

Interference study

Generally, five solutions were prepared to measure the interference of the ions, all in 100 ml volumetric flask. All solutions were spiked with the 5.0 mg l^{-1}Se sub-stock solution to a final concentration of 5.0 or 2.5 μg l^{-1} of Se. The first solution served as a "blank" and received no interference ion. Each subsequent solution was spiked with an increasingly larger aliquot of the ion solution. The final ion concentrations of the five solutions ranged from 0- 1000 mg l^{-1} or 0 – 100 mg l^{-1}, depending on the ion. Ions tested at 0, 1, 10, 100, and 1000 mg l^{-1} were K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, NO_3^-, NO_2^-, PO_4^{3-}, and SO_4^{2-}. The ions tested at 0, 0.1, 1, 10, and 100 mg l^{-1} were Ba^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, and Al^{3+}.

The effect of HNO_3 on the analytical performance of this technique was also tested at higher concentrations. Four 5.0 μg l^{-1} Se standard solutions were prepared, in which acid matrices were varied to ensure a hydrogen ion concentration of 3 mol l^{-1}. The compositions of the four solutions were as follows: solution 1, 1 ml HNO_3 in 99 ml HCl (3 N); solution 2, 5 ml HNO_3in 74 ml HCl (3 N), diluted to 100 ml with DDI water; solution 3, 10 ml HNO_3 in 48 ml HCl (3 N), diluted to 100 ml with DDI water; and solution 4, 19 ml HNO_3, diluted to 100 ml with DDI water. These four solutions were analyzed against the standard calibration curve obtained using 3 N HCl as matrix.

Pond water was used to determine the effect, if any, of dissolved organic carbon (DOC) on Se detection. A large sample of pond water was collected from which two solutions were prepared. The first solution was prepared by diluting 25 ml of 12.1 N HCl to 100 ml with the pond water, thus making a 3 N HCl solution of acidified pond water. The second solution was prepared exactly as the one above, except that it was spiked with 200 μl of the 5.0 mg l^{-1} Se sub-stock solution, thus making a 100 ml solution of acidified pond water with a known Se concentration of 10.0 μg l^{-1}. The two solutions were then tested for Se by HG-AFS.

RESULTS AND DISCUSSION

Sample digestion

By using hydride generation technique, selenite (Se(IV)) can be determined directly, but other species of selenium must be converted to Se (IV) prior to analysis. Although thermodynamic calculations indicate selenate (Se(VI)) can also be directly converted to hydrogen selenide (H_2Se) using $NaBH_4$, the required high acid concentration results in complete hydrolysis of borohydride without

selenate reduction.[20] In biological and environmental samples, the total selenium may be composed of selenite, selenate, and selenide (organic and inorganic forms). All those chemical species must be converted to selenite. Therefore, two step digestions, one oxidation and other reduction, are generally used.

Two oxidation procedures, combinations of HNO_3/H_2O_2 and $HClO_4/HNO_3$, were investigated in this study for selenium analysis in DORM-2. Both acidic matrices digested the sample very well. However, a higher blank level was found with the second digestion method. Although different acids can be used as digestion matrices, one must keep in mind that the purity of the reagents is a critical issue in using the high sensitive HG-AFS technique.

Once different species of selenium are converted to selenate by oxidation, heating the sample solution acidified with HCl is a common step to convert selenate to selenite.

$$SeO_4^{2-} + 2HCl \rightleftharpoons SeO_3^{2-} + Cl_2 + H_2O \quad [1]$$

In order to avoid the potential loss of selenium during heating, digestion at 70°C for 20 minutes was first tested using SRM 1643d. It was found that constantly low selenium concentrations (15% lower than expected) were obtained with or without the watch glass cover on the digestion beaker, indicating an uncompleted conversion under these conditions. However, digestion at 100 °C for 15 minutes was found to be a satisfactory procedure. It should be noted that the digestion was carried out in an open beaker without a watch glass cover. Covering the beaker during digestion may cause the back oxidation of selenite to selenate by Cl_2 produced during the digestion procedure.[19]

Interferences

It is well known that hydride generation techniques based on the reaction between $NaBH_4$ and acidified analyte are susceptible to severe interference caused by the presence of transition metals such as Ni^{2+}, Co^{2+}, and Cu^{2+}.[21,22] All of the hydride forming elements suffer interferences from this technique, but selenium seems to suffer the most.[22] Several mechanisms have been proposed for the mode of interference, and it seems clear that the major interference occurs after the hydride has been formed.[22] The $NaBH_4$ reduces the element in question to its hydride first. Once the hydride is formed, it is speculated that the analyte hydride then reacts with reduced forms of the metals or, after their conversion to the corresponding metal borides. Kinetically, the formation of the hydride takes place much more rapidly than the reaction with the metal borides, and so several techniques have been devised to remove the hydrides as soon as they are formed, thereby eliminating the interference.[22,23] It has also been

reported that the magnitudes of the observed effect appear to be highly dependent on the exact nature of the apparatus and experimental conditions employed and should be evaluated for individual applications.[21–23]

The design of the instrument used in this study requires the presence of a mixing valve and a gas-liquid separator where analyte is reacted with $NaBH_4$. Under these conditions, the hydrides remain in the presence of interfering species for a relatively extended amount of time and considerable signal suppression will be experienced. Figure 1 shows the results for the first group of ions, which were tested at concentrations of 0, 1, 10, 100, and 1000 mg l^{-1}. It is clearly indicated from these results that, at regular concentration levels occurring in environmental and biological samples, K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, and SO_4^{2-} do not interfere the analysis of selenium at all under current experimental conditions. It has been reported that one of the most significant interferences can arise from the presence of nitrite, which is readily produced by the reduction of NO_3^- during digestion of solid sample with nitric acid.[22–24] However, in acidic solutions, nitrite ion decomposes to NO and NO_2 gases, which escape the solution in the second digestion step with HCl. As such, the nitrite ion does not interfere with the analysis of selenium.

The effects of the second group of ions, which were tested at concentrations of 0, 0.1, 1, 10, and 100 mg l^{-1}, are shown in Figure 2. No interfering effects were found for Sr, Ba, Fe, Zn and Al. However, a clear decrease in selenium fluorescence signal was observed with increased Co concentration. A 73% suppression of signal resulted from the presence of 100 mg l^{-1} Co. A stronger interference was observed from the presence of Ni. The presence of 10 mg l^{-1} of Ni in the sample solution produced 50% signal suppression, while 100 mg l^{-1} can lead to total signal disappearance. The results clearly show that of all the ions tested, the selenium fluorescence signal suffers most from the presence of Cu. When Cu is present at a concentration of only 1 mg l^{-1}, a 24% suppression of signal occurs, as shown in Figure 2; at concentrations higher than 10 mg l^{-1} no fluorescence signal can be measured. These results are in agreement with those studies using hydride generation coupled with AAS or ICP [22,23]. A number of methods, such as varying concentration of $NaBH_4$ and use of masking reagents etc., have been tested to reduce the interferences of these transition metals for other detection techniques (AAS and ICP). Those methods may be also used for HG-AFS. A detailed investigation of those procedures is beyond the scope of this study.

Although it is believed that concentrations of more than 1000 mg l^{-1} of nitrate is unlikely to exist in natural waters, extremely high levels can be experienced in the analysis of environmental and biological samples because nitric acid is the most widely used digestion reagent. The results obtained at different concentrations of nitric acid are shown in Figure 3. It is clear that, with the presence of

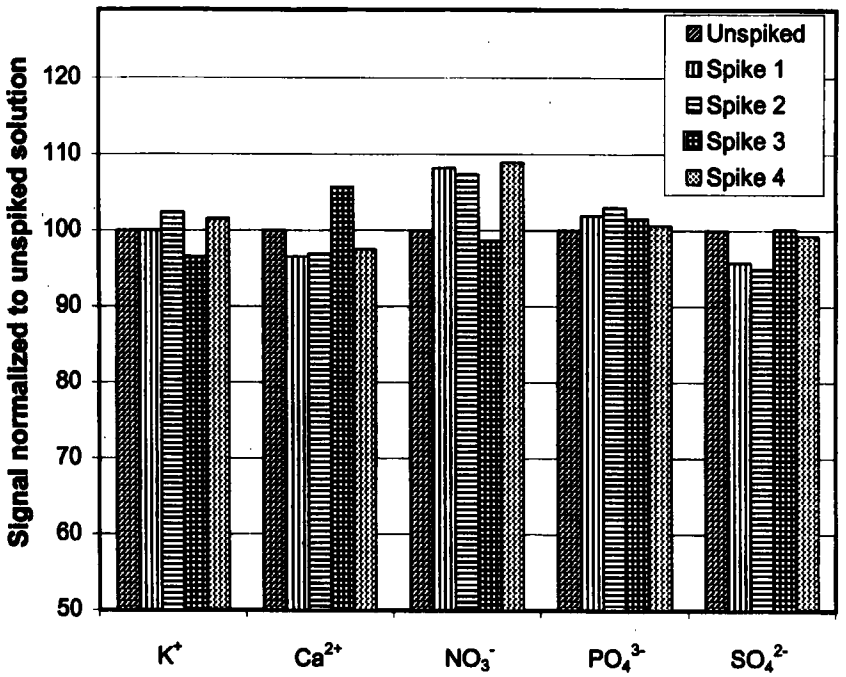

FIGURE 1 Interfering effects of group 1 ions on the determination of selenium using HG-AFS. Signals are normalized to the unspiked solution. Selenium concentrations are 5 μg l^{-1} for K^+, Ca^{2+}, and NO_3^-, and 2.5 μg l^{-1} for PO_4^{3-} and SO_4^{2-}. Unspiked: no interference ions; Spike 1: 1 mg l^{-1} of interference ions; Spike 2: 10 mg l^{-1}; Spike 3: 100 mg l^{-1}; Spike 4: 1000 mg l^{-1}

nitric acid, the 5.0 μg l^{-1} Se spiked solutions (solutions 1 to 4) all reported selenium concentrations of lower than 5.0 μg l^{-1}. Solutions 1 (1 ml HNO_3) and 2 (5 ml HNO_3) were reasonably close to 5.0 ppb, but solutions 3 (10 ml HNO_3) and 4 (19 ml HNO_3) showed a marked decrease in quantitative measurements. This clearly indicates that nitric acid causes measurable interference, and may hamper detection if used in digestion procedures for environmental and biological samples.

In order to determine the loss of HNO_3 during sample digestion, twelve 125 mL Erlenmeyer flasks containing 10 mL HNO_3 were prepared. Each of them was heated in a sandbath at 100 °C. At 10 minute intervals for first 60 minutes, one flask was removed from the sandbath. Then 50 mL of 6N HCl was added to the remaining six flasks. These continued to be heated. They were then each removed at 10 minute intervals over the next 60 minutes. Each sample was removed at its proper time and placed in a water bath to cool the solution. They were then quantitatively transferred to a 100 mL volumetric flask diluted with

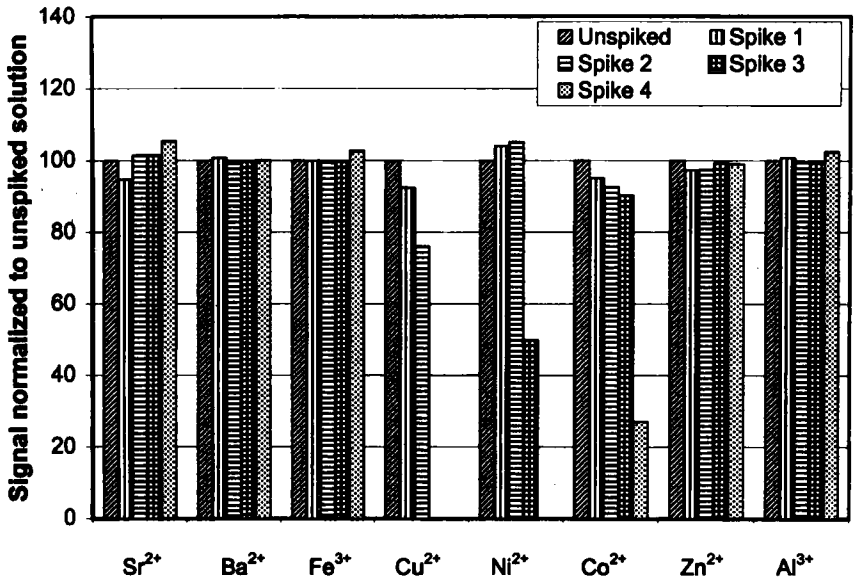

FIGURE 2 Interfering effects of group 2 ions on the determination of selenium using HG-AFS. Signals are normalized to the unspiked solution. Selenium concentrations are 5 μg l^{-1} for Sr^{2+}, Cu^{2+}, Ni^{2+}, and Co^{2+}, and 2.5 μg l^{-1} for Ba^{2+}, Fe^{3+}, Zn^{2+}, and Al^{3+}. Unspiked: no interference ions; Spike 1: 0.1 mg l^{-1} of interference ions; Spike 2: 1 mg l^{-1}; Spike 3: 10 mg l^{-1}; Spike 4: 100 mg l^{-1}

distilled water. The concentrations of these solutions were measured using ultraviolet (UV) spectrometry at 220 nm wavelength.[25] It can be seen from Figure 4 that about 25% HNO_3 was lost during the first 60-minute digestion. Although HNO_3 concentration was further reduced in the second digestion step with the addition of HCl, the final solution still contained about 45% HNO_3.

The pond water in the DOC effect experiment had a DOC concentration of 10.0 ± 0.2 mg l^{-1}. There was no measurable selenium in the pond water. The 10.0 μg l^{-1} Se spiked pond water showed a concentration of 9.8 ± 0.17 μg l^{-1} using a quantitative method of external calibration curve, indicating only a slight change in signal under the experimental conditions.

Validation of the method

Validation of the analytical procedure was carried out by analyzing several water, sediment, and biological SRMs. The results are summarized in Table I. The digestion procedures mentioned in the Experimental Section were utilized. The measured values using HG-AFS was in good agreement with the certified values.

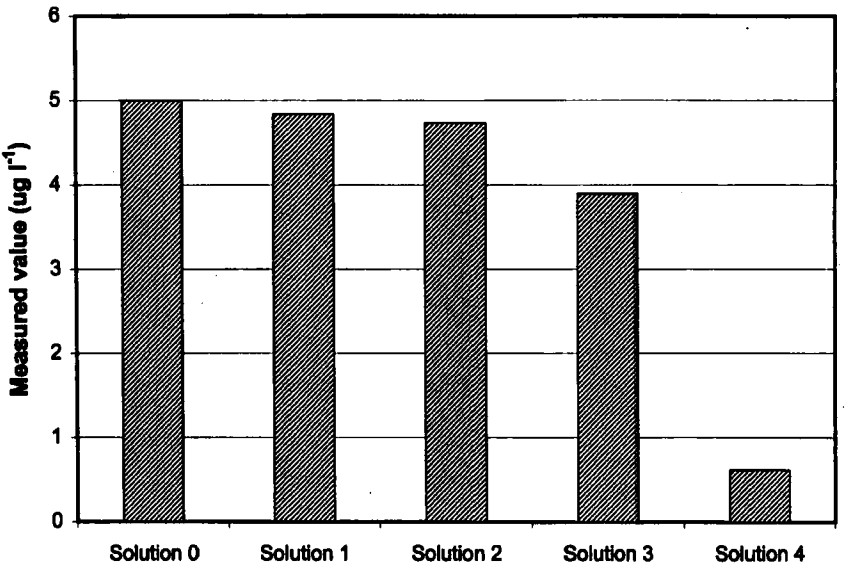

FIGURE 3 Interfering effects of nitric acid on the determination of selenium using HG-AFS. Selenium concentration is 5 μg l^{-1}. Solution 0: 100 mL of HCl (3N); Solution 1: 1mL of HNO_3 in 99 mL of HCl (3N); Solution 2: 5mL of HNO_3 in 74 mL of HCl (3N), diluted to 100 mL with DI water; Solution 3: 10 mL of HNO_3 in 48 mL of HCl (3N), diluted to 100 mL with DI water; Solution 4: 19 mL of HNO_3, diluted to 100 mL with DI water

TABLE I Determination of selenium in some standard reference materials (SRMs) using hydride generation atomic fluorescence spectrometry

SRMs	*Value obtained (n=3)*	*Certified value (n=3)*
1643 (natural fresh water)	21.85 ± 0.03 μg kg^{-1}	21.96 ± 0.51 μg kg^{-1}
1643d (simulating fresh water)	11.43 ± 0.23 μg l^{-1}	11.43 ± 0.17 μg l^{-1}
Dorm-2 (dogfish muscle)	1.33 ± 0.05 μg g^{-1}	1.40 ± 0.09 μg g^{-1}
1646a (estuarine sediment)	0.198 ± 0.013 μg g^{-1}	0.193 ± 0.028 μg g^{-1}

DORM-2 is dogfish reference material with certified selenium concentration of 1.40 ± 0.09 μg g^{-1}. Ten ml of nitric acid was used for the digestion of 100 mg DORM-2. After dissolution of the material, about 5–7 ml of nitric acid was usually left over. This solution was further subjected to second step treatment, i.e. heating in 6 N HCl at 100 °C for 60 min. The selenium concentration obtained using the external calibration curve from this digested solution was found to be about 15 % lower than the certified value, indicating an incomplete digestion or matrix effect to the hydride generation step. When matrix match (preparing

standard curve with 5% nitric acid) or standard addition methods were used, however, the results were in good agreement with the certified value. From this result, it appears that the nitric acid left in the digestion solution is the major cause of the lower recovery.

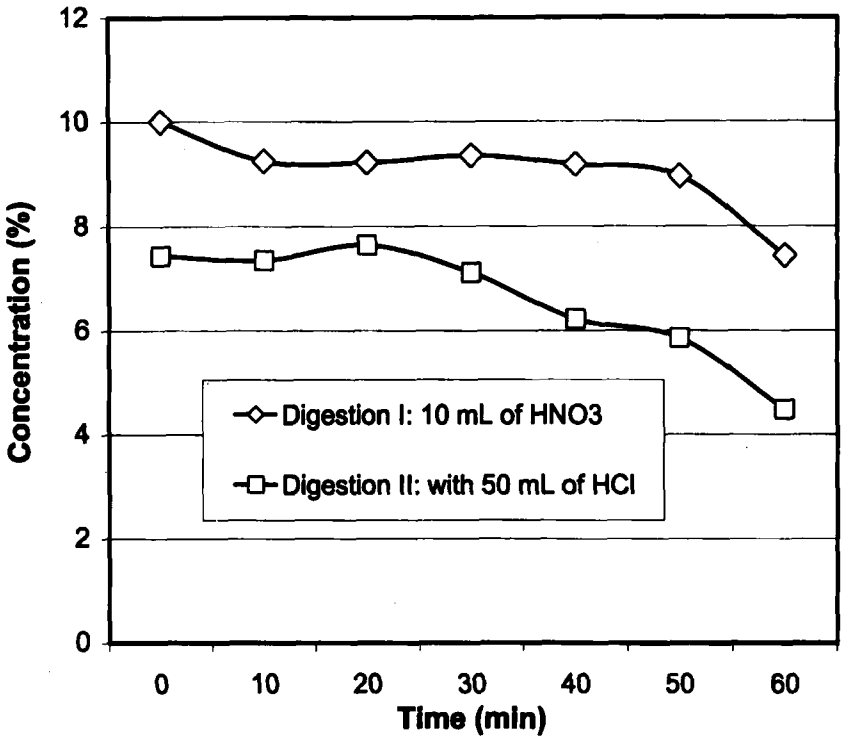

FIGURE 4 Changes of HNO_3 concentrations vs. time of digestion (see text for explanations of digestion I and II). The Y-axis represents the concentrations of HNO_3 in the final solutions

Real sample analysis

The HG-AFS method was used for the determination of selenium in varied biological samples and the results are summarized in Table II. It was observed that both fish samples and aquatic invertebrates were totally digested using the procedure proposed here. The concentrations of selenium found in fish samples ranged from 0.139 to 0.287 $\mu g\ g^{-1}$ wet weight, while that in aquatic invertebrates ranged from 0.136 to 0.975 $\mu g\ g^{-1}$ wet weight. Selenium in three human urine samples was also analyzed with concentrations ranging from 25.09 to 48.0 $\mu g\ l^{-1}$. These results demonstrate that the HG-AFS is a simple and sensitive analytical tool for

selenium determination analysis in biological samples. The concentration detection limit of the system is a function of the sample size. If 0.1 g of tissue is used, the estimated limit of detection (LOD) is 0.05 $\mu g\ g^{-1}$ wet weight. For aqueous sample, 0.05 $\mu g\ l^{-1}$ can be obtained under optimized conditions.

TABLE II Determination of selenium in fish and aquatic invertebrates collected from the Florida Everglades and in three human urine samples

Sample type	*Wet weight used (g)*	*Concentration ($\mu g\ g^{-1}$) (n=3)*
Gambusia sp. (1)[a]	0.1189	0.165 ± 0.009
Gambusia sp. (2)	0.6896	0.139 ± 0.024
Gambusia sp. (3)	0.4078	0.287 ± 0.018
Gambusia sp. (4)	0.0204	0.163 ± 0.001
Procambarus (1)[b]	1.6043	0.137 ± 0.005
Procambarus (2)	2.3556	0.224 ± 0.024
Palaenonetes (1)[b]	2.1213	0.975 ± 0.343
Palaenonetes (2)	1.1304	0.189 ± 0.002
Anisoptera	0.2346	0.261 ± 0.024
Dolomedes	0.1056	0.136 ± 0.017
Pelocoris	0.0755	0.255 ± 0.001
Snail	0.5694	0.139 ± 0.041
Pamacea	7.6832	0.154 ± 0.058
Urine sample 1[c]	10 ml	48.00 ± 0.12 $\mu g\ l^{-1}$
Urine sample 2	10 ml	25.09 ± 0.05 $\mu g\ l^{-1}$
Urine sample 3	10 ml	45.40 ± 0.04 $\mu g\ l^{-1}$

a. Four individuals were analyzed.
b. Two individuals were analyzed.
c. Urine samples were diluted five times before analysis.

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