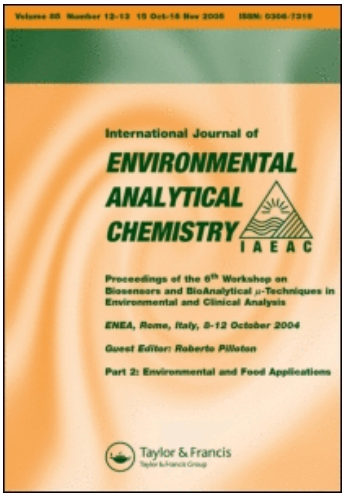

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Stability and Storage Problems in Selenium Speciation from Environmental Samples

José L. Gómez-Ariza[a]; José A. Pozas[b]; Inmaculada Giráldez[a]; Emilio Morales[a]
[a] Departamento de Química y Ciencia de los Materiales, Escuela Politécnica Superior, Universidad de Huelva, Huelva, Spain [b] Departamento de Bioquímica, Bromatología y Toxicología, Facultad de Medicina, Universidad de Sevilla, Sevilla, Spain

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STABILITY AND STORAGE PROBLEMS IN SELENIUM SPECIATION FROM ENVIRONMENTAL SAMPLES

JOSÉ L. GÓMEZ-ARIZA[a*], JOSÉ A. POZAS[b], INMACULADA GIRÁLDEZ[a] and EMILIO MORALES[a]

[a]*Departamento de Química y Ciencia de los Materiales, Escuela Politécnica Superior, Universidad de Huelva, 21819-La Rabida, Huelva, Spain and* [b]*Departamento de Bioquímica, Bromatología y Toxicología, Facultad de Medicina, Universidad de Sevilla, 41003-Sevilla, Spain*

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The stability of selenite and selenate at 0.3 and 100 μg L^{-1} concentrations, preserved at different pH values (pH 2, 4 and 8) and stored in different containers (Teflon, polypropylene and polyethylene) at various temperatures (from −20 to 40°C) was studied for one year period. Both species were stable in acidified samples at pH 2 with HCl at − 20°C in Teflon containers for the twelve months tested. However, losses of selenite were observed after 6 months in river and tap water samples. Selenate was more stable than selenite and higher concentrations were more stable than lower concentrations. The order of decreasing stability was Teflon > polyethylene >polypropylene, pH 2 > pH 4 > pH 8 and −20°C > 4°C > 25°C > 40°C. The stability of four volatile organic selenium species in seawater spiked at concentrations of 50 μg L^{-1} for both DMSe and DESe and at concentrations of 0.50 μg L^{-1} for both DMDSe and DEDSe, stored at two temperatures (4°C and −20°C) in three different container materials (Teflon, polyethylene and polystyrene) was studied. The four species were only stable for 24 h. The order of decreasing stability was DMDSe > DESe > DEDSe > DMSe, Teflon > polyethylene > polystyrene and −20°C > 4°C.

Keywords: Storage; organoselenium; selenite; selenate; speciation; water

INTRODUCTION

Selenium is present in aquatic systems at different oxidation states: selenide (both in inorganic and organic compounds), selenite and selenate. Selenium concentration in water is low, ranging from 0.02 to 1 μg L^{-1} in most drinking and fresh waters[1] and from 0.004–0.06 μg L^{-1} in sea water[2]. However, pore water from seleniferous soil in semiarid areas may contain up to hundreds or thousands

* Corresponding author. Fax: +34–959–350311. E-mail: ariza@uhu.es

of micrograms of dissolved selenium per litre[3]. Selenium is a minoritary element in the environment but is biologically essential. Low levels of selenium are necessary for human metabolism, but higher concentrations of this element may cause damage in the health[4]. Organic species of selenium have a different toxicity than inorganic ones, being methylation an effective detoxification mechanism[5–8]. As a consequence analytical methods for the chemical speciation of selenium including both inorganic (selenite and selenate) and organic species (dialkylselenide and dialkyldiselenide) in environmental samples are necessary. However, the accuracy of selenium species determination in natural samples is not only dependent on the measurement step, but sampling, storage and sample preparation affect the reliability of the results, as far as several processes such as volatilization, adsorption, interconversion of species, precipitation or contamination may alter the initial composition of the sample.

Variations in the selenium species concentrations in environmental samples during storage have been recently reviewed[9,10]. Alkylated selenium compounds are volatile and losses by volatilization may occur even at room temperature in airtight containers within a day[11–13]. Adsorption and desorption phenomena are really important at the low selenium concentrations usually found in environmental samples, and depend on pH, ion strength, container material and the ratio of container surface area per unit of volume[14–16]. Moreover, a modification in the oxidation states between selenite and selenate during storage has been observed by several authors, being selenate the most stable species[17,18]. Otherwise, selenium losses due to precipitation processes are more important for selenite than for selenate[19]. The selenium stability is affected by algal growth which must be avoided[17–20]. Finally, an increase in selenium concentrations leads to a decrease in selenium losses[21,22]. Several storage variables including the nature of the matrix, the presence of dissolved gases and preservative agents, light action, temperature, container materials and shaking have been evaluated, resulting in the following conclusions: a higher stability of selenium is obtained at higher ionic strengths, in solutions acidified with HCl concentrations lower than 1 mol L^{-1} and stored at low temperatures in PTFE containers with the smaller specific surface, being advisable both a purge with nitrogen to remove the oxygen and the chlorine and dark conditions[14,17,18,22–24]. Shaking to rehomogenize the sample is necessary prior to the aliquot removing from the parent sample for analysis[25]. However, some controversial points remain: modification in the oxidation states of species have been observed in acidified samples with HCl at pH 2 after two months[26] and in unacidified samples stored at 4 °C in polyethylene containers after a month[19]. Moreover, most of the studies have been carried out in deionized water spiked with selenite and selenate species[18,21,22], whereas both synthetic solutions and contaminated natural waters

should preferably be tested[12]. Finally, non-volatile organoselenium compounds (selenocystine, selenomethionine and trimethylselenonium) were found to be stable in the dark over a one year period in an aqueous matrix at pH 4.5[27].

The aim of this study is to assess the stability of selenite, selenate, dimethylselenide (DMSe), diethylselenide (DESe), dimethyldiselenide (DMDSe) and diethyldiselenide (DEDSe) in natural samples during storage under different conditions such as pH, container material and temperature. In addition, the concentration variations of selenite and selenate in five samples collected from the southwest Spain, including sea water and river water samples has been evaluated after a 1-year storage period.

EXPERIMENTAL

Reagents, calibrants and apparatus

The reagents used in the experiments were analytical grade and obtained from Merck and Sigma. SAX (600 mg of sorbent) and C-18 (600 mg of sorbent) cartridges were obtained from Alltech and Waters, respectively. Pesticide grade solvents were purchased from Merck. Stock solutions of Se(IV) and Se(VI) (1000 mg of Se L^{-1}) were prepared from analytical-reagent grade selenium dioxide and sodium selenate (Merck), respectively. Organoselenium stock solutions were prepared at a concentration of *ca.* 100 mg (as Se) L^{-1} in benzene from DMDSe (Aldrich), DESe, DMSe (Pfaltz & Bauer, inc.) and DEDSe (syntetized by the authors) and were kept in a refrigerator. Aqueous working solutions were prepared daily. Water used in the experiments was double-distilled and deionized and gave blank readings in all the analysis.

Selenium species analysis was carried out using an HP Model 5890 gas chromatograph, HP Model 5970 mass detector and a fused silica capillary column, 25 m length, 0.20 mm i.d. and a film thickness of 0.33 μm HP-1 crosslinked methyl-silicone gum, as previously reported[28].

Diethyldiselenide synthesis and purity of the organoselenium calibrants

Diethyldiselenide was synthesized by a modification of the procedure proposed by Ganther and Kraus for DMDSe[29,30]. A 0.5 g of selenourea was placed in a round-bottomed flask and 25 ml of water was added. Then 3 ml of ethyl iodide was added and the mixture was refluxed at 200 °C under continuous stirring for 2 h. The excess of reagent was removed by rotatory evaporation (40–50 °C), and then 25 ml of n-heptane and 40 ml of 5 mol L^{-1} NaOH aqueous solution were added. The mixture was refluxed for 1 h and then allowed to cool. The upper yel-

low heptane layer was dried over anhidrous Na_2SO_4 and filtered. Finally, the heptane was removed by rotatory evaporation (30 °C). The resulting extract was a brown-yellowish oily liquid, which purity was studied by GC-MS and FAAS.

Purities of 97±3%, 98±2%, 98±3% and 97±3% were assessed for DEDSe, DESe, DMDSe and DMSe, respectively.

Inorganic selenium analysis

Se(IV) was separated from Ss(VI) by means of a solid phase extraction based on a method proposed in the literature[31]. Briefly, an anion-exchange resin (SAX cartridge) was conditioned with 10 ml of 3 mol L^{-1} HCl and 10 ml of distilled water at a flow rate of 5 ml min^{-1}. A 500 ml sample of water (pH adjusted at 7–8 using HCl or NaOH) was passed through the cartridge at 8 ml min^{-1}. Se(IV) and Ss(VI) were successively eluted with 25 ml of both 1 mol L^{-1} formic acid and 3 mol L^{-1} of hydrochloric acid, respectively, using a flow rate of 5 ml min^{-1}. The extract containing Se(VI) was transferred to a Pyrex tube (50 ml) and quantitatively reduced to Se(IV) with 10 ml of boiling 5 mol L^{-1} HCl for 30 min. After allowing the residual solution to cool, the pH was adjusted to 2.1 and selenium was derivatized.

5 ml of 0.1% 4-Cl-*o*-phenylendiamine in 0.1 mol L^{-1} HCl was added to the solution containing Se(IV) and heated at 75 °C for 7 min to form the corresponding piaselenol. After cooling to room temperature, the selenium derivative was extracted twice with 1 ml of toluene for 1 min. The organic phase was separated, reduced to just dryness under a N_2 stream and the residue was dissolved in 50 µl of hexane containing fluorodinitrobenzene (FDNB) as internal standard (230 ng L^{-1}). Aliquots of 1 µl were analyzed by GC-MS using the following oven temperature program: 40 °C for 1 min after injection, followed by a 60 °C min^{-1} ramp to 125 °C and isothermal maintenance for 1 min. Then a second heating ramp of 10 °C min^{-1} up to 250 °C and a final isotherm for 1 min. The injector block was heated at 240 °C.

Organic selenium analysis

A C-18 cartridge was conditioned with 10 ml of CS_2 and 10 ml of distilled water at 5 ml min^{-1}. A 500 ml sample of water was passed through the cartridge at 10 ml min^{-1}. Then, the cartridge was dried under a N_2 stream and the organic selenium species were eluted with 2 ml of CS_2 at 1 ml min^{-1}. 200 µl of a solution of 2.0 µg L^{-1} of 2,6-diisopropylphenol (propofol) as internal standard in CS_2 was added and the solution was analyzed by GC-MS using the following oven temperature program: 35 °C for 2 min after injection, followed by a 10 °C min^{-1} ramp to 180 °C and isothermal maintenance for 5 min. The injector block was heated at 240 °C and the injection volume was 1 µl.

To improve the sensitivity of the chromatographic method, a derivatization step for the dialkyldiselenide species was introduced[28]. The apparatus used in the derivatization step is depicted in Figure 1. A suitable aliquot of CS_2 extract containing the organoselenium compounds was placed into the volatilization vial and 350 mg of zinc dust and 3–4 drops of n-octanol were added. The trapping vial contained 0.4 ml of double-distilled water, 0.6 ml of dimethylformamide (DMF), 1 ml of freshly prepared FDNB in DMF (1% v/v) and 14 mg of $NaHCO_3$. A N_2 stream (as carrier gas) was passed through the volatilization manifold to remove the oxygen. Then 3 ml of 12 mol L^{-1} HCl was injected into the volatilization vial through a septum with a syringe. A N_2 flow rate of 100 ml min^{-1} during 10 min was used to complete the reaction in the trapping vial. The selenium derivatives were then extracted with 4 ml of ethyl acetate (3 times) for 15 min, the solvent removed under a N_2 stream, the residue dissolved with 50 µl of toluene containing 200 µg L^{-1} of propofol and analyzed by GC-MS using the following oven temperature: 40 °C for 1 min after injection, followed by a 60 °C min^{-1} ramp to 125 °C and isothermal maintenance at this temperature for 1 min. Then a second heating ramp of 10 °C min^{-1} up to 250 °C and a final isotherm for 1 min. Injector block temperature, 250 °C.

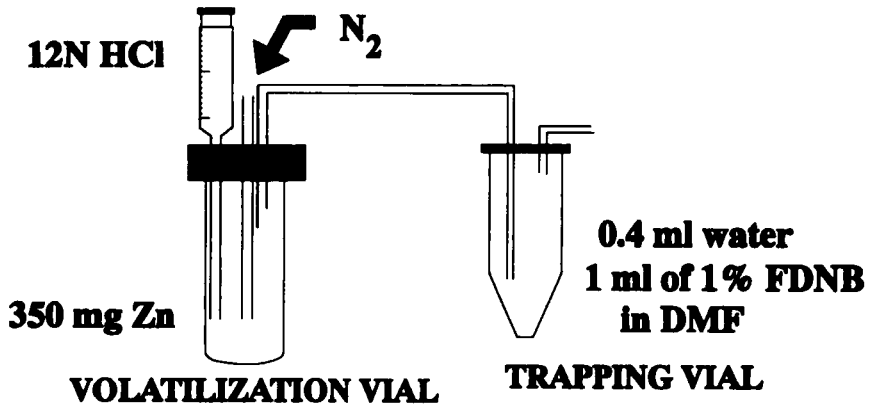

FIGURE 1 Volatilization and trap device

Calibration

Selenium concentrations were deduced from calibration curves derived from standard solutions in clean seawater using peak heights. Standard addition procedures were performed to avoid possible matrix effects. The repeatability of the technique for the different selenium species (5 replicate analysis of standard

solutions in water in a day), the reproducibility of the method over a month, the detection limits (evaluated as 3 × standard deviation of the mean + the value for the mean standard blank, for n=10 standard blank runs), the sensitivities (slope of the calibration curve) and the correlation coefficients are presented in Table I.

TABLE I Detection limits (DL), correlation coefficient (r^2). sensitivity (S), repeatability (r) and reproducibility (R) of selenium analysis in water samples. The repeatability and the reproducibility were assessed in solutions containing 0.300 μg L^{-1} of Se(IV), 0.300 μg $L^{-1}$of Se(VI), 33.3 μg L^{-1} of DMSe, 35.6 μg L^{-1} of DESe, 0.480 μg L^{-1} of DMDSe and 0.975 μg L^{-1} of DEDSe (as Se)

Species	*D.L. (ng L^{-1})*	*S (L μg^{-1})*	r^2	*r (%)*	*R (%)*
Se(IV)	1.4	2.35	0.996	5.5	8.5
Se(VI)	4.5	2.06	0.992	5.4	7.0
DMSe	170	0.015	0.995	4.4	6.5
DESe	102	0.023	0.999	4.5	9.4
$DMDSe_d$*	2.9	1.21	0.991	5.6	8.5
$DEDSe_d$*	19	0.494	0.995	6.4	10.2

* Organselenium derivative.

Sample collection and preservation

Water samples including both river and seawater were collected in several areas from the southwest Spain (Table II). They were placed in Teflon containers and frozen for transporting to the laboratory. Then, they were filtered through 0.2 μm membrane filter, analyzed for selenium species, acidified at pH 2 with HCl and stored at –20 °C in Teflon containers. Oxygen was removed from solutions by bubbling with N_2. The stability of selenite and selenate at low selenium concentrations (about 0.3 μg L^{-1} of Se for each species) during a year was studied using unspiked aliquots of the seawater sample collected from Portil boatyard (Spain). The influence of higher concentrations of selenium on the preservation of Se(IV) and Se(VI) in seawater samples was studied by spiking aliquots of this sample with both selenite and selenate (controlled weighing) at concentrations of 100 μg L^{-1} of selenium. The stability of the inorganic selenium species at three different pH values (pH 2 and 4 by adding HCl and pH 7.6) in containers made in Teflon, polyethylene and polypropylene was studied at a temperature of 25 °C. These containers were 500 ml in volume and were kept in the dark. The influence of the temperature on the preservation of selenite and selenate was evaluated using samples acidified at pH 2 and stored in Teflon containers at –20, 4, 25 and 40 °C. These species were quantified after 1 week, 2 weeks, 1 month, 3 month, 6 months and 1 year.

TABLE II Stability of selenite and selenate after 180 and 360 days in water samples acidified at pH 2 with HCl and stored at −20° in 500 ml Teflon containers secure from light, expressed as ng of Se L^{-1} (mean ± standard deviation, n=3)

Location	*Type of water*	*Se(IV)*			*Se(VI)*		
		Initial	*180 days*	*360 days*	*Initial*	*180 days*	*360 days*
Portil	seawater	0.343 ± 0.018	0.322 ± 0.026	0.333 ± 0.028	0.311 ± 0.025	0.329 ± 0.21	0.307 ± 0.033
Puntaumbria	seawater	270 ± 17	301 ± 27	253 ± 21	81.0 ± 5.7	86.9 ± 4.7	72.1 ± 4.5
Ayamonte	seawater	5.67 ± 0.28	5.16 ± 0.23	5.99 ± 0.34	3.42 ± 0.29	3.08 ± 0.18	3.33 ± 0.18
Niebla	river water	0.033 ± 0.003	0.037 ± 0.002	<DL*	0.025 ± 0.003	0.022 ± 0.002	0.028 ± 0.002
Seville	tap water	980 ± 65	1040 ± 80	599 ± 44	430 ± 22	469 ± 31	407 ± 25

* Below detection limit.

The stability of the organic selenium species was studied during a month period using the seawater sample collected in Portil boatyard spiked at concentrations of 50 μg L^{-1} for both DMSe and DESe and at concentrations of 0.50 μg L^{-1} for both DMDSe and DEDSe. Previously, oxygen was removed from bottles. Samples were unacidified (pH 7.6) and stored in the dark in Teflon, polyethylene and polystyrene containers (500 ml in volume) at two temperatures (-20 and 4°C). Organoselenium species were quantified after 1 day, 1 week, 2 weeks and 1 month. First use bottles were only used. The samples were manually homogenized prior to each analysis. The results (as selenium) given are the mean of three independent analysis performed in different days for selenite and selenate but in the same day for organic selenium species.

Statistical treatment

The data were analyzed statistically for differences using factorial analysis of variance (ANOVA). Prior to analysis, all the data were tested for homogeneity of variance using the Barlett and Levene tests[32]. Parametric statistical test (Student's t-test) was applied to different hypothesis. An α-value of 0.05 was adopted as the critical level for all statistical testing giving a 95 % confidence level (CSS: STATISTICA™).

RESULTS AND DISCUSSION

Stability of inorganic selenium species in seawater at pH 2 stored at –20 °C in Teflon containers

Excellent stability of selenium has been observed by several authors using different storage conditions of acidity, container materials and temperature. However, these studies were sometimes performed using selenium concentrations much higher than that found in most of environmental samples, simple matrix and short test periods. Shendrikar and West[25] preserved inorganic selenium in aqueous solutions using different acid concentrations from 0.5% v/v nitric acid to pH 7 in Pyrex, flint-glass and polyethylene beakers. However, they used selenium concentrations of 1 mg L^{-1} in distilled water for 15 days. Otherwise, optimum conditions of preservation up to four months were found in natural and distilled water at the more relevant levels of 1 and 10 μg L^{-1} when samples were acidified to pH 1.5 with sulphuric acid and stored in polyethylene or Pyrex containers at room temperature[14]. However, the sulphuric acid interferes with some analyti-

cal methods, and selenium losses or interconversion between selenium forms have been observed[12,18,33]. Both selenite and selenate at concentrations of 10 μg L^{-1} were found stable for one year in unacidified distilled samples with and without 100 mg L^{-1} of chloride (as NaCl) when they were stored in polyethylene containers at –20 °C[18]. However, acidification of samples must be used to prevent precipitation, flocculation or complexation in natural samples, being selenite more liable than selenate to this type of phenomenon[19]. Moreover, traces of algae appeared after six weeks in natural samples at pH 5.4–7.2 and as a consequence a decrease of selenite concentration in solution has been observed[14]. Finally, no significant change in concentration neither oxidation states was observed in seawater samples acidified to pH 2 with hydrochloric acid stored in either glass or polyethylene containers over a period of 4.5 months[23].

In the current work, we studied the inorganic selenium stability in natural water for a long period of time, using drastic storage conditions such as low temperature (-20 °C) and low pH. Two working solutions, constituted by weakly and highly contaminated seawater samples (with concentrations of 0.3 and 100 μg L^{-1} of each selenium species, respectively) were used to verify the stability of Se(IV) and Se(VI) solutions stored at –20 °C. In both cases the results showed an optimum preservation of both inorganic selenium species during one year (ANOVA $p>0.45$). Consequently, results from the present work and from literature show that the inorganic selenium species are well preserved in seawater samples stored at low temperatures and low pH values. However, sample preservation using an acid medium may cause analytical interferences and the use of low temperature during storage may be a serious space problem for environmental laboratories and for sample transport. Therefore, the effect of both temperature and acid on the selenium stability was studied in further experiments.

Effect of temperature on the stability of inorganic selenium species in seawater at pH 2 stored in Teflon containers

Variations of selenite and selenate concentrations in the weakly contaminated seawater as a function of the storage time at four temperatures (-20, 4, 25 and 40°C) in the dark are summarized in Figure 2. Selenate concentration remained fairly constant in samples stored at –20 and 4 °C (ANOVA, $p=0.60$), whereas the samples stored at 25 and 40 °C displayed a significant decrease in selenate concentration after 6 months (t-test, $p=0.0034$) and 15 days (t-test, $p=0.0012$), respectively. The concentration of selenite decreased over the period of the experiment in samples stored at temperatures higher than –20 °C. Significant losses of selenite started after 30, 15 and 7 days for samples stored at 4 °C (t-test, $p=0.036$), 25 °C (t-test, $p=0.011$) and 40 °C (t-test, $p=0.024$), respectively. Less

marked losses of inorganic selenium were systematically observed in high polluted seawater sample. Selenate concentration remained constant over the period of the experiment (1 year) for samples stored at 4 °C (ANOVA, p=0.88) and over 6 months at 25 °C (ANOVA, p=0.27). However, significant losses of selenate started after 90 days when the samples were stored at 40 °C (t-test, p=0.028). Selenite concentrations lowered faster than those for selenate. Significant losses started after 90, 15 and 30 days for samples stored at 4 °C (t-test, p=0.041), 25 °C (t-test, p=0.005) and 40 °C (t-test, p=0.0009), respectively.

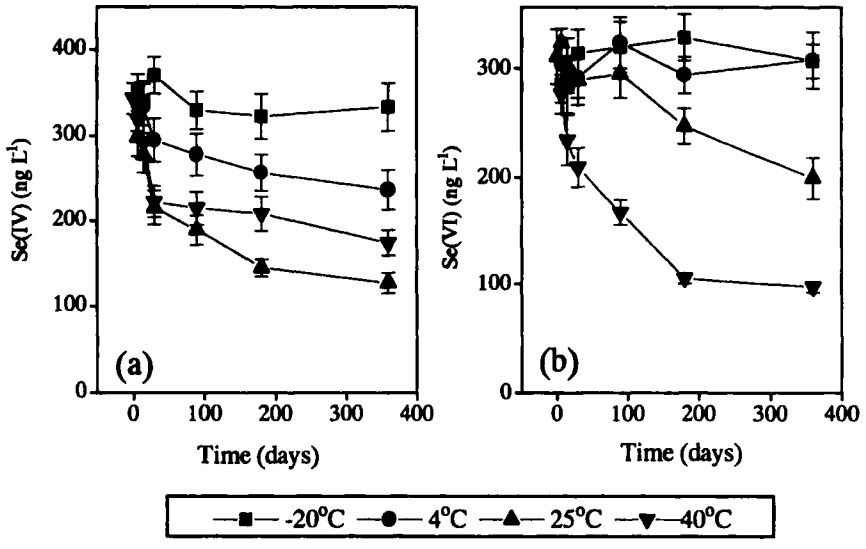

FIGURE 2 Stability of (a) selenite and (b) selenate in seawater collected from the Portil boatyard, acidified at pH 2 with HCl and stored in teflon containers at different temperatures

The losses of selenium observed in samples stored at temperatures higher than −20 °C indicated a strong temperature dependence of selenium stability. The same behaviour has been observed by several authors, being selenium more stable in samples stored at lower temperatures[14,18,21,22,26]. Otherwise, preservation studies at room temperature are necessary when interlaboratory quality control samples have to be transported. Significant losses of selenium were observed in the current experiments after 15 days in seawater samples stored in 500 ml Teflon containers although they were acidified to pH 2 which has been reported to minimize both the losses of selenium through adsorption onto container walls and the microbiological activity[34]. Cobo *et al.*[18] found losses of selenite in samples spiked with selenium to a concentration of about 10 μg L^{-1} and acidified at pH 2 with sulphuric acid after 1 month of storage in 500 ml pol-

yethylene containers. However, Sheam and Agemian[14] found that selenium species were stable for 4 months in natural water spiked with Se(IV) and Se(VI) at concentrations near 1 $\mu g\ L^{-1}$ stored unacidified in 25 gallon polyethylene barrels and acidified at pH 1.5 with sulphuric acid in 500 ml pyrex and polyethylene bottles at room temperature. This is in contrast with the finding of Cobo *et al.* and with the results of the present study. An explanation of the improved preservation of selenium reported in the study of Sheam and Agemian may be the use of smaller specific surface containers or the different nature of the acid used by the authors. According to the findings of several authors selenate was more stable than selenite[14,18,33], which agrees with our results. However, selenate losses were observed in samples acidified at pH 2 and stored at temperatures over 4 °C which may be due to the reduction of this species by the HCl used for acidification[35]. A subsequent increase in selenite concentration was not noted, which may be attributed to the adsorption of this species onto the container wall. Finally, an increase in selenium concentrations in the current experiments led to a decrease in selenium losses as it has been demonstrate by several authors using different storage conditions[14,21–23].

Effect of container material and pH preservation on the stability of inorganic selenium species in seawater stored a 25 °C

A total of 324 storage containers (Teflon, polypropylene and polyethylene) were filled with the two working solutions (weakly and highly contaminated seawater samples) at three pH values (pH= 2, 4 and 7.6) and kept at 25 °C in the dark for 1 year. The Se(IV) and Se(VI) concentrations were periodically measured. Figures 2–3 summarize the trends of the inorganic selenium species stability in the weakly contaminated seawater as a function of the storage time for the different storage conditions determined by the pH and type of container material. Selenate concentration remained fairly constant over a year for unacidified samples or for samples at pH 4 (ANOVA, $p > 0.36$). The results were independent of the container material used in the experiment (ANOVA, $p=0.80$ for unacidified samples and $p=0.64$ for samples at pH 4). However, the samples at pH 2 displayed a decrease in selenate concentration after 180 days (t-test, $p<0.008$). A similar trend was observed for the highly contaminated seawater sample. Different results were obtained for selenite which showed significant changes over the period of experiment, particulary for low levels of selenium. Selenite displayed losses during the first month in Teflon containers and during the first six months in polyethylene and polypropylene containers, and stabilised afterwards. Selenite concentrations decreased faster at higher pH values, independent of the container material. For instance, selenite losses of 36% occurred within 15 days for unaci-

dified samples stored in Teflon containers and were higher than the losses observed for samples acidified at pH 4 (21%) and pH 2 (15%). Otherwise, the stability of selenite in Teflon containers was higher than that in polypropylene or polyethylene containers. For instance, selenite losses of 60 and 81% were obtained for samples acidified at pH 2 stored in Teflon, polyethylene and polypropylene containers, respectively, after a year of storage.

The increasing stability of selenite in acid solutions has been observed by several authors and may be explained by the pernicious effect of the acid on the algae growth and on the bacterial activity. Moreover the addition of acids prevents flocculation and precipitation or hydrolysis of metals and increases the ionic strength of the solution which may minimize the adsorption of selenium onto container walls[9]. Acids of different nature have been used and a good preservation of samples acidified with sulphuric acid[14], nitric acid[21] and hydrochloric acid[23] was observed. However a drawback of acidification is that the anions of the acids used may be a source of interference for the analytical method. Nitrate interferes in fluorometry, chromatography or HG-AAS, chloride in HG-AAS, chromatography and ICP-MS and sulphate may interfere with chromatographic methods[14,36,37]. Therefore, the nature of the acid used for preservation of selenium species must be chosen avoiding interferences for the analytical method.

The stability of inorganic selenium in the different containers materials has been reported: Teflon > silanized glass > borosilicate glass = pyrex > quartz > polyethylene > glass[9]. In our experiments, selenium species stored in Teflon containers were more stable than stored in polypropylene and polyethylene containers. According with our findings, Cobo *et al.*[18], reported a maximum time of sample storage in Teflon containers longer than that in polyethylene containers. However, a partial oxidation of selenite to selenate has been observed in samples acidified with sulphuric acid and stored in Teflon containers[33].

Influence of the matrix on the stability of the inorganic selenium species

The stability of selenite and selenate was studied for a year period in several natural water samples including fresh and seawater, collected from the southwest Spain area. After acidification (at pH 2 with HCl) and filtration (0.2 μm membrane filter), samples were stored in Teflon containers at −20 °C. They were analyzed for selenate and selenite after 6 and 12 months (Table II). Selenate was stable for a whole year (ANOVA, $p > 0.06$). However, selenite losses were clearly observed in fresh water (river and tap water) after a year of storage (t-test, $p < 0.001$) but this species was stable for a year in seawater samples (ANOVA, $p > 0.07$), showing a higher stability at high ionic strengths. Some authors have

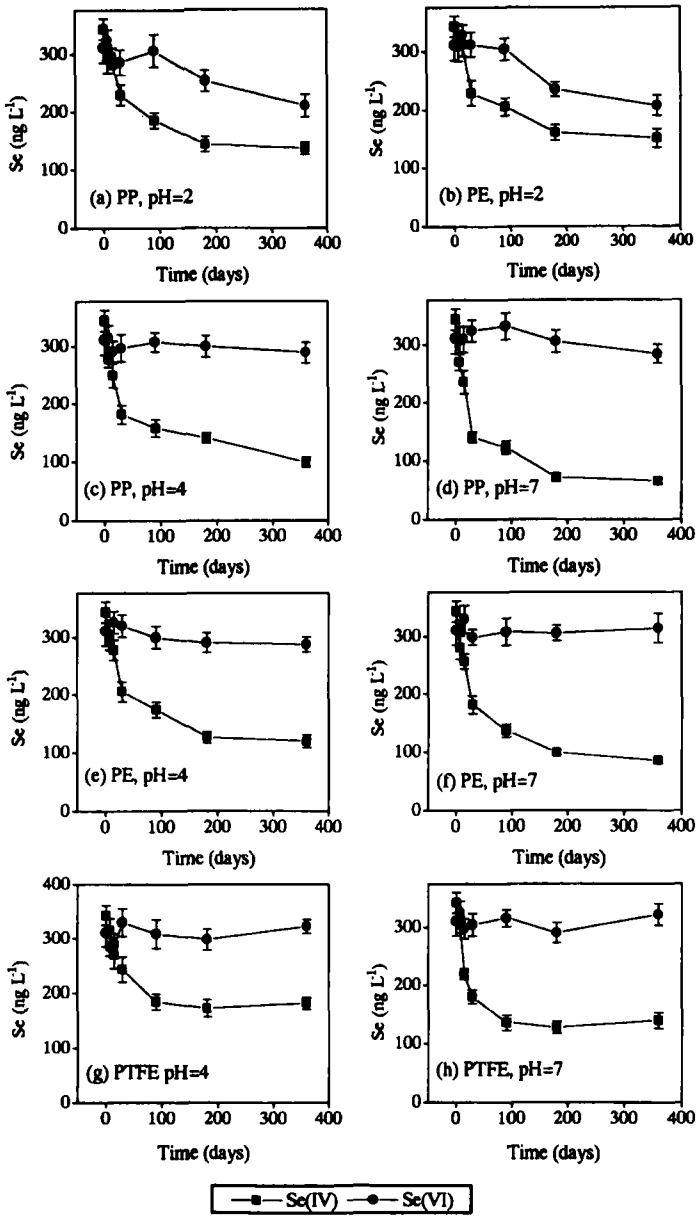

FIGURE 3 Stability study of selenite and selenate in a seawater sample collected from the Portil boatyard under different storage conditions at 25°C: (a) pH 2, polypropylene containers; (b) pH 2, polyethylene containers; (c) pH 4, polypropylene containers; (d) pH 7, polypropylene containers; (e) pH 4, polyethylene containers; (f) pH 7, polyethylene containers; (g) pH 4, Teflon containers; (h) pH 7, Teflon containers

shown that selenium is most stable in natural water than in deionized water[14,22] and that the presence of chloride decreases the risk of selenite losses[15,18,26,33].

Effect of container and temperature on the preservation of organic selenium species

Initial concentration of organic selenium species in all of the samples collected were below detection limits. Therefore, the stability study was performed on spiked samples with known amounts of each organic selenium species. A total of 75 storage containers (Teflon, polystyrene and polyethylene) of 500 ml in volume were filled with the working solution containing the organoselenium and kept at two temperatures (4 and −20 °C) in the dark for 1 month. The DMSe, DESe, DMDSe and DEDSe concentrations were measured after 1, 7, 15 and 30 days. Figure 4 summarizes the trends of the organoselenium concentrations as a function of the storage time for different storage conditions. The four organic selenium species studied in the present work were stable for 24 h at both 4 °C (t-test, $p > 0.52$) and − 20 °C (t-test, $p > 0.32$) using the three container materials tested in this work: Teflon (ANOVA, $p > 0.14$), polystyrene (ANOVA, $p > 0.49$) and polyethylene (ANOVA, $p > 0.34$). DESe and DMDSe were more stable than DMSe and DEDSe, and no significant losses were observed after 7 days when they were stored in Teflon containers at −20 °C (ANOVA, $p = 0.10$ and 0.62 for DESe and DMDSe, respectively). The stability at −20 °C was significantly higher than that at 4 °C for all of the species considered in this work (t-test, $p < 0.05$). The order of decreasing stability of the organic selenium species in diffe- rent container materials was Teflon > polyethylene > polystyrene. For example, DMDSe losses of 27% occurred within 15 days in Teflon containers at −20 °C, whereas percentages of losses increased to 40% and 58% in polyethylene and polystyrene, respectively. Therefore, a maximum storage period of 24 h is man- datary, which allows transporting the samples from field to the laboratory to be analyzed immediately after receiving. According with our findings, a maximum storage period of one day for methyl selenium species was reported by Cutter[13], who used air-tight containers. Freezing is the best storage method of volatile organoselenium compounds[12].

CONCLUSIONS

Numerous preservation studies concerning aqueous samples containing inorganic selenium have been reported in the literature but they are scarce for organic

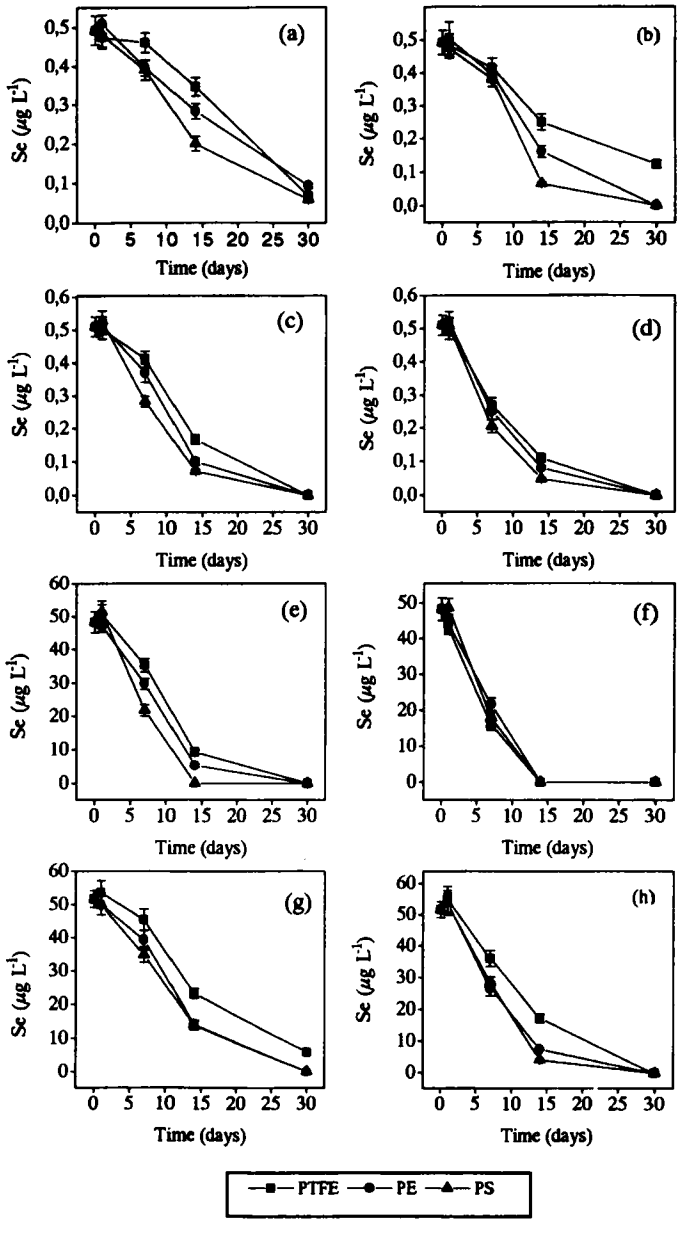

FIGURE 4 Stability study of organic selenium species in a sea water sample collected from the Portil boatyard spiked at concentrations of 50 μg L^{-1} for both DMSe and DESe and at concentrations of 0.50 μg L^{-1} for both DMDSe and DEDSe and stored at 4 and −20°C in teflon, polystyrene and polyethylene containers: (a) DMDSe at −20°C; (b) DMDSe at 4°C; (c) DEDSe at −20°C; (d) DEDSe at 4°C; (e) DMSe at −20°C; (f) DMSe at 4°C; (g) DESe at −20°C; (h) DESe at 4°C

selenium species. The stability of selenium in environmental aqueous samples differs drastically from organic to inorganic selenium species and depend on the matrix nature. Selenate is more stable than selenite and this later is more stable in seawater than in river or tap water samples. The maximum time of storage for inorganic selenium species was 6 months in fresh water but they were stable for at least 12 months in seawater samples acidified at pH 2 with HCl and stored at –20 °C in Teflon containers. This result indicates that storage procedure is suitable for environmental monitoring purposes, but further studies would be necessary for long term storage needed for a CRM candidate. This fact has been constated by Camara *et al.*[38] for inorganic selenium species in freshwater reference materials. The maximum time for storage of organoselenium species in water samples was 24 h using temperatures of 4 and – 20 °C, which allows the transport of samples from field to laboratory but analysis must be performed immediately after reception of the samples.

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