Ultrasound Accelerated Solid-Liquid Extraction for the Determination of Selenium in Biological Samples by Electrothermal Atomization Atomic Absorption Spectrometry

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A novel method for sample preparation by ultrasound-accelerated solid-liquid extraction prior to selenium determination in the phosphate-rich biological samples by electrothermal atomization atomic absorption spectrometry (ETAAS) with continuum-source background correction and nickel nitrate as a chemical modifier was established and compared with the results of conventional sample wet decomposition (however, this last item was not the object of this study). Diluted (4%) nitric acid was used as an extraction medium. The evaluation of the analytical results for biological Certified Reference Materials indicates that the results of a selenium determination after wet acid digestion (HNO_3+HCl+H_2O_2) agreed well with the certified values, whereas the proposed solid-liquid extraction procedure can be used for simple and rapid control of the selenium levels.

Keywords Ultrasound accelerated extraction, wet decomposition, selenium, nickel nitrate modifier, biological samples, electrothermal atomization atomic absorption spectrometry

Although selenium is known as an essential trace element for humans and animals, it is also potentially toxic. An adequate and safe range of Se for adults ranges between 50 and 200 μg daily.[1] The determination of selenium in samples of biological origin (especially food samples) is an important analytical task.

Atomic absorption spectrometry, both a hydride-generation technique (HGAAS) and electrothermal-atomization technique (ETAAS) is widely applied to the determination of selenium at trace levels. Selenium is among the most difficult elements to determine by electrothermal atomization AAS, as a result of its volatility, reactivity and possible interferences. In particular, phosphorus is known to interfere with the determination of selenium by this technique.[2-5] The interference presumably originated from such molecules as PO, PO^+, and P_2 within the narrow vicinity of the selenium line.[4] Interference problems arising from a phosphate-rich matrix can be reduced by using an adequate matrix modifier. Two commonly used chemical modifiers are palladium (palladium–magnesium)[6-10] and nickel.[11-14] To date, numerous chemical modifiers have been reported; *e.g.* recently a mixture of nickel and strontium nitrates as a modifier for the determination of selenium in urine was proposed.[15] However, there is no universal modifier for all sample matrix, and the chemical forms of selenium in a sample must be considered. The isolation of the selenium absorption signal from interferences can be performed by a combined study of the thermal pretreatment temperature and the chemical modification.[16]

The next analytical problem is adequate sample digestion. Basically, prior to an ETAAS determination of selenium in biological samples a wet digestion procedure is presently used, which is often accelerated by microwave energy.[17,18] However, the high volatility of selenium creates additional problems prior to its analytical determination. Losses of this element may be encountered in conventional ashing and digestion procedures (see, *e.g.* ref. 19). The wet decomposition of solids also increases the risk of sample contamination. A method of ultrasound accelerated solid-liquid extraction for the direct determination of Cd, Co, Mn and Pb in powdered biological samples has recently been described.[20] Therefore, an ultrasound accelerated extraction was tested as a rather simple method of sample pretreatment. Solid-liquid extraction is based on a

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Table 1 Basic instrumental settings and operational parameters

Drying temp./°C	95 (15 s)
	120 (20 s)
Thermal pretreatment temp./°C	650 (30 s)
	900 (5 s)
Atomization temp./°C	2000 (0.5 s)
	2400 (2.5 s)
Cleaning temp./°C	2600 (4 s)
Cool down step	No
L'vov platform	Yes
Integration time/s	3
Inert gas	Ar
Injected sample volume/μl	20
Injected modifier volume/μl	10

In parentheses: hold time of temperature–time program steps.

quantitative acidic extraction of the analyte from the solid sample directly into the liquid phase.[21]

The main purpose of this study was to establish a short and simple analytical method to control the selenium level in some powdered biological samples containing relatively high amounts of phosphorus.

Experimental

Apparatus

A Varian SpectrAA-20+ (Varian, Australia) atomic absorption spectrometer with a GTA-96 graphite-furnace atomizer and a PSC-56 programmable autosampler was used. A selenium hollow cathode lamp (Photron, Australia) operated at 8 mA was used as the primary radiation source. A spectral band pass of 1 nm and a spectral line of 196.0 nm were selected. A deuterium lamp background-correction system was employed throughout all measurements. Pyrolytically coated graphite tubes with pyrolytically coated L'vov platforms (forked type) were used. The optimum experimental settings and operational conditions of the graphite furnace are summarized in Table 1. Samples were injected into the furnace using an autosampler. The temperatures of the furnace were additionally controlled up to 1000°C by means of a thermocouple. An ultrasonic bath (FX-12, Unisonic, Australia) and Vortex type mixer (MT-19, Chiltern Scientific, Australia) were used for sample mixing.

All of the glassware and Teflon tubes were cleaned with HNO_3 and rinsed with Milli-Q water before use.

Reagents and samples

Distilled Milli-Q water, hydrogen peroxide (Specpure, Merck, Germany), and nitric and hydrochloric acids of spectral purity (Aristar, BDH, Australia) were used for sample preparation. Nickel nitrate, palladium nitrate and magnesium nitrate of high purity were applied as chemical modifiers. Working standards of selenium (IV) were obtained from a stock solution (Merck, Germany) by serial dilution with a 0.25% nitric acid solution. Pure KH_2PO_4 was used in some experiments.

The certified reference materials used in the study were prawn sample (AGAL-3) from Australian Government Analytical Laboratories (Sydney, Australia) and bovine liver NIST 1577b from the National Institute of Standards and Technology (Gaithersburg, USA).

Extraction procedure

The samples were initially ground (manually) in a glass mortar for 12 min and weighed (about 250 mg of sample) into polypropylene screw-cap bottles, to which *ca.* 10 g of nitric acid (different concentrations of HNO_3 were used throughout this study) was added as an extraction medium; the bottle was then weighed again, after which the samples were treated for approx.18 min in an ultrasonic bath at a temperature of *ca.* 65 – 68°C (maximum obtainable temperature). The ultrasonic bath was preheated to a temperature of 70°C. After vacuum filtration of the solid residue the liquid phase was collected, 1 ml of the sample supernatant was transferred into the autosampler vial and an aliquot of 20 μl was injected into the graphite furnace. For a comparison, only vortex sample mixing instead of ultrasonic treatment was also tested.

Wet digestion procedure

An appropriate amount of the sample (typically 0.2 – 0.5 g) was weighed into a 50 ml Teflon test tube with a screw cap. After the addition of 6 ml of concentrated nitric acid and 2 ml of concentrated hydrochloric acid, the Teflon tubes were closed and each sample was kept overnight on a water bath (temperature about 90°C). Each sample was heated until the volume was reduced to about half the initial volume. After cooling the tubes and releasing their pressure, 4 ml of H_2O_2 was added to the samples, and the tubes were heated for *ca.* 1 h at 100°C. The tubes were then cooled, and each solution was transferred to a calibration flask and made up to 25 ml with water.

Results and Discussion

Temperature–time program

The instrumental parameters and details of the graphite-furnace heating program used are presented in Table 1. It was observed that drying and charring samples with a series of gradually elevated temperatures proved to be slightly more effective in preventing selenium volatilization than rapid ramping to the highest temperature of each cycle and holding for a longer period of time. The recovery of Se measured for a series of experiments was 1.4 to 2.3% better. The gradual stepwise temperature increase was important for obtaining more reproducible (relative standard deviation was reduced about 1.5%) results. The thermal pretreatment

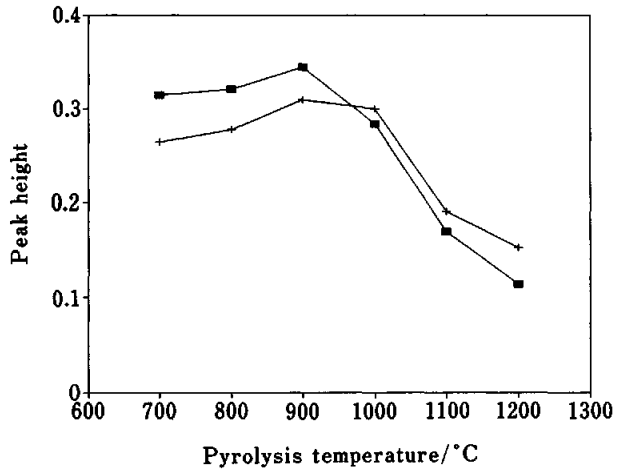

Fig. 1 Influence of the thermal pretreatment temperature on the selenium peak height (2 ng of Se in the graphite furnace) in the presence of nickel (■) and palladium (+).

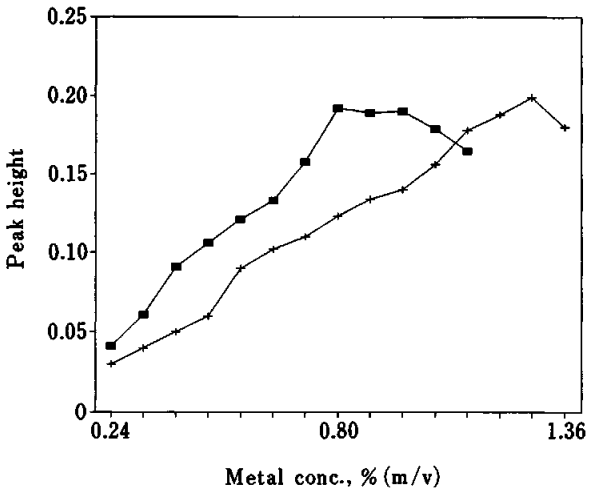

Fig. 2 Influence of the nickel nitrate (■) and palladium nitrate (+) modifier concentration on the analytical signal of selenium in the presence of phosphorus. The thermal pretreatment temperature was 900°C for Ni and 1000°C for Pd modifier.

temperature was optimized; the results are presented in Fig. 1. Finally, a thermal pretreatment step at a temperature of 600°C and an additional shorter step at a temperature of 900°C was applied with a nickel nitrate modifier. Atomization at the temperature recommended by the manufacturer of the spectrometer and with the maximum power heating was used. Since there was no measurable difference in the peak shape, area or height when a cool-down step was used, it was omitted.

The use of air and Ar+1%O_2 as alternate gases during the thermal pretreatment steps was studied, but no significantly positive effect was observed. On the other hand, the life-time of the graphite tube was shortened. Finally, only pure argon as a purge gas (300 ml min^{-1}) was used, and the gas flow was interrupted during the atomization step.

Phosphorus interference

In biological samples the amount of phosphorus is often very high. In the samples which we studied the amount of phosphorus was about 11000 μg g^{-1} for bovine liver and about 6700 μg g^{-1} for prawn tissue. Such an amount of phosphate causes the deuterium arc background-correction system to tend to overcompensate at the selenium resonance lines.

The choice of a proper modifier and its concentration is very important because the mechanism of selenium atomization is very complex, and various chemical forms of selenium (and from different matrices) can atomize in a very different way.[22–24] Fortunately, Johannessen *et al.*[24] observed that the proper increase in the mass of the chemical modifier (Ni, Cu or Pd modifier) injected into the graphite furnace significantly reduced the difference in the characteristic masses for the four studied selenium forms. In this study, the concentration of nickel and palladium modifiers was optimized in order to reduce the phosphorus interference, and stabilize the selenium

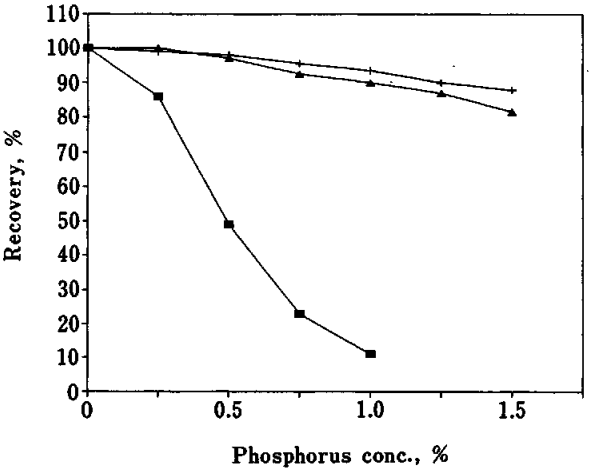

Fig. 3 Effect of Ni and Pd nitrates on the selenium (2 ng) recovery in increasing amounts of phosphorus: (■) without a modifier, (▲) with palladium, and (+) with nickel.

thermally. Experiments involving different chemical modifiers were carried out with new graphite tubes and platform. The effect of nickel and palladium nitrates on the analytical signal of selenium in a 100 ng ml^{-1} standard containing 1.0% of phosphorus (as KH_2PO_4) is presented in Fig. 2. It was stated that for the effective reduction of phosphorus interference in our experimental system a much more concentrated palladium nitrate (1.28% mass/volume of Pd) over that of nickel nitrate (0.80% mass/volume of Ni) must be used. Figure 3 displays the effect of Ni and Pd on the recovery of selenium in increasing amounts of phosphorus. A slightly better recovery for a higher concentration (in the range 1 – 1.5%) of phos-

phorus was observed with a nickel chemical modifier. For this reason a nickel modifier was used; however, with palladium at the optimum concentration a slightly better (5–6%) characteristic mass could be obtained. The addition of magnesium nitrate to a nickel and/or palladium modifier was not advantageous in the studied case. Noteworthy differences in the peak shape and characteristic mass were not obtained. Shan and Bei[25] also reported that the same maximum pyrolysis temperature (1000°C) for a thermal pretreatment without Se loss was permissible, and that the same characteristic mass for this element was obtained with a palladium modifier either alone or in combination with ascorbic acid or magnesium nitrate.

Selenium extraction

Nitric acid was studied as an extraction medium in the range of concentrations which may be directly introduced into the graphite tube of an electrothermal atomizer. Such acids as HCl or $HClO_4$ were not used because of the interference effect of chlorine with ETAAS determinations. The extraction of selenium from the tested samples with nitric acid was successful, and the use of an ultrasonic treatment significantly accelerated the extraction compared to that in vortex mixing. Table 2 displays the fact that the selenium signal increases with increasing the ultrasonication time. The plateau of the analytical signal was obtained with 20 min of ultrasonication at room temperature. Extraction at a higher temperature (about 70°C) was more efficient (see also Table 2) and faster, and the analytical signal was close to the maximum obtained within 17–18 min. Table 3 provides the results for the solid-liquid extraction of selenium from the studied certified reference materials. These results depend on the nitric acid concentration. As shown in this table (Table 3) the percent of extraction is almost constant for an acid concentration of 3.5% and higher. A relatively low acid concentration (4%) was chosen because the more concentrated acid has a stronger deterioration effect on the pyrolytic graphite platform and the tube surface.

The measured extraction efficiencies were similar (a slightly lower for bovine liver) for both samples. This may have been due to a similarity in the forms of the selenium present in these materials. The results may also indicate some similarity of particle size and sample homogeneity. Because the recovery of selenium extracted from spiked samples was good (better than 95%, also see Table 4), the eventual presence of any extracted organic materials was regarded as having no effect.

Table 2 Influence of the ultrasonication time on the selenium extraction from a sample of bovine liver in 3.5% HNO_3

Time of ultrasonication/ min	Percent of Se extracted at:	
	Room temperature	70°C
0.0[a]	59.8	59.8
5.0	64.9	65.1
10.0	69.2	71.2
12.5	69.9	73.6
15.0	72.8	76.9
17.5	73.9	84.9
20.0	75.0	84.2
22.5	74.9	85.0
25.0	75.2	84.4

a. Only short (15–20 s) vortex mixing.

Table 3 Effect of the nitric acid concentration on the extraction of selenium into the liquid phase

HNO_3 conc., %	Se extraction (% of recovery)[a]	
	Prawn	Bovine liver
0.5	42.8	34.0
1.0	74.6	65.2
2.5	84.9	80.0
3.5	87.9	84.9
5.0	86.8	85.2
6.5	88.0	85.1

a. Certified reference value = 100%.

Standardization and quantitative analysis

A comparison of the calibration curves based on the peak height and peak area revealed that the use of the

Table 4 Results of the selenium determination in biological certified reference materials

Sample	Selenium concentration/μg g^{-1}				
	Reference value	After extraction[a]	Recovery[c]	After digestion[b]	Recovery[c]
AGAL-3 Prawn	2.74±0.37	2.41±0.16	96.3	2.51±0.14	109
NIST 1577b Bovine Liver	0.73±0.06	0.62±0.05	95.2	0.76±0.05	106

a. Mean plus standard deviation based on three independent solid-liquid extractions and three measurements on each extraction. b. Mean plus standard deviation based on three independent digestions and three measurements on each digestion. c. Recovery (in %) for spiked samples (spike for prawn sample was 50 ng, for bovine liver sample 25 ng of Se).

peak height gave a better sensitivity and better signal-to-noise ratio. The characteristic mass (with Ni chemical modifier) calculated for the peak height was 24.6 pg (the mass of analyte which produces a defined peak absorbance of 0.0044 A), whereas that for a peak area was only about 30.5 pg of Se (the mass of analyte which provides a net peak area equal to 0.0044 As). The detection limit (based on 3σ criterion) was found to be about 7.5 $\mu g\,l^{-1}$ of selenium. The calibration graph (concentration *vs.* peak height), based on aqueous standards, was linear to about 120 $\mu g\,l^{-1}$ of selenium. The aqueous-solution calibration and standard additions methods were both tested. Since the slope of the aqueous-solution calibration curve only represented 84 – 88% of the slope of the standard additions graphs (the same situation was observed for both digested and extracted samples), the standard additions method was used for quantification. Table 4 shows the analytical results obtained for the determination of selenium in two biological certified reference materials after ultrasound accelerated solid-liquid extraction with 4% HNO_3 and after conventional acid wet digestion compared with the certified values. The results for digested samples were in good agreement with the reference values. The recovery of spiked selenium was slightly higher than 100%. Although the results obtained after ultrasound-accelerated extraction were lower than the certified values, they were still within the confidence levels.

In conclusion, an optimized temperature–time program and a carefully chosen amount of nickel modifier can accommodate high concentrations of phosphorus in a sample, even for an atomic absorption spectrometer with a deuterium-lamp background-correction system. The solid-liquid extraction of selenium by diluted nitric acid as a liquid medium accelerated by ultrasound allows one to separate a matrix of the sample, and can be used as a simplified, rapid and relatively inexpensive "first-action" method of sample pretreatment prior to a selenium determination in some biological samples, especially when very high accuracy is not required.

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