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Method development and optimization for the determination of selenium in bean and soil samples using hydride generation electrothermal atomic absorption spectrometry

Abdallah A. Shaltout[a,b,c], Ivan N.B. Castilho[a], Bernhard Welz[a,d,*], Eduardo Carasek[a], Irland B. Gonzaga Martens[e], Andreas Martens[f], Silvia M.F. Cozzolino[g]

[a] Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil
[b] Spectroscopy Department, Physics Division, National Research Center, El Behooth Str., 12622 Dokki, Cairo, Egypt
[c] Physics Department, Faculty of Science, Taif University, 21974 Taif, P.O. Box 888, Saudi Arabia
[d] Instituto Nacional de Ciência e Tecnologia do CNPq, INCT de Energia e Ambiente, Universidade Federal da Bahia, 40170-115 Salvador, BA, Brazil
[e] Departamento de Nutrição, Universidade Federal de Pará, Belem, PA, Brazil
[f] Institute of Inorganic and Analytical Chemistry, University of Technology, Braunschweig, Germany
[g] Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, São Paulo, SP, Brazil

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ABSTRACT

The present investigation is the first part of an initiative to prepare a regional map of the natural abundance of selenium in various areas of Brazil, based on the analysis of bean and soil samples. Continuous-flow hydride generation electrothermal atomic absorption spectrometry (HG-ET AAS) with in situ trapping on an iridium-coated graphite tube has been chosen because of the high sensitivity and relative simplicity. The microwave-assisted acid digestion for bean and soil samples was tested for complete recovery of inorganic and organic selenium compounds (selenomethionine). The reduction of Se(VI) to Se(IV) was optimized in order to guarantee that there is no back-oxidation, which is of importance when digested samples are not analyzed immediately after the reduction step. The limits of detection and quantification of the method were 30 ng L^{-1} Se and 101 ng L^{-1} Se, respectively, corresponding to about 3 ng g^{-1} and 10 ng g^{-1}, respectively, in the solid samples, considering a typical dilution factor of 100 for the digestion process. The results obtained for two certified food reference materials (CRM), soybean and rice, and for a soil and sediment CRM confirmed the validity of the investigated method. The selenium content found in a number of selected bean samples varied between 5.5 ± 0.4 ng g^{-1} and 1726 ± 55 ng g^{-1}, and that in soil samples varied between 113 ± 6.5 ng g^{-1} and 1692 ± 21 ng g^{-1}.

1. Introduction

Selenium is an essential micronutrient for humans and animals [1]. It has an important role as a substance with anti-cancer properties and its ability to prevent heart disease. Furthermore, selenium possesses antineoplasmatic properties since studies have demonstrated that dietary selenium supplementation can inhibit chemically induced tumors in rats [2]. The US Food and Nutrition Board established dietary reference intakes for selenium, calculated from the recommended dietary allowance (RDA) for certain groups with special physiological requirements [3], which are between 15 and 20 μg/day for infants and 70 μg/day for lactating mothers. An upper limit for safe intake was set at 400 μg/day. Nowadays, application of selenium-containing mineral supplements is very common for cattle breeding in the higher developed south and south east of Brazil.

Different types of food, such as biological materials and dairy products, are important sources of selenium in the human diet and its uptake depends on its chemical form. Various plants growing on selenium-rich soil absorb and accumulate this element [4]. Selenomethionine has been shown to be the predominant form of selenium in wheat, soybeans and selenium-enriched yeast [5].

Because of the importance of this element selenium maps are already available for many countries in the world (Selenium World Atlas) [6]; however, there is essentially nothing known about the natural abundance of selenium in areas of the Brazilian territory. In order to close this gap, a sampling campaign has already been completed to collect bean and soil samples from various states from the north to the utmost south of Brazil [7]. Bean samples have been chosen because they are grown all over Brazil, and they also represent a major part of the daily diet in this country. The purpose of the

* Corresponding author at: Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil. Fax: +55 48 3721 6850.
E-mail address: welz@qmc.ufsc.br (B. Welz).

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present work was to develop and validate a reliable routine method for the determination of selenium in a large number of bean and soil samples, taking into consideration all stages of the analytical procedure.

Because of the usually low concentration of selenium in biological and soil samples and the often complex matrix it is necessary to apply sensitive and selective analytical techniques for its determination. Electrothermal atomic absorption spectrometry (ET AAS) and hydride generation atomic absorption spectrometry (HG AAS) are frequently used for this purpose. While the response of HG AAS is strongly dependent on the selenium species present, ET AAS is adequate for the determination of both organic and inorganic selenium compounds [8]. Nevertheless, the accurate determination of selenium in biological materials and soil is still a major challenge [9–11]. In HG AAS the incomplete mineralization of refractory organic selenium compounds, such as selenomethionine, is one of the major challenges, whereas selenium losses by volatilization were often the cause for erroneous results in ET AAS. Furthermore, there are three nearby iron lines at 195.950 nm, 196.061 nm and 196.147 nm, which might cause spectral interference at the most sensitive selenium line at 196.026 nm when conventional line-source AAS is used [12]. This line is also in the range of strong molecular absorption due to PO and NO bands with pronounced fine structure, which might cause spectral interference due to under- or overcorrection in ET AAS [11,12].

In situ trapping of a hydride (arsine) in a pre-heated graphite tube was for the first time proposed by Drasch et al. [13] in 1980, and further refined by Sturgeon et al. [14]. Sturgeon and co-workers investigated carefully the sequestration of various hydride-forming elements, including selenium [15] in 'used' graphite tubes, which had a more active surface for trapping the hydrides at elevated temperature. In 1989 two papers were published almost simultaneously, in which a treatment of the graphite tubes with palladium was proposed in order to avoid the rather unpredictable behavior of a 'used' graphite tube [16,17]. This treatment was applied successfully by several authors to pre-concentrate and atomize selenium, and to combine the advantages of HG AAS and ET AAS [18–20]. The disadvantage of palladium was its low thermal stability in the atomization stage, so that the coating lasted only for one determination and had to be renewed before each measurement. In order to solve this problem, Shuttler et al. [21] proposed the use of iridium as a permanent modifier for the trapping of selenium and other hydride-forming elements in the graphite tube, a practice that was applied successfully by others later on [22–24]. Iridium applied as a permanent modifier was found to be a much more economic alternative; as such tubes could be used for several hundred measurements without any re-coating. The use of iridium coating also significantly improved the sensitivity of the method and the efficiency of hydride deposition in comparison with those obtained with other kind of modifiers [25].

Nevertheless, the first step of this combined hydride generation electrothermal atomic absorption spectrometric technique (HG-ET AAS) is the generation of the gaseous SeH_2, which requires that the selenium is present as inorganic Se(IV), i.e., selenite. This means that any organic selenium compounds have to be completely mineralized, which might require quite harsh conditions [26], and all Se(VI) has to be reduced to Se(IV) prior to hydride generation. Different methods have been suggested to convert the total organo-selenium in biological materials to inorganic selenium under varying oxidizing conditions. The digestion of human body fluids with only HNO_3 at 160 °C under pressure gave low selenium recoveries, but digestion with nitric, sulfuric and perchloric acids at a maximum temperature of 310 °C under reflux gave recoveries of 97–104% [26,27]. Comparison of several digestion methods for Se determination in biological samples has shown that only the $HNO_3 + HClO_4$ or $HNO_3 + H_2SO_4 + H_2O_2$ mixtures yielded complete recovery of Se [28]. Microwave-assisted wet digestion based on a mixture of HNO_3 and H_2O_2 followed by UV irradiation was successful to recover the total Se content in fish [29], but failed for shellfish samples. After digestion, boiling the solution with 5 or 6 mol L^{-1} hydrochloric acid under reflux for 15–30 min was found to be sufficient to reduce all hexavalent to tetravalent selenium [30–32].

The goal of this work has been to develop a reliable routine procedure for the determination of selenium in a large number of bean and soil samples in order to establish the selenium status in as many states of Brazil as possible. Time and reagent consumption obviously played a major role, and all stages of the analytical procedure had to be optimized.

Table 1
Graphite furnace temperature program for the deposition of the iridium permanent modifier on the graphite platform; argon flow rate 1 L min^{-1} in all stages.

Step	Temperature (°C)	Ramp (°C s^{-1})	Hold time (s)
1	90	5	40
2	110	1	40
3	130	1	40
4	1200	300	25
5	2100	500	10
6	2100	0	5

2. Experimental

2.1. Instrumentation

All measurements were carried out using a Model AAS 5 EA atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with deuterium background correction, equipped with a transversely heated graphite tube atomizer and a Model HS1 hydride generation system (Analytik Jena). A selenium hollow cathode lamp (Analytik Jena) was used as the radiation source and operated with a current of 8.0 mA (wavelength 196.0 nm, spectral bandwidth 1.2 nm). The spectrometer was interfaced to an IBM PC/AT compatible computer. The hydride generation system was used in the continuous-flow mode and the gaseous hydrides were introduced into the pre-heated graphite tube using a Model MPE 50 furnace autosampler (Analytik Jena), equipped with a stainless steel capillary. A four-channel hose pump with snap-in hose cartridges was used for transporting the sample, the reducing agent and the acid to the reaction coil; the fourth channel was used to transport the waste from the gas–liquid separator into the receptacle. The gas–liquid separator was a modified 25-mL pointed flask with larger dead volume, which was half filled with 5-mm diameter glass beads. Pyrolytically coated graphite tubes without platform (Analytik Jena Part No. 407-A81.011) were used exclusively, and coated with 200 μg Ir, depositing five portions of 40 μL of a 1000 mg L^{-1} Ir stock solution and executing the temperature program shown in Table 1 after each injection. The tube treated in this manner could be used for about 500 measurements without any re-coating. The graphite furnace temperature program for trapping the hydrogen selenide and atomization of selenium is shown in Table 2. In order to avoid loss of the permanent modifier at high temperatures, the

Table 2
Graphite furnace temperature programs for the sequestration of hydrogen selenide and atomization of selenium.

Stage	Temperature (°C)	Ramp (°C s^{-1})	Hold time (s)	Argon flow rate (mL min^{-1})
Collection	500	100	30	300
Atomization	2100	2000	5	0
Cleaning	2200	100	3	300