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Microwave Digestion of Environmental and Natural Waters for Selenium Speciation

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A microwave preparation procedure is proposed for selenium speciation in natural and drinking waters. Different chemical reagents were tested, and the conditions for Se speciation were optimized. The effect of the different reagents on various oxidation states of selenium under microwave digestion conditions was investigated. Most of the Se(−II) was converted to selenite when digested with HNO_3 and <20% to selenate. The digestion with H_2O_2/H_2SO_4 can change most Se species into Se(IV). The concentration of Se(IV) in the samples was then determined by HPLC with a fluorescence detector after derivatization with 2,3-diamino-naphthalene (DAN). The microwave preparation procedure allows Se speciation in water samples. Se(IV) was determined after concentrating the sample under nitrogen protection. The amount of Se(IV) and Se(VI) was measured by adding an equal volume of concentrated hydrochloric acid to water sample to reduce Se(VI) to Se(IV). Then the amount of Se(VI) can be calculated by subtraction. 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 concentrated hydrochloric acid. Selenium (−II, 0) was calculated by subtracting inorganic Se(IV+VI) from the total. Detection limits of 0.0066 ng and 0.0096 ng Se were obtained for HNO_3 and H_2O_2/H_2SO_4 as digestion reagents, respectively. The total Se in the four water samples tested range from 0.20 to 0.90 $\mu g\ L^{-1}$. Among them the dominant form was Se(VI) with the exception of pond waters where Se(−II) predominated.

Selenium is an interesting element in natural systems because of its dual role as both an essential nutrient at low concentrations and a toxic substance at higher levels. The concentration range between Se as an essential element and a toxic one is moreover, rather narrow.[1] However, the safety factor of selenium, or the amount required versus toxic levels, is wider than vitamins A and D and copper for sheep. Food is the main source of human body selenium, and drinking water is responsible for a significant fraction of the total intake.[2] Thus, accurate knowledge of the Se content in the environment, particularly in food products, is mandatory. In addition, the chemical form in which Se exists in the diet can have an important impact on its physiological behavior[3] through influencing its toxicity and bioavailability, its distribution in the organism, and its metabolism in the biological system.[4] For instance, levels of certain Se-containing proteins, such as glutathione peroxidase when its activity is below the saturation level, could better describe Se nutritional states in the human body than by the body burden.

The determination of Se in environmental samples usually requires the destruction of the matrix and the transformation of the organic Se into inorganic forms. In many studies reporting Se determination, a time-consuming conventional wet digestion method is used. In the wet digestion process, the samples are highly exposed to contamination, and volatile Se compounds can be lost[5] causing large errors in the final determination. A mixture of nitric acid and perchloric acid is generally employed to destroy the organic matrix in traditional wet digestion procedures. The use of perchloric acid can often result in an explosion or a fire during digestion if the mixture becomes dry. Furthermore, the use of manifold digesting reagents increases the possibility of sample contamination. Recently, there has been an increasing interest in using microwave digestion methods to speed up the dissolution of a variety of biological materials. The merits of pressurized acid digestion in closed vessels with microwave heating, particularly the increased speed and reduced losses of volatile elements, are widely recognized.[1] Additionally, the sample dissolution can be achieved using microwave digestion with HNO_3 alone.[6] Thus, the method can effectively avoid sample contamination resulting from the environment and reagents.

In this paper, an approach to the speciation of selenium in environmental and drinking water has been proposed. The approach includes a microwave preparation of water samples and Se(IV) measurement by high performance liquid chromatography (HPLC) with fluorimetric detection (FLD) after derivatization of the 2,3-diaminonaphthalene (DAN).[7−8]

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EXPERIMENTAL SECTION

Instrumentation. A CEM microwave digestion system (model MDS-2000) with maximum microwave power of 630 W, highest operating temperature 200 °C, and highest operating pressure of 1.38 MPa (14.08kg cm^{-2}) was used in the experiment. The equipment has a turntable on which 12 100-mL Teflon PFA digestion vessels with a safety pressure-relief disks can be assembled. The system allows programming both the pressure and time in a maximum of five steps. Concentration of selenium was measured using a normal phase HPLC (Waters 201 HPLC W/510) with a Shimadzu RF-535 fluorescence detector and a μ-porasil $C_{18}-NH_2$ column after a precolumn derivatization procedure.

Reagents and Materials. All beakers, funnels, and calibrated flasks, together with other glassware used for experiments, were soaked in nitric acid (ca. 7 M) for 24 h and cleaned sequentially with neutral detergent, tap water, deionized water, and double-deionized distilled water before use. The ultrapure chemical reagents used for digestion were 15 M HNO_3, 12 M $HClO_4$, 9.3 M H_2O_2, 15 M H_3PO_4, and selenium-free sulfuric acid, which was prepared using the following procedure. Fifteen mL ultrapure concentrated hydrobromic acid was added to 100 mL of ultrapure sulfuric acid. The mixture was heated on an electric heater until fumes appeared; it was then allowed to stand for 30 min. After cooling, the prepared solution was stored in a clean glass bottle. All of the other chemicals were of analytical grade or higher (Beijing Chemical Co), and double-deionized water was used throughout. A reference material, NIES-10 (rice flour from Japan Environmental Agency) with a content of 60 ng Se g^{-1} was used as a biological standard reference material. The stock solution of Se(−II), Se(IV), and Se(VI) (100 mg L^{-1} Se) was prepared by dissolving weighted amounts of selenomethionine (Sigma Co.), sodium selenite (GR, Beijing Chemical Co.), and sodium selenate (GR, Beijing Chemical Co.), respectively, in 0.1 M hydrochloric acid. The solution was diluted to a final concentration of 1 ng mL^{-1} before use. A 0.1% DAN solution was prepared in 0.1 M HCl, then it was extracted with 25 mL cyclohexane (spectroscopic grade), and this procedure was repeated five times. The solution was stored in a cool and lightproof condition and covered with 2 cm of cyclohexane.

Sampling. Tap water was sampled directly from the laboratory. Rainwater was collected on the roof of the main building of the Research Center of Eco-Environmental Sciences, Beijing, in July 2000 using a hyetometer. Spring water was sampled in a mountain spring located in the suburbs of Beijing. Pond water was sampled 1 m beneath the surface at Shuangqing Villa, Beijing. All water samples were filtered through a 0.45 μm nylon filter membrane (Millipore Co.), and Se determinations and speciation were carried out within 1 day after sampling.

Microwave Digestion and Speciation Procedure. In microwave digestions, 2 mL filtered water sample was combined with 0.5 mL of concentrated nitric acid or 0.5 mL of concentrated H_2O_2/H_2SO_4 mixture (v:v = 5:1) in a Teflon digestion vessel. The vessels were placed in symmetrical positions on the turntable. Then the samples were digested according to conditions shown in Table 1. After digestion using concentrated nitric acid, the reduction of Se(VI) to Se(IV) was achieved by adding an equal volume of concentrated hydrochloric acid and heating the tubes in a water bath (100 °C) until the fumes of nitrogen dioxide disappeared. Then it was cooled to room temperature before measurement. For the samples digested with the H_2O_2/H_2SO_4 mixture, it is necessary to add 2 drops of 0.1 M $MnCl_2$ and heat for 5min on a hot plate to remove the excessive H_2O_2. A hot-plate digestion technique developed in our laboratory for Se analysis of biological samples was used for comparison[8] in which samples were digested using a mixture of nitric acid and perchloric acid (v:v = 5:2, GR, Shanghai Chemical Co.) and heated to 80 °C for 2 h, then the temperature was elevated to 150 °C for 5 h and to 200 °C until 30 min after white smoke appeared and then left.

Table 1. Working Program of the Microwave System

step	power, %	pressure, MPa	time, min	notes
1	100	0.55	5	rising of temp
2[a]	80	0.55		constant pressure
3	0		10	to restore pressure
4	100	0.55	5	rising of temp
5[a]	80	0.55		constant pressure

[a] 10 and 15 min of digestion time for HNO_3 and H_2SO_4/H_2O_2, respectively.

The amount of Se(IV) present in the digested solution was first derived with DAN[8] and then extracted by 0.5 mL cyclohexane. To measure the amount of Se(IV) in water samples, 20 mL of water was mixed with 1 mL of concentrated H_3PO_4. After evaporation of the mixture to ~2 mL under nitrogen protection, the amount of Se(IV) in the sample was determined. Se(VI) in the sample was first reduced to Se(IV), then the total inorganic selenium, Se(VI) and Se(IV), was determined, and Se(IV) was obtained by subtraction. The error on Se(VI) values was estimated as the root of the summed squares of SD values of Se(IV) and of total inorganic selenium. Total selenium was determined after microwave dissolution of the samples and transformation of all forms of selenium into Se(IV). Se(−II, 0) was calculated by subtracting the amounts of total inorganic selenium from total selenium. The error of the selenium(−II, 0) amount was calculated as the root of the summed squares of the SD values of the total selenium and the total inorganic selenium.

After extraction, the concentration of naphthoselenodiazol (NSD) in cyclohexane is measured by a normal phase HPLC with a fluorescence detector fixed at an excitation wavelength of 366 nm, an emission wavelength of 520 nm, and a μ-porosil $C_{18}-NH_2$ column. An injection volume of 100 μL is applied. The mobile phase consists of a mixture of cyclohexane:tetrahydrofuran (9:1 = v:v). Typical HPLC chromatography for determination of Se in water samples was illustrated elsewhere.[8] The signal for NSD in the chromatogram was characterized by its retention time and co-chromatography with a NSD standard prepared by our laboratory. The relative fluorescence intensity was recorded as the peak height for calculating selenium content in the samples according to the calibration curve. The calibration curve was obtained by adding 0−1.5 ng Se (as selenite) to distilled water, followed by the same procedure as that for the determination of selenium in samples. Calibration was carried out together with sample measurements. The validation of the method for quantitative analysis was performed with the certified reference material, NIES-

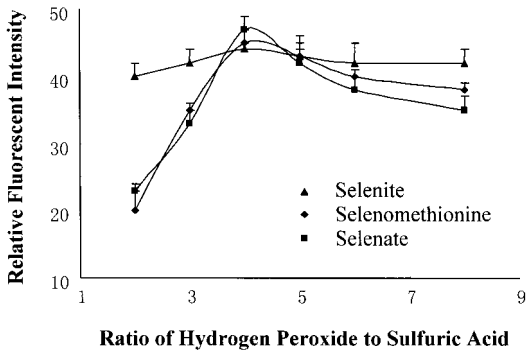

Figure 1. Influences of the volume ratios of H_2O_2 to H_2SO_4 on the determination of Se added as different chemical forms (selenite, selenate, and selenomethionine).

10 (rice flour containing 60 ng Se g^{-1}, from the Japan Environmental Agency). The validation of the method for the speciation of selenium in water samples was performed by an addition of a known amount of selenite, selenate, and selenomethionine standard solution to rainwater. To assess the interference from the whole procedure, 10 replicated determinations were performed on 2 mL of distilled water. The measured blank was 0.021 ± 0.002 ng Se per sample using concentrated nitric acid and 0.052 ± 0.003 ng Se per sample using the H_2O_2/H_2SO_4 mixture (v:v = 5:1). This is obviously better than the measured blank using hot plate digestion (0.08 ± 0.02 ng Se per sample).[8]

RESULTS AND DISCUSSION

Digestion Reagents. The selection of digestion reagents is a key factor for a successful microwave digestion. HNO_3 is used for its excellent dissolving capability. It can decompose organic materials effectively at room temperature, and complete mineralization can be achieved at high temperature and high pressure.[9] HNO_3 is a unique acid that can be used alone, and a simple reagent composition is beneficial to trace analysis. A mixture of H_2O_2/H_2SO_4 is another common digestion reagent. An advantage of employing H_2O_2/H_2SO_4 is that residual oxidant that can affect the relative fluorescence intensity of the NSD can be easily eliminated by heating. There is no research to demonstrate the effect of digestion reagents on Se oxidation state or digestion efficiency for different Se forms. The influence of different ratios of H_2O_2 to H_2SO_4 on the relative fluorescence intensity of the NSD derived from selenite, selenate, and selenomethionine was tested, and the results are shown in Figure 1. The maximum relative fluorescence intensity of various Se species was obtained at H_2O_2/H_2SO_4 ratios of 4:1 to 5:1. The parallelism of the relative fluorescence intensity for different oxidation states of Se was satisfactory when the ratio was 5:1.

Digestion Time. The digestion time determines the completeness of the digestion. An appropriate time is necessary not only for a complete sample digestion but also for saving time. The influence of the digestion time on the completeness of the digestion was tested using selenomethionine, which is known to be one of the most acid-resistant Se(−II) species,[10] and rice flour as samples. HNO_3 or H_2O_2/H_2SO_4 (5:1) was used as the reagent. The results of the digestion time test are shown in Table 2. When the heating time was less than 15 min, the recoveries of selenium in both selenomethionine and rice flour were comparatively low, and the rice flour solution remained blue and turbid, likely indicating an incomplete digestion. In contrast, the recoveries of selenium in both samples were increased to satisfactory, and clear, colorless solutions were obtained after 20 min of microwave heating with HNO_3 or 30 min of heating for H_2O_2/H_2SO_4. Thus, the proper choice of digestion time for HNO_3 was 20 min, and that for H_2O_2/H_2SO_4 was 30 min. Actually, the heating procedure was separated by a break of 10 min to let the pressure in the vessels drop to the normal level.

Effect of Digestion Reagents on Se Oxidation State. Digestion reagents affect Se valence in digested solutions. The changes of selenium oxidation states in digested process were studied using HNO_3 and H_2O_2/H_2SO_4 as microwave digestion reagents and 0.6 ng Se (selenomethionine, selenite, and selenate) in samples. The results are shown in Table 3. Results obtained when using H_3PO_4 with evaporation of the water sample and when using hot plate digestion are also listed in Table 3. When digested with HNO_3, Se(IV) could be detected in digested solutions of selenomethionine and selenite, but not in that of selenate. The recoveries of various Se compounds without the reduction step were not satisfactory (92% for selenomethionine, 80% for selenite, and 15% for selenate). It indicates that, with the present operating conditions, more than 80% of selenomethionine or selenite was found in the tetravalent state after microwave digestion with HNO_3. After reducing the Se(VI) in the digested solutions with an equal volume of concentrated hydrochloric acid, the recoveries reached 100.0% for selenomethionine, 98.3% for selenite, and 103.3% for selenate, respectively. This indicates that most of both selenomethionine and selenite were oxidized to or remained as Se(IV) by a microwave digestion with HNO_3, and part of them as Se(VI). Therefore, when digestion of the sample with HNO_3 was used for the fluorimetric determination of the total Se, a reduction step is necessary. The results likely indicate also that the Se(−II) could be transformed into Se(IV) almost quantitatively and that most of Se(IV) remained unchanged under these conditions. The total amount of Se(−II) and Se(IV) could thus be determined directly after sample digestion and derivation.

When the H_2O_2/H_2SO_4 mixture was used, Se(IV) could be measured directly after microwave heating, and the recoveries of different Se compounds were satisfactory. A reduction procedure could not further significantly improve the recoveries. The results showed that H_2O_2/H_2SO_4 could oxidize Se(−II) and reduce Se(VI) to Se(IV) quantitatively. Thus, total selenium can be measured directly after digesting the sample with H_2O_2/H_2SO_4, followed by a derivation procedure. This is surprising, considering the expected strong oxidizing power of the H_2O_2/H_2SO_4 mixture. One possible explanation could be that the combined action of high temperature and high pressure of the microwave treatment limits the oxidizing capacity of the mixture and maintains the oxidation state of selenium at Se(IV). This temperature/pressure effect could also explain why most of the digested Se in various forms

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Table 2. Amount and Recovery of Se/ng in Solutions after Various Digestion Times

sample[a]	reagent	digestion time, min 10	15	20	30
Se(−II)-seleno-methionine	HNO_3 [b]	0.41 ± 0.08 (68.3)[c]	0.55 ± 0.03 (91.7)	0.60 ± 0.02 (100.0)	0.59 ± 0.01 (98.3)
	H_2O_2/H_2SO_4	0.36 ± 0.10 (60.0)	0.48 ± 0.06 (80.0)	0.53 ± 0.02 (88.3)	0.62 ± 0.01 (103.3)
rice flour	HNO_3 [b]	0.35 ± 0.05 (58.3)	0.54 ± 0.10 (90.0)	0.59 ± 0.01 (98.3)	0.60 ± 0.01 (100.0)
	H_2O_2/H_2SO_4	0.31 ± 0.07 (51.7)	0.41 ± 0.10 (68.3)	0.50 ± 0.04 (83.3)	0.59 ± 0.02 (8.3)

[a] 0.60 ng of Se was added in each sample ($N = 3$). [b] A reduction process was performed after digestion with HNO_3. [c] Data in parentheses show the percentile recoveries of Se.

Table 3. Amount and Recovery of Se in Digested Solution/ng

Se forms added[a]	digestion reagent H_2O_2/H_2SO_4	HNO_3	H_3PO_4 [c]	$HNO_3/HClO_4$ [d]
Se(−II), selenomethionine	0.62 ± 0.01 (0.60 ± 0.02)[b]	0.55 ± 0.01 (0.60 ± 0.02)	0.02 ± 0.00 (0.03 ± 0.04)	0.03 ± 0.02 (0.58 ± 0.06)
Se(IV), selenite	0.60 ± 0.01 (0.59 ± 0.02)	0.48 ± 0.03 (0.59 ± 0.03)	0.60 ± 0.03 (0.61 ± 0.03)	0.07 ± 0.04 (0.61 ± 0.07)
Se(VI), selenite	0.58 ± 0.04 0.62 ± 0.02)	0.09 ± 0.01 (0.62 ± 0.01)	0.05 ± 0.01 (0.59 ± 0.01)	0.08 ± 0.04 (0.57 ± 0.05)
rice flour	0.59 ± 0.02	(0.60 ± 0.01)		

[a] 0.6 ng Se was added in each sample ($N = 3$). [b] Data in parentheses was obtained after a reduction process. [c] H_3PO_4 was used in the evaporation of water sample under N_2 protection. [d] Hot plate digestion was employed.

was recovered as Se(IV) and not Se(VI) when the oxidizing HNO_3 was used.

It is reported that Se in a water sample could be lost during storage under high pH and at high-temperature conditions.[11] H_3PO_4 can be used to prevent selenium loss and scale depositing in sample evaporation and storage when measurement of Se(IV) is performed. Our results show that there was no Se loss or significant change of the selenium oxidation states occurred during the heating procedure when H_3PO_4 was used and the system was under N_2 protection (Table 3). The hot plate digestion with $HNO_3/HClO_4$ (v:v 5:2) can transform all states of selenium into Se(VI). When the method is combined with a reduction procedure, total Se can be measured. However, it suffers from procedural contamination and comparatively large errors (as shown in Table 3.) and is time-consuming.

Calibrating and Se Determination. The calibration curves of selenium determination using HNO_3 and H_2O_2/H_2SO_4 as microwave digestion reagents or using the hot plate digestion technique to prepare samples all show good correlation coefficients ($R^2 > 0.997$). Linear relationships between dosage of Se(IV) and fluorescence intensity can be observed in a range of 0−1.5 ng Se.

For a sensitive analysis method, the blank has to be considered. In the proposed procedure for selenium determination, interference may be introduced from fluorescent impurity in DAN and traces of selenium in chemical reagents. Samples can also be contaminated during the pretreatment of samples and the subsequent selenium determination. The interference coming from the fluorescent impurity can be eliminated by DAN purification and an HPLC separation.[8] To assess the interference from reagents other than DAN and the whole procedure, 10 duplicate determinations were performed on 2.0 mL of quartz-distilled water. The results were 0.0205 ± 0.0022, and 0.0521 ± 0.0032 ng of selenium per sample respectively, which corresponds to a detection limit (3Φ) of 0.0066 and 0.0096 ng of selenium for HNO_3 and H_2O_2/H_2SO_4 as digestion reagents, respectively. The blank is comparatively high for H_2O_2/H_2SO_4 because of the trace amounts of Se present in concentrated H_2SO_4. The detection limit is 0.06 ng for the hot plate digestion technique.[8] The microwave digestion technique can supply a lower detection limit. This is a benefit to the improvement of accuracy and precision of determination. A biological standard reference material, rice flour from Japan Environmental Agency (NIES 10), was employed to validate the total selenium analytical process. The results are shown in Table 3. The recoveries were 99.4 ± 2% for HNO_3 as digesting reagent and 97.8 ± 2.5% when the H_2O_2/H_2SO_4 mixture was used ($N = 3$). In addition to reducing the treatment time, the microwave digestion technique performed in closed vessels can avoid contamination of samples and eliminate the uncontrolled loss of volatile molecular species, thus reducing errors of measurement. A microwave digestion with HNO_3 combines the advantage of a simple reagent, a short sample treatment, and the lowest detection limit.

Speciation of Natural Waters. When appropriate reagents are used, the transformation of different selenium species into Se(IV) can be achieved, and Se(IV) can be easily determined by HPLC-FLD after DAN derivation. This makes possible the speciation of selenium in natural and environmental samples.

The proposed methodology was used for the speciation of Se in four water samples. A 20-mL sample was required for Se(IV)

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Table 4. Selenium Speciation in Different Types of Water Samples from Beijing (μg L^{-1})

	tap water	spring water	pond water	rainwater
total Se[b]	0.20 ± 0.01	0.90 ± 0.03	0.55 ± 0.02	0.54 ± 0.03
Se(IV+VI)[c]	0.19 ± 0.01	0.89 ± 0.05	0.23 ± 0.01	0.42 ± 0.02
Se(IV)[d]	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.20 ± 0.01
	(10.0)[a]	(2.2)	(1.8)	(37.0)
Se(VI)[e]	0.17 ± 0.01	0.87 ± 0.05	0.22 ± 0.01	0.22 ± 0.02
	(85.0)	(96.7)	(40.0)	(40.7)
Se(−II, 0)[e]	0.01 ± 0.01	0.01 ± 0.06	0.32 ± 0.02	0.12 ± 0.04
	(5.0)	(1.1)	(58.2)	(22.0)

[a] Data in parentheses is % of Se species with respect to the total Se ($N = 3$). [b] Total Se was determined by HNO_3 digestion followed by a reduction process. [c] Inorganic Se was determined after a reduction process. [d] 20 mL of water was mixed with 1 mL of concentrated H_3PO_4. After evaporation of the mixture to ~2 mL under nitrogen protection, the amount of Se(IV) in the sample was determined. [e] Calculated data by subtraction.

Table 5. Recovery of Spiked Se in Different Forms from the Rainwater Sample

Se forms added[a]	Se in sample ng	Se added ng	Se found ng	recovery %
Se(IV)	0.20 ± 0.01	0.20	0.38 ± 0.02	95.0
Se(IV, VI)	0.42 ± 0.02	0.40	0.84 ± 0.03	102.4
Se(−II, IV, VI)[b]	0.54 ± 0.03 (0.55 ± 0.02)	0.55	1.10 ± 0.03 (1.13 ± 0.04)	99.1 (103.7)
Se(VI)[c]	0.22 ± 0.02	0.20	0.46 ± 0.04	109.5
Se(−II, 0)[c]	0.12 ± 0.04 (0.13 ± 0.03)	0.15	0.26 ± 0.04 (0.28 ± 0.05)	96.3 (100)

[a] To 1.0 mL of the rainwater sample (Se speciation see Table 4), an equal amount of Se in different forms was spiked. [b] Data show results obtained by using HNO_3; data in parentheses show results obtained by using H_2SO_4/H_2O_2. [c] Calculated data by addition or subtraction.

measurement and a 2.0-mL sample was sufficient for total inorganic Se(IV, VI) or total Se determination. The results obtained are shown in Table 4 ($N = 3$). In most natural waters, total Se levels range from 0.04 to 5 μg L^{-1}.[12] Selenium in water can exit in different oxidation states (−II, 0, IV, VI); Se(IV) and Se(VI) are the most common forms.[13] For the water samples investigated in this study, total Se levels ranged from 0.2 to 0.90 μg L^{-1}. Among its four valences, Se(VI) was the most common state, and Se(0) was not discriminated from Se(−II) in most Se speciation procedure. The total Se concentration in tap water was the lowest of our four samples at 0.20 μg L^{-1}. About 85% of Se was present as Se(VI), 5% as Se(−II, 0), and 10% as Se(IV), which is slightly different from the values (total Se 0.15 μg L^{-1}; Se(VI) 68%, Se(IV) 32%) in its source water (Huairou Reservoir, Miyun Reservoir, Beijing) which was reported previously.[14] In pond water, the total Se concentration was at 0.55 μg/L. There was more selenide than selenate, with 58.2% of the total Se being in the form of Se(−II, 0) and 40% of total Se as Se(VI). It has been reported that reduced species can be produced from biotic process in surface water, and Se(−II, 0) is correlated with indicators of biological activity, such as chlorophyll, bioluminescence, and primary productivity.[15,16] Phytoplankton is abundant in this pond, which could explain the dominance of Se(−II, 0). In spring water, the total Se content was 0.90 μg L^{-1}. Almost all of total Se was in Se(VI) form, which may partly be explained by the Se form present in bedrock. Similar results were reported in our previous work.[12] Se content in rainwater was 0.53 μg L^{-1}, and not one of the four selenium species was obviously predominant. The total Se level in rainwater in the Beijing area has been reported to range from 0.03 to 0.43 μg L^{-1}, and more generally in the 0.25−0.35 μg L^{-1} range,[17] which is lower than the value measured in this present work. Many factors can affect the Se content and species in rainwater, such as weather conditions, rainfall, atmospheric pollution, and microorganism activity in the soil.[17,18] A higher Se content in rainwater may result from more serious air pollution.

To validate the proposed speciation approach, a recovery test was performed. In the absence of a proper standard reference material for selenium speciation in natural waters, known amounts of selenomethionine, selenite, and selenate were spiked to a water sample. For the test, 1.0 mL of the rainwater containing 0.12 ng of Se(−II, 0), 0.20 ng of Se(IV), and 0.22 ng of Se(VI) was spiked with equal amounts of different selenium compounds (selenomethionine, selenite, and selenate). The percentage of recovery of each species was calculated by eq 1 using the Se amount determined and the combined mass of sample Se and spiked Se. The results showed that the recoveries of the different selenium species were 95.0−109.5%, as shown in Table 5.

$$\text{Recovery (\%)} = \text{Se measured}/(\text{Se in sample} + \text{Se added}) \times 100 \quad (1)$$

CONCLUSIONS

A microwave preparation procedure for selenium speciation in natural waters was established. The optimized procedure for 2 mL water consists of using 1.0 mL of HNO_3 or of a mixture of H_2O_2/H_2SO_4 (5:1 v:v) as the reagent, heating the solution 20 min with HNO_3 or 30 min with H_2O_2/H_2SO_4 with the microwave power at 80% and a pressure of 0.55 Mpa. The digestion with H_2O_2/H_2SO_4 can transform all Se species to selenite; digestion with HNO_3, to selenite and selenate. Selenite, Se(IV), can be determined using a normal phase HPLC-FLD after a precolumn derivatization with DAN, and selenium speciation in a water sample can be carried out. The proposed procedure was applied to four different water samples from the Beijing area. Total Se ranged from 0.20 to 0. 90 μg L^{-1} with Se(VI) being the dominant species in samples, with the exception of pond water, in which Se(−II, 0) was predominant.

The proposed methodology combines the advantages of a shorter digestion time, a lower risk of sample contamination, and

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a minimized amount of sample requirement. Detection limits of 0.0066 and 0.0096 ng Se were obtained for HNO_3 and H_2O_2/H_2-SO_4, respectively. The optimized microwave preparation procedure was also applied to rice flour, and recoveries reached 99.4 ± 2% and 97.8 ± 2.5% for HNO_3 and H_2O_2/H_2SO_4, respectively, as digestion reagents, confirming that the procedure can also be used to measure total Se in biological samples.

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