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Analysis of organic and inorganic selenium anions by ion chromatography–inductively coupled plasma atomic emission spectroscopy

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Abstract

We report analysis of both inorganic and amino acid forms of selenium by ion chromatography with inductively coupled plasma atomic emission spectroscopic detection. Three chromatographic systems are compared; effects of representative sample matrices on the separations are investigated. We are unable to resolve selenate and seleno-cystine using the Dionex AS4A column. Elution of seleno-cystine and seleno-cysteine is strongly suppressed in samples of bacterial cell extract matrix analyzed with the Dionex AS10 column; this interference is not observed with the Dionex AS11 column. Synthetic sea water sample matrix has little effect on analytical results. Quantitation parameters are reported. © 1997 Elsevier Science B.V.

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1. Introduction

Selenium is widely but variably distributed on earth, having an average abundance in the lithosphere of about 0.05 ppm [1]. Both organic and inorganic selenium compounds are present in the oceans [2]. Oceanic selenium concentration increases with depth, and concentrations as high as 0.2 μg/l are found in deep ocean waters. The element is found in surface waters at an average concentration of 2 ng/l or less, and may be present in ground waters and polluted waters at much higher concentrations [3–5]. The aquatic chemistry of selenium is complicated since it can exist in four different oxidation states and in a variety of inorganic and organic compounds [6,7]. Microorganisms play an important role in controlling the chemical speciation of selenium in the aquatic environment [8–10].

Research in the forms, metabolism and physiological role of selenium compounds in organisms has been conducted since the early 1950s [11–18]. Studies have shown that both selenite and selenate ions in soil are taken up by vegetation, and that selenite is converted to amino acids [19,20]. The most common seleno-amino acids in nature appear to be seleno-methionine, seleno-cysteine, and seleno-cystine [17]. Seleno-amino acids are present at part per million levels in most human tissues [21]. Seleno-amino acids are the main dietary form of selenium [22].

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The most popular method for determining total selenium, selenite, and selenate has been hydride generation-atomic absorption spectrometry (HGAAS). This method has a detection limit of 2–3 μg/l and is subject to few interferences [23]. However, two separate analyses are required to distinguish selenite and selenate by HGAAS, and individual organic selenium species cannot be distinguished by hydride generation analysis alone.

Inorganic selenium has been analyzed by ion chromatography (IC) with conductometric detection. This technique is limited by interferences by inorganic ions commonly found in natural samples [23]. These interferences can be avoided by pretreatment of samples with Ba^{2+} and Ag^{+} ion-exchange resins [5,24], or by detection with fluorometry [4], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [25,26], flow injection hydride generation ICP [5,27], IC–HGAAS [28], or direct current plasma atomic emission spectroscopy [29]. Inorganic selenium has also been analyzed by capillary electrophoresis with ICP–MS detection [30,31].

Several sensitive methods for determining seleno-amino acids have been developed. Seleno-cysteine has been analyzed by mass spectrometry following derivatization with Sanger's reagent [15]. Reversed-phase high-performance liquid chromatography (HPLC) of seleno-amino acids with electrochemical detection [32] and with neutron activation analysis detection [33] have been reported.

Beilstein and Whanger simultaneously analyzed ^{75}Se-labeled selenite, selenate, and seleno-amino acids by IC with γ-ray detection [16]. Their chromatographic method requires an analysis time of about 75 min. Potin-Gautier et al. attempted to determine both inorganic selenium and seleno-amino acids in the same chromatographic analysis by ion-pair HPLC with electrothermal atomic absorption spectrometry (ETAAS) as an element-specific detector [17]. They were able to resolve seleno-methionine and seleno-cystine, but were unable to adequately resolve selenate and selenite from each other or from the amino acids. Gilon et al. resolved seleno-cystine and seleno-methionine from selenite and selenate by IC–ETAAS, with an analytical time of about 30 min [34]. Maher et al. distinguished seleno-amino acids and trimethylselenonium from inorganic selenium by combining results of both IC and ion-pair HPLC analyses, utilizing ETAAS detection [35]. Bektas et al. resolved selenourea from inorganic selenium using IC–ETAAS [36].

We have compared the performance of three analytical anion-exchange columns in resolving selenite, selenate, seleno-methionine, seleno-cystine and seleno-cysteine. To minimize interferences by non-selenium anions, we used element specific detection by ICP-AES.

2. Materials and methods

A Beckman Model 110A pump with an Altex 210 sample injection valve (250-μl loop) was used in all experiments. Two detection systems were employed in developing chromatographic methods reported here. Eluent development studies were performed with a conductivity detector (Dionex Model 10) connected to either an anion micromembrane suppressor (Dionex AMMS-II) or a suppressor column (Dionex). A Perkin-Elmer Model 5000 atomic absorption spectrophotometer, configured with a hydrogen/argon flame, was also used in development studies. Eluent suppression was not employed in using either the atomic absorption or emission detectors.

IC–ICP-AES studies were conducted using a Perkin-Elmer Optima 3000XL ICP with a 'Gem Tip' cross-flow pneumatic nebulizer. The nebulizer gas flow-rate was set at 0.8 l/min. The crystal-controlled radio frequency (RF) generator was operated at 27.12 MHz, with ICP power of 1375 W. Acquisition and processing of data were performed with the Perkin-Elmer Optima 3000XL software, with an integration time of 0.1 s, and data collection over 5-s intervals by the 'peak area' function. Selenium was monitored with the 196.0-nm atomic emission line. ICP-AES peak areas were obtained by manually summing the emission counts included in the observed chromatographic peaks.

Detection limits (DLs) were obtained by averaging DL values for three sets of seven emission counts of blanks, corresponding to typical widths of the selenium ion peaks. DL values calculated using the following equation: $DL=3s/m$, where s is the standard deviation of the blank readings, and m is the slope of the calibration line.

The anion-exchange columns were interfaced with the ICP nebulizer by a piece of PTFE tubing of 20 cm×0.84 mm I.D. (dead volume of 0.11 ml). An eluent flow-rate of 1.0 ml/min was used to match that of the nebulizer uptake.

The analytical columns compared in this study were Dionex AS4A, AS10, and AS11. The AS4A column is commonly used in the analysis of inorganic anions. The AS10 column has a relatively high capacity, to accommodate analysis of ions in concentrated matrixes. It and the AS11 column were recommended by Dionex representatives for this study. Phenomenex Star-Ion A300, Dionex AG10, and AG11 guard columns were used, respectively, with these columns.

All eluents were degassed by continuous bubbling of helium. At least three replications with the same conditions were performed in each experiment.

ASTM Type I water was used to prepare all solutions. All chemicals used were ACS reagent grade from Fisher Scientific except for seleno-DL-methionine ($C_5H_{11}NO_2Se$), seleno-(DL)-cystine ($C_6H_{12}N_2O_4Se_2$), sodium selenite ($NaHSeO_3$), and sodium selenate (Na_2SeO_4), which were obtained from Sigma.

Seleno-cysteine stock standard solution was prepared from seleno-cystine by reduction with sodium borohydride ($NaBH_4$) [13]. Following reduction, the mixture was acidified to pH 5.0–5.5 with concentrated acetic acid, to destroy the excess $NaBH_4$, and diluted with water. This procedure was performed in a glove box with a nitrogen atmosphere.

To prevent decomposition of selenium species, standards were prepared just before analysis and stored in a refrigerator at 4°C after injection. Seleno-cysteine was stored in valved sealed vials (Supelco 'Miniinert'). This compound was found to be unstable when mixed with the other selenium anions except seleno-cystine, hence it was analyzed separately.

Synthetic seawater matrix was prepared from 'Instant Ocean' salt (Aquarium Systems, Mentor, OH, USA) according to package instructions. Cell extract was prepared by culturing *E. coli* in sterile Luria-Bertani dry media (tryptone 10 g, yeast extract 5 g, sodium chloride 10 g). The resulting cells were isolated by centrifugation, sonicated in an ultrasonic dismembrator (Fisher Sonic Dismembrator 550) and centrifuged again. The supernatant extract was diluted in 50 m*M* trishydroxyaminomethane at pH 7. Bulk protein concentration was determined by the method of Bradford [37]. Small volumes of selenium standards were added to the synthetic seawater and to the cell extract.

3. Results and discussion

3.1. IC separation

Retention of seleno-methionine, selenite, selenate and seleno-cystine (eluted in this order) with the Dionex AS4A column was found to vary regularly with the concentration of carbonate ion in the eluent. Our best resolution selenate and seleno-cystine was inadequate (resolution <1.0), obtained with an eluent of 1.0 m*M* CO_3^{2-} having pH 10.1 and with a flow-rate of 2.0 ml/min. Resolution of these peaks worsened when higher eluent carbonate concentrations were tried to allow use of an eluent flow-rate of 1.0 ml/min, appropriate for ICP interfacing. This result caused us to investigate use of the AS10 and AS11 columns.

The chromatographic parameters we determined to adequately separate the selenium species using the columns we compared are given in Table 1. We obtained baseline resolution of seleno-cystine and selenate peaks with the AS10 column and 100 m*M* NaOH eluent. (A gradient elution with NaOH eluent is recommended by Dionex for separation of selenite, selenate and other anions with this column [38]). We found that the Na_2CO_3 eluent produces a more evenly spaced chromatogram than NaOH in separating selenium anions with this column (Fig. 1). NaOH (10 m*M*) produces adequate resolution of selenium ions with the AS11 column, though selenite and seleno-cystine are not totally resolved (Fig. 2). Seleno-cysteine co-elutes with seleno-cystine with both these systems.

The elution order of selenium ions obtained with the columns is seleno-methionine, selenite, seleno-cystine and seleno-cysteine, and selenate. This is nearly the reverse of the elution order obtained with conventional IC methods for analysis of amino acids, in which acidic eluents are used [12,16]. Seleno-methionine, having the lowest negative charge

Table 1
Linear regression parameters for IC–ICP-AES of selenium anions

Anion	AS10[a]				AS11[b]			
	Slope (peak area/mg/l Se)	r^2	Relative response[c]	Detection limit (mg/l Se)	Slope (peak area/mg/l Se)	r^2	Relative response[c]	Detection limit (mg/l Se)
Se-methionine	106	0.993	1.00	0.37	121	0.994	1.00	0.32
$HSeO_3^-$	76.8	0.990	0.72	0.48	87.5	0.996	0.72	0.45
Se-cystine	60.0	0.987	0.57	1.2	82.3	0.988	0.68	0.62
SeO_4^{2-}	72.5	0.996	0.68	0.68	93.6	0.941	0.77	0.54

[a]Eluent: 1.0 ml/min 10 m*M* Na_2CO_3, pH 11.0.
[b]Eluent: 1.0 ml/min 10 m*M* NaOH, pH 11.8.
[c]Slope relative to that of Se-methionine.
[d]Injection volume: 50 μl.

(−1.0) with our buffers, has the shortest retention time. Selenite, seleno-cystine, seleno-cysteine, and selenate all have virtually the same total negative charges (−2.0). The small size of selenite may account for it eluting before seleno-cystine. We were unable to resolve seleno-cysteine and seleno-cystine. Both ions have negative charges at both ends. It may also be that seleno-cystine is decomposing to seleno-cysteine in the column, which would explain the similarity in chromatographic behavior of these ions in cell extract matrix (discussed below). As seleno-cysteine is the amino acid present in reduced protein hydrolysis mixtures [16], failure to resolve these two amino acids should not be a shortcoming in many biological applications.

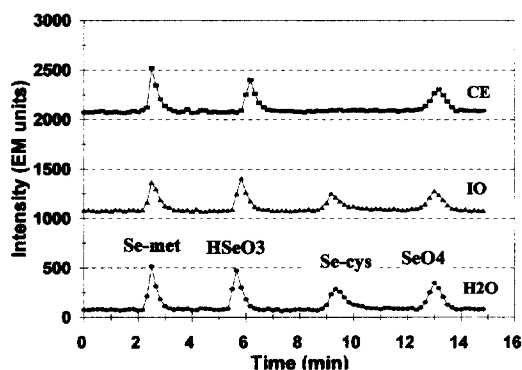

Fig. 1. IC–ICP of selenium anions with a Dionex AS10 column; 10 m*M* Na_2CO_3, 1.0 ml/min. Amounts injected (μg Se): Se-methionine 0.48, selenite 0.57, Se-cystine 0.86, and selenate 0.57. Sample matrices: H_2O, Type I water; IO, synthetic seawater; CE, cell extract.

3.2. Quantitation

IC–ICP-AES peak area response curves were determined for each compound by injection of serial standard solutions made by directly diluting stock solutions (Table 1).

Statistical comparison indicates that the slopes of seleno-methionine and seleno-cystine differ ($P<0.005$) from that of selenate on both AS10 and AS11 columns. On both columns, selenite and selenate slopes are not found to differ. The response factors of the selenium ions, relative to that of seleno-methionine, are given in Table 1.

The concentrations of the standards analyzed were determined by analysis by ICP-AES using the same selenate standard, hence the differences in sen-

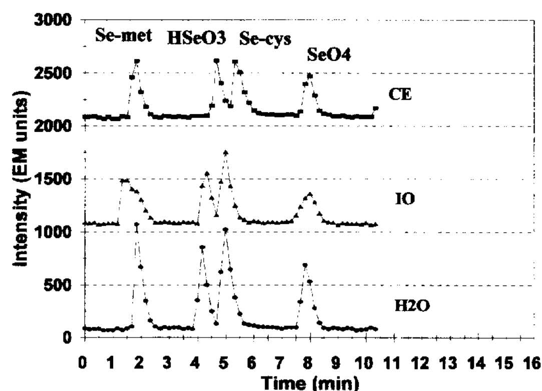

Fig. 2. IC–ICP of selenium anions with a Dionex AS11 column; 10 m*M* NaOH, 1.0 ml/min. Amounts injected and abbreviations are the same as in Fig. 1.

sitivities among the species are due to the chromatographic process. (Such differences in sensitivity have been observed in chromatographic analysis of arsenic anions [39].) The moderate decrease in sensitivity with retention time among the selenium ions are likely due to diminished ability to distinguish the ICP-AES response at the wings of the broader, later-eluting peaks. Broader peaks lead to somewhat lower detection limits being obtained with the AS10 compared with the AS11 system. Secondary adsorption of seleno-cystine may be responsible for the markedly diminished sensitivity for this ion analyzed with the AS10 system (see Section 3.3). Baseline signal (noise) was very similar with both chromatographic systems.

The sensitivity of the IC–ICP-AES technique is limited to a large degree by the inefficiency of the nebulizer. With the conventional cross-flow nebulizer, only 1–3% of the aspirated sample reaches the plasma. Alternative nebulization methods, such as thermospray, ultrasonication, and direct injection, could be employed with these columns to lower detection limits to low microgram per liter levels. Larger injection volumes or use of a preconcentrator column would also decrease detection limits of the selenium anions.

3.3. Applicability and interferences

We investigated potential matrix interferences to the AS10 and AS11 chromatography systems by analyzing the selenium anions in solutions of 'Instant Ocean' synthetic seawater (IO) and bacterial cell extract. The IO sample was prepared according to the manufacturer's instructions for preparing aquarium water. The cell extract matrix is typical of a cell-free extract used in biochemical studies. Selenium salts were added to these matrices to prepare samples for injection containing 10–17 mg/l (as Se) of each anion.

The concentrations of metal ions and anions in the IO and cell extract matrices were determined by ICP-AES and IC analyses (Table 2). Fe, Mn and F were not detected in either matrix (<0.005, <0.005 and <0.1 mg/l, respectively). Cr, Zn and Cu were not detected in the IO matrix (<0.005, <0.005 and <0.01 mg/l, respectively), but were detected near their detection limits in the CE matrix. B and Si were present in both matrices at concentrations below 2.5 mg/l.

Table 2
Composition of interference study matrices

Constituent	IO (mg/l)	Cell extract (mg/l)
Ca	227	1.33
K	470	72
Mg	960	0.35
Na	800	800
Cl	15 000	9100
SO_4^{2-}	2100	10
Protein	—	42

Minor changes in retention times of selenite and seleno-cystine with matrix were observed (Figs. 1 and 2). (For clarity, IO and cell extract matrix chromatograms have been offset by successive increments of 1000 EM units in the figures.) Compared with the AS11 system, these changes are less with the AS10 system.

The chromatograms obtained by IC–ICP-AES with the AS10 column display no seleno-cystine or seleno-cysteine peaks with cell extract matrix. Seleno-cystine and seleno-cysteine are eluted from the AS11 column when injected in cell extract matrix, though this matrix causes pronounced tailing of the seleno-cysteine peak. The reason for the interference in analysis of seleno-cystine and seleno-cysteine by cell extract when using the AS10 column is not known. This column has a macro-porous structure, with a pore diameter of 2000 Å [40]. It is possible that the seleno-cystine and seleno-cysteine may be interacting with protein or other macromolecules in the cell extract, and that the resultant complexes are detained in the macropores.

Potential effects of the matrices on sensitivity were evaluated by determining that the peak areas fall within the 95% prediction confidence intervals of the calibration lines. Although peak shapes changed for some anions in IO and cell extract matrixes, with neither column did we find a significant effect on peak area for selenium anions in these two matrices compared with the anions in Type I water. For selenium ions, all responses in the matrices were within 95% confidence limits for values predicted using the Type I water calibration lines.

4. Conclusions

Three Dionex anion-exchange columns, AS4A, AS10, and AS11 have been evaluated for analysis of selenium anions with ICP-AES detection. Our results show that in analysis of selenium anions, the Dionex AS4A column with carbonate buffer gives poor resolution of selenate and seleno-cystine. Dionex AS10 and AS11 columns with carbonate and hydroxide eluents, respectively, separate both organic and inorganic selenium anions. Neither of these columns resolves seleno-cystine from seleno-cysteine with the eluents tried in this study. The AS10 chromatographic system reported here produces an evenly spaced chromatogram of the anions studied. Somewhat better detection limits are obtained with the AS11 system.

In almost all cases, synthetic seawater and cell extract matrices have no significant effect on the integrated chromatographic response of selenium anions. The exception is a strong interference by the cell extract on elution of seleno-cystine and seleno-cysteine with the AS10 column. In cell extract matrix, all selenium anions are eluted from the AS11 column.

Compared with conductimetric detection, detection of selenium anions by ICP-AES is subject to much less interference by common inorganic ions. High concentrations of inorganic salts in samples do not interfere with detection by ICP.

Applicability of the chromatographic methods presented here may be improved by use of a detection system which distinguishes seleno-cystine from seleno-cysteine. We are conducting study of preconcentration of selenium anions to allow analysis of environmental and biochemical samples by IC–ICP-AES detection. Determination of the nature of, and means of eliminating the interference of protein matrix on analysis of seleno-cysteine and seleno-cystine with the AS10 column would be useful.

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