Efficient Interface for Online Coupling of Capillary Electrophoresis with Inductively Coupled Plasma–Mass Spectrometry and Its Application in Simultaneous Speciation Analysis of Arsenic and Selenium

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S Supporting Information

ABSTRACT: A simple and highly efficient online system coupling of capillary electrophoresis to inductively coupled plasma–mass spectrometry (CE-ICP-MS) for simultaneous separation and determination of arsenic and selenium compounds was developed. CE was coupled to an ICP-MS system by a sprayer with a novel direct-injection high-efficiency nebulizer (DIHEN) chamber as the interface. By using this interface, six arsenic species, including arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC) and five selenium species (such as sodium selenite (Se(IV)), sodium selenate (Se(VI)), selenocysteine (SeCys), selenomethionine (SeMet), and Se-methylselenocysteine (MeSeCys)) were baseline-separated and determined in a single run within 9 min under the optimized conditions. Minimum dead volume, low and steady sheath flow liquid, high nebulization efficiency, and high sample transport efficiency were obtained by using this interface. Detection limits were in the range of 0.11–0.37 $\mu\text{g L}^{-1}$ for the six arsenic compounds (determined as ^{75}As at m/z 75) and 1.33–2.31 $\mu\text{g L}^{-1}$ for the five selenium species (determined as ^{82}Se at m/z 82). Repeatability expressed as the relative standard deviations (RSD, $n = 6$) of both migration time and peak area were better than 2.68% for arsenic compounds and 3.28% for selenium compounds, respectively. The proposed method had been successfully applied for the determination of arsenic and selenium species in the certified reference materials DORM-3, water, urine, and fish samples.

Arsenic and selenium are widely distributed in the environment and have toxic properties. The release of those metalloids into the environment could cause exposure to the public population and pose human health risk. Arsenic is a concern as a poison and carcinogen, and a variety of health problems could be caused by it, even at very low levels of exposure. Paradoxically, arsenic derivatives have been used as therapeutic agents for various diseases including cancers, among which arsenic trioxide has been approved as an effective drug for the treatment of acute promyelocytic leukemia by the U.S. Food and Drug Administration in 2000.¹ Selenium is known as an essential trace element for biological systems. It is a nutrient at low concentration and recognized as an important antioxidant and anticancer element. However, it is also a potential toxicant at high doses, with a narrow range between human dietary deficiency (<40 $\mu\text{g day}^{-1}$) and excess levels (>400 $\mu\text{g day}^{-1}$).² The toxicity of arsenic and selenium is dependent not only on their total amounts, but also on their chemical forms and oxidation states. Inorganic arsenic species are more toxic than the methylated organic species, such as MMA and DMA, while inorganic As(III) is the most toxic arsenic species. Regarding selenium, the majority of selenium

species in environmental matrices include two inorganic species (selenite Se(IV) and selenate Se(VI)) and some selenoamino acids (selenocysteine (SeCys), selenomethionine (SeMet), and Se-methylselenocysteine (MeSeCys)).³ Inorganic Se(IV) is the most toxic species.⁴ Selenoamino acids, which participate in the biological selenium cycle, are considered to be less toxic than the inorganic Se(IV) and Se(VI).⁵ Furthermore, it has been found that there was an antagonistic effect between arsenic and selenium in biological systems that caused detoxification.^{6,7} In view of their various toxicity, the chemical forms and quantities of arsenic and selenium are important information for estimating the environmental impact and potential health risk, studying the antagonistic interactions and probing the possible metabolic pathways in biological system. Thus, simple, rapid, and sensitive analytical methods for simultaneous speciation of arsenic and selenium compounds in biological samples are necessary and crucial for supporting these studies.

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Several techniques based on high-performance liquid chromatography (HPLC), ion chromatography (IC), monolithic capillary microextraction (MCE), and capillary electrophoresis (CE), coupled with sensitive detection techniques, have been reported for the speciation of arsenic and selenium.^{3,5,8–14} Compared with other chromatographic techniques, CE can offer advantages of high separation efficiency, comparatively mild separation conditions, short analysis time, low sample volume requirement, and minimal buffer consumption. In recent years, the application of CE for separation has grown rapidly and has been widely used for single-elemental speciation analysis, such as sulfur, arsenic, selenium, mercury, platinum, and other metal ions, as well as their complexes with biomolecules.^{15–23} However, the application of CE hyphenated systems for simultaneous speciation of arsenic and selenium is limited. The significant advantages of CE, such as high separation efficiency and low sample volume requirements, make it more suitable for element speciation, especially for biological samples. The minimal buffer consumption averts the detrimental effects on the signal caused by the large amounts of solvents and salts introduced into the detectors. However, the low sample injection volume (approximately nanoliter (nL) level) restricts the detection limits of commercial UV–vis detectors. Therefore, different element-specific detection techniques, such as atomic fluorescence spectrometry (AFS), atomic absorption spectrometry (AAS), inductively coupled plasma–optical emission spectrometry (ICP-OES), and inductively coupled plasma–mass spectrometry (ICP-MS) were coupled to CE.^{24–28} Among these options for online coupling with CE, ICP-MS has received particular attention, because of its extremely low detection limits, multielemental detection specificity, wide linear dynamic range of detection, and rapid analysis. Therefore, the combination of CE with ICP-MS could provide a powerful technique for the simultaneous separation and determination of arsenic and selenium species.

In the coupling of CE with ICP-MS, the interface must fulfill several requirements: (i) maintain a steady electrical connection at the end of the CE capillary, (ii) match the flow rate of the CE capillary ($\mu\text{L min}^{-1}$ level) with the vastly different uptake rate of the ICP-MS (mL min^{-1} level) for the stable operation, and (iii) efficiently introduce analytes from CE capillary to the plasma. Until now, three commonly used types of interfaces have been reported to combine CE with ICP-MS: sheath-flow interface, no-sheath-flow interface, and hydride generation interface. Among these three interfaces, the sheath-flow interface has received the most attention. The commercially available microconcentric nebulizers have been studied and used as sheath-flow interfaces for CE-ICP-MS.^{27,29,30} The addition of sheath flow liquid fulfills the two requirements of the interface: (i) closing the electrical circuit of CE and (ii) compensating the low flow rate of CE with an adequate flow rate. However, there are considerable disadvantages of using the commercial nebulizer, such as big dead volume and unstable high flow rate of sheath flow liquid resulting from self-aspiration. The dead volume could result in peak broadening and tailing, while the high flow rate of sheath flow liquid could cause excessive dilution of analytes from the CE capillary and eventually reduce the separation efficiency and the detection sensitivity. Besides, the standard spray chamber configuration of the ICP-MS instrument causes a loss and dilution of analytes on their way toward the plasma and introduces an additional dead volume into the separating

system, resulting in a decrease in sensitivity and an increase in peak tailing.^{31,32} All these issues compel additional work to improve the CE-ICP-MS interface. A high-efficiency CE-ICP-MS interface composed of low-dead-volume spray chamber and optimized fluid dynamics is needed to efficiently introduce trace analytes into ICP-MS without degradation of CE separation.

The objective of this work is to develop a simple and high-efficiency interface for coupling of CE with ICP-MS by using a sprayer and a homemade direct-injection high-efficiency nebulizer (DIHEN) chamber and to establish a rapid method for simultaneous separation and determination of six arsenic and five selenium compounds using the online hyphenated CE-ICP-MS system with the developed interface. Analytical conditions of the coupled CE-ICP-MS system by the proposed interface were optimized and discussed. The established method was applied for the separation and determination of arsenic and selenium species in water, fish, and urine samples.

■ EXPERIMENTAL SECTION

Chemicals and Reagents. Sodium arsenite (As(III)), sodium arsenate (As(V)), and a solution of arsenocholine (AsC) in water (GBW08671, 28.0 mg L^{-1}) was obtained from the Chinese Reference Material Center (Beijing, China). Arsenobetaine (AsB) was purchased from Fluka (Italy), and sodium selenite (Se(IV)) was purchased from Alfa (USA). DMA, MMA, sodium selenate (Se(VI)), SeCys, SeMet, and MeSeCys were obtained from Sigma (USA). Stock standard solution of 1000 mg L^{-1} SeCys was prepared by dissolving above SeCys solid in 0.2% NaOH solution. The 1000 mg L^{-1} stock solutions of As(III), As(V), DMA, MMA, AsB, Se(IV), Se(VI), SeMet, and MeSeCys were prepared by dissolving each of the above standard compounds in deionized water. All the stock standard solutions were stored in darkness at 4°C . Analytical working solutions were prepared daily by diluting appropriate stock solutions to the desired concentrations with the buffer solution prior to analysis.

Sodium dihydrogen phosphate dehydrate (NaH_2PO_4) obtained from Beijing Chemical Reagents Company (Beijing, China) and boric acid (H_3BO_3) obtained from Sigma (France) were diluted to prepare various concentrations of running buffer solutions. The pH of the running buffer solutions were adjusted by 0.1 mol L^{-1} sodium hydroxide (NaOH, Sigma, Sweden) solutions. The sheath flow liquid of $100 \mu\text{g L}^{-1}$ rhodium solution was prepared by diluting rhodium standard solution (10 mg kg^{-1} ; Fluka, Switzerland), which also used as internal solution in ICP-MS, in the 6% (v/v) methanol (J.T. Baker, USA) of HPLC grade. Nitric acid (65%, Merck, USA) and hydrogen peroxide (30%, Beijing Chemical Reagent, Beijing, China) were used for microwave-assisted digestion. All other reagents and chemicals used were of analytical grade or better. Deionized (DI) water ($18.2 \text{ M}\Omega \text{ cm}$) used throughout the experiment was prepared using a Milli-Q Advanced A10 system (Millipore, Bedford, MA, USA).

CE-ICP-MS system. The instrumental setup of CE-ICP-MS system used in this study consisted of a HP^{3D} CE (Agilent Technologies, Germany) and an Agilent 7500ce ICP-MS (Agilent Technologies, USA). The operating parameters were summarized in Table 1. The torch position and argon flow rate were optimized with a standard solution of $10 \mu\text{g L}^{-1}$ Li, Y, Ce, and Tl, prior to hyphenation. Signals at m/z of 75 and 82 were both monitored using time-resolved analysis mode for the detection of ^{75}As and ^{82}Se .

Table 1. Equipment and Operating Conditions of CE-ICP-MS

parameter	value/remark
ICP-MS Parameters	
RF power	1500 W
sample depth	8.0 mm
plasma gas flow rate	1.5 L/min
carrier gas flow rate	1.05 L/min
makeup gas flow rate	0.10 L/min
dynamic reaction cell	off
monitored isotope(<i>m/z</i>)	⁷⁵ As, ⁸² Se
CE Parameters	
fused silica capillary	75 μ m id $\times$ 60 cm length
buffer	NaH ₂ PO ₄ (6 mM), H ₃ BO ₃ (9 mM), pH 9.0
voltage	+25 kV
temperature	25 $^{\circ}$ C
sample injection	hydrodynamic 10 s (50 mbar), 72.6 nL
preanalysis rinse	0.1 M sodium hydroxide (2 min)
deionized (DI) water (2 min)	
running buffer (2 min)	
Interface	
nebulizer	CE-ESI-MS sprayer
sheath liquid	100 μ g L ⁻¹ Rh(NO ₃) ₃ , 6% methanol
sheath flow rate	4 μ L/min

The separation was achieved on the HP^{3D} CE system with a 60 cm length $\times$ 75 μ m i.d. $\times$ 365 μ m o.d. fused-silica capillaries (Yongnian Optical Fiber Company, Hebei Province, China). New capillary was initialized by flushing with 1 mol L⁻¹ NaOH for 60 min, 0.1 mol L⁻¹ NaOH for 60 min, H₂O for 30 min, and running buffer solution for 60 min, sequentially. Between each run, the capillary was flushed with 0.1 mol L⁻¹ NaOH, H₂O and running buffer for 2 min, respectively. At the beginning of each day, the capillary was reconditioned by purging with 0.1 mol L⁻¹ NaOH and H₂O for 10 min. Samples were injected into the capillary hydrodynamically at 50 mbar for 10 s. The applied voltage and cassette temperature were set

at 25 kV and 25 $^{\circ}$ C with room temperature regulated in 25 $^{\circ}$ C by an air conditioner.

CE-ICP-MS Interface. The interface used in this work was configured on the basis of our previous work,¹⁹ and the schematic diagram was illustrated in Figure 1. Briefly, a CE-ESI-MS sprayer (G1607A, Agilent Technologies, USA) was used as the interface for coupling CE with ICP-MS as well as the nebulizer for the ICP-MS. The interface was installed on a homemade DIHEN chamber and the sprayer tip was directly inserted into the chamber. The CE capillary passed through the inner stainless steel capillary of the sprayer and the outlet end of the CE capillary was extended 0.1 mm beyond the sprayer tip. Sheath flow liquid was added in the gap between the CE capillary and the inner stainless steel capillary and sprayed together with sample elution. Carrier gas was added in the outer tube of the sprayer for nebulization. The sheath flow liquid of 100 μ g L⁻¹ rhodium nitrate in 6% (v/v) methanol was introduced into system by an Agilent 1200 series quaternary pump (Agilent Technologies, Germany) and a built-in active 1:100 flow-splitter (Agilent Technologies, USA), offering a steady and low flow rate sheath liquid of 4 μ L min⁻¹. The stainless-steel shell of the sprayer was grounded to maintain a steady separation voltage.

After sample injection, analytes were separated under the high voltage in the CE capillary and then migrated to the end of the capillary, where they were mixed with the sheath flow liquid and directly nebulized by the carrier gas without further transport. The nebulized analytes were then directly transported into the ICP-MS torch for ionization and further detection. The entire system was easy to install and provided good stability, low dead volume, and good repeatability.

Sample Preparation. Two groundwater samples were obtained from different tube wells from the town of Shanyin (Shanxi province, China), while the tap water was collected stochastically from the laboratory (Beijing, China). The urine sample was collected from a volunteer living in Shanyin. The fish-1 sample (*Paralichthys olivaceus*) was collected from Dalian (Liaoning province, China), and the fish-2 sample (*Racoma*

Figure 1. Schematic diagram of the interface for coupling capillary electrophoresis (CE) with inductively coupled plasma–mass spectrometry (ICP-MS).

biddulphi Gunther) was collected from the Tibetan plateau (Tibet, China).

To determine the total concentrations of As and Se, the experimental details are described in the Supporting Information.

For speciation measurements, the water samples and urine sample were filtrated through 0.22 μm nylon filter to remove particulates. Fish samples were lyophilized and homogenized before the extraction procedure. For the aqueous extraction, 0.3–0.5 g DORM-3 and/or fish power was placed to the centrifuge tubes and 5 mL of deionized water was added. The tube was vortex mixed for 2 min and extracted with sonication for 120 min. The suspensions were then centrifuged at 4000 rpm for 10 min and the collected supernatant was filtrated through 0.22 μm nylon filter. All extracted solutions were diluted with running buffer solution before injection and further measurements.

RESULTS AND DISCUSSION

Interface Design. In the previous reports, the interface between CE and ICP-MS usually consisted of a tee or cross fitting and a commercial nebulizer with an extra sheath flow liquid. The nebulizer was often installed on a regular Scott double pass spray chamber, which resulted in a huge dead volume. Since the nebulizer, the flow rate of sheath flow liquid, and the chamber could significantly affect the analytical performance of this hyphenated system, interface design for high sample introduction efficiency and low dead volume is needed.

Generally, with the use of commercial nebulizer as the interface, the sheath flow liquid would mix with the separated analytes at the outlet end of the CE capillary and then be transported to the nebulizer. Thus, a large liquid dead volume of the CE-ICP-MS system is inevitable. In this work, a CE-ESI-MS sprayer was used as the injection nebulizer to couple CE with ICP-MS, as described in the previous work.¹⁹ The sprayer interface could eliminate the liquid dead volume of the CE-ICP-MS system, offer high sample introduction efficiency, and simultaneously maintain high CE resolution. With the use of the proposed interface (Figure 2B), the migration times and the peak width of arsenic species decreased, resulting in peak sharpening and higher separation efficiency compared with the use of the commercial MicroMist nebulizer (Figure 2A).

When the regular Scott double pass spray chamber was used in the CE-ICP-MS system, the long distance from the nebulizer to the plasma results in larger gas phase dead volume, which would cause a significant reduction of the peak height, as well as band broadening, lower sensitivity, and longer wash-out times.^{32,33} In this work, a miniaturized DIHEN chamber was designed and used for ICP-MS in order to minimize the gas phase dead volume and maintain the high resolution of CE. The sprayer was fit on this homemade chamber as showed in Figure 1. With the use of the DIHEN chamber, the dead volume of the system and the dilution of analytes decreased significantly. As the centerlines of the sprayer, the designed chamber and the torch as well as the sample cone were in a straight line, the sample transport efficiency was greatly improved, ensuring highly sensitivity. It was found that the peak intensity of arsenic increased by a factor of ~ 2 –4, and the detection limits decreased with the use of the DIHEN chamber (Figure 2C), compared to the regular Scott double pass spray chamber (Figure 2B), mainly due to the increased sample transport efficiency. To compare the separation efficiency of the

Figure 2. Comparison of electropherograms of three different types of CE-ICP-MS interfaces: (A) a homemade interface of a cross design fitting coupled with a MicroMist nebulizer installed on a Scott double pass spray chamber (sheath flow liquid: self-aspiration, arsenic concentration = $400 \mu\text{g L}^{-1}$); (B) a CE-ESI-MS sprayer interface installed on a regular Scott double pass spray chamber (arsenic concentration = $400 \mu\text{g L}^{-1}$); and (C) a CE-ESI-MS sprayer installed on a novel direct-injection high-efficiency nebulizer (DIHEN) chamber as the interface (arsenic concentration = $100 \mu\text{g L}^{-1}$). Other conditions were kept constant.

three interfaces, the resolutions between arsenic peaks were calculated. The resolutions between the closest two peaks (peaks of AsB and As(III)) obtained in Figure 2A, 2B, and 2C were 0.85, 1.00 and 1.20, respectively. This results indicated

that a better resolution and higher separation efficiency was achieved by using the proposed interface.

In order to maintain a closed electrical circuit at the end of the CE capillary and increase the total liquid flow to match the nebulizer flow rate, a sheath flow liquid is needed and added in the interface. The flow rate of the sheath liquid is a key factor, because it has significant influence on separation and sensitivity. Generally, the sheath flow liquid could reduce the laminar flow, which was caused by the suction flow in the CE capillary and dispersion. With the increase of the flow rate of sheath liquid, peak sharpening would be realized, because of the suppression of the laminar flow, while the analyte transport efficiency would be decreased.³⁴ The effect of the sheath liquid flow rate on the CE resolution and sensitivity was studied from $1 \mu\text{L min}^{-1}$ to $7 \mu\text{L min}^{-1}$. There was no significant extension of residence time of the analytes under different flow rate. Considering both the peak area and the peak height of the arsenic and selenium compounds, the flow rate of sheath liquid was chosen as $4 \mu\text{L min}^{-1}$.

Research has shown that the transport efficiency of aerosol at low sheath flow rates of $5\text{--}10 \mu\text{L min}^{-1}$ could approach 100%, regardless of the type of nebulizer that was used.³⁵ With the use of the direct injection nebulizer and the DIHEN chamber in this experiment, analytes were directly nebulized after being separated in the CE capillary and then $\sim 100\%$ transported into the ICP-MS plasma. The steady and continuous sheath liquid added in the interface ensures a stable atomization and ionization efficiency. With above features, the improved interface keeps a minimum dead volume and a stable low flow rate of sheath liquid, thereby completely avoiding laminar flow. The advantages of higher sensitivity and better electrophoretic resolution make the improved interface convenient and suitable for the separation and routine analysis of different charged compounds.

Optimization of ICP-MS Conditions. ICP-MS conditions were optimized for arsenic and selenium determination to obtain higher signal-to-noise ratio. Determination of ^{80}Se and ^{78}Se , the two most abundant Se isotopes (49.6% and 23.8%, respectively), by quadrupole ICP-MS is interfered by the argon dimers $^{40}\text{Ar}_2$ and $^{40}\text{Ar}^{38}\text{Ar}$. Although the isobaric interferences could be eliminated by the dynamic reaction cell (DRC), research has shown that a decrease in the sensitivities for both ^{75}As and ^{78}Se signals (up to 50-fold) was observed by reaction with the methane in the DRC.³⁶ Considering the sensitivities of both arsenic and selenium, the DRC was therefore not used in this experiment. When ^{75}As , ^{78}Se , ^{80}Se , and ^{82}Se was simultaneously monitored by ICP-MS without the DRC, it was found that ^{82}Se (8.73% abundance) could provide a stable low background and a better signal-to-noise ratio than the more-abundant ^{78}Se and ^{80}Se isotope (see Figure S1 in the Supporting Information). Therefore, ^{75}As and ^{82}Se were selected as the target isotopes for the quantification work by ICP-MS.

The signal intensity for arsenic and selenium could be enhanced by the addition of suitable amounts of carbon as methanol to the aqueous solutions; this is called the carbon enhancement effect.³⁷ The increased amount of C^+ by the addition of organic solvent can lead to electron transfer from the molecule of the analyte to the carbon-containing ion, thus improving the degree of ionization of the analytes and subsequently enhancing the signal intensity. Therefore, in this experiment, methanol was added in the sheath liquid and the influence of methanol concentration (v/v, 0%, 2%, 4%, 6%, 8%)

on the ICP-MS signal intensities of arsenic and selenium was studied. Results in Figure 3 demonstrated that peak areas of all

Figure 3. Influence of methanol concentration in the sheath flow liquid on the ICP-MS signal intensities for arsenic and selenium compounds. The data were obtained by determining $100 \mu\text{g L}^{-1}$ arsenic and $200 \mu\text{g L}^{-1}$ selenium standard solutions under the optimized CE-ICP-MS conditions.

arsenic and selenium species increased with the increase of the methanol concentration in the range of 0% to 6% and decreased when 8% methanol was added. The observed signal suppression of arsenic and selenium under higher methanol concentration results from the local cooling of the central channel of the plasma caused by the high concentration of carbon compounds, which subsequently affects the processes of desolvation, atomization, and ionization of the analytes in plasma.^{38,39} Therefore, 6% was chosen as the methanol concentration in the sheath flow liquid.

Optimization of CE Conditions. The CE separation of arsenic and selenium compounds was affected by several operation parameters, such as chemical components, concentration and pH of buffer solution, and the separation voltage. In our previous work,¹⁹ the best resolution and lower retention time for arsenic speciation were obtained when the mixture of phosphate and borate were used as the electrophoretic buffer. This buffer system has been examined in this experiment and the composition of electrolyte buffer was optimized for the simultaneous speciation of arsenic and selenium. It was found that the six arsenic and five selenium species could be separated by the mixed buffer solution when the phosphate:borate ratio was 1:4, 2:3, 1:1, 3:2, and 4:1 (mole concentration). A better electropherogram, based on the best resolution and lower retention times, was obtained when the phosphate:borate ratio was 2:3. Therefore, the mixture of phosphate and borate (2:3, mole concentration) was selected as the electrophoretic buffer for the following experiment.

The effect of buffer concentration was studied by using different concentrations of phosphate–borate buffer solution (10, 15, 20, 25, 30 mmol L^{-1} , phosphate:borate ratio = 2:3). With the increase of the buffer concentration, the high ionic strength would decrease the electro-osmotic flow (EOF) and then increase the migration time of the species in the capillary. As shown in Figure 4, the migration times of the compounds became longer and the separation efficiency improved with the increase of the buffer concentration. However, the broadening

Figure 4. Effect of buffer concentration on the CE separation of six arsenic and five selenium compounds. The data was obtained by determining $100 \mu\text{g L}^{-1}$ arsenic and $200 \mu\text{g L}^{-1}$ selenium standard solution with CE-ICP-MS under different buffer concentrations while keeping other conditions constant. Peaks: 1, AsC; 2, AsB; 3, As(III); 4, DMA; 5, MMA; 6, As(V); 7, SeMet; 8, MeSeCys; 9, SeCys; 10, Se(IV); and 11, Se(VI).

of the peaks and degradation of resolution were observed if the concentration of the buffer solution was too high. This could be due to the increase of the conductivity of the electrolyte and the increase of the temperature caused by the Joule heating.⁴⁰ The ICP-MS signals of the detected compounds also decreased as a result of low nebulization efficiency at high buffer concentration. To achieve a better separation, lower retention time and higher ion signals, 15 mM phosphate-borate solution was selected as the electrophoretic buffer.

The pH of buffer solution significantly influences the CE separation of selenium and arsenic by affecting the EOF and the electrophoretic mobility of analytes. In a positive polarity configuration of CE, the migration velocity of an anionic analyte depends upon both its electrophoretic mobility which is toward the positive electrode and the EOF rate of the buffer solution, which is toward the negative electrode. The As and Se anions would reach the detection window by the EOF and would be separated because of their different electrophoretic mobilities. With a decrease of pH, the weak acid anions of the buffer system could be partially protonated and the EOF would become slower. Therefore, the observed mobility of the anions was faster and the migration time decreased. In this study, the effect of the pH of the buffer solution on the separation was investigated with a 15 mM phosphate-borate buffer solution in the pH range of 8.6–9.4 (see Figure S2 in the Supporting Information). The results illustrated that arsenic and selenium species could be baseline-separated at different pH values. The separation efficiency improved and the migration times of the compounds slightly increased with the increase of the buffer pH. However, the peak height decreased significantly at higher pH. Considering the separation and sensitivity, a buffer of pH 9.0 was finally chosen for separation in the experiment.

The influence of applied voltage on resolution and migration time was investigated in the range of 19–30 kV with a 15 mM phosphate-borate buffer at pH 9.0. The migration times of the arsenic and selenium species under different voltage were shown in Figure S3 in the Supporting Information. The migration time and peak widths decreased and the CE resolution also improved as the applied voltage increased.

Although the analysis time was less at the higher voltage of 30 kV, this high voltage lead to increased temperature, degradation of resolution and poor stability due to Joule heating. So, +25 kV was selected as the separation voltage, because of its lower retention times and better resolution.

The effect of injection time on the separation and sensitivity was also studied. A series of injection times (1, 2, 5, 10, 15, and 20 s) were investigated under the hydrodynamic injection pressure of 50 mbar. Peak areas of each species increased linearly with the increase of sample injection time (Figure 5).

Figure 5. Influence of injection time on the intensities of arsenic and selenium compounds. The data were obtained by determining $100 \mu\text{g L}^{-1}$ arsenic and $200 \mu\text{g L}^{-1}$ selenium standard solutions under the optimized CE-ICP-MS conditions.

However, the peak height of MMA decreased slightly in the range of 10–25 s and the electrophoretic resolution decreased under longer injection times. The peaks of SeMet and MeSeCys were overlapped and the two species could not be baseline-separated when the injection time was longer than 10 s. Accordingly, 10 s was chosen as the sample injection time. A summary of the final optimum operating conditions of the CE system is shown in Table 1.

Analytical Performance. Under the optimized conditions, six arsenic and five selenium compounds including AsB, AsC, As(III), DMA, MMA, As(V), SeMet, MeSeCys, SeCys, Se(IV), and Se(VI) were all baseline-separated within 10 min. The analytical features are summarized in Table 2. The calibration curves based on the peak area were linear with all correlation coefficients (r^2) better than 0.998 in the range of 5–200 $\mu\text{g L}^{-1}$ for arsenic species and 10–400 $\mu\text{g L}^{-1}$ for selenium species. For a mixture solution of 100 $\mu\text{g L}^{-1}$ arsenic species and 200 $\mu\text{g L}^{-1}$ selenium species, the precisions (RSD, $n = 6$) of the migration time for the 11 compounds ranged from 0.72% to 2.68% and the precisions (RSD, $n = 6$) of the peak area were in the range of 0.71%–3.28%, indicating good reproducibility of the method. For a lower concentration solution (10 $\mu\text{g L}^{-1}$ arsenic species and 20 $\mu\text{g L}^{-1}$ selenium species), the precisions (RSD, $n = 4$) of the migration time and peak area for the 11 species were in the range of 0.81%–6.44% and 1.92%–8.25%, respectively. The detection limits (3σ) ranged from 0.11 $\mu\text{g L}^{-1}$ to 0.37 $\mu\text{g L}^{-1}$ for the suite of As compounds and 1.27 $\mu\text{g L}^{-1}$ to 2.31 $\mu\text{g L}^{-1}$ for the suite of Se compounds (see Table 2). These values indicated that the method was suitable for the direct speciation of arsenic and selenium in real samples. Figure

Table 2. Analytical Performance of the Developed CE-ICP-MS Method

analyte	linear range ($\mu\text{g L}^{-1}$)	correlation coefficient	Limit of Detection, LOD		RSD ^c (%)	
			($\mu\text{g L}^{-1}$) ^a	(fg) ^b	retention time	peak area
AsC	5–200	0.9997	0.21	15.6	0.74	0.88
AsB	5–200	0.9995	0.37	26.5	0.72	0.72
As(III)	5–200	0.9995	0.25	17.8	1.02	2.22
DMA	5–200	0.9999	0.21	15.5	0.76	3.28
MMA	5–200	0.9996	0.11	7.8	1.04	1.71
As(V)	5–200	0.9989	0.22	15.9	1.23	0.91
SeMet	10–400	0.9999	1.48	107.5	1.07	2.09
MeSeCys	10–400	0.9998	1.33	96.4	1.40	1.39
SeCys	10–400	0.9992	1.27	91.9	1.48	1.72
Se(IV)	10–400	0.9999	2.31	167.8	2.68	0.71
Se(VI)	10–400	0.9999	2.23	161.6	1.87	0.89

^aCalculated using $3\sigma/S$ based on the peak height measurement. ^bAbsolute detection limits (fg) based on a 72.6 nL sample injection. ^cStandard concentration, 100 $\mu\text{g L}^{-1}$ (As) and 200 $\mu\text{g L}^{-1}$ (Se), $n = 6$.

6 shows a typical electropherogram of the six arsenic species at 100 $\mu\text{g L}^{-1}$ and five selenium species at 200 $\mu\text{g L}^{-1}$ levels obtained at the optimized conditions.

Figure 6. Electropherogram of the mixed standard solutions of six arsenic species (100 $\mu\text{g L}^{-1}$) and five selenium species (200 $\mu\text{g L}^{-1}$) under the optimized conditions.

Application to Real Samples. In order to verify the applicability of the established method and evaluate the accuracy of the proposed method, different matrix samples were analyzed by CE-ICP-MS under the optimized conditions shown in Table 1. The typical mass selective (^{75}As and ^{82}Se) electropherograms of the samples are shown in Figure 7 and analytical results are listed in Table 3.

Ground water samples were collected from Shanyin (Shanxi province, China), which is an area seriously affected by arsenic-contaminated groundwater. Inorganic arsenic was the predominant species in the water samples and organic arsenic or selenium species were not detectable. As(III) and As(V) were detected to be $357.7 \pm 4.3 \mu\text{g L}^{-1}$ and $126.3 \pm 1.3 \mu\text{g L}^{-1}$ in the Ground Water-1 sample, while in the Ground-Water-2 sample they were detected to be $85.5 \pm 1.4 \mu\text{g L}^{-1}$ and $201.1 \pm 2.9 \mu\text{g L}^{-1}$, respectively. In the tap water, only As(V) ($2.04 \pm 0.02 \mu\text{g L}^{-1}$) was detected. Recovery tests have been performed by spiking suitable amounts of the species to the groundwater 1 and the electropherogram was showed in Figure S4 in the

Supporting Information. The recoveries of individual arsenic and selenium species in the groundwater were in the range of 93.0%–99.7% and 88.9%–99.8%, respectively (see Table 3).

Fish samples, such as reference material DORM-3, Fish-1 (collected from Dalian, Liaoning province), and Fish-2 (collected from the Tibetan plateau) were analyzed by the proposed method for arsenic and selenium speciation. DMA ($0.43 \pm 0.01 \mu\text{g g}^{-1}$), MMA ($0.45 \pm 0.06 \mu\text{g g}^{-1}$), As(V) ($0.32 \pm 0.02 \mu\text{g g}^{-1}$), and AsB ($5.11 \pm 0.09 \mu\text{g g}^{-1}$) were detected in the DORM-3 and the sum concentration of individual arsenic species ($6.31 \pm 0.13 \mu\text{g g}^{-1}$) agreed well with the certified value of total arsenic ($6.88 \pm 0.3 \mu\text{g g}^{-1}$). AsB ($4.87 \pm 0.23 \mu\text{g g}^{-1}$), DMA ($0.21 \pm 0.01 \mu\text{g g}^{-1}$) and SeMet ($1.56 \pm 0.02 \mu\text{g g}^{-1}$) were found in the Fish-1 sample, while AsB ($0.61 \pm 0.04 \mu\text{g g}^{-1}$), MMA ($0.16 \pm 0.01 \mu\text{g g}^{-1}$) and As(V) ($0.13 \pm 0.01 \mu\text{g g}^{-1}$) were detected in Fish 2 sample. The selenium compounds were only detected in the Fish-1 samples. Standard addition validation tests were conducted with sample DORM-3 (see Figure S5 in the Supporting Information) and the recoveries of individual species were in the range of 96.2%–106.3% for arsenic and 93.7%–105.8% for selenium.

Speciation analysis of arsenic and selenium in the urine sample collected from a volunteer in Shanyin was performed in order to test the developed method in a high chloride matrix sample. AsB ($314.3 \pm 24.5 \mu\text{g L}^{-1}$), As(III) ($50.5 \pm 5.7 \mu\text{g L}^{-1}$), DMA ($150.4 \pm 8.7 \mu\text{g L}^{-1}$), MMA ($85.1 \pm 1.1 \mu\text{g L}^{-1}$), and As(V) ($19.0 \pm 1.2 \mu\text{g L}^{-1}$) were measured and selenium species were not observed. The electropherogram of urine sample with spiked species was showed in Figure S6 in the Supporting Information and the recoveries for arsenic and selenium species were in the range of 94.9%–110.2% and 93.3%–110.1%, respectively.

CONCLUSIONS

In this work, a method for the fast and simultaneous speciation of arsenic and selenium was developed based on the hyphenated capillary electrophoresis coupled with inductively coupled plasma–mass spectrometry (CE-ICP-MS) system by using a CE-ESI-MS sprayer and a novel direct-injection high-efficiency nebulizer (DIHEN) chamber as the interface. The interface decreases the dead volume of the system, avoids laminar flow in the CE capillary, and provides high analyte transport efficiency to the plasma, leading to higher sensitivity and better electrophoretic resolution. Baseline separation of six arsenic and five selenium species was achieved within 9 min

Figure 7. Electropherogram of arsenic and selenium species obtained by CE-ICP-MS in environmental samples: (A) Ground Water-1, (B) DORM-3, (C) Fish-2, and (D) Urine sample.

Table 3. Concentrations and Recoveries of Arsenic and Selenium Species in Real Samples Quantified by CE-ICP-MS

compound	Water Samples ($\mu\text{g L}^{-1}$)				Fish Samples ($\mu\text{g g}^{-1}$)				Urine Sample ($\mu\text{g L}^{-1}$)	
	Ground water-1	Ground water-2	tap water	recovery ^a (%)	DORM-3 ^b	Fish-1	Fish-2	recovery ^c (%)	Urine	recovery (%)
AsC	nd		nd	93.0	nd	nd	nd	97.9	nd	94.9
AsB	nd		nd	99.0	5.11 $\pm$ 0.09	4.87 $\pm$ 0.23	0.61 $\pm$ 0.04	106.3	314.3 $\pm$ 24.5	110.2
As(III)	357.7 $\pm$ 4.3	85.5 $\pm$ 1.4	nd	99.7	nd	nd	nd	100.4	50.5 $\pm$ 5.7	106.6
DMA	nd		nd	99.3	0.43 $\pm$ 0.01	0.21 $\pm$ 0.01	nd	96.4	150.4 $\pm$ 8.7	95.9
MMA	nd		nd	97.1	0.45 $\pm$ 0.06	nd	0.16 $\pm$ 0.01	101.1	85.1 $\pm$ 1.1	100.6
As(V)	126.3 $\pm$ 1.3	201.1 $\pm$ 2.9	2.04 $\pm$ 0.02	99.4	0.32 $\pm$ 0.02	nd	0.13 $\pm$ 0.01	96.2	19.0 $\pm$ 1.2	98.4
SeMet	nd	nd	nd	99.8	nd	1.56 $\pm$ 0.02	nd	102.7	nd	103.4
MeSeCys	nd	nd	nd	96.2	nd	nd	nd	105.8	nd	100.8
SeCys	nd	nd	nd	88.9	nd	nd	nd	93.7	nd	110.1
Se(IV)	nd	nd	nd	97.5	nd	nd	nd	102.0	nd	97.5
Se(VI)	nd	nd	nd	98.3	nd	nd	nd	103.0	nd	93.3
total As	499.1 $\pm$ 2.3	310.8 $\pm$ 3.7	3.3 $\pm$ 0.06		6.73 $\pm$ 0.13	5.90 $\pm$ 0.06	1.04 $\pm$ 0.02		637.0 $\pm$ 10.2	
total Se	nd	nd	nd		2.98 $\pm$ 0.17	2.38 $\pm$ 0.05	0.01 $\pm$ 0.001		nd	

^aSpiked 100 $\mu\text{g L}^{-1}$ arsenic and 200 $\mu\text{g L}^{-1}$ selenium species on groundwater-1. ^bCertified value of the total arsenic ($6.88 \pm 0.3 \mu\text{g g}^{-1}$). ^cSpiked 50 $\mu\text{g L}^{-1}$ arsenic and 100 $\mu\text{g L}^{-1}$ selenium species on DORM-3.

with detection limits in the range of 0.11–0.37 $\mu\text{g L}^{-1}$ for the arsenic species and 1.33–2.31 $\mu\text{g L}^{-1}$ for the selenium species. The proposed method was successfully applied to quantify arsenic and selenium species in real samples and good recoveries were obtained for different matrices. The low sample consumption of CE combined with the high sensitivity of ICP-MS makes this CE-ICP-MS hyphenated system an attractive tool for simultaneously evaluating the environmental concentration, distribution, and toxicity of trace arsenic and selenium in aqueous biological samples.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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