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Talanta 66 (2005) 858–862
Talanta
www.elsevier.com/locate/talanta

Determination of selenium in water and soil by hydride generation atomic absorption spectrometry using solid reagents

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Received 28 May 2004; received in revised form 9 November 2004; accepted 17 December 2004
Available online 16 March 2005

Abstract

A hydride generation method for the determination of traces of selenium at ng mL^{-1} concentration ranges has been introduced using a solid mixture of tartaric acid and sodium tetrahydroborate. Atomic absorption spectrometry (AAS) has been used as the detection system. Several parameters such as the ratio of tartaric acid to sodium tetrahydroborate, type and amount of acid, and the reaction temperature were optimized by using 640 ng mL^{-1} (16 ng per 25 μL) of Se(IV) standard solution. The calibration curve was linear from 20 to 1200 ng mL^{-1} (0.5–30 ng Se(IV) per 25 μL). The relative standard deviation (%R.S.D.) of the determination was 1.93% and the detection limit was 10.6 ng mL^{-1} (265 pg per 25 μL) of Se(IV). The reliability of the method was checked using different types of environmental samples, such as several types of water, a sample of soil and also in a kind of calcium phosphate sample by standard addition method. For conversion of Se(VI) present in real samples to Se(IV), L-cysteine was added to $NaBH_4$ and tartaric acid mixture. The results showed good agreement between this method and other hydride generation techniques.

Keywords: Solid reagents; Hydride generation; Selenium determination

1. Introduction

Toxic elements such as arsenic, selenium, tellurium, antimony and lead can be determined by hydride generation method. Flame atomic absorption and inductively coupled plasma are usually used as detection systems [1–7].

Selenium as a nonmetallic chemical element, has received high attention of biologists because of its dual role as an essential trace nutrient and a toxic element. This dual effect has been recognized for many naturally occurring chemicals. This element is an essential trace mineral in the human body. It is an important part of antioxidant enzymes that protects cells against the effects of free radicals that are produced during normal oxygen metabolism. Selenium is also essential for normal functioning of the immune system and thyroid gland. Vegetables are the major dietary sources of selenium [4,8–12]. The amount of selenium in soil, which varies by region, determines the amount of selenium in the vegetables that are grown in that soil [13].

Several analytical techniques have been applied to the determination of selenium in biological sample. The most important are fluorometry, voltammetry, hydride generation atomic absorption spectrometry (HGAAS), hydride generation atomic fluorescence spectrometry, graphite furnace atomic absorption spectrometry, mass spectrometry and neutron activation analysis [1,14–18]. HGAAS is usually the method of choice where simplicity, sensitivity and short analysis time is required [19–25].

HGAAS technique involves the generation of metallic hydride species in order to separate the analytes from the matrix and concentrate them in the analyzing volume, and finally permitting not only the determination of the total content of the elements but also the content of their species [26–29].

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doi:10.1016/j.talanta.2004.12.053

Many batch methods were introduced in which an acidic sample was added to sodium tetrahydroborate solution. The hydride is transferred to the detection system by an inert gas. To achieve higher precision, accuracy, and decreasing the analysis time flow methods were used [18]. Various multichannel segmented or continuous flow methods have been used. Flow methods are more suitable for large number of samples. High volumes of reagents are consumed particularly in those methods that steady state signals are obtained. The instrumentation is also more complex than batch methods. In these techniques in order to achieve higher sensitivity in hydride generation of selenium, more concentrated $NaBH_4$ is used and also concentrated NaOH solution is needed.

In the analysis of elements, reagents in solid phase usually cause simplicity of the method, less interference effects, and reduction in pretreatment steps of the analysis [30].

In this study, it is intended to develop a simple and precise technique using solid reagents for the determination of selenium species in environmental samples by HGAAS technique. This type of HGAAS technique was previously used for the determination of lead [31].

2. Experimental

2.1. Reagents

All the reagents used in this study were of analytical reagent grade. Triple distilled water was used throughout. Se(IV) stock solution (1000 μg mL^{-1}) was prepared by dissolving 0.14053 g SeO_2 (Merck, Darmstadt, Germany) in 100 mL volumetric flasks. A minimum amount of NaOH was added to dissolve the selenium dioxide before dilution to the mark with distilled water. The fresh standard solutions were prepared daily by successive dilution from the stock solution. Standard solution of each interfering ion was prepared from its analytical soluble salt. Tartaric acid and $NaBH_4$ were of analytical grade (Merck, Darmstadt, Germany).

2.2. Apparatus and operating conditions

An AA9 Pye-Unicam AA spectrometer was used. Analog output of the spectrometer was connected to the input of a laboratory made RS-232 interface. Absorbance data were transferred to the computer via the interface. The data were collected and processed using programs written in Visual Basic.

The setup of the system is shown in Fig. 1. It consists of a straight 12 cm (i.d. 8 mm) quartz tube as the cell. This tube was fixed at an optimum height on the top of the slot burner. The quartz tube is connected to one side of a Teflon-T connector. A piece of quartz window was attached to the opposite side of the Teflon-T. The side arm of the Teflon-T was connected through a piece of tygon tubing to the side arm of another brass or Teflon-T connector. The other two ends of the T connector were connected to the sample tube and a

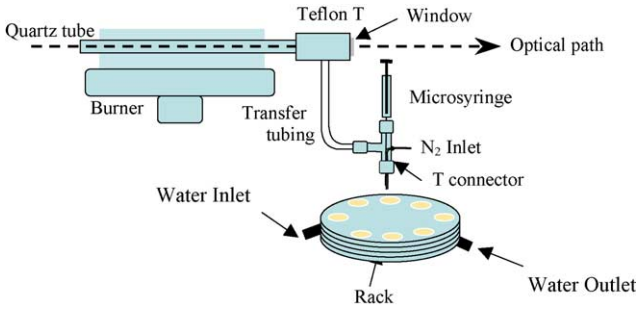

Fig. 1. Schematic diagram of the apparatus.

septum for injection of the sample. A small hole was drilled in the second T connector and a piece of Teflon tubing was inserted to the sample tube through this hole. This tubing was glued to the T connector. This tubing is used as the carrier gas inlet. A thermostated six-cell sample rack was also used as shown in Fig. 1.

2.3. Procedure

For selenium(IV) determination, $NaBH_4$ and tartaric acid, were dried separately in a microwave oven oven at about 80% of full power for 20 s. Then, about 0.2 g of $NaBH_4$ and about 0.18 g of tartaric acid were placed in each cell. The cells were heated to 38 °C via circulation of water. Nitrogen flow rate was 750 mL min^{-1}.

For total selenium determination (in real samples), about 0.12 g L-cysteine was also added to the above solid mixture. After thermal equilibration 25 μL of selenium sample solution was injected and the absorbance was recorded.

2.4. Real sample pretreatment

In this study total selenium content in soil, two types of water samples, and a kind of polyphosphate sample was determined by standard addition method.

Exactly 0.2 g of sodium polyphosphate was transferred into a beaker. Then 10 mL of concentrated HNO_3 was added and the sample was digested completely by heating to about 80 °C for 5 min. The solution was transferred to 100 mL volumetric flask and diluted to the mark.

Samples of soils were grinded to obtain a uniform, fine powder. One gram of this was transferred into a beaker. Then 10 mL of HNO_3:HCl (3:1) was added and was allowed to digest completely by heating to 80 °C for 4 min. Then, the solution was filtered and poured into a 50 mL volumetric flask and diluted to the mark with distilled water. For each sample, the determinations were made using standard addition method each result was an average of three or four analyses.

For water samples 5 mL of each was acidified by 1 mL of concentrated HNO_3 and heated to about 70 °C for 3 min and was transferred to 100 mL volumetric flask and diluted to the mark. This sample was analyzed by the recommended procedure, described for total selenium measurement. It should

be mentioned that for the analysis of all these real samples, 0.12 g of L-cysteine was added to $NaBH_4$ and tartaric acid mixture in order to make sure that all Se(VI) in the samples are first converted to Se(IV).

3. Results and discussion

3.1. Optimizations of operating parameters

In selenium determination, several parameters were optimized. The parameters having influence on the sensitivity of selenium hydride (SeH_2) generation are system design, the amount of sodium tetrahydroborate as reducing agent, the type and amount of the acid, the generation temperature. The optimization procedures were carried out by measuring the absorbance of 25 μL injection of 640 ng mL^{-1} of Se(IV).

In designing the system, two kinds of connectors (metallic and Teflon connectors) were compared with each other. Some temperature fluctuations and also some interference in selenium hydride formation were observed when metallic connectors were used. Thus, Teflon-T connector was used as a part of apparatus system.

The temperature of the generation cell was changed to reach the maximum sensitivity. The maximum sensitivity was observed at 38 °C. At temperatures higher than 40 °C, a decrease in sensitivity was observed due to the decomposition of selenium hydride. Thus, 38 °C was selected as the optimum temperature. Special precaution must be taken to avoid the presence of metallic selenium in the generation tubes. Metallic selenium is produced by further reduction, which is mostly due to the high temperature of the cell.

The effect of the nitrogen flow rate as the carrier gas was also investigated. The optimum nitrogen flow rate was 750 mL min^{-1}. At higher nitrogen flow rates, decrease in residence time causes loss of sensitivity. At nitrogen flow rates above 850 mL min^{-1}, solid particles of sodium tetrahydroborate were transferred to the quartz tube and scattering of light by these particles causes a less reproducible signal. Placing some glass wool in the connecting tube prevents the transfer of the particles to the tube.

The height of the quartz tube from the slot burner did not have any significant effect on the results. A height of 1.5 cm from the burner was selected.

In selenium determination, the choice of the acid and its concentration is very important. Since in this study, it was intended to use reagents in pure solid form, the effects of several solid acids such as oxalic, tartaric, adipic, salicylic and some amino acids on the sensitivity of hydride generation were evaluated. Tartaric acid was selected, because it gave the highest sensitivity. The blank signal was also lower when tartaric acid was used. The optimum amount of tartaric acid is related to the volume of selenium solution introduced and to the volume of the generation cell. For about 0.18 g of tartaric acid mixed with a suitable reducing agent, six injections of 25 μL selenium solution are possible with about 3 min delay between injections.

Sodium tetrahydroborate is considered as the most suitable reductant for hydride generation. One of the advantages of this reductant is its relatively short reaction times coupled with the rapid transfer of the products to the gas phase [32–34]. In this analysis, different weights of pure $NaBH_4$ were tested. About 0.2 g of $NaBH_4$ powder and 0.18 g of tartaric acid were selected as the optimum amount.

When using solid reagents, the moisture of the reagents would cause some errors in weighing and also in the efficiency of the selenium hydride formation during addition of small volumes of selenium solution (25 μL). In this study, solid reagents such as $NaBH_4$ and tartaric acid, were dried separately in a microwave oven at about 80% of full power for about 20 s. Higher microwave power or longer exposure can decompose the $NaBH_4$ reagent.

The total selenium can be determined after digestion with H_2O_2/H_2SO_4, or after digestion with HNO_3 followed by reduction with suitable reducing agent [35]. In this study, poor reduction of Se(VI) to Se(IV) was seen for selenium(VI) determination. Therefore, it is only necessary to add some suitable reducing agents to reduce Se(VI) to Se(IV) prior to hydride generation formation. Se(VI) can be reduced to Se(IV) by addition of an equal volume of concentrated hydrochloric acid to the real sample [35]. As in this study, our aim was to use solid reagents, the effects of L-cysteine and other solid reductants for Se(VI) reduction were investigated. The study showed that, the presence of L-cysteine has lower background absorbance and higher sensitivity. The optimum amount was about 0.12 g L-cysteine. The amount of Se(IV) can be determined by subtraction of the Se(IV) content from total selenium.

3.2. Analytical figures of merit

The calibration curve for Se(IV) solutions was linear from 20 to 1200 ng mL^{-1} (0.5–30 ng per 25 μL) as given in Table 1. In this study, the calibration sensitivity is not very high due to the injection of only 25 μL of the selenium solution. By increasing the amount of the solid reagent and using higher volumes of selenium solution, the sensitivity will be improved.

The reproducibility of selenium hydride generation method was determined by six injections of 25 μL of 640 ng mL^{-1} of Se(IV) solution to a cell containing the reductant mixture. The relative standard deviation was obtained as 1.93% (peak heights were measured). Intermediate precision was also obtained by six injections of 640 ng mL^{-1} of Se(IV) into six different cells containing the same amount of

Table 1
Analytical figures of merit for Se(IV) determination

Linear range (ng mL^{-1})	20–1200 Se(IV) (0.5–30 ng per 25 μL)
Regression equation	$A = 8E{-}5C + 0.0181$
Correlation coefficients (R)	0.9992

A is absorbance (peak height) and C is the selenium concentration (ng mL^{-1}).

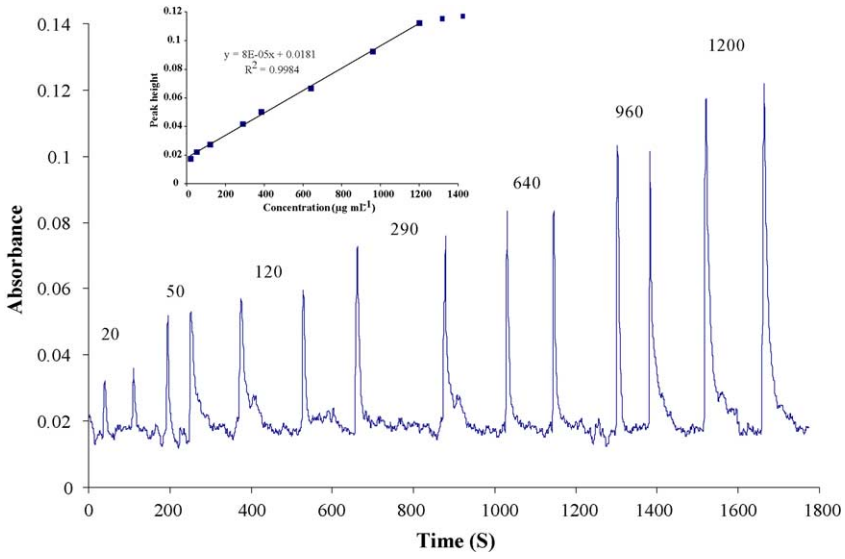

Fig. 2. The absorbance trace of Se(IV) solutions from 20 to 1200 ng mL^{-1}.

the reductant. The relative standard deviation for this determination was obtained as 1.38%.

The detection limit is defined as the concentration corresponding to triple value of the standard deviation of the blank plus the blank signal. In selenium determination study, the detection limit was 10.6 ng mL^{-1} (265 pg per 25 μL) of selenium. Fig. 2 illustrates the absorbance trace of Se(IV) standard solutions from 20 to 1200 ng mL^{-1}.

3.3. Interferences

Every determination using hydride generation technique reported so far has shown interferences that influence the determination to various degrees [36]. Interferences may occur by a number of mechanisms, including consumption of reducing agent, and formation of metals, which decompose the hydride [1,37,38]. The interfering effects of some metals for the determination of selenium were investigated (Table 2). In these cases standard addition method can be used to tolerate higher concentrations of cobalt and lead as interfering ions.

Table 2
Effect of some heavy metals on the determination of 640 ng mL^{-1} Se(IV)

Element	Mass ratio of interfering ion to selenium	Effects
Cr(III)	1000	No interference
Ba(II)	500	No interference
Co(II)	10	Increasing effect
Pb(II)	10	9.2% Decrease
Fe(III)	1000	No interference
Ce(III)	10	3.6% Decrease
Cd(II)	10	10% Decrease
Cu(II)	100	No interference
Hg(II)	100	7.0% Decrease
Ag(I)	100	No interference
Ni(II)	10	9.1% Decrease

The results of the interfering effects of some metal ions for the determination of selenium are shown in Table 2. In this study, 25 μL of 640 ng mL^{-1} of selenium solution containing ions such as 6.4 μg mL^{-1} of lead and 64 μg mL^{-1} of mercury resulted in about 9.2% and 7.0% errors, respectively by the proposed method. Increasing effect has also been observed for some elements such as Co(II). The interferences in solid forms of reagents are less severe and are due to a shorter residence time of selenium hydrides in the reaction cell as compared to solutions [30].

3.4. Analysis of real samples

The reliability of the method was evaluated by determining selenium contents of different samples as shown in Table 3. Standard addition method was used on a 640 ng mL^{-1} standard selenium solution and good agreement between results obtained by the proposed method and segmented flow HGAAS determination was observed. The calculated result for testing the reliability was 638.47 ng mL^{-1} with percent relative error of 1.53%.

To validate the proposed speciation approach, a recovery test was performed. Known amounts of selenium were spiked to different samples. The percentage of the recovery of each species was calculated using the selenium determined and the combined mass of sample and spiked selenium [5]. The results are shown in Table 4.

Table 3
Determination of total selenium in real samples

Sample	Se(IV) (ng mL^{-1})
River water	33 ± 2
Soil	353 ± 3
Sodium polyphosphate	183 ± 1

Error was estimated as 2S.D. (three measurements).

Table 4
Recovery of spiked selenium in different samples

Selenium sample	Selenium present in sample (ng mL^{-1})	Selenium added (ng mL^{-1})	Selenium found (ng mL^{-1})	Recovery (%)
River water	33 ± 2	5.0	39 ± 1	101
Soil	353 ± 3	5.0	357.6 ± 0.4	100
Sodium polyphosphate	183 ± 1	5.0	188.9 ± 0.8	101

Table 5
Comparisons of the proposed method with conventional method in selenium determination

Parameters	Proposed method	Flow method
Instrumental components needed	Heated cell, connectors	Gas–liquid separator, several valves, mixing chamber, multi-channel peristaltic pump [39]
Reagents	Pure solid	Fresh solutions
Linear range	20–1200 Se(IV) (0.5–30 ng per 25 μL)	0–1.5 ng [40]
Volume of metal hydride forming sample injected	25 μL	>25 mL [32]
Residence time in the reaction cell	Short	High
Automation	Less amenable	Amenable

Table 5 shows a comparison between the proposed method and conventional flow methods.

4. Conclusion

Compared to conventional hydride generation techniques, this method needs lower amounts of reagents. It is not necessary to make fresh solutions or do extra reagent preparation. By using homogenous mixtures of solid reagents, the problems of solubility and the effect of dilution during the analysis are less important. In this method, the analyst only needs to mix correct ratio of pure solid reagents together. In comparison to the conventional hydride generation techniques, this method is simple and precise and also has higher linear range and less interference. Also, lower volumes of selenium sample solution are needed for the analysis.

Acknowledgement

The authors wish to acknowledge the support of this work by the Shiraz University Research Council.

References

[1] G. Komaromy-Hiller, Anal. Chem. 71 (1999) 338R.
[2] B.K. Mandal, Y. Ogra, K.T. Suzuki, Chem. Res. Toxicol. 14 (2001) 371.
[3] Y. Shiobara, Y. Orga, K.T. Suzuki, Chem. Res. Toxicol. 14 (2001) 1446.
[4] I. Polyi, Z. Stefánka, P. Fodor, Anal. Chim. Acta 435 (2001) 367.
[5] Z. Wang, Y.X. Gao, N. Belzile, Anal. Chem. 73 (2001) 4711.
[6] Yu. Lu, H. Sun, Ch. Yuan, X. Yan, Anal. Chem. 74 (2002) 1525.
[7] K.T. Suzuki, B.K. Mandal, Y.S. Orga, Talanta 58 (2002) 111.
[8] G.F. Combs, L. Clark, Selenium and Cancer Prevention, Antioxidant Nutrients and Disease Prevention, CRC Press, Boca Raton, NY, 1997.
[9] L.C. Clark, B. Dalkin, A. Konrad, et al., Br. J. Urol. 81 (1998) 730.
[10] P. Knekt, J. Marniemi, L. Teppo, M. Heliovara, A. Aromaa, Am. J. Epidemiol. 148 (1998) 975.
[11] L. Bonelli, M. Conio, P. Massa, et al., Cancer Prev. Contr. 100 (1998) 351.
[12] M.P. Rayman, BMJ 314 (1997) 387.
[13] M.-Q. Yu, G.Q. Liu, Q. Jin, Talanta 30 (1983) 265.
[14] Y.K. Lu, H.W. Sun, J. Insturm, Anal. Chim. Acta 19 (2000) 190.
[15] I. Marawi, J. Wang, J.A. Caruso, Anal. Chim. Acta 291 (1994) 127.
[16] H. Elfering, J.T. Anderson, K.G. Poll, Analyst 123 (1998) 669.
[17] D. Beauchemin, A.F. J. Anal. At. Spectrom. 13 (1998) 1.
[18] P.N. Vijan, G.R. Wood, Analyst 101 (1976) 966.
[19] R.S. Braman, L.L. Justen, C.C. Foreback, Anal. Chem. 44 (1972) 2195.
[20] E.A. Crecelius, Anal. Chem. 50 (1978) 826.
[21] R.S. Braman, D.L. Johnson, C.C. Foreback, J.M. Ammons, J.L. Bricker, Anal. Chem. 49 (1977) 621.
[22] P. Smichowski, S. Faryas, Mikrochem. J. 67 (2000) 147.
[23] M.O. Andreae, J.-F. Asmode, P. Foster, L. Van't dack, Anal. Chem. 53 (1981) 1766.
[24] A.G. Howard, C. Salou, A.F. J. Anal. At. Spectrom. 13 (1998) 683.
[25] M. McDaniel, A.D. Shendrikar, K.D. Reszner, P.W. West, Anal. Chem. 14 (1976) 2240.
[26] J. Mierzwa, S.B. Adeloju, H.S. Dhindsa, Analyst 122 (1997) 539.
[27] W. Holak, Anal. Chem. 41 (1969) 1712.
[28] F.J. Schmidt, J.L. Royer, S.M. Muier, Anal. Lett. 8 (1975) 123.
[29] J.Y. Cabon, W. Erler, Analyst 123 (1998) 1565.
[30] I. Lopez-Garc, Yu.J. Arroyo-Cortez, M. Hernandez-Cordoba, At. Spectrosc. 14 (1993) 144.
[31] N. Maleki, A. Safavi, Z. Ramezani, J. Anal. Atom. Spectrosc. 14 (1999) 1227.
[32] R.E. Sturgeon, J. Liu, v.J. Boyko, V.Y. Luong, Anal. Chem. 68 (1996) 1883.
[33] C. Moor, J.W.H. Lam, R.E. Sturgeon, J. Anal. At. Spectrom. 15 (2000) 143.
[34] A.S. Luna, R.E. Sturgeon, R.C. De Campos, Anal. Chem. 72 (2000) 3523.
[35] Z. Wang, Y.X. Gao, N. Belzile, Anal. Chem. 73 (2001) 4711.
[36] A. Raab, H.R. Hansen, L. Zhuang, J. Feldmann, Talanta 58 (2002) 67.
[37] N.H. Bings, A. Bogaerts, J.A.C. Broekaert, Anal. Chem. 74 (2002) 2691.
[38] K.W. Jackson, D.R. Thomerson, Anal. Chem. 70 (1998) 363R.
[39] Z. Gong, X. Lu, M. Ma, C. Watt, X.C. Le, Talanta 58 (2002) 77.
[40] H. Hasegawa, T.Y. Sohrln, M. Matsul, M. Hojo, T. Kawashlmal, M. Kawashlmal, Anal. Chem. 66 (1994) 3247.