http://dx.doi.org/10.5935/0103-5053.20130287

J. Braz. Chem. Soc., Vol. 25, No. 2, 229-237, 2014.
Printed in Brazil - ©2014 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Dispersive Liquid-Liquid Microextraction-Solidified Floating Organic Drop Combined with Spectrophotometry for the Speciation and Determination of Ultratrace Amounts of Selenium

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Neste estudo, um método simples e barato de microextração líquido-líquido dispersiva com solidificação da gota orgânica flutuante (DLLME-SFOD) foi desenvolvido para separação e especiação de Se inorgânico previamente à sua determinação espectrofotométrica. O método está baseado na formação do complexo amarelo piazoselenol entre Se(IV) e 3,3'-diaminobenzidina (DAB) seguido por sua extração em 1-undecanol e a medida da absorção molecular em 434 nm. O Se inorgânico total foi determinado após a redução do Se(VI) a Se(IV). A concentração de Se(VI) é calculada pela diferença entre o Se total e Se(IV). Nas condições otimizadas, foram obtidos um fator de enriquecimento de 133 e um limite de detecção de 1,6 μg L^{-1}. O desvio padrão relativo foi de 2,1% para uma solução contendo 50,0 μg L^{-1} (n = 6) e a curva analítica de calibração foi linear no intervalo de 5,0-600,0 μg L^{-1} para a pré-concentração de 20,0 mL da amostra aquosa. O método foi aplicado com sucesso na determinação de Se em alho, em pastilhas de selênio e em amostras de água. A exatidão foi avaliada usando experimentos de adição-recuperação e uma amostra de água certificada.

In this study a simple and inexpensive dispersive liquid-liquid microextraction-solidified floating organic drop (DLLME-SFOD) has been reported for the separation and speciation of inorganic selenium prior to its spectrophotometric determination. The method is based on the formation of a yellow piazselenol complex between the Se(IV) and 3,3'-diaminobenzidine (DAB) followed by its extraction into 1-undecanol and the measurement of its absorption at 434 nm. Total inorganic selenium is determined after the reduction of Se(VI) to Se(IV). The concentration of Se(VI) is calculated by the difference between the total selenium and Se(IV). Under the optimized conditions, an enrichment factor of 133, a detection limit of 1.6 μg L^{-1}, a relative standard deviation of 2.1% at 50.0 μg L^{-1} (n = 6) and a linear dynamic range of 5.0-600.0 μg L^{-1} for the preconcentration of 20.0 mL of aqueous sample was obtained. The method was successfully applied to the determination of selenium in garlic, selenium plus tablet and water samples. The accuracy was evaluated through the recovery experiments and the analysis of a certified water sample.

Keywords: inorganic selenium speciation, 3,3'-diaminobenzidine, dispersive liquid-liquid microextraction-solidified floating organic drop, spectrophotometry

Introduction

Selenium as a trace element plays an important role in the environmental and the health studies.[1-3] It is both a toxic and an essential element with a narrow concentration range between the two contrary effects. In high concentration, selenium is toxic while its shortage can cause heart disease. At trace levels, selenium has a role in protecting from several heart diseases, preventing the toxic effects of heavy metal, offering anti-carcinogenic activity, functioning as the immune altering agent, viral suppression and AIDS.[4,5] Selenium can be released by natural processes such as weathering of minerals and the anthropogenic activities such as agriculture, industry, fossil fuel combustion, and metallurgical processes, especially from mining activities of sulfide ores.[6] The chemical species of selenium have different behaviors in the environmental and industrial processes. Mobility, distribution, biological availability and toxicity of selenium all depend on its chemical form and oxidation state. In water samples, selenium exists mainly in the inorganic forms (Se(VI) and Se(IV)) which are more toxic than the organic ones and the toxicity of Se(VI) is more serious than Se(IV) for human beings and most of the other mammals.[7] Thus, the development

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of a sensitive method for the determination of inorganic species of selenium in water samples is extremely important. Different analytical techniques including X-ray fluorescence,[8] voltammetry,[9] UV-VIS spectrophotometry,[10] atomic absorption spectrometry,[11] high performance liquid chromatography atomic fluorescence spectroscopy (HPLC-AFS),[12] and gas chromatography[13] have been used for the determination of selenium. However, the concentration levels of selenium in water samples are often lower than the detection limits of most of these techniques; thus, an extraction/preconcentration step prior to its determination is required. Different methods such as solid phase extraction (SPE),[14-17] cloud point extraction (CPE),[18-20] solid phase microextraction (SPME)[21-23] and liquid phase microextraction (LPME)[24-28] have been used for the separation, preconcentration and speciation of selenium.

LPME has attracted many researchers because of its advantages such as the consumption of small volumes of toxic organic solvents, ease of the operation and the possibility of obtaining a high enrichment factor. Since the introduction of the first LPME method by Liu, Dasgupta[29] and Jeannot and Cantwell,[30] different modes of LPME including headspace-single drop microextraction (HS-SDME), hollow-fiber LPME (HF-LPME), dispersive liquid-liquid microextraction (DLLME), solidified floating organic drops microextraction (SFODME) and DLLME-SFOD have been reported for the separation and preconcentration of various analytes. Among these techniques, DLLME-SFOD has the advantages of both DLLME and SFODME techniques, i.e., it provides a vast contact area between the extractant and the sample, fast mass transfer and short extraction time, simplicity, high efficiency and consumption of very small volumes of the organic solvent in green operation. In DLLME-SFOD method, the immiscible extracting solvent with a melting point near to the room temperature (10-30 °C) is mixed with the disperser solvent and is rapidly injected into the aqueous sample where a cloudy solution is formed and the analyte is extracted into the organic solvent. After centrifugation of the mixture, the extraction vial is placed in an ice bath until the organic drop is solidified. The solidified organic drop is then removed and the amount of the analyte in the melted drop is determined.[31-38]

DLLME combined with X-ray fluorescence spectrometry,[1] gas chromatography,[39] electrothermal atomic absorption spectrometry[40] using diethyl dithiocarbamate or ammonium pyrrolidinedithiocarbamate as the complexing agent and SFODME coupled to ultrasound-assisted back extraction and hydride generation atomic fluorescence spectrometry[41] has been used for the separation and preconcentration of selenium. However, to the best of our knowledge, there is no report on the use of the DLLME with relatively the inexpensive and easy handling spectrophotometry method for the separation and determination of the inorganic species of selenium. In this paper, DLLME-SFOD technique combined with spectrophotometry is used for the separation/preconcentration and determination of selenium in garlic and selenium plus tablet and its inorganic species in water samples. The method relies on the formation of a yellow complex between the 3,3'-diaminobenzidine and Se(IV) followed by the extraction of the complex into an small amount of 1-undecanol and the measurement of its absorption at 434 nm. The total inorganic selenium is determined after the reduction of Se(VI) to Se(IV) upon the addition of concentrated hydrobromic acid and heating the sample prior to the application of the DLLME-SFOD method. The concentration of Se(VI) is calculated by the difference between the total selenium and Se(IV).

Experimental

Reagents

3,3'-diaminobenzidine hydrochloride with 99% purity was obtained from Sigma-Aldrich company (St. Louis, MO, USA). All the other chemicals used were of the analytical grade reagent obtained from the Merck Company (Darmstadt, Germany). Doubly distilled deionized water was used for all the sample preparations. Stock standard solutions of Se(VI) and Se(VI) (1000 mg L^{-1}) were prepared by dissolving proper amounts of Na_2SeO_3 and Na_2SeO_4 into a 100 mL flask and diluting to the mark with distilled water. The working standard solutions were prepared by the appropriate dilution of stock solution. The solution of 3,3'-diaminobenzidine hydrochloride in water (0.023 mol L^{-1}) was prepared daily. 1-undecanol was used as the extracting solvent.

Apparatus

A single-beam spectrophotometer model JENWAY-6300 (Jenway, Essex, UK) equipped with a 1 cm quartz microcell with 200 µL capacity was used for all the absorbance measurements at 434 nm. An Avantes photodiode array spectrophotometer model AvaSpec-2048 matched with a source model of Ava Light-DH-S-BAL (Avantes, Eerbeek, The Netherlands) was used for recording the absorbance spectra. All the measurements were made against a reagent blank solution. The pH measurements were carried out with a Metrohm pH meter (model 827, Herisau, Switzerland) with a combined glass calomel electrode. The centrifuge

(Hitachi, Universal 320, Tuttlingen, Germany) was used for the phase separation process.

Sample preparation

Water samples

The water samples were filtered through a 0.45 μm Millipore filter and passed through a cation exchange column to remove the possible interfering cations. Then, the pH was adjusted and the concentrations of selenium species in the samples were determined according to the given procedure.

Selenium plus tablet

Ten selenium plus tablets were grinned and homogenized.[18] To 0.01 g of it, 2 mL of hydrochloric acid solution (6 mol L^{-1}) was added and the mixture was heated in an open vessel for ten minutes. The solution was then passed through a 0.45 μm Millipore filter and diluted with distilled water to 50.0 mL in a volumetric flask. Finally, the amount of selenium in 20 mL of it was measured according to the given procedure.

Garlic samples

The garlic was digested according to the given procedure in the literature,[42] i.e., the garlic was peeled, the bulbs were washed with distilled water to remove all the possible residues from the soil and were dried for 1 day in an oven at 70 °C. Ten milliliter of HNO_3:$HClO_4$ (1:1) was added to 2.5 g of dried garlic samples and the mixture was left overnight. The samples were then heated using a Bunsen flame nearly dried until the nitrogen oxide fumes were given off. Then, 10.0 mL HNO_3 was added and the heating was continued with the same temperature until the nitrogen oxide fumes were completely evolved. Then, 3.0 mL $HClO_4$ was added and the solution was heated until the volume was reduced to approximately 1 mL, the process was repeated to the point that the digestion was completed and a clear solution was obtained. Finally, 1.0 mL of concentrated hydrobromic acid (47%) added and the mixture was heated for 15 min to convert all the extracted selenium to Se(IV). The digested sample solution was cooled to the room temperature and diluted to 20 mL in a volumetric flask and total concentration of selenium was measured according to the given procedure.

Procedure

Determination of Se(IV)

To an aliquot of the sample or standard solution containing not more than 12 μg of Se(IV), 0.25 mL of formic acid solution (20%) and 0.7 mL of DAB solution (0.023 mol L^{-1}) was added. The mixture was then transferred into a ca. 25 mL sample vial, the pH was adjusted to 2.0-2.5 by diluted hydrochloric acid solution and was left aside for 30 min until the formation of a yellow piazselenol complex was completed.[43,44] Then, the solution was neutralized (pH 6.0-7.0) with diluted ammonia solution and a mixture of 100 μL 1-undecanol as an extraction solvent and 150 μL ethanol as the dispersive solvent was rapidly injected into the aqueous sample which caused the formation of a cloudy solution. At this stage, the piazselenol complex was quickly extracted into the fine droplets of 1-undecanol. The mixture was then centrifuged at 1500 rpm and the organic solvent containing the selenium complex floated on the top of the aqueous solution. The sample vial was transferred into an ice bath and the organic solvent was solidified after 5 min. Then, with a special designed spatula, the solidified solvent was transferred into a conical vial where it melted immediately; its viscosity decreased upon the addition of 50 μL of ethanol and was transferred into the microcell where its absorption was measured at 434 nm against a reagent blank.

Determination of total selenium and Se(VI)

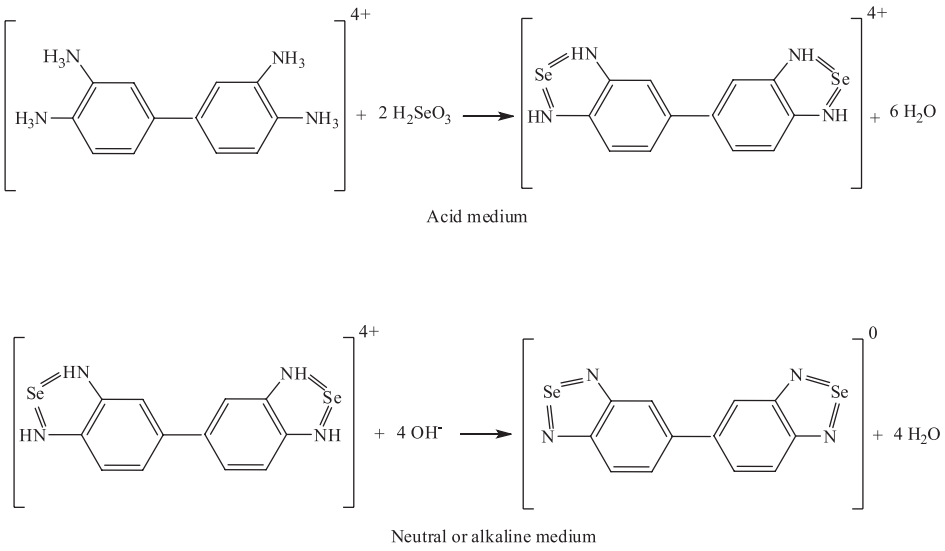
Total concentration of selenium in the sample and standard solution was determined by the effective reduction of Se(VI) to Se(IV) upon the addition of 1 mL concentrated hydrobromic acid (47%) and heating the solution in boiling water for 15 min according to the given procedure in the literature.[45] The solution was then treated according to the given procedure for the determination of Se(IV). The concentration of Se(VI) was determined from the difference in the concentration of the total selenium and Se(IV).

Results and discussion

3,3'-diaminobenzidine (DAB) is known as a selective ligand for the spectrophotometric determination of Se(IV). DAB forms a yellow piazselenol complex with Se(IV) according to the following reaction (Scheme 1) which is relatively insoluble in the aqueous phase but is soluble in the organic solvent.[46]

The initial experiments showed that the Se(IV)-DAB complex in aqueous phase can be extracted into 1-undecanol. Therefore, a DLLME-SFOD method for the selective separation and preconcentration of Se(IV) by DAB ligand was designed and the parameters affecting the complex formation, extraction and determination of the analyte were optimized by using the univariable method.

The spectra of the extracted Se(IV)-DAB complex as well as the DAB are shown in Figure 1a and 1b, respectively, indicating that the Se(IV)-DAB complex absorbed in the region of 400-500 nm with a maximum absorption at



Scheme 1.

434 nm, whereas the absorption of the ligand is negligible in this region. Thus, all the measurements were made against the reagent blank at 434 nm. The percentage of the extraction and the enhancement factor were calculated according to the aforementioned equations 1 and 2.[2,47-49]

$$\text{Percentage of the extraction} = (C_O V_O / C_{aq} V_{aq}) \times 100 \quad (1)$$

$$\text{Enrichment factor} = C_O / C_{aq} \quad (2)$$

where V and C are the volume and the concentration and the suffixes O and aq stand for the organic and aqueous phases, respectively. C_O was calculated from the calibration graph of the standard solution of the Se(IV)-DAB complex in ethanol.

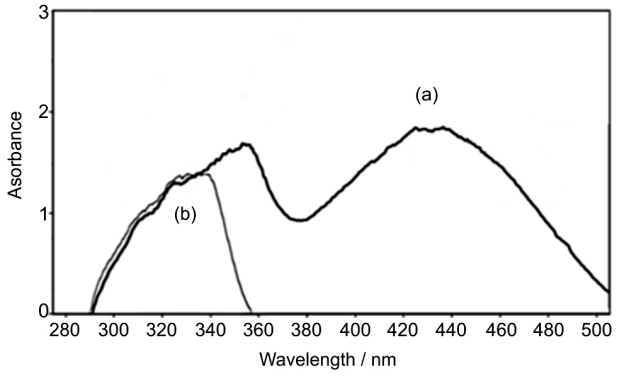


Figure 1. Absorption spectra of the extracted Se(IV)-DAB complex (a) and DAB (b) against the blank solvent.

Effect of the nature of the extraction solvent

In order to achieve the high recovery and an enrichment factor in the DLLME-SFOD method, the extractant solvent must have low solubility in water and high solubility in dispersive solvent. It also must have a density lower than water, a melting point near to the room temperature, low volatility, low toxicity, and must not interfere with the analytical technique of the measurement of the analyte. Thus, 1-undecanol, 1,10-dichlorodecane, *n*-hexadecane and 1-dodecanol were examined as the extractant solvents. The extraction efficiency was found to be higher with 1-undecanol, while with 1-dodecanol, the extraction was about 59% of 1-undecanol. *n*-Hexadecane was ruled out as the extractant solvent due to its low solubility in the dispersive solvent and the formation of an emulsion. The solubility of 1,10-dichlorodecane in the aqueous phase was relatively high and the recovery and the collection of the organic drop was difficult. Thus, in the present study, 1-undecanol was selected as the extracting solvent because of its stability, low water solubility and low vapor pressure.

Effect of the nature of the dispersive solvent

In the DLLME-SFOD, the dispersive solvent must be miscible with both organic solvent and water. Accordingly, four types of dispersive solvents, ethanol, methanol, acetonitrile and acetone were considered. It was found that the analyte signal was higher with ethanol than the other solvents. Ethanol has a dipole moment close to that of water and so it has a higher capability of dispersing 1-undecanol into water. Therefore, ethanol was selected as the most suitable dispersive solvent in the further experiments.

Effect of sample pH

For the extraction of metal ions by the DLLME-SFOD method, a hydrophobic complex with high solubility in extracting solvent must be formed. Thus, as the pH of the

sample has an important effect on the formation of metal complex, it has an important role in the extraction method. So, the effect of the sample pH on the extraction of Se(IV)-DAB complex in the pH range of 1.0-9.0 was investigated. The pH was adjusted by either diluted hydrochloric acid or ammonia solution and the other variables were kept constant. It was found that the formation of the complex and its extraction are dependent on the pH of the solution. In the pH range of 6.0-7.0, the extraction and consequently the absorbance were maximum and constant (Figure 2). At pH > 7.0, the solution became turbid due to the precipitation of selenium as selenium hydroxide and measurement of the absorption was not possible, while the progressive decrease in the extraction of selenium at pH < 6.0 is due to the protonation of the complex which increases its solubility in the aqueous phase. Therefore, a pH range of 6.0-7.0 was selected as the optimum pH for the subsequent studies.

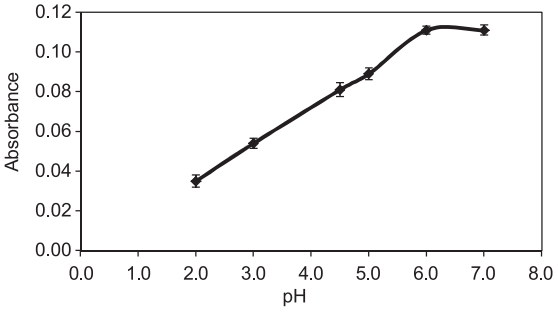


Figure 2. Effect of pH sample on Se(IV) extraction. Aqueous phase volume, 10 mL; [Se(IV)], 100.0 μg L^{-1}; formic acid 20%, 0.25 mL; DAB, 1.6×10^{-3} mol L^{-1}; extracting solvent, 100.0 μL; dispersive solvent, 150.0 μL; n = 3.

Effect of DAB concentration

The concentration of ligand is one of the important factors affecting the selenium extraction. For the extraction of a given amount of metal ions, the stronger the complex, the less amount of ligand is required for its quantitative extraction. Thus, the effect of the DAB concentration on the extraction and the absorption signal of the Se-DAB complex was investigated in the concentration range of 2.3×10^{-4}-4.7×10^{-3} mol L^{-1}. It was found that the absorption signal of the Se(IV) ion was increased with an increase in the DAB concentration up to 1.6×10^{-3} mol L^{-1} and became constant at a higher concentration. Therefore, an optimum concentration of 1.6×10^{-3} mol L^{-1} of DAB was employed for the subsequent studies.

Salt effect

The addition of salt increases the ionic strength of the solution and may affect the solubility of the extracting solvent in water which consequently affects the efficiency of the liquid-liquid microextraction. Thus, several experiments were performed by varying the concentration of NaCl in the range of 0.0-1.5 mol L^{-1}. The results indicated that the increase in the concentration of NaCl up to 1.0 mol L^{-1} has no significant effect on the extraction efficiency. However, further increase in the concentration of NaCl caused a decrease in the absorbent signal which can be related to the increase of 1-undecanol solubility in the aqueous phase at high ionic strength. Thus, the method is suitable for the separation and preconcentration of selenium from the solutions with salinity up to 1.0 mol L^{-1}.

Effect of the volume of extracting and dispersing solvent

The volume of the extracting solvent is one of the important factors which affect the enrichment factor and the extraction efficiency. A decrease in the ratio of the volume of the organic phase to the aqueous phase will increase the preconcentration factor, but it may reduce the extraction efficiency in a given extraction time. The influence of the volume of 1-undecanol on the extraction was examined by performing several experiments with the volume of 1-undecanol in the range of 20-150 μL. The final extract was diluted to 150 μL with ethanol and then the absorption was measured. The results showed that an increase in the volume of the 1-undecanol up to 100 μL caused an increase in the absorption signal and then became constant at a higher volume. Thus, the maximum preconcentration factor and the extraction efficiency were obtained using 100 μL of 1-undecanol and it was chosen as the optimum volume of the extracting solvent.

The effect of the dispersive solvent on the extraction efficiency in the range of 50-300 μL of the ethanol was investigated. When the volume of the ethanol was less than 150 μL, the 1-undecanol was not completely dispersed in the aqueous phase and the extraction was not completed. However, when the volume of the ethanol was in the range of 150-300 μL the absorbance signal reached its maximum and became constant. Therefore, in further studies, an optimum volume of 150 μL of ethanol was selected.

Effect of sample volume

Sample volume is an important factor which demonstrates the capability of the extraction method for the separation and preconcentration of the trace amounts of analyte from the large sample volume and the achievement of high preconcentration factor. For this purpose, different sample volumes containing 1.0 μg of

selenium were subjected to the developed procedure. It was found that up to a sample volume of 20.0 mL, the extraction efficiency is maximum but a further increase in the sample volume causes a decrease in the extraction. Thus, the method is capable of the quantitative extraction and determination of the analyte up to a volume of 20.0 mL of the sample.

Interference study

In order to consider the selectivity of the proposed method for the determination of selenium, the effect of the common ions present in the environmental samples was studied. The experiments were performed with 20 mL of the sample containing 50 μg of Se(IV) and various amounts of interfering ions at an initial mole ratio of ion/selenium of 1000. The tolerance limit of the interfering ions was defined as the largest amount making a variation of less than 5% in the determination of selenium. In the preliminary interfering ion study, it was observed that most of the cations interfere with the determination of selenium. Thus, in order to remove the interference of the cations, the solutions were passed through a cation exchange resin prior to the application of the procedure. The results (Table 1) showed that with the given procedure, the cations and anions have no obvious interference with the determination of Se(IV).

Analytical performance

Performance characteristics of the method were obtained by processing the standard solution of Se(IV). The calibration curve was constructed by processing 20 mL of the standard solution (in triplicate) under the optimum conditions of DLLME-SFOD. The graph of absorbance versus selenium concentration was linear over the range of 5.0-600.0 μg L^{-1} of selenium. The equation of the calibration graph was $A = 0.002C + 0.0088$ (where A is the absorbance and C is the concentration of Se (μg L^{-1}) in the aqueous phase). The preconcentration factor determined as the ratio of the volumes of the sample to the extract was found to be 133. The relative standard deviation of the method for 6 replicate measurements at 50.0 μg L^{-1} of Se(IV) was ± 2.1%. The limits of the detection and the quantification defined as the three and ten times of the ratio of the standard deviation of the blank extract measurement (n = 5) to the slope of the calibration graph were 1.6 and 5.3 μg L^{-1}, respectively.

Determination of total selenium and Se(VI)

The capability of the DLLME-SFOD method in the extraction and determination of Se(IV) and Se(VI) was considered by analyzing several standard solutions containing different amounts of selenium species. The results (Table 2) indicate that the recovery of both species

Table 1. Tolerance limits of diverse ions on the determination of 50.0 μg L^{-1} Se(IV)

Foreign ion	Molar ratio (ion/Se(IV))	Recovery / %	Foreign ion	Molar ratio (ion/Se(IV))	Recovery / %
Mg^{2+}	1000	98.7 ± 1.6	K^{+}	1000	96.8 ± 1.9
Pb^{2+}	1000	97.3 ± 3.8	Ca^{2+}	1000	95.4 ± 2.8
Al^{3+}	1000	102.6 ± 3.6	Ni^{2+}	1000	97.3 ± 2.0
Cr^{3+}	1000	95.8 ± 3.8	Fe^{3+}	1000	96.6 ± 4.8
Cd^{2+}	1000	104.6 ± 2.2	Cu^{2+}	1000	94.6 ± 1.4
Mn^{2+}	1000	104.2 ± 4.5	Cl^{-}	1000	95.8 ± 3.8
Zn^{2+}	1000	97.7 ± 3.3	SO_4^{2-}	1000	98.7 ± 1.6
Co^{2+}	1000	103.7 ± 1.3	NO_3^{-}	1000	97.7 ± 3.3
Ba^{2+}	1000	101.3 ± 1.8			

Table 2. Speciation of selenium in synthetic water sample

Added / (μg L^{-1})		Found / (μg L^{-1})[a]		Recovery / %	
Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
200	0	199.1 ± 1.4	< LOD[b]	99.5	–
0	200	< LOD[b]	192.5 ± 2.8	–	96.2
100	100	96.2 ± 4.2	95.4 ± 5.04	96.2	95.4
50	150	49.6 ± 1.4	144.1 ± 4.4	99.2	96.1
150	50	147.3 ± 2.8	48.6 ± 3.1	98.2	97.2

[a]Mean and standard deviation of three independent determinations; [b]limit of detection.

Table 3. Determination of selenium in water samples

Sample	Added / (μg L^{-1})		Found / (μg L^{-1})[a]		Recovery / %	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
Persian Gulf Sea water	–	–	n.d.[b]	n.d.	–	–
	10	10	9.8 ± 0.1	9.5 ± 0.3	98.0	95.0
	50	50	49.2 ± 0.7	48.4 ± 1.6	98.4	96.8
River water	–	–	n.d.	n.d.	–	–
	10	10	10.1 ± 0.3	9.7 ± 0.4	101.0	97.0
	50	50	49.3 ± 1.4	48.9 ± 1.9	98.6	97.8
Caspian Sea water	–	–	n.d.	n.d.	–	–
	10	10	9.6 ± 0.4	9.5 ± 0.4	96.0	95.0
	50	50	48.1 ± 0.7	48.1 ± 1.0	96.2	96.2
Mineral Water	–	–	n.d.	n.d.	–	–
	10	10	9.8 ± 0.1	9.6 ± 0.2	98.0	96.0
	50	50	48.6 ± 1.4	49.2 ± 2.0	97.2	98.4
Waste water	–	–	5.2 ± 0.1	4.5 ± 0.3	–	–
	10	10	14.9 ± 0.4	14.1 ± 0.2	97.0	96.0
	50	50	53.5 ± 1.6	52.3 ± 3.1	96.6	95.6

[a]Mean and standard deviation of three independent analyses; [b]not detected.

is quantitative and at 95% confidence level selenium species are completely separated.

Analysis of real samples

The procedure was applied to the determination of the inorganic selenium species in waste water, mineral water, river water, the Caspian Sea water and the Persian Gulf Sea water samples and the total selenium in garlic samples. The accuracy of the method was evaluated by spiking the samples with two levels of selenium. The results along with the recoveries for the spiked samples are given in Table 3 and Table 4 indicating that the recoveries of the spiked samples are almost quantitative (95.0-101.0). The method was also applied to the determination of Se(IV) in the selenium plus tablet. The amount of Se(IV) in the selenium plus tablet was found to be 195.6 ± 2.1 μg. According to the *t*-test, at 95% confidence level there is no significant difference between the claimed value (200 μg) and the measured one (t_{exp} = 3.6 and t_{crit} = 4.3). The accuracy of the proposed method was further evaluated by the application of the procedure to the determination of selenium in a standard reference material from the NIST (trace elements in water, SRM 1643e). The concentration of selenium in the sample was found to be 11.4 ± 0.2 μg L^{-1} which is in good agreement with the certified value (11.97 ± 0.14 μg L^{-1}).

Comparison of the proposed method with other methods

The proposed method was compared with some of the previously reported spectrophotometric methods for the determination of selenium. The results shown in Table 5 revealed that the detection limit of the proposed method is less than the other methods and its linear range is wider. Furthermore, the DLLME-SFOD separated and preconcentrated the selenium from the matrix constituents.

Table 4. Determination of total selenium in garlic samples

Sample	Added / (μg g^{-1})	Found / (μg g^{-1})[a]	Recovery / %
Garlic sample 1	–	0.968 ± 0.042	–
	0.1	1.065 ± 0.035	97.0
	0.5	1.450 ± 0.052	96.4
Garlic sample 2	–	1.848 ± 0.076	–
	0.1	1.946 ± 0.063	98.0
	0.5	2.341 ± 0.086	98.6

[a]Mean and standard deviation of three independent analyses.

Conclusions

In this study, a simple, sensitive and easy DLLME-SFOD combined with the spectrophotometry method was developed for the preconcentration, separation and determination of the inorganic selenium species using the 3,3'-diaminobenzidine (DAB) ligand. The method is based on the formation of the yellow Se(IV)-DAB complex and its extraction into 1-undecanol. Compared with the previously reported spectrophotometric methods, the detection limit of the proposed method is lower while its linear dynamic range is wider. The method is capable of speciation of selenium in complex matrices such as sea water. The main benefits

Table 5. Comparison of the proposed method with the other spectrophotometric methods

Method	Reagent	λ_{max} / nm	Linear range / (μg L^{-1})	LOD[a] / (μg L^{-1})	PF[b]	Ref
Spectrophotometry	Trifluoroacetic acid (TFA)	380	1.0×10^5-7.4×10^6	–	–	50
Spectrophotometry	Potassium iodide	290	1000-10000	110	–	51
Kinetics	Toluidine blue and sodium sulfide	630	20-240	–	–	52
Kinetics	EDTA, $NaNO_3$ and $(NH_4)FeSO_4$	440	5-200 and 200-2000	2	–	53
DLLME-SFOD/ Spectrophotometry	3,3'-Diaminobenzidine	434	5-600	1.6	133.3	This work

[a]Limit of detection; [b]preconcentration factor.

of the system are: cost effectiveness, high sensitivity and accuracy, simplicity, high preconcentration factor, reduction in the use of toxic organic solvents, and the use of available and low cost spectrophotometer.

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Submitted: August 6, 2013
Published online: December 10, 2013