SHORT COMMUNICATION

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Rapid speciation of Se(IV) and Se(VI) by flow injection–capillary electrophoresis system with contactless conductivity detection

Abstract A flow injection–capillary electrophoresis system with contactless conductivity detection and hydrostatic-pressure-generated flow was used for the fast and sensitive speciation of Se(IV) and Se(VI). The sample throughput was 25 samples per hour using a background electrolyte solution containing 8.75 mM L-histidine (His) adjusted to pH 4.00 with acetic acid. The repeatability of peak areas (n=8) was better than 1.41% and the limits of detection were 190 μg L^{-1} and 7.5 μg L^{-1} for Se(IV) and Se(VI), respectively. The interference from carbonate, typically present in water samples, was eliminated by using a low-pH electrolyte in which carbonate is uncharged and migrates at the EOF front. The method was applied to the analysis of Se(IV) and Se(VI) in soil samples that were spiked with both selenium species and the results for recovery of both selenium species were in good agreement with their introduced concentrations.

Keywords Flow injection analysis · Capillary electrophoresis · Contactless conductivity detection · Selenium · Inorganic anions · Speciation

Introduction

Selenium is generally recognized to be a trace mineral of great importance. It is a unique element in that there is a small margin of safety between levels of Se compounds that will cause dietary deficiency and those that result in toxicity. It is found naturally in food and soils, the atmosphere, the aquatic environment and organisms. However a significant portion of the total selenium found in the environment is anthropogenic. It is for instance used in electronics, the manufacture of glass, drugs, as a fungicide and as a feed additive.

Selenium exists in both inorganic and organic forms. Inorganic forms of selenium include selenite and selenate anions, elemental selenium and sparingly soluble metal selenides. Organic selenium compounds, such as alkylselenides, selenoaminocarboxylic acids, selenium-containing peptides and derivatives of nucleic acids are found in biological systems [1] and play important roles in cell biochemistry and nutrition. Their reactivity and bioavailability depends on the physicochemical form of existence and its concentration; inorganic selenium species are more toxic than organic species.

Selenates and selenites are among the most mobile Se compounds in the environment due to their high solubility in water and are characterized by high bioavailability to plants and animals. Se deficiency in soils, agricultural products and foodstuffs can cause serious diseases [2] and inorganic selenium compounds are often added to animal feeds and fertilizers in regions with selenium-deficient soils. The monitoring of the content of Se in the environment and speciation of low concentrations of inorganic Se compounds is an important task, because it is the water-soluble, inorganic Se that contributes to the contamination of drinking and underground water sources.

Capillary electrophoresis (CE) proved to be an efficient tool in speciation analysis [3, 4] due to its high separation efficiency and robustness to samples with complicated matrices, for instance bacterial suspensions [5]. Although coupling of CE with ICP-MS remains the mainstay in the electrophoretic selenium speciation/analysis due to its good sensitivity and selectivity [6, 7, 8, 9, 10], other detection methods such as UV [11, 12, 13, 14, 15, 16], fluorescence [17] or conductivity [18] can be alternatively used. Field-amplified sample stacking is often used to increase the detection sensitivity with UV methods [15,

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16] that are generally less sensitive than fluorescence or conductivity detection. The limits of detection (LODs) of tens to hundreds of $\mu g\,L^{-1}$ were achieved with sample stacking and UV detection that are comparable to the fluorescence [17] and direct conductivity [18] detection.

In this paper, a flow injection–capillary electrophoresis (FI-CE) system with contactless conductivity detection (CCD) is used for rapid, sensitive and selective speciation of inorganic selenium species. The method is then applied for quantitation of Se(IV) and Se(VI) in the spiked soil samples.

Experimental

Chemicals, solutions, samples and procedures

All chemicals were of reagent grade and deionised water was used throughout. Stock solutions ($1\,g\,L^{-1}$) of Se(IV) and Se(VI) were prepared from sodium selenite and sodium selenate decahydrate (both from Sigma-Aldrich, Steinheim, Germany), respectively. Stock solutions of other anions ($1\,g\,L^{-1}$) were prepared from the corresponding sodium or potassium salts (all chemicals were purchased from Pliva-Lachema Ltd., Brno, Czech Republic). All multi-ion standard solutions were then freshly prepared from these stock solutions.

The electrolyte solutions were prepared from $120\,mM$ stock solution of L-histidine (His, Merck, Darmstadt, Germany). The pH of all His-based electrolyte solutions was adjusted with 4% or 10% (v/v) acetic acid (Pliva-Lachema Ltd., Brno, Czech Republic). For most of the analyses an electrolyte solution of pH 4.00 was used. All electrolyte solutions were degassed in an ultrasonic bath for 5 min prior to use.

Sample treatment

Aqueous samples and standard solutions were injected without any treatment except dilution. Soil samples were prepared as follows: 2 g of soil sample was mixed and homogenized with 2 mg of Se(IV) and 1 mg of Se(VI) and subsequently diluted in 1 L of deionised water. Solutions were stirred well and ultrasonicated for 30 min in an ultrasonic bath. The samples were filtered and small portions (typically 10 mL, used for the injection into the FI-CE system) were further filtered through a 0.45-μm nylon filter. A 9-mL portion of the filtered solution was mixed with 1 mL of $25\,mg\,L^{-1}$ solution of NO_2^- (internal standard).

CE components

The CE part of the system comprised a high-voltage supply unit (Spellman CZE1000R, Spellman, USA) operated at a potential of 15 kV during all runs, a function generator (Model GFG 8219A, Goodwill Instruments Co. Ltd, Taiwan) providing a sinusoidal input signal to the detection cell of a contactless conductivity detector of varying amplitude (typically 10 Vpp), peak to peak, and the contactless conductometric detector as described previously [19, 20, 21]. The function generator was operated at frequency of 290 kHz. The data were processed in a professional data system (CSW v. 1.7, Data Apex, Prague, Czech Republic). A polyimide-coated fused silica capillary (Supelco, Bellefonte, PA, USA; 50-μm i.d., 363-μm o.d.) of total length 65 cm was used; the effective capillary length from the injection end to the detection cell was 35 cm. The capillary was preconditioned with 1 M NaOH for 10 min, deionised water for 5 min, 1 M HCl for 10 min, deionised water for 10 min and finally with electrolyte solution for 15 min. Standard solutions in the electrolyte optimisation study were injected hydrodynamically (HD) by elevating one capillary end to the height of 20 cm

for 20 s. Standard solutions and samples in the FI-CE system were injected electrokinetically (EK). All experiments were performed at the constant laboratory temperature of 24±1 °C, measured inside the CE protective box with the fused silica capillary and the detection cell. The FI-CE interface was machined in a Plexiglass block (2×2×3 cm) as described elsewhere [22]. The flow rate of the electrolyte solution used in the FI system was $300\,\mu L\,min^{-1}$ and the volume of injected samples was $4\,\mu L$.

Results and discussion

Choice of the background electrolyte for the separation of Se(IV), Se(VI) and other common inorganic anions

The electrophoretic separation of Se(IV) and Se(VI) with conductivity detection was previously demonstrated in alkaline electrolytes containing 2-(cyclohexylamino)ethane-sulfonic acid (CHES) of pH 9.4, in which both species are fully ionised [18]. We have studied the electrophoretic behaviour of a number of inorganic anions in acidic electrolytes containing His and acetic acid in the pH range 3–6 [23, 24, 25]. These electrolytes are suitable for the determination of both fast-migrating anions (such as Cl^-, NO_3^-, SO_4^{2-}) and slow-migrating anions (such as anions of organic acids) without modification of the electroosmotic flow (EOF) as is commonly practiced in analysis of inorganic anions [26]. When electrophoretic speciation of Se(IV) and Se(VI) was performed in an alkaline background electrolyte, serious problems were encountered due to the co-migration of Se(IV) and carbonate that is typically present in various aqueous samples in relatively high concentrations [12, 13]. One important property of acidic His-based electrolyte is that it efficiently eliminates the carbonate interference. At the pH used, carbonate is uncharged (pK_{a1}=6.37, pK_{a2}=10.25 [27]) and migrates at the EOF front and does not interfere with the determination of other anions, including Se(IV).

Effect of pH of the electrolyte solution

A 10 mM concentration of His was initially chosen for the evaluation of different pH values on the migration order and behaviour of selected inorganic anions. The pH of the electrolyte solution was adjusted by addition of appropriate amount of 4% or 10% (v/v) acetic acid. Simultaneously with Se(IV) and Se(VI), other anions were analysed (Cl^-, SO_4^{2-}, NO_3^-, NO_2^-) that are likely to be present in the real samples. Nitrite was included in this set of anions as an internal standard for the quantitative analysis by the FI-CE system. A standard solution containing $15\,mg\,L^{-1}$ of Se(IV) and $2.5\,mg\,L^{-1}$ of another five anions was prepared and used in all experiments.

Figure 1 shows the effect of pH in the range 3.5–5.5. The electrophoretic behaviour of Cl^-, NO_3^-, SO_4^{2-} and Se(VI) is similar in the whole pH range and the migration times decrease slightly with decreasing pH due to the decrease in the EOF velocity. The pH of the electrolyte does not influence the charge of these anions, since they all are
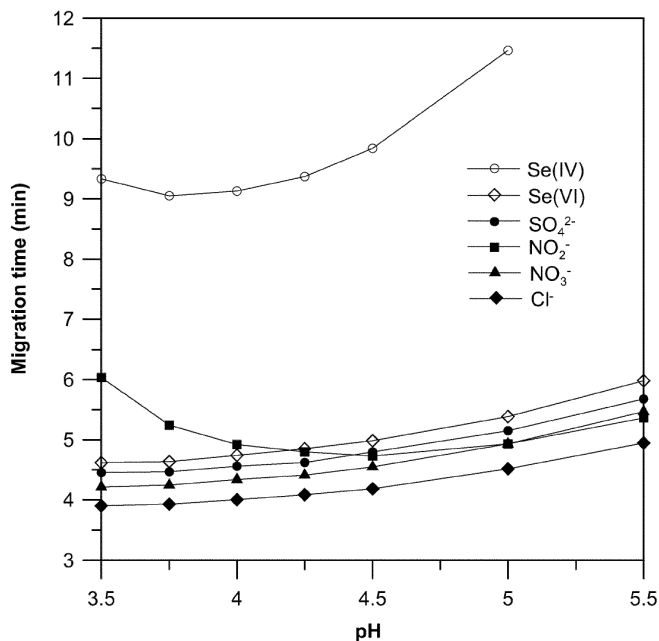
Fig. 1 The effect of the pH on the migration behaviour of selected anions. Electrolyte composition 10 mM His; separation voltage + 15 kV; hydrodynamic injection, capillary end elevated to a height of 20 cm for 20 s

Fig. 2 The effect of the concentration of His on migration behaviour of selected anions. Electrolyte pH 4.00 (adjusted with 10% (v/v) acetic acid). Injection and separation conditions for anions are the same as in Fig. 1

anions of strong acids (for instance, the pK_{a2} of selenic acid is 1.92 [27]). Since the electrolyte does not contain any EOF modifier, the anions migrate against the EOF. Lowering the pH will cause the EOF decrease due to the protonation of the capillary wall and consequently the effective velocity of the anions will increase.

The behaviour of selenite and nitrite was significantly different from that observed for other anions. The migration time for selenite in the studied pH range was about twice the migration time of selenate, which indicates that it migrates as a monovalent anion, $HSeO_3^-$. This observation is in agreement with the pK_a values for selenious acid (pK_{a1}=2.46, pK_{a2}=7.31 [27]) and indeed in the pH range 3.5–5.5 the dominant Se(IV) species is $HSeO_3^-$. Similarly the migration behaviour of NO_2^- can be explained by using the pK_a of nitrous acid (pK_a=3.37 [27]) and the effective ionic mobility and acid–base equilibria according to ref. [28]. The shortest migration times of Se(IV) were achieved in the pH range 4.25–3.5. When resolution of sulfate and Se(VI) and the resolution of Se(VI) and NO_2^- were considered, the best results (R_s≥1.7) were achieved at pH 4.00 and therefore this pH was used in further optimisation procedures.

Effect of His concentration

The effect of the concentration of His in the electrolyte solution on separation selectivity of selenium species and other anions was studied in the range 6–15 mM. The pH of each electrolyte solution was adjusted to 4.00 with 10% (v/v) acetic acid.
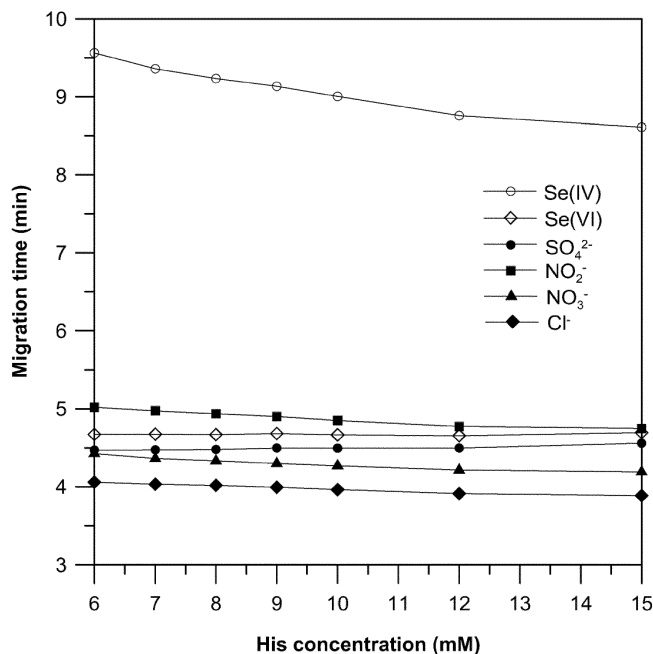
The results are presented in Fig. 2. Partial co-migration of Se(VI) and SO_4^{2-} and Se(VI) and NO_2^- occurred in the electrolyte solutions containing concentrations of His higher than 12 mM. The concentration of His below 7 mM resulted in co-migration of nitrate and sulfate peaks, and additionally the peak shape of Se(IV) was slightly deteriorated. The optimal concentration of His was found to be 8.75 mM. The electrolyte solution consisting of 8.75 mM His adjusted to pH 4.00 with acetic acid was used for the quantitative determination of both selenium species with the FI-CE system.

Parameters of the method for FI-CE determination of Se(IV) and Se(VI)

For the determination of inorganic selenium species an FI-CE system with hydrostatic-pressure-generated flow was used [23]. This system allows automated, repeated injections of samples in one electrophoretic run that results in lowering the total analysis time. Further, on-line sampling from a solution can be performed in an automated fashion by using an FI-CE system and may be useful in monitoring studies such as sorption or bacterial intake/reduction of selenium anions [5].

First we evaluated the repeatability of the FI-CE system by sequential injections of a standard solution containing 15 mg L^{-1} of Se(IV) and 2.5 mg L^{-1} of another five inorganic anions. The injection-overlapping technique described in ref. [29] was used to increase the sample throughput. Briefly, the injection of a subsequent sample
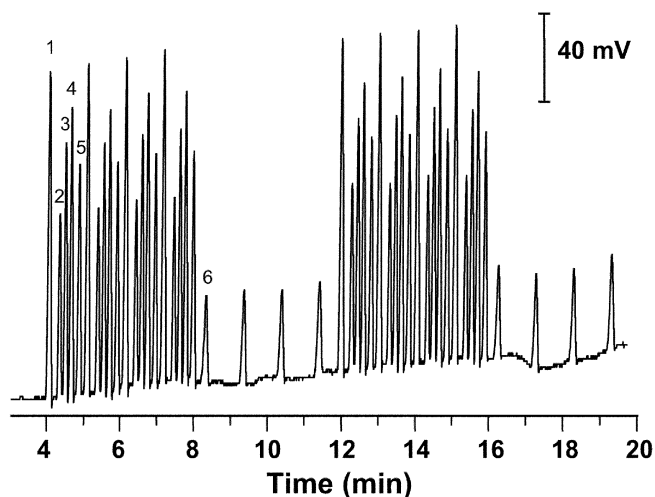
Fig. 3 FI-CE determination of Se(IV) and Se(VI) and common inorganic anions. Concentration of Se(IV) 15 mg L^{-1}, concentration of all other anions 2.5 mg L^{-1}. Electrolyte composition 8.75 mM His, pH 4.00, separation voltage + 15 kV. Peak description: *1* chloride, *2* nitrate, *3* sulfate, *4* selenate, *5* nitrite (IS), *6* selenite
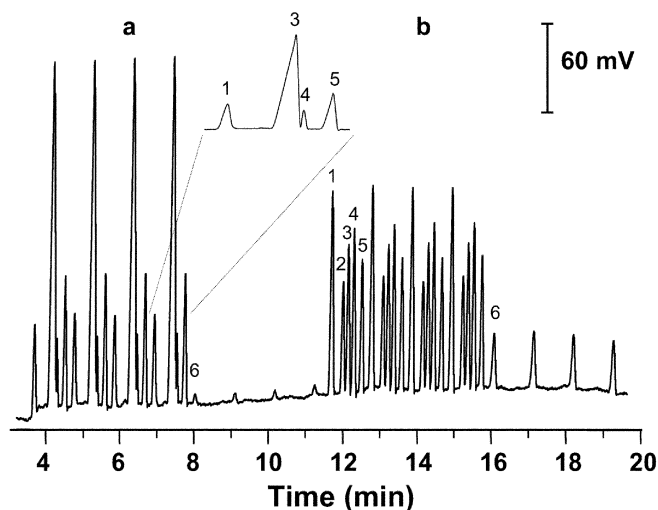

Fig. 4a,b FI-CE analysis of Se(IV) and Se(VI) in spiked soil sample (**a**) and analysis of standard solution (**b**) for the quantitative determination of selenium species in soil sample. CE and FI conditions are the same as in Fig. 3

can be performed before all the analytes from the previous injection had migrated through the detector. Thus, four injections of the standard solution containing six anions were performed every 62 s. Then, the injection sequence was interrupted for 255 s, the time needed for the slowest analyte (Se(IV)) from the previous four injections to migrate through the detection cell. A typical electropherogram of eight consecutive injections of standard solution is depicted in Fig. 3. Note that peak 6 (Se(IV)), belonging to the first injection, appears after the five peaks that belong to the fourth injection. The sample throughput using this injection technique was calculated to be 25 samples per hour, which represents approximately three- to fivefold reduction in analysis time compared to the previously published methods [12, 13, 18].

The parameters of the method for the determination of Se(IV) and Se(VI) are summarized in Table 1. The repeatability was evaluated as the relative standard deviation (RSD) of peak heights (PH) and peak areas (PA) for eight consecutive injections of standard solution. The RSD values were better than 1.5% and 1.4% for the peak height and peak area, respectively. An internal standard (IS) method was used for quantitative evaluation to compensate for the bias in the electrokinetic injection intro-

duced in the FI-CE system [23]. The relationship between the concentration and IS-corrected peak area was calculated for both selenium species. The calibration curves (using NO$_2^-$ as IS) were found to be linear (five point calibration, r^2=0.9997 and 0.9998, respectively) over the range 1–15 mg L^{-1} for Se(IV) and 0.165–2.5 mg L^{-1} for Se(VI).

The limit of detection (LOD) values, defined as the concentration of analyte giving a signal equal to three times the standard deviation for the blank (3 S/N) were estimated for Se(IV) and Se(VI). The LOD values were obtained through repetitive runs of model solutions, which were diluted with deionised water in steps until the condition of the LOD definition was fulfilled. The standard deviation calculation for the blank was based on more than 100 data points. The LODs are summarized in Table 1.

Analysis of soil samples

The method for the determination of Se(IV) and Se(VI) was applied to the analysis of pretreated soil samples (see "Experimental" section) that were spiked with both inorganic selenium species and internal standard (IS).

The concentrations of both selenium species were calculated. Four consecutive injections of a soil sample spiked with Se(IV) and Se(VI) (panel a) and four injections of a standard solution (panel b) used for the quantitative analysis are depicted in Fig. 4.

The results obtained for the two selenium species by the FI-CE system were in good agreement with the concentrations introduced into the samples; the recovery for the Se(IV) and Se(VI) was 90–98% and 96–101% (*n*=4), respectively. The method allowed precise determination of both selenium species in the presence of other inorganic anions and can potentially be used for the rapid screening of inorganic selenium in soil samples.

Table 1 Analytical parameters for the determination of Se(IV) and Se(VI)[a]

Anion	RSD (%), *n*=8		RSD (%), *n*=8		LOD (µg L^{-1})	r^2
	PH	PA	PH (IS)[b]	PA (IS)		
Se(IV)	1.1	1.41	1.5	1.13	190	0.9997
Se(VI)	0.57	1.26	0.68	0.66	7.5	0.9998

[a]Concentration of anions is 2.5 mg L^{-1} except Se(IV), 15 mg L^{-1}
[b]PH (IS) and PA (IS) are the corrected peak height and peak area calculated using internal standard (NO$_2^-$), respectively

Adsorption of Se(IV) and Se(VI) onto soil particles

The presence of Se(IV) and Se(VI) species in alkaline and acidic soils, respectively, and fast equilibrium establishment among selenium species (Se(II), Se(0), Se(IV) and Se(VI)) and fast and irreversible sorption of Se species on Mn and Fe sesquioxides in soils were reported [30, 31]. In a preliminary study the adsorption of both selenium species onto the soil particles was investigated. The dissolved soil sample spiked with Se(IV) and Se(VI) was stirred using a magnetic stirrer for 24 h and five samples were taken at different times. The first sample was taken at 30 min and the next samples 1, 2.5, 4 and 24 h after the spiked soil was dissolved. The sample was filtered using an in-line filter, mixed with an internal standard and injected in the FI-CE interface. Concentrations of Se(IV) and Se(VI) were calculated. However, it was found that no adsorption of selenium species occurred within 24 h. The adsorption of selenium anions depends on the type of soil and pH. For instance kaolinite, montmorillonite and calcite-containing soils were shown to readily adsorb selenium anions [32]. An on-line monitoring of sorption of Se in different types of soil using the FI-CE system is underway.

Conclusions

The proposed flow injection–capillary electrophoresis system with contactless conductivity detection and hydrostatic-pressure-generated flow provides a sensitive, rapid and inexpensive method for speciation of Se(IV) and Se(VI) and simultaneous determination of other common inorganic anions. The interference from carbonate typically present in water samples is eliminated by using a low-pH electrolyte, in which carbonate is uncharged and migrates at the EOF front. The system can potentially be used for on-line sampling and allow continuous monitoring of the content of inorganic selenium species, as required for instance in oxidation/reduction studies or determination of the adsorption rate of the analytes.

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