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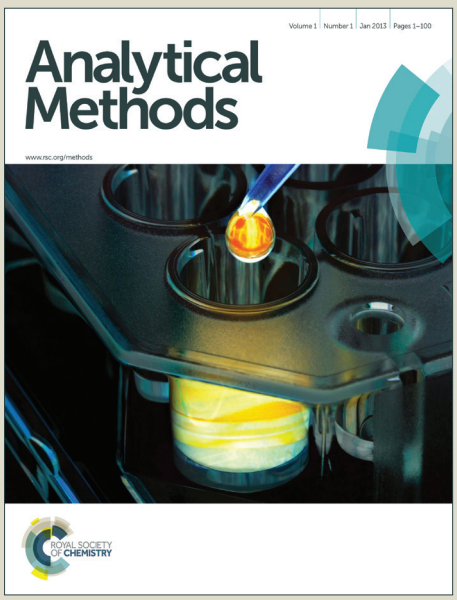

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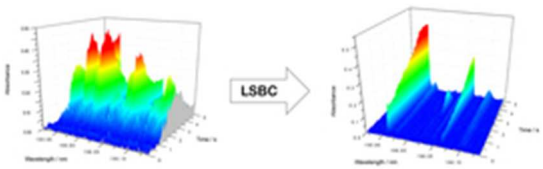

Least Squares Background Correction (LSBC) corrects for spectral interference of the NO and PO molecules in the determination of Se.
23x7mm (300 x 300 DPI)

Determination of selenium in soil samples using high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample analysis

Ivan N.B. Castilho,[a] Éderson R. Pereira,[a] Bernhard Welz,*[ab] Abdallah A. Shaltout,[ac] Eduardo Carasek,[a] Irland B. Gonzaga Martens[d]

[a] *Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil. E-mail:* w.bernardo@terra.com.br (B. Welz); *Fax: +55 48 3721 6850*

[b] *Instituto Nacional de Ciência e Tecnologia do CNPq, INCT de Energia e Ambiente, Universidade Federal de Bahia, 40170-115 Salvador, BA, Brazil*

[c] *Spectroscopy Department, Physics Division, National Research Center, Cairo, Egypt*

[d] *Departamento de Nutrição, Universidade Federal de Pará, Belem, PA, Brazil*

A method has been developed for the determination of Se in soil samples using high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample analysis. The most sensitive absorption line at 196.026 nm has been used for all determinations. Ruthenium, apart from being deposited on the platform as permanent modifier, was also added in solution on top of each solid sample. All soil samples exhibited a fine structured background caused primarily by the NO and PO molecules. Reference spectra of these molecules were recorded using nitric and phosphoric acid, respectively, which were used for least squares background correction. The limits of detection and quantification were 30 ng g^{-1} Se and 100 ng g^{-1} Se, respectively. Soil samples were collected from untreated experimental farming areas (without addition of fertilizers) in order to obtain representative values of natural selenium distribution. Appropriate areas for sampling were selected in 12 of the 27 Brazilian states, covering 61% of the total area of the country. The results of the Certified Reference Materials MURST-ISS-A1, MESS-3 and PACS-2 Marine Sediments confirmed the validity of the proposed method. The selenium content found in the soil samples varied between 130±10 ng g^{-1} and 630±15 ng g^{-1}. The repeatability of the measurements was between 3% and 10% (n = 5).

Keywords: Selenium; High-resolution continuum source AAS; Direct solid sample analysis; Ruthenium chemical modifier; Least squares background correction.

1. Introduction

Selenium plays a key role in the prevention of chronic and degenerative diseases in humans and animals, in its function in the immune system to the threat of free radicals and its detoxifying effect in the contamination by mercury and other toxic metals. Selenium is a component of the enzyme *glutathione peroxidase*, which is responsible for the removal of hydrogen peroxide from cells.[1] In spite of being an essential element, the variation between deficiency and toxicity of selenium is the smallest of all the essential elements. Thus, knowledge about its natural distribution is a fundamental pre-condition in an attempt to prevent harm to human health and animals and develop appropriate prevention.[1]

Different types of foods are important sources of selenium in the human diet and the metabolism depends on its chemical form. Besides that, routine determination based on monitoring sub-μg g^{-1} Se levels in biological materials should be carried out regularly. Several plants growing on soils rich in selenium absorb and accumulate this element[2]. Selenomethionine is the predominant form of selenium in wheat, soy and selenium-enriched yeast.[3] Ingestion of 55 μg/day selenium currently recommended by the "U.S. Dietary Reference Intake" for adults is considered insufficient by many researchers in relation to human need.[4]

Because of the usually low concentration of selenium in biological samples and soil, and the complexity of the matrix, it is necessary to apply sensitive and selective analytical techniques for its determination. Atomic absorption spectrometry with a graphite furnace atomizer (GF AAS), or hydride generation (HG AAS) are often used for this purpose. The response of HG AAS is strongly dependent on the selenium species present, whereas GF AAS is suitable for the determination of both organic and

inorganic selenium compounds.[5] Nevertheless, the precise determination of selenium in biological materials and soil is still a major challenge.[6,7] In HG AAS, incomplete mineralization of refractory organic selenium compounds, such as selenomethionine, selenocysteine and the trimethyl selenonium ion, is the major problem, whereas the loss of selenium by volatilization and spectral interferences are among the main causes for erroneous results in GF AAS. There are three iron lines at 195.950 nm, 196.061 nm and 196.147 nm, which can cause spectral interference at the most sensitive selenium line at 196.026 nm when conventional line source AAS (LS AAS) is used.[8] Molecular absorption bands of NO and PO with rotational fine structure are observed as well in this spectral region, which can cause spectral interference in LS GF AAS.[8]

The combination of hydride generation with trapping and atomization in a graphite furnace (HG-GF AAS) appears to be the ideal solution for the determination of Se. Shuttler *et al*.[9] 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.[10-12] However, the first step of this combined HG-GF AAS technique is the generation of the hydride, which means that it is essential that selenium is present in its inorganic form, Se(IV), or selenite. This also means that any organic compound of selenium must be completely mineralized, which may require extreme conditions,[13] and all Se(VI) has to be reduced to Se(IV) before hydride generation.

In a previous work of our group,[14] a method was developed for the determination of selenium in soil samples using hydride generation with collection and atomization of the analyte in a graphite tube furnace. The method proved to be reliable, however, as it required a microwave-assisted acid digestion, followed by a reduction of

Se(VI) to Se(IV), it was quite time-consuming and not appropriate for the routine analysis of a large number of samples.

The determination of an element using direct solid sample (SS) analysis with GF AAS appears as an attractive alternative.[15-18] Nevertheless, Se still is problematic when conventional line source GF AAS is used because of the above-mentioned spectral interferences.[8] High-resolution continuum source AAS (HR-CS AAS) offers a sophisticated and efficient correction sysyem for almost any kind of background absorption.[8,19] Background that is continuous within the spectral range covered by the array detector is corrected automatically using correction pixels on both sides of the analytical line, whereas structured background can be corrected using reference spectra and a least-squares algorithm.[8,18-20]

The goal of this study has been the development of a fast and reliable method for the determination of total selenium in a large number of soil samples that were collected in 12 states of Brazil already several years ago. GF AAS with direct SS analysis has been envisaged because of its sensitivity, robustness and speed of analysis, as essentially no sample preparation is required. HR-CS AAS has been chosen because it was expected that this technique could cope best with the notorious spectral interferences that are associated with the determination of this element in complex matrices, such as soil samples. One of the goals of method development was also to use aqueous standard solutions for calibration because of simplicity and speed of analysis.

2. Experimental

2.1. Instrumentation

All measurements were carried out using a contrAA 600 high-resolution continuum source atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with a transversely heated graphite tube atomizer. The instrument is equipped with a 300 W xenon short-arc lamp, operating in a hot-spot mode, as continuous radiation source for the wavelength range from 185 - 900 nm; a high-resolution double monochromator, consisting of a prism pre-monochromator and an echelle grating monochromator, providing a spectral bandwidth per pixel of about 1.6 pm at 200 nm; and a linear charge coupled device (CCD) array detector with 588 pixels, 200 of which are used for analytical purposes, displaying the vicinity of the analytical line at high resolution. The most sensitive absorption line at 196.026 nm was used for the determination of Se. All measurements were made with 300 scans per reading and an integration time of 10 ms each.

The graphite furnace technique was used exclusively for all measurements. A manual solid sampling system SSA 6 (Analytik Jena), consisting of a rail and a pre-adjusted pair of tweezers was used to introduce the SS platforms (Analytik Jena Part No. 407-152.023) into SS graphite tubes (Analytik Jena Part No. 407-A81.303). The samples were weighed on an M2P micro balance (Sartorius, Göttingen, Germany) directly onto the SS platforms. Pyrolytically coated graphite tubes with integrated PIN platform (Analytik Jena Part No. 407-A81.025) and an MPE 60 autosampler were used for the measurement of the aqueous standards. The integrated absorbance of three pixels has been added (peak volume selected absorbance, PVSA, $A_{\Sigma 3,int}$),[21] as it resulted in the

best signal-to-noise ratio. Argon 99.996% (Air Liquid, Florianópolis, Brazil) was used as purge and protective gas. The temperature program used for the determination of Se is shown in Table 1.

Table 1. Temperature program for the determination of Se in soil samples; Argon gas flow rate 2.0 L min^{-1} in all stages except during atomization, where the gas flow rate was turned off.

Stage	Temperature / ºC	Ramp / °C s^{-1}	Hold Time / s
Drying	110	5	10
Pyrolisis	1400	300	15
Atomization	2100	3000	5
Cleaning	2500	500	6

2.2. Reagents and standard solutions

All reagents used in this work were at least of analytical grade. Nitric acid (Merck, Darmstadt, Germany) was further purified by double sub-boiling distillation in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). Distilled and deionized water obtained from a Model Mega ROUP purification system (Equisul, Pelotas, Brazil) with a specific resistivity of 18 MΩ cm was used throughout for preparation of calibration solutions. All bottles were decontaminated with 30% v/v nitric acid for 24 hours and then rinsed with deionized water three times before use.

The standard solutions used were prepared by serial dilution of a 1000 mg L^{-1} Se stock solution (Merck, Darmstadt, Germany) with water. For the determination of

selenium, Ru has been used as a permanent modifier. A stock solution containing 1000 mg L^{-1} Ru (Fluka, Buchs, Switzerland) has been used as provided for coating of the platform. Ten repetitive injections of 40 µL of the stock solution, each one followed by a five-step temperature program with previously optimized ramp and hold times,[19] have been used for coating the platform with a total of 400 µg of Ru modifier. The same procedure was followed with two alternate modifiers: Pd 1.000 mg L^{-1} (Merck) and Zr 1.000 mg L^{-1} (Merck). The mixture of 0.05% w/v Pd and 0.03% w/v Mg, both as the nitrates (Merck), with 0.05% v/v Triton X-100 (Union Carbide) was used as a modifier in solution, injected directly over the solid samples.

The certified reference materials (CRM) MURST-ISS-A1, "Antarctic Marine Sediment" (Italian Programma Nazionale di Ricerche in Antartide, Instituto Superiore di Sanita, Rome, Italy), MESS-3 and PACS-2 Marine Sediments (National Research Council Canada, Ottawa, Canada) were used to check the accuracy of the proposed method.

2.3. Samples and sample preparation

The samples were collected in polyethylene bags and stored at -18 °C. In the laboratory the samples were dried in an oven at a temperature of 50 to 60 °C for 24 h, allowed to cool down over silica gel and stored in polyethylene cups. First, all samples were ground manually in an agate mortar until the material passed a sieve of 0.5 mm. Roots and plant material were removed during this procedure. Further grinding was done in a planetary ball mill (Fritsch Pulverisette Model 05.102) with agate cups, lids and balls for 20 min at 300 rpm. After that samples were passed again through laboratory sieves of 0.1 mm and stored in polyethylene cups until they were analyzed. A number of soil samples from the collection campaign have been analyzed to show the

applicability of the developed method for real samples. No attempt has been made to correlate the concentration of selenium found in the samples with the location where they were collected.

3. Results and discussion

3.1. Pyrolysis and atomization curves

The pyrolysis and atomization curves for selenium, using an aqueous standard solution and various modifiers are shown in Figure 1. The Pd + Mg modifier added in solution clearly showed the best results, followed by the permanent modifiers Ru and Ir. Palladium used as a permanent modifier could stabilize Se only to a pyrolysis temperature of 900 °C, which is much lower than the 1400 °C that could be used with the other modifiers. The use of Zr as a permanent modifier clearly resulted in the lowest sensitivity for Se.

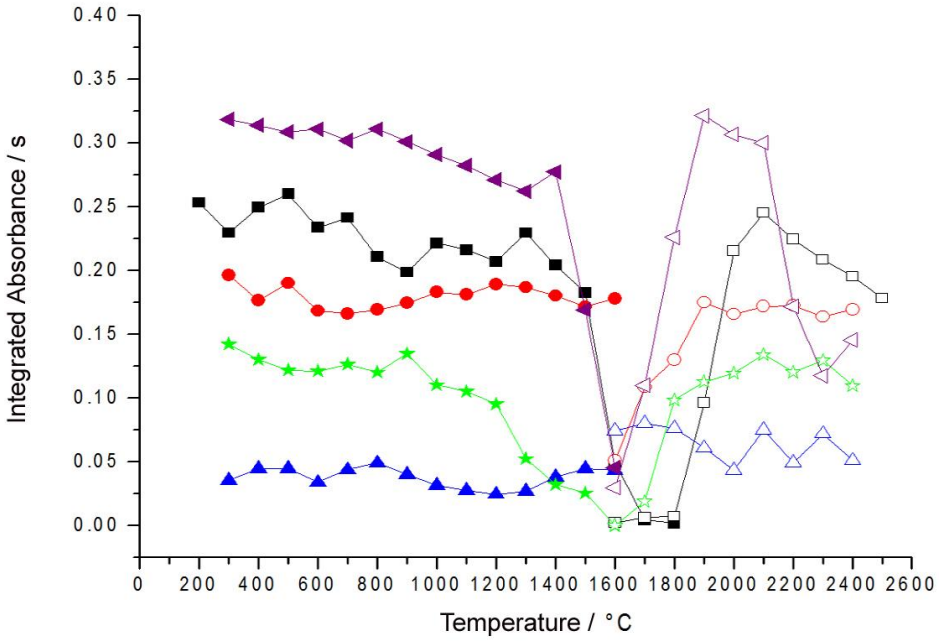

Figure 1. Pyrolysis (full symbols) and atomization (open symbols) curves obtained with HR-CS GF AAS for Se with the modifiers Ru (■,□), Zr (▲,Δ), Ir (●,○), Pd (★,☆) and Pd + Mg in solution (◀,◁). Atomization temperature, T_{at} = 2100 ºC for pyrolysis and pyrolysis temperature, T_{pyr} = 1400 ºC for atomization, except for Pd, where T_{pyr} = 900 ºC.

Using the data obtained with the aqueous standard solution, pyrolysis and atomization curves were also established for a solid soil sample using the modifiers that provided the best sensitivity: Pd + Mg in solution and Ru as a permanent modifier, respectively. However, the results have been extremely non-reproducible at all temperatures and for both modifiers, so that no meaningful data could be obtained to establish pyrolysis and atomization curves. To solve this problem, different strategies have been investigated; one of them has been using ruthenium both as a permanent modifier deposited on the platform, and as a modifier in solution added on top of the

solid soil sample. Figure 2 shows the results of this experiment, which appeared to be appropriate for the determination of selenium in soil samples, so that these conditions, i.e., a pyrolysis temperature of 1400 °C and an atomization temperature of 2100 °C, have been used for all further investigations.

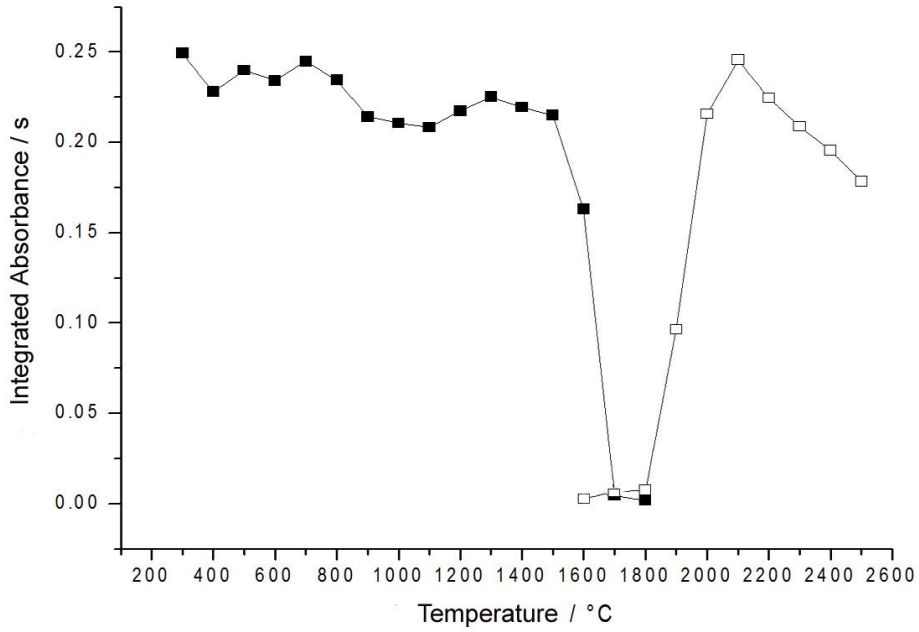

Figure 2. Pyrolysis (■) and atomization curves (□) obtained with HR-CS GF AAS for Se in a soil sample, using Ru as a permanent modifier and also added in solution over the solid sample; T_{at} = 2100 ºC for pyrolysis and T_{pyr} = 1400 ºC for atomization.

3.2. Background correction

All soil samples exhibited high discontinuous background absorption, as is shown in Figure 3A. This background has been identified as being primarily due to the NO and PO molecules. Pure reference spectra for these two molecules were established

using 20 µL each of nitric and phosphoric acid, respectively, and the same temperature program as shown in Table 1. These two spectra were then subtracted sequentially from the spectra recorded for the soil samples, using the least squares background correction (LSBC) algorithm provided by the software of the HR-CS AAS equipment.

Figure 3B shows the absorbance spectrum for the same soil sample after subtraction of the spectra for NO and PO using LSBC. The remaining corrected spectrum only shows the selenium signal and the well-known iron lines in its vicinity. The latter ones, however, are well separated in wavelength from the analytical line, so that they cannot cause any interference in HR-CS GF AAS. Varying quantities of iron have been found in all the soil samples investigated in this study.

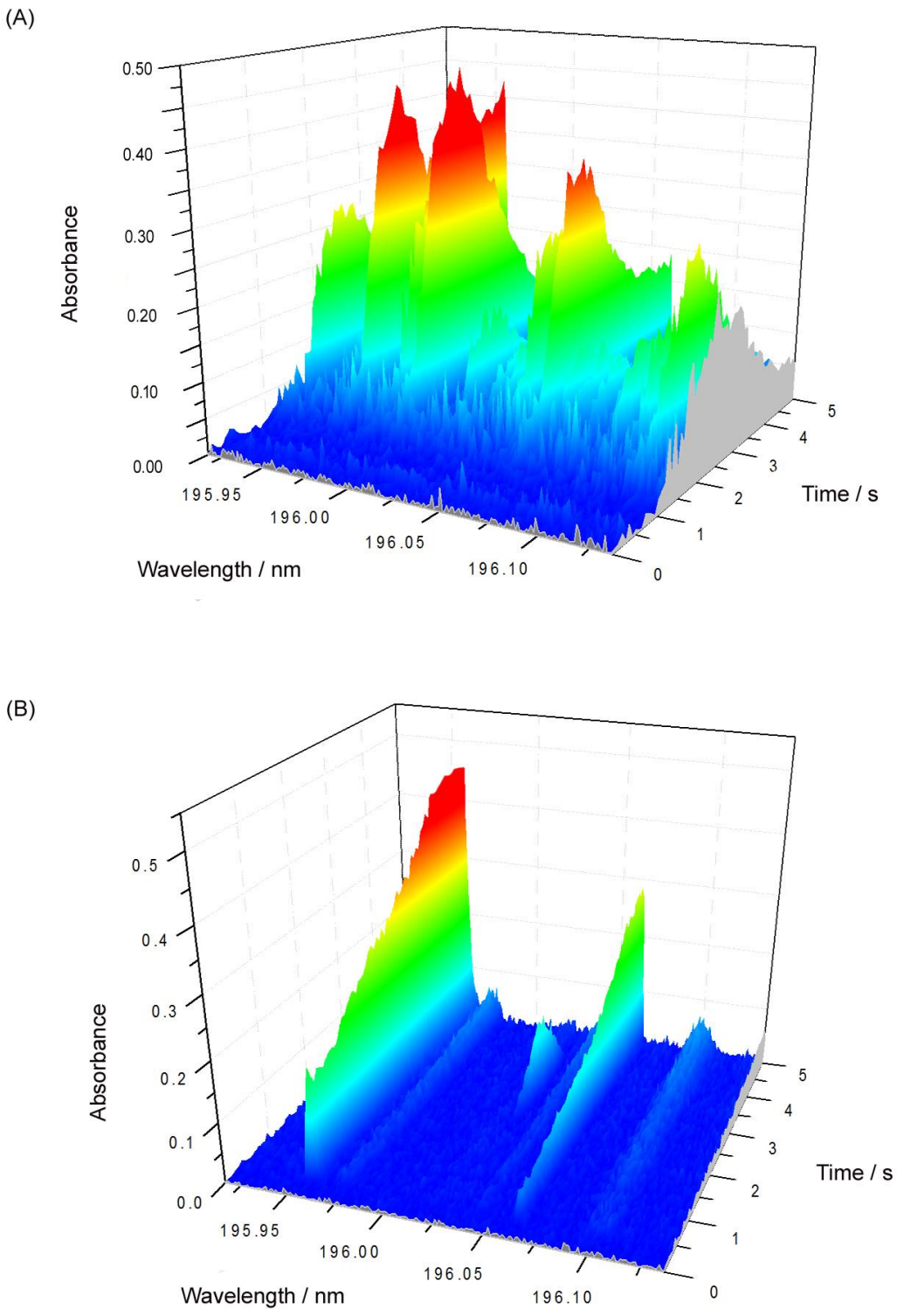

Figure 3. Absorbance spectrum over time for a soil sample in the vicinity of the selenium line at 196,026 nm; T_{pyr} = 1400 ºC; T_{at} = 2100 ºC. **A:** after automatic correction for continuous background only; **B:** After sequential subtraction of the reference spectra for NO and PO using LSBC.

3.3. Figures of merit

After all the analytical conditions have been optimized, such as temperature program and modifiers, a calibration curve has been established and the analytical figures of merit determined. The linear regression equation, the coefficient of correlation (R), the characteristic mass (m_0), limit of detection (LOD) and quantification (LOQ) for Se are shown in Table 2. The characteristic mass is the mass of an analyte required to generate an integrated absorbance of 0.0044 s. The value of 50 pg found in this work is close to the value of 40 pg reported in the literature for a transversely heated graphite tube.[8] The LOD and LOQ have been calculated as 3 and 10 σ/S (n = 10), respectively, where σ is the standard deviation of a blank, and S is the slope of the calibration curve. In direct SS analysis σ is usually calculated according to the 'zero mass response', inserting repeatedly an empty SS platform that only contains the modifier, and running a complete temperature program.[22]

Table 2. Figures of merit for the determination of Se with HR-CS SS-GF AAS.

Parameter	Se
Regression equation	$A_{int} = 0.0937\ m_{Sb} + 0.0191$
R	0.9989
LOD / ng g^{-1}	30
LOQ / ng g^{-1}	100
m_0 / pg	50

3.4. Analysis of soil samples and a CRM

The results obtained for selenium in different soil samples and three CRM using HR-CS GF AAS and direct SS analysis are shown in Table 3. Also shown are the results obtained in previous work of our group for the same samples after microwave-assisted digestion and using HG-GF AAS.[14] There is no significant difference between the two sets of results, based on a Student t-test on a 95% confidence interval. The result obtained for the three CRM also confirmed that the proposed method using HR-CS GF AAS and direct SS analysis with calibration against aqueous standard solutions provides accurate results. The precision, expressed as RSD, was better than 10% in all cases, which is acceptable for direct SS analysis, considering sample inhomogeneity and the small sample portions used for the determination.

Actually, the precision obtained with the comparative method, HG-GF AAS, is not significantly different from that for the proposed method, although acid digested samples were used in that case. This is most likely due to the relatively large number of sample preparation steps involved in this method. Another aspect is the number and quantity of reagents that have to be used for HG-GF AAS in comparison with HR-CS GF AAS and direct SS analysis, which only requires a chemical modifier.

Last, but not least, there is the question about the sample throughput. Using HR-CS GF AAS with direct SS analysis, a single measurement takes about five minutes, which comes to about 15 min for a triplicate analysis, and a sample throughput of about 25-30 samples per day, including time for calibration, coating of platforms etc. This throughput is roughly a factor of five greater than that with HG-GF AAS, mostly due to the extensive sample preparation necessary for the latter one, which means that the

proposed method meets the expectation for a fast routine determination of Se in a large number of soil samples.

Table 3. Results obtained for the determination of Se in soil samples and CRM; all values are average ± standard deviation of n = 3 determinations.

Sample	Certified value / mg kg^{-1}	Concentration found / ng g^{-1}		RSD / %
		HG-GF AAS[14]	HR-CS SS GF AAS	
S01 (F/IR/AM)	−	604 ± 15	630 ± 15	2.5
S02 (C/CG/MS)	−	113 ± 6.5	130 ± 10	7.7
S03 (F/TA/PA)	−	419 ± 18	405 ± 20	5.0
S04 (C/VA/RS)	−	248 ± 11	280 ± 20	7.1
S05 (C/LA/SC)	−	262 ± 20	250 ± 25	10
S07 (C/PA/CE)	−	599 ± 24	550 ± 20	3.6
S08 (F/FL/SC)	−	406 ± 19	420 ± 20	4.8
S09 (C/SH/GO)	−	215 ± 21	200 ± 20	10
S10 (C/CU/PR)	−	370 ± 12	350 ± 10	2.9
MURST-ISS-A1	2.2 ± 0.1*	2076 ± 129	2050 ± 150	7.3
MESS-3	0.72 ± 0.05	−	690 ± 30	4.3
PACS-2	0.92 ± 0.22	−	880 ± 50	5.7

*****Informed value, not certified

3.5. Potential limitations of the proposed method

The background correction problems, particularly those due to diatomic molecules, such as NO and PO that hamper the determination of Se with LS GF AAS, or even make it impossible, do not present any difficulty in HR-CS GF AAS, as they can be eliminated using LSBC. The presence of iron does not pose a problem either, as the three lines in the vicinity of the Se line are well separated in wavelength from the latter one in HR-CS GF AAS. Silica, the only other substance that is often found at high concentration in soils, does not present any problem either, as the very pronounced absorption band system of the SiO molecule is located between 210 and 260 nm,[8] and no absorption has been observed below 200 nm.

Actually we encountered only one problem during these investigations, which has not yet been mentioned. Originally we had another CRM included in our investigations, BCR-142, "light sandy soil" (European Community Bureau of Reference, Brussels, Belgium); however, we did not succeed to determine Se in this CRM. Immediately upon the addition of the Ru modifier in solution over the sample, strong foam formation and bubbling was observed, and after the reaction had ceased, we could not detect any Se signal. We did not investigate this problem in detail, as none of our samples had shown a similar effect; however, we suspect that the CRM contains a significant amount of limestone and the high acid concentration of the Ru modifier added over the solid sample caused the evolution of CO_2. Somehow the Se must have been lost during this violent reaction, although we do not have any proposal for a potential mechanism. Nevertheless, a warning has to be issued that the proposed procedure might not be applicable to all kind of soils.

4. Conclusion

A procedure for the determination of selenium in soil samples has been developed using high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample analysis. A clear advantage of direct analysis of solids is that the method requires essentially no toxic or corrosive acid and produces no harmful discharge to the environment. In addition, it is fast and requires no sample preparation except the usual crushing and sieving, which, however, has to be used as well prior to sample digestion. The calibration can be performed with aqueous standards, which further simplifies the procedure.

No forecast should be given here if the proposed procedure could be applicable for other analytes and matrices, as this might not be the case. Nevertheless, we consider this work as a break-through, as the determination of selenium in soils and sediments using GF AAS and direct SS analysis has kept us busy for at least one decade before we succeeded, and to the best of our knowledge, there is no other publication in the literature on this topic.

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