Shu-Yu Lin
Guan-Ren Wang
Quan-Pin Huang
Chuen-Ying Liu

Department of Chemistry,
National Taiwan University,
Taipei, Taiwan

Received March 30, 2006
Revised May 19, 2006
Accepted May 19, 2006

Research Article

Speciation of selenium compounds by open tubular capillary electrochromatography-inductively coupled plasma mass spectrometry

We introduce a T-type interface and a crossflow nebulizer to find ways to combine CEC with inductively coupled plasma MS (ICP-MS) detection for selenium speciation. For CEC separation, we employed a macrocyclic polyamine-bonded phase capillary as the separation column and a bare fused-silica capillary filled with the make-up liquid (0.05 M HNO_3). The effect of nebulizer gas flow rate, make-up liquid flow, type, concentration and pH of the mobile phase on the separation have been studied. Tris buffer of 50 mM at pH 8.50 gave the best performance for selenium speciation. The reproducibility of the retention time indicated that sample injection by electrokinetic and nebulizer gas flow was better than that by self-aspiration alone. The detection limits for selenate, selenite, selenocystine and selenomethionine were found to be 2.40, 3.53, 12.86 and 11.25 ng/mL, respectively. Due to the high sensitivity and element-specific detection, as well as the high selectivity of the bonded phase, quantitative analysis of selenium speciation in urine was also achieved.

Keywords: Crossflow nebulizer / Inductively coupled plasma mass spectrometry / Macrocyclic polyamine / Open-tubular capillary electrochromatography / Selenium speciation DOI 10.1002/elps.200600186

1 Introduction

Recently, elemental speciation has become an important topic of research, and efforts have been made to couple various separation and preconcentration methods [1–3]. CE is a powerful separation technique. Inductively coupled plasma MS (ICP-MS) is a multielement, highly sensitive and specific detector. Much effort therefore has been devoted to interfacing CE with ICP-MS. CE–ICP-MS couplings have been based on commercial nebulizers, including conventional concentric [4–6], ultrasonic [7, 8] and crossflow [9–12] nebulizers. However, problems derived from nebulizer suction on column or poor nebulization/transport efficiency have been widely documented [1, 2, 13]. Such a limitation has prompted the use of micronebulizers such as MicroMist [14, 15] and high-efficiency nebulizers [16]. Microconcentric nebulizers [16, 17] and CEI-100 [18] are better matched to the very low flows emerging from the CE capillary. However, the easy clogging, breakage and comparatively high costs of micronebulizers have limited its widespread use. Thus, on-line detection with ICP-MS in CE or CEC remains a challenging task for analytical chemists.

Michalke [19] has reported advantages and improvements in selenium speciation with CE–ICP-MS. Selenium is an essential nutrient at low concentrations, but is toxic to humans and animals at high doses with a relatively narrow margin between effective and toxic levels. The toxic dose and bioavailability depend on its chemical form and oxidation state [20]. Selenoamino acids are considered less toxic than inorganic selenium forms while their bioavailability is higher. The toxicity of selenite is greater than selenate. Speciating selenium provides a more accurate toxicity based risk assessment than an analysis based on total selenium.

Interfacing CE with ICP-MS by direct injection high-efficiency nebulizer [21], MCN-100 [22, 23] and MicroMist [23] was also accomplished for the selenium speciation

Correspondence: Professor Chuen-Ying Liu, Department of Chemistry, National Taiwan University, 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan
E-mail: cyliu@ntu.edu.tw
Fax: +886-2-23638543

Abbreviations: [28]ane-N_6O_2, 4,8,12,18,22,26-hexaaza-1,15-dioxacyclooctaeicosane; **ICP-MS**, inductively coupled plasma MS; **SeCys**, seleno-DL-cystine; **SeMet**, seleno-DL-methionine

study. Bendahl and Gammelgaard [24] investigated selenosugars in nutritional supplement tablets by CE separation using capillaries coated dynamically with poly(vinyl sulfonates) and detected by ICP-MS.

Macrocyclic polyamine-bonded phases have been studied extensively as stationary phases for CEC [12, 25–31]. The phases have been applied to the separation of oxyanions, polycarboxylates and polyphosphates. A highly selective property has been attributed to anion coordination, anion exchange and reversal of the EOF provided by the wall-bonded functional groups. Supercomplexation formation resulting from the second sphere interaction between metallocyanide and the polyamine has also been observed [26]. In this study, we evaluated the feasibility of separation of selenium compounds, including oxyanions and neutral species, with the macrocyclic-bonded phase, and on-line detection with ICP-MS.

2 Materials and methods

2.1 Chemicals

All chemicals were of analytical reagent grade from E. Merck (Darmstadt, Germany), unless stated otherwise. Purified water (18 MΩ-cm) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all solutions. Phosphoric acid, sodium phosphate monobasic, dibasic and tribasic, phenylarsine oxide (TCI, Tokyo, Japan), seleno-DL-cystine (SeCys) and seleno-DL-methionine (SeMet) (Sigma, St. Louis, MO, USA), sodium selenite and sodium selenate (E. Merck) were purchased as indicated. Stock solutions of 100 μg/mL were prepared by dissolving selenium compounds in pure water and diluted appropriately prior to use. All solvents and solutions for CEC analysis were filtered through a 0.45 μm PTFE (Millipore) or cellulose acetate membrane (Whatman, Maidstone, Kent, UK).

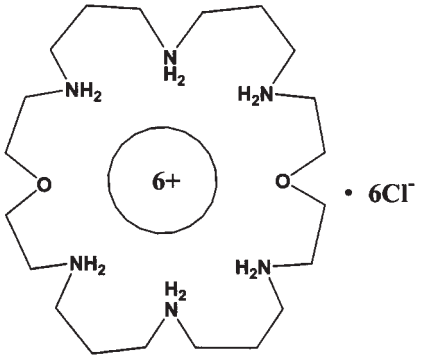
2.2 Instrumentation

2.2.1 ICP-MS

An ICP-MS (Elan 6000, Perkin-Elmer, Norwalk, CT, USA) with crossflow nebulizer, Scott-type Ryton double pass spray chamber, and quadrupole mass analyzer was interfaced to the CEC capillary. Flow rates of plasma and auxiliary gas were 15 and 1.2 L/min, respectively. Dwell time *per* mass was 100 ms. Selenium was determined at *m/z* 82.

2.2.2 CEC

All experiments were carried out in a laboratory-built unit, consisting of a ±30 kV high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA). Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an id of 100 μm and an od of 375 μm were used. A 28-membered macrocyclic polyamine, 4,8,12,18,22,26-hexaaza-1,15-dioxacyclooctaeicosane ([28]ane-N_6O_2) (Fig. 1) bonded phase capillary column (160 cm long) was employed for the CEC separation. A bare fused-silica capillary with an id of 150 μm and column length of 50 cm was used for filling the make up liquid (0.05 M nitric acid). The inlet end of the capillary was held at a negative potential, while the outlet end was grounded. A platinum wire (0.5 mm diameter, 5 cm long) was used as the electrode. The make up liquid was infused with a peristaltic pump (Minipuls 3, Gilson, Villiers-le-Bel, France).



4,8,12,18,22,26-hexaaza-1,15-dioxacyclooctaeicosane
(28ane-N_6O_2)

Figure 1. Structure of the macrocyclic polyamine covalently bound to the inner wall of the fused-silica capillary.

2.3 Preparation of macrocyclic polyamine-bonded phase capillary column

The column preparation was done as described in [26]. After pretreatment with NaOH (1 mM), HCl (1 mM) and pure water, the bare fused-silica capillaries were purged with nitrogen (20 min), then dried at 110°C overnight. For coating, a capillary washing kit was used to fill the capillary with a 10% w/v solution of γ-glycidoxypropyltrimethoxysilane in toluene under a pressure of 30 psi for 20 min. The capillary was kept for 3 h at 110°C for silylization. After purging with toluene (30 min) to remove unreacted reagent, the capillaries were dried in a vacuum oven (20 min). The capillary was then filled with a 1% w/v solution of the macrocyclic compound [28]ane-$N_6O_2 \cdot 6HCl$ in *N*,*N*-dimethylformamide. After standing for 10 h at 120°C for functionalization, the dried capillaries were purged with ethanol and pure water sequentially (30 min) to remove unreacted

reagent before equilibration with buffer solution. They were then ready for use. When the columns were not in use, they were stored only in pure water.

2.4 Capillary electrochromatographic conditions

Before analysis, the column of the macrocyclic polyamine-bonded phase was preconditioned with the running buffer. It was rinsed with pure water and buffer between runs at 1 or 2 min intervals. The samples were injected electrokinetically (−10 kV, 20 s) and nebulizer gas flow rate was of 1.02 L/min. EOF was measured with phenylarsine oxide as neutral marker.

3 Results and discussion

In a previous work, a Y-tube and crossflow nebulizer for the coupling of CEC and ICP-MS demonstrated good performance for the metal analysis even in high concentration of chloride matrix [12]. But the Y-tube was found to slacken easily in operation. In this study, a Tee and crossflow nebulizer were used instead as shown in Fig. 2.

3.1 Evaluation of the CEC–ICP-MS interface

Here, nitric acid was employed as the make-up electrolyte. It was introduced into the nebulizer with the help of a peristaltic pump to give a continuous flux of current. Kinzer *et al.* [32] reported that the suction produced by the nebulizer could induce laminar flow and resulting in a broad peak in a CE–ICP-MS connection system. The pressure difference is dependent on the type and gas flow rate of nebulizer, inner diameter and length of the capillary tube, the placement of the capillary within the nebulizer, as well as the flow rate of the make-up liquid.

Here, a Tee and a crossflow nebulizer were in use. With phosphate buffer (pH 8, 20 mM) and a nebulizer gas flow rate of 0.95 L/min for a given dimension of the bonded phase column, optimization of the make-up liquid flow was investigated. Phenylarsine oxide, a neutral compound was used as the flow index. The monitored mass is ^{75}As. It is a unique isotope. No significant difference for the elution time, but an increase of signal was observed as the rotation rate decreased from 0.08 to 0.02 mL/min (Table 1). As the rotation rate increases, more dilutes by the make-up liquid makes the signal decrease, while a broad asymmetric lower peak was shown as the rotation rate further decreased to less than 0.01 mL/min. Sample dispersion became evident in this case, we therefore selected 0.02 mL/min for subsequent experiments.

Table 1. Effect of the rotation rate of peristaltic pump on retention time and peak intensity of phenylarsine oxide with CEC–ICP-MS[a)]

Rotation rate (mL/min)	Retention time (s)	Peak intensity (counts/s)
0.08	544	1695
0.04	523	1791
0.02	515	2450
0.01	519	2262

a) ICPMS: nebulizer: crossflow nebulizer; nebulizer gas flow: 0.95 L/min; lens voltage: 6.0 V; ICP Rf power: 1050 W; CE system: [28]ane-N_6O_2-bonded phase (160 cm × 100 μm id); BGE: phosphate buffer (20 mM, pH 8); sample injection: electrokinetic (−10 kV, 20 s) and nebulizer gas flow of 0.95 L/min; applied voltage: −15 kV; sample concentration: 1 μg/mL.

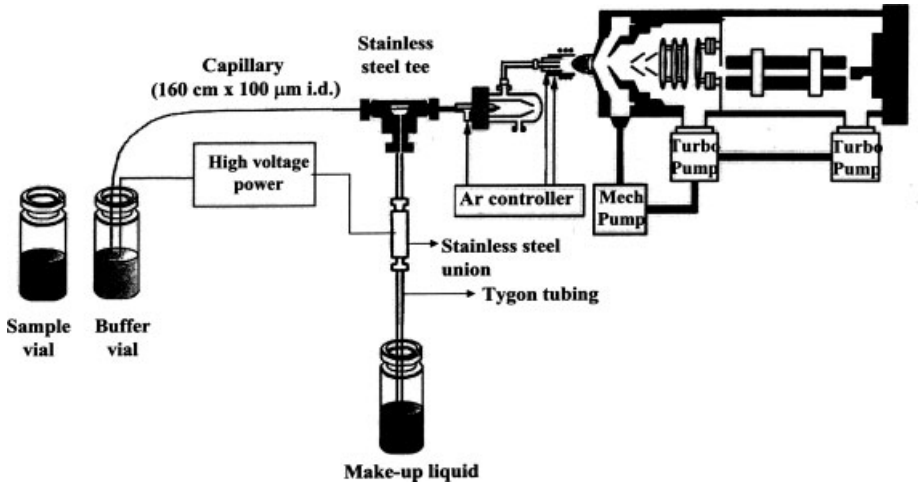


Figure 2. Schematic diagram of CEC–ICP-MS interface (T-type).

3.2 Effect of pH on the separation of selenium speciation

The pH of the mobile phase was found to have a significant influence on the protonation of the bonded phase and the electrophoretic mobility of the analytes, as well as the buffer species. A pH range from 2.25 to 9.00 of phosphate buffer (20 mM) was investigated (Fig. 3). The analyte was injected from the negative end. Selenate was more negative than selenite. No significant difference was observed for the effective charge between SeCys and SeMet when the pH was below 6.00 (Table 2). Since EOF is reversal, selenate is eluted the earliest among the analytes. Complete resolution was observed for the pair of selenate and selenite, but no resolution was achieved for SeCys and SeMet at pH 2.25 (Fig. 3a).

Increasing the pH led to a decrease of the protonated amino group on the bonded phase. This will yield a smaller EOF and a longer retention for the analytes. Contrary to the expected results, faster elution of the analytes – especially for the later eluted ions – was observed (Fig. 3b). Overlapping for the pair of selenate and selenite was also indicated. In addition, both electrostatic attraction and anion exchange with the bonded phase became evident as the pH increased (Table 2). That is, the overlapping for the pair of selenate and selenite was the result of the concomitant increase in electrophoretic mobility and the affinity with the bonded phase. While SeCys and SeMet carry positive charge and eventually approach neutral as pH increases, faster elution is in accordance with expected results.

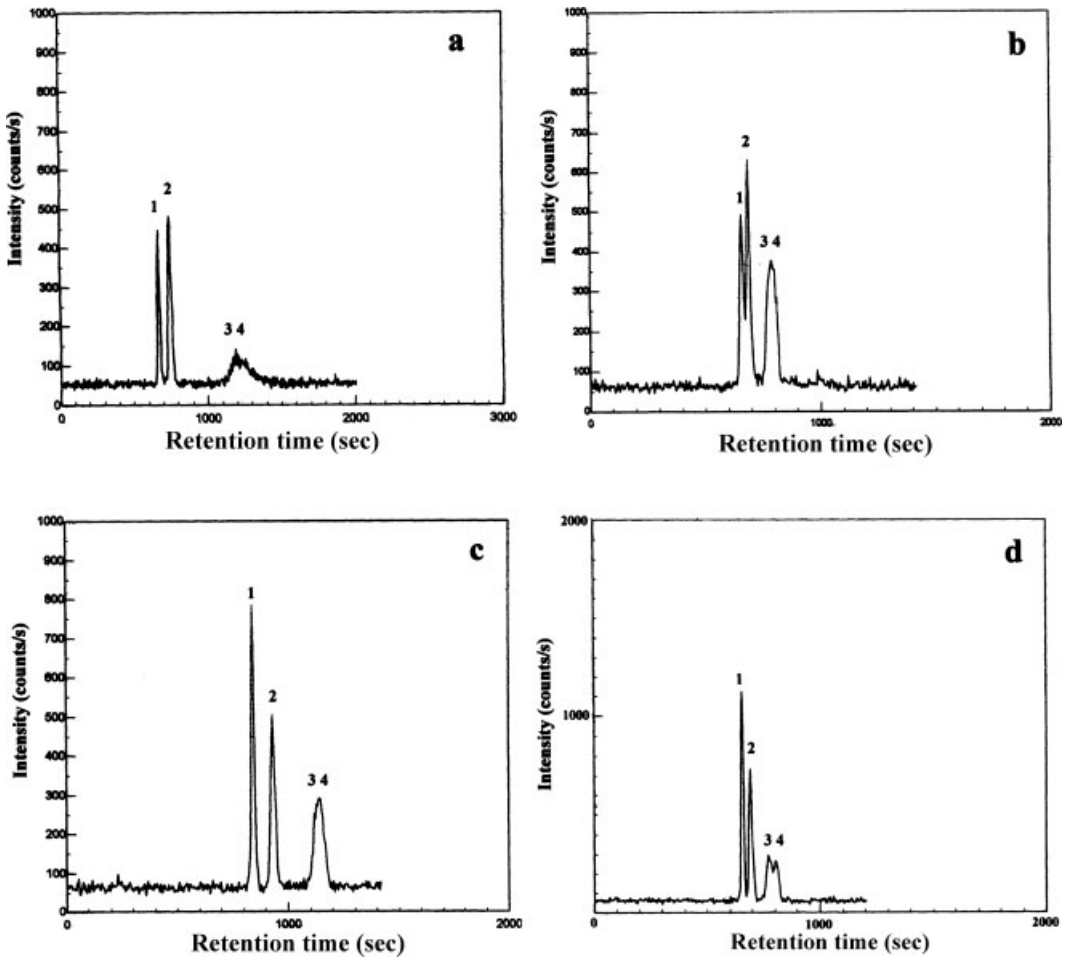
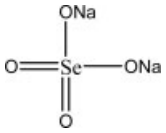
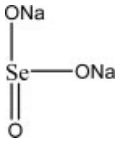
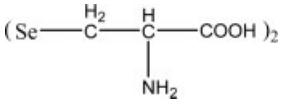
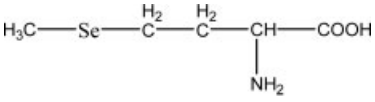


Figure 3. Electrochromatographic separation of selenium species with CEC–ICP-MS at different pH. Column: [28]ane-N_6O_2-bonded phase (100 μm id × 160 cm); sample injection: electrokinetic (−10 kV, 20 s) and nebulizer gas flow rate of 0.95 L/min; applied voltage: −15 kV; sample concentration: 1 μg/mL; rotation rate of peristaltic pump: 0.02 mL/min; mobile phase: phosphate buffer (20 mM) at pH (a) 2.25, (b) 4.00, (c) 6.00 and (d) 8.50. Peak id: 1, Se(VI); 2, Se(IV); 3, SeCys; 4, SeMet.

Table 2. Chemical and physical properties of selenium compounds and other related compounds studied in this work

Compounds	Formula	pK_a	Effective charge						
			pH 2.25	pH 4	pH 5	pH 6	pH 8	pH 8.5	pH 9
Selenate (Se(VI))		<1; 2	−1.61	−2	−2	−2	−2	−2	−2
Selenite (Se(IV))		2.6; 8.3	−0.33	−0.96	−1	−1	−1.33	−1.61	−1.83
Selenocystine (SeCys)		1 et 2.1 8.02 et 8.71	+0.46	+0.01	0	0	−0.65	−1.13	−1.57
Seleno-methionine (SeMet)		2.28; 9.21	+0.48	+0.02	0	0	−0.06	−0.16	−0.38
Phosphate		2.12; 7.20; 12.46	−0.58	−0.99	−1.01	−1.06	−1.86	−1.95	−1.98
Borate		9.23	0	0	0	0	−0.06	−0.16	−0.37
Carbonate		10.3	0	0	0	0	0	−0.02	−0.04
Tris		8.1	0	0	0	−0.01	−0.44	−0.72	−0.89
HEPES		7.5	0	0	0	−0.03	−0.76	−0.91	−0.97
MES		6.1	0	0	−0.07	−0.44	−0.99	−1	−1

pK_a values of analytes are from [41].

The elution profile at pH 6.00 (Fig. 3c) was different from that observed at lower pH levels. Considerably better resolution for selenate and selenite, but a longer retention time was indicated. Higher ionic strength made the elution slower. But beyond this pH, faster elution was seen again. A probable reason might be due to less interaction force with the bonded phase. In other words, the distinct inflection point at pH 6.00 could be a correlation with the protonation constant of the bonded phase [31].

Although less protonated amino groups on the bonded phase led to the decrease of EOF, most analytes had greater mobility as the pH increase except the fully dissociated selenate (Table 2). Better resolution for SeCys and SeMet was indicated at pH above 8.50 (Fig. 3d), but some overlapping was shown for Se(VI) and Se(IV) at pH 9.00. pH 8.50 was selected for subsequent experiments.

3.3 Effect of phosphate buffer concentration on separation efficiency

The influence of phosphate buffer concentration on separation was investigated at pH 8.50. Increasing buffer concentration would result in an increase of the retention time. However, only a slightly longer retention time was seen. The most probable reason is that sample retention is controlled not only by CEC mechanism but also by self-aspiration of the nebulizer. The resolution for SeCys and SeMet improved as the buffer concentration increased from 10 to 20 mM. Also, signals were found to increase. However, as it was further increased to 40 mM, the peaks of SeCys and SeMet were overlapping. The bonded macrocyclic polyamine is a good anion coordinator [26]. A possible explanation for this phenomenon is that the selenium species with carboxylate moiety have a higher affinity than phosphate to the bonded phase. The displacement reaction therefore occurred only at higher

concentration of phosphate ion. Based on these results, phosphate buffer of 20 mM was chosen as the optimum condition.

3.4 Effect of applied voltage on separation

The separation voltage was optimized for the purpose of this study. With phosphate buffer (pH 8.50, 20 mM), sample injection with electrokinetic mode (−10 kV, 20 s) coupled with the nebulizer gas flow rate of 1.02 L/min, peristaltic pumping rate of 0.02 mL/min, and applied voltage of −15 kV, four peaks were found although not completely resolved. After decreasing the applied voltage to −10 kV, only one broad peak was seen, which could be explained by slower motion resulting in longitudinal dispersion. Thus a higher voltage of −20 kV was applied. As a result, greater peak height was observed, but the mixture of Se(IV), SeCys and SeMet were coeluted as two peaks. In light of these results, we found −15 kV to be the optimum voltage.

3.5 Effect of organic buffer on separation efficiency

As described above, phosphate buffer (pH 8.50) was chosen as the optimal mobile phase but it was found to have poor reproducibility with decreased separation performance after long-term use. Therefore, other commonly used organic buffers such as Tris, HEPES and MES were used instead.

The order of the effective charge of these buffers (phosphate >MES >HEPES >Tris) (Table 2) can be used for the explanation of the retention affinity. Buffer with higher affinity towards the bonded phase displaced the analytes more easily. Therefore, the retention time decreased in the following order: Tris >HEPES >MES >phosphate. Results indicate that selenate and selenite can be completely separated by either phosphate or Tris buffer (Figs. 4a and b). Both Tris and MES buffer showed better differentiating ability for SeCys and

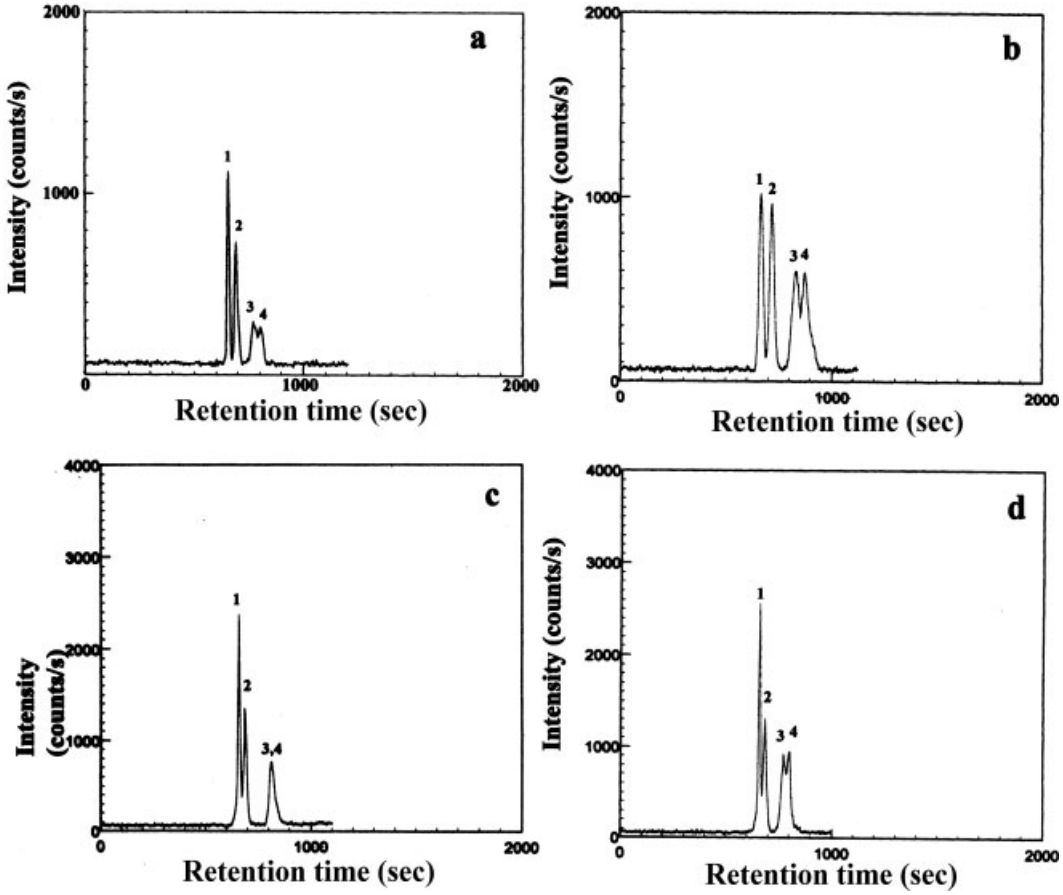


Figure 4. Electrochromatographic separation of selenium species with CEC–ICP-MS at different organic buffers. Conditions as in Fig. 3, except for the nebulizer gas flow rate of 1.02 L/min and mobile phase (20 mM, pH 8.50): (a) phosphate (for the comparison), (b) Tris, (c) HEPES and (d) MES. Peak identity: 1, Se(VI); 2, Se(IV); 3, SeCys; 4, SeMet.

SeMet (Figs. 4b and d). But no baseline separation for selenate and selenite was revealed under the latter condition. SeCys and SeMet was coeluted with the HEPES buffer (Fig. 4c). The probable reason for the best recognition of the bonded phase under Tris buffer might be due to more hydrogen bonding formation with it than the other buffers.

3.6 Effect of Tris buffer concentration on separation efficiency

By varying the concentration of Tris buffer (pH 8.50) from 20 to 50 mM, it was found that retention time increased slightly with increasing buffer concentration (Fig. 5). But, as the concentration increased to 75 mM, less retention time and greater peak height were observed (Fig. 5d). The phenomenon was similar to that in phosphate buffer, *i. e.*, Tris buffer was also a guest ion for the anion coordination with the bonded phase but a higher concentration of Tris buffer (75 *vs.* 40 mM) was necessary to get the comparable behavior. These results indicate that the affinity of phosphate to the bonded phase is stronger than that of Tris buffer. Therefore, we selected a mobile phase of 50 mM as the optimal condition.

3.7 Reproducibility of the established method

Repeatability data (%RSD) of different injection methods in the CEC–ICP-MS system are shown in Table 3. It was less than 1.38%, evaluated in terms of retention time for seven consecutive injections, while the RSD was less than 1.79% as the sample injection was attributed by the self-aspiration alone. In the latter case, retention time was slightly longer. This is rational, since separation was occurring simultaneously to sample injection.

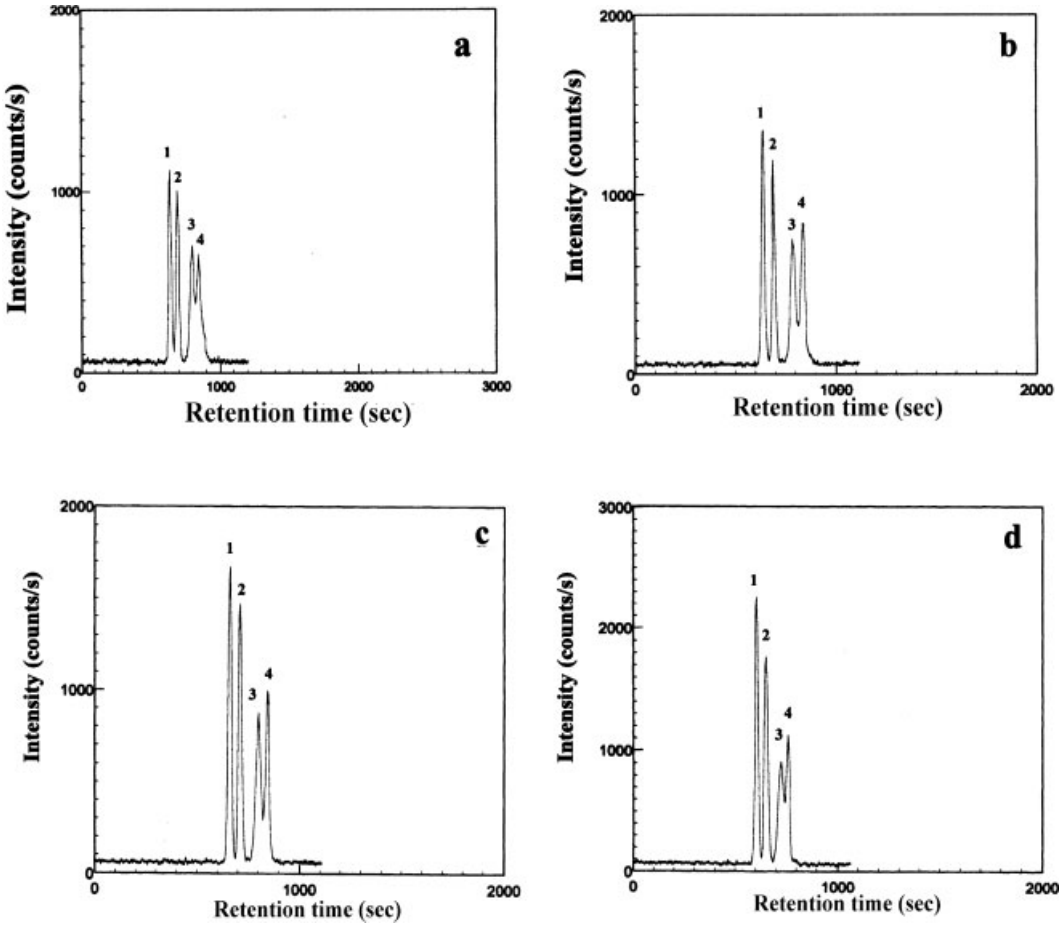


Figure 5. Electrochromatographic separation of selenium species with CEC–ICP-MS at different Tris buffer concentrations. Conditions as in Fig. 4, except for Tris buffer concentrations: (a) 20, (b) 30, (c) 50 and (d) 75 mM. Peak identity: 1, Se(VI); 2, Se(IV); 3, SeCys; 4, SeMet.

Table 3. Comparison the repeatability of different injection methods in the CEC–ICP-MS separation of selenium species[a)]

Analyte	Retention time (s)		Mobility (μ_{app}-cm^2/Vs)		RSD (%)	
	Electrokinetic[b)]	Self-aspiration[c)]	Electrokinetic	Self-aspiration	Electrokinetic	Self-aspiration
Se(VI)	662.27	708.77	2.58×10^{-3}	2.41×10^{-3}	1.38	1.42
Se(IV)	713.47	768.17	2.39×10^{-3}	2.22×10^{-3}	1.13	1.79
SeCys	804.70	869.11	2.12×10^{-3}	1.96×10^{-3}	0.82	1.60
SeMet	850.59	919.92	2.01×10^{-3}	1.86×10^{-3}	1.00	1.69

a) ICP-MS: nebulizer: crossflow nebulizer; nebulizer gas flow: 1.02 L/min; lens voltage: 6 V; ICP Rf power: 1200 W. CEC system: column: [28]ane-N_6O_2-bonded phase (160 cm × 100 μm id); BGE: Tris buffer (50 mM, pH 8.5); rotation rate of peristaltic pump: 0.02 mL/min; sample concentration: 1 μg/mL.
b) Sample injection: electrokinetic (−10 kV, 20 s) and nebulizer gas flow of 1.02 L/min, seven consecutive injections.
c) Sample injection: nebulizer gas flow (1.02 L/min, 20 s), seven consecutive injections.

3.8 Analytical application

The equations for the calibration graphs by CEC–ICP-MS analysis of mixed standard solutions of Se(IV), Se(VI), SeCys and SeMet are indicated in Table 4. Using optimized conditions, the detection limits evaluated by S/N 1 = 3 were found to be 2.40, 3.53, 12.86 and 11.25 ng/mL for Se(VI), Se(VI), SeCys and SeMet, respectively. After careful consideration of the CE–ICP-MS conditions for the analysis of selenium species [8, 21–23, 33–39], we note that the addition of surfactant to the BGE is often needed. In order to prove that the system was viable for practical analysis, a 50-fold dilution of urine sample was injected directly without any pretreatment, and a stable baseline was obtained (Fig. 6). No mass interference was indicated. With 250 ng/mL for each species spiked into the urine sample, complete separation of all analytes was

Table 4. Quantitation of selenium species with macrocyclic polyamine-bonded phase by CEC–ICP-MS[a)]

Analyte	Linear equation *x*, peak height/counts/s; *y*, concentration/ng/mL)			Detection limit (ng/mL)[b)]
	Slope	Intercept	r^2	
Se(VI)	0.8929	468.67	0.9950	2.40
Se(IV)	0.9598	318.04	0.9902	3.53
SeCys	0.6054	113.45	0.9985	12.86
SeMet	0.7384	83.64	0.9982	11.25

a) ICP-MS: as in Table 3. CEC system: column: [28]ane-N_6O_2-bonded phase (160 cm × 100 μm id); BGE: Tris buffer (50 mM, pH 8.5); sample injection: electrokinetic (−10 kV, 20 s) and nebulizer gas flow of 1.02 L/min; rotation rate of peristaltic pump: 0.02 mL/min; sample concentration: 75–1000 ng/mL.
b) S/N = 3.

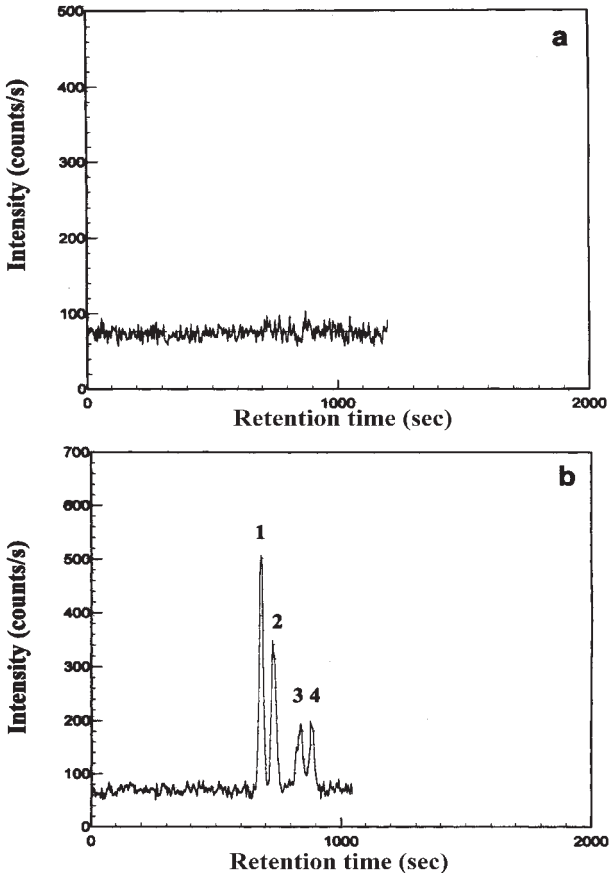


Figure 6. Electrochromatographic separation of selenium species spiked in urine with CEC–ICP-MS. Column: [28]ane-N_6O_2-bonded phase (100 μm id × 160 cm); sample injection: electrokinetic (−10 kV, 20 s), nebulizer gas flow rate of 1.02 L/min; rotation rate of peristaltic pump: 0.02 mL/min; mobile phase: Tris buffer (50 mM, pH 8.50); applied voltage: −15 kV. Peak identity: 1, Se(VI); 2, Se(IV); 3, SeCys; 4, SeMet. (a) Sample: 50-fold dilution of urine (blank). (b) Sample: 250 ng/mL selenium species spiked in 50-fold dilution of urine.

demonstrated. However, recovery of these species was 18% for Se(VI), 13% for Se(IV), 53.9% for SeCys and 63% for SeMet.

4 Concluding remarks

Anion coordination chemistry is a rapidly growing field. Macrocyclic polyamine, when protonated, binds a variety of organic as well as inorganic anions [40]. The protonation of the designed receptors can be carried out at neutral and even weak alkaline pH. For monitoring all relevant species including a mixture of anions of different oxidation state, cations as well as neutral species in a single run (Table 2), the macrocyclic polyamine-bonded phase was applied. The stationary phase was eminent as it can provide reversal EOF, anion coordination as well as anion exchange for the CEC separation. Compared with the conditions of CE–ICP-MS shown in the literature [8, 21–23, 33–39]), advantages of the method proposed in this study include: no need for expensive equipment, only a Tee and crossflow nebulizer serve as the interface, and no need for surfactant (which would affect the detection limit), as the mobile phase for the separation of neutral species. For high sensitivity and element-specific detection, as well as high selectivity of the bonded phase, quantitative analysis of selenium speciation in a complex matrix sample (such as urine) can be done directly without sample pretreatment.

The authors thank the National Science Council of Taiwan for financial support (grant No. NSC 94-2113-M-002-015).

5 References

[1] Michalke, B., *Electrophoresis* 2005, *26*, 1584–1597.

[2] Kannamkumarath, S. S., Wrobel, K., Wrobel, K., B'Hymer, C., Caruso, J. A., *J. Chromatogr. A* 2002, *975*, 245–266.

[3] Chamoun, J., Hagège, A., *J. Anal. At. Spectrom.* 2005, *20*, 1030–1034.

[4] Lu, Q., Bird, S. M., Barnes, R. M., *Anal. Chem.* 1995, *67*, 2949–2956.

[5] Olesik, J. W., Kinzer, J. A., Olesik, S. V., *Anal. Chem.* 1995, *67*, 1–12.

[6] Silva da Rocha, M., Soldado, A. B., Blanco-González, E., Sanz-Medel, A., *J. Anal. At. Spectrom.* 2000, *15*, 513–518.

[7] Lu, Q., Barnes, R. M., *Microchem. J.* 1996, *54*, 129–143.

[8] Kirlew, P. W., Castillano, M. T. M., Caruso, J. A., *Spectrochim. Acta B* 1998, *53*, 221–237.

[9] Majidi, V., Miller-Ihli, N. J., *Analyst* 1998, *123*, 803–808.

[10] Majidi, V., Qvarnström, J., Tu, Q., Frech, W., Thomassen, Y., *J. Anal. At. Spectrom.* 1999, *14*, 1933–1935.

[11] Baker, S. A., Miller-Ihli, N. J., *Appl. Spectrosc.* 1999, *53*, 471–478.

[12] Chen, W. H., Lin, S. Y., Liu, C. Y., *Anal. Chim. Acta* 2000, *410*, 25–35.

[13] Álvarez-Llamas, G., Fernández de laCampa, M. del R., Sanz-Medel, A., *Trends Anal. Chem.* 2005, *24*, 28–36.

[14] Kuczewski, B., Marquardt, C. M., Seibert, A., Geckeis, H., Kratz, J. V., Trautmann, N., *Anal. Chem.* 2003, *75*, 6769–6774.

[15] Wuilloud, R. G., Shah, M., Kannamkumarath, S. S., Altamirano, J. C., *Electrophoresis* 2005, *26*, 1598–1605.

[16] Gammelgaard, B., Bendahl, L., *J. Anal. At. Spectrom.* 2004, *19*, 135–142.

[17] Lee, T. H., Jiang, S. J., *Anal. Chim. Acta* 2000, *413*, 197–205.

[18] Lee, T. H., Jiang, S. J., *J. Anal. At. Spectrom.* 2005, *20*, 1270–1274.

[19] Michalke, B., *Spectroscopy* 2000, *15*, 30–33.

[20] Viñas, P., López-García, I., Merino-Meroño, B., Campillo, N., Hernández-Córdoba, M., *Anal. Chim. Acta* 2005, *535*, 49–56.

[21] Bendahl, L., Gammelgaard, B., Jøns, O., Farver, O., Hansen, S. H., *J. Anal. At. Spectrom.* 2001, *16*, 38–42.

[22] Mounicou, S., McSheehy, S., Szpunar, J., Potin-Gautier, M., Lobinski, R., *J. Anal. At. Spectrom.* 2002, *17*, 15–20.

[23] Casiot, C., Donard, O. F. X., Potin-Gautier, M., *Spectrochim. Acta B* 2002, *57*, 173–187.

[24] Bendahl, L., Gammelgaard, B., *J. Anal. At. Spectrom.* 2004, *19*, 143–148.

[25] Hsu., J. C., Chen, W. H., Liu, C. Y., *Analyst* 1997, *122*, 1393–1398.

[26] Liu, C. Y., Chen, W. H., *J. Chromatogr. A* 1998, *815*, 251–263.

[27] Chen, W. H., Liu, C. Y., *J. Chromatogr. A* 1999, *848*, 401–416.

[28] Lin, S. Y., Chen, W. H., Liu, C. Y., *Electrophoresis* 2002, *23*, 1230–1238.

[29] Lin, S. Y., Liu, C. Y., *Electrophoresis* 2003, *24*, 2973–2982.

[30] Chen, W. H., Lin, C. C., Chen, T. S., Misra, T. K., Liu, C. Y., *Electrophoresis* 2003, *24*, 970–977.

[31] Lin, S. Y., Liu, C. Y., *J. Incl. Phenom. Macrocycl. Chem.* 2004, *48*, 103–109.

[32] Kinzer, J. A., Olesik, J. W., Olesik, S. V., *Anal. Chem.* 1996, *68*, 3250–3257.

[33] Liu, Y., Lopez-Avila, V., Zhu, J. J., Wiederin, D. R., Beckert, W. F., *Anal. Chem.* 1995, *67*, 2020–2025.

[34] Michalke, B., Schramel, P., *J. Chromatogr. A* 1996, *750*, 51–62.

[35] Magnuson, M. L., Creed, J. T., Brockhoff, C. A., *Analyst* 1997, *122*, 1057–1061.

[36] Michalke, B., Schramel, P., *J. Chromatogr. A* 1998, *807*, 71–80.

[37] Prange, A., Schaumlöffel, D., *J. Anal. At. Spectrom.* 1999, *14*, 1329–1332.

[38] Michalke, B., Schramel, O., Kettrup, A., *Fresenius' J. Anal. Chem.* 1999, *363*, 456–459.

[39] Day, J. A., Kannamkumarath, S. S., Yanes, E. G., Montes-Bayón, M., Caruso, J. A., *J. Anal. At. Spectrom.* 2002, *17*, 27–31.

[40] Potvin, P. G., Lehn, J. M., in: Izatt, R. M., Christensen, J. J. (Eds.), *Synthesis of Macrocycles: The Design of Selective Complexing Agents*, John Wiley and Sons, New York 1987.

[41] Albert, M., Demesmay, C., Rocca, J. L., *Fresenius' J. Anal. Chem.* 1995, *351*, 426–432.

