The Analyst

Speciation of selenium in aqueous and biological matrices by microbore ion chromatography coupled with electrothermal atomic absorption spectrometry *via* ultra low volume fraction collection†

Håkan Emteborg*‡, Guy Bordin and Adela R. Rodriguez

European Commission—Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium

A robust, user-friendly, sensitive and affordable speciation method based on microbore anion-exchange chromatography (IC) for the separation of selenium species and Zeeman-effect ETAAS for element specific detection is described. By exploiting the very low flow rates normally employed in microbore chromatography, the analytical usefulness of chromatography coupled to the sensitive but discontinuous ETAAS detector has been increased in comparison with couplings incorporating normal bore liquid chromatography. The flow rate of the mobile phase in this particular IC system was 80 µl min^{-1}. A highly reproducible and automated collection of 20 µl fractions in sampler cups was used for interfacing the chromatographic separation with ETAAS. The IC–ETAAS results were also directly compared with those obtained by IC-direct injection nebulizer ICP-AES for assessing the chromatographic resolution of the proposed method. It is possible to separate selenomethionine, selenite, selenate and selenocystine in 6 min. The trade-off in chromatographic resolution and time consumption in the detection step using IC–ETAAS is compensated for by a high degree of simplicity and the high specificity and sensitivity of the Zeeman-effect ETAAS resulting in relative detection limits of 2.8–4.1 ng ml^{-1} (42–61 pg absolute). These detection limits are comparable to those for published HPLC–ICP-MS methods. The method was evaluated by injecting aqueous standards, unspiked and spiked sample extracts from the biological certified reference material CRM 402. The eluent was injected along with a palladium–magnesium nitrate modifier since the pyrolysis curves for trimethylselenonium, selenomethionine, selenite, selenate and selenocystine revealed a similar response for all species by using this modifier, *i.e.* all species are thermally stabilized up to 1000 °C. Without adding the modifier, the species showed very different volatilities during the thermal pre-treatment step.

Keywords: *Speciation; selenium; microbore ion chromatography; electrothermal atomic absorption spectrometry; discontinuous detection mode*

Selenium is an essential trace element exhibiting a complex impact on humans and animals depending on the concentrations, too low an intake can lead to various deficiency syndromes whereas too high an intake is toxic or even lethal.[1] Selenium exists in many different chemical forms in the environment and biota such as the inorganic oxyanions, selenite and selenate and the organic selenoamino acids, selenomethionine and selenocystine. Trimethylselenonium is another important species since it may be found in urine and is consequently a metabolite of selenium.[1] For more detailed information concerning analytical methods for speciation of selenium, readers may find useful information in three comprehensive reviews by Kölbl *et al.*[2], Cámara *et al.*[3] and Muñoz Olivas *et al.*[4]

ICP-MS demonstrates very high sensitivity for most elements but there are exceptions. Selenium is one of those elements because of significant isobaric overlaps on its most abundant isotopes (^{78}Se, ^{80}Se), rendering ^{82}Se the most suitable isotope, having an abundance of only 9%.[5] Moreover, a high ionization energy (9.75 eV) also reduces the sensitivity compared with other elements. Less costly techniques such as ETAAS have absolute detection limits for selenium close to those with ICP-MS, *i.e.*, approximately 10–20 pg. Since this instrumentation is more widespread, development of a speciation method for selenium using ETAAS would give more laboratories the possibility of performing speciation work at a reasonable cost. By constructing a special interface and with continuous heating of the furnace, on-line detection of tin species has been demonstrated.[6] This approach, however, calls for special equipment which is not readily available. A number of papers have indeed been published describing selenium speciation by coupling normal bore chromatography to ETAAS without continuous heating of the furnace.[7–13] In those studies, two to five selenium species were separated on various normal bore columns with separation times ranging from 10 to 35 min. The reported detection limits are from 1.2 to 50 ng ml^{-1} for the investigated selenium species.[7–13] Possibilities and limitations in general of using ETAAS for detection in chromatography have been briefly summarized by Laborda *et al.*[8] Interference from other anions such as chloride, phosphate and sulfate on the separation of selenite and selenate was studied by Chakraborti *et al.*[7] and the element specific ETAAS detector was found to be superior to the general conductivity detector for obvious reasons. It should be noted that an excess of other anions did not disturb the chromatographic separation of selenite and selenate.[7]

With growing interest in elemental speciation at trace levels in real samples, it is also important to realize that many analytical techniques that have been developed for speciation are based on very expensive and sophisticated combinations of instrumentation such as HPLC coupled with ICP-AES,[14–16] ICP-MS[17–22] or even high resolution ICP-MS.[23] On the one hand, this is obviously in line with the analytical challenge that this field offers, but on the other, few less expensive and uncomplicated analytical methods have been presented. The desire to use ICP-MS is mainly associated with the on-line detection capability suitable for coupling with chromatography and the high sensitivity for most elements. Another aspect of

† Presented at The Third International Symposium on Speciation of Elements in Toxicology and in Environmental and Biological Sciences, Port Douglas, Australia, September 15–19, 1997.

‡ Present address: ABB Corporate Research, 721 78 Västerås, Sweden. E-mail: hakan.emteborg@secrc.abb.se

developing mainly very expensive and sophisticated analytical methods for speciation is that the time to transfer this concept from the research community to routine laboratories, legislators and industry is slowed. This is unfortunate since it is generally agreed that species determinations give a much better possibility of assessing the impact of an element in vital areas such as nutrition, environment, health and working sectors than does a total determination of an element. It is consequently of importance to develop rapid, sensitive, less expensive and less complex analytical instrumental set-ups. This would allow and speed up the transfer of the speciation concept to a wider range of laboratories. The possibility of comparing and assessing simpler procedures with more sophisticated analytical instrumentation is thus already possible. This paper describes one approach to realizing such an objective, namely by coupling microbore ion chromatography (IC) to ETAAS for the speciation of selenium. The IC–ETAAS method was compared with on-line detection using IC–direct injection nebulizer (DIN) ICP-AES, for which results have been reported in a previous paper.[24]

Experimental

Instrumentation

The IC system consisted of an inert [all wetted parts metal-free and made from poly(ether ether ketone), (PEEK)] GP-40 gradient HPLC pump in a microbore version (Dionex, Sunnyvale, CA, USA) which was operated in flow control mode at a flow rate of 80 μl min^{-1}. Initially the accuracy of the actual flow rate delivered compared with the instrumental setting of the pump was assessed by weighing the effluent. At this stage the pump was recalibrated following the instructions given by the manufacturer. Two 250 × 2 mm id dummy columns (not shown in Fig. 1) were installed in series prior to the injection valve in order to build up a back-pressure of approximately 125 bar for obtaining optimum operating conditions for the pump. The HPLC pump incorporated an internal vacuum de-gas module and eluent flasks pressurized with helium for degassing of the eluent. An electrically actuated inert valve internally made from PEEK, which was purchased as a part of a direct injection nebulizer system (Cetac, Omaha, NE, USA), was connected to the pump and the column as depicted in Fig. 1 using PEEK tubing. The valve was equipped with a 15 μl loop and was loaded by using a 1 ml plastic syringe with a Luer connection.

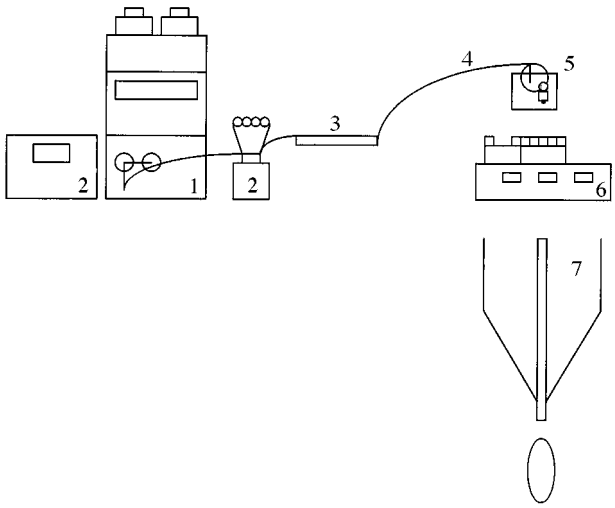

Fig. 1 Instrumental set-up of IC fraction collection system: 1, microbore HPLC pump; 2, inert valve with 15 μl loop and control unit; 3, separation column, 50 × 2 mm id PAX 100; 4, fused-silica capillary, 50 μm id, coated with PTFE, 1.6 mm od; 5, orifice of capillary and fraction collector in drop counting mode, circle indicates blow-up of orifice depicted below; 6, fraction collector and sampler cups; 7, blow-up of capillary orifice consisting of fused-silica and PTFE.

Two different separation columns made from PEEK were used, namely PAX-100 and Ion Pac AG-10, 50×2 mm id (Dionex), originally intended to be guard columns. Both stationary phases are compatible with organic solvents and pH values ranging from 0 to 14. The eluent must always contain at least 1% of organic modifier as the stationary phase otherwise would be destroyed. A Retriever II fraction collector, from ISCO (Lincoln, NE, USA) was used for collecting 20 μl fractions of the eluent from the IC system in a highly reproducible manner (*i.e.*, a time resolution of 15 s). The fraction collector could be operated in the drop counting mode based on a light path being blocked as soon as a droplet fell from the orifice. The high reproducibility achieved for the collection of such low volumes was effected by counting seven drops emerging from the tip of a 50 μm id fused-silica capillary contained in and protected by 1.6 mm od PTFE tubing obtained from Cetac. Initially, the tubing and capillary were cut perpendicular using a razor blade and the PTFE tubing surrounding the capillary was cut away to allow the fused-silica capillary to protrude and form the tip as depicted in Fig. 1. This was found to be necessary in order to minimize the size of the droplets emerging from the IC system, which led to a more reproducible fraction collection. (Accurate counting of the number of drops and the drop size of the seven small droplets was much more reproducible than collection of one large droplet, an approach tried in initial experiments.)

The 20 μl portions were collected in this manner in sampler cups, which were then transferred to an AS-70 autosampler carousel, which was part of a Perkin-Elmer (Überlingen, Germany) Model 5100 ZL ETAAS system, for the detection of selenium. The PE 5100 ETAAS system incorporates the concept of transversely heated pyrolytic graphite-coated platform-equipped graphite tubes and incorporates Zeeman effect background correction. An electrodeless discharge lamp (EDL) for selenium was used as an element specific line source at 196 nm and operated at 215 mA in the pulsed mode using a Perkin-Elmer System 2 EDL power supply. The sample volume injected was 15 μl, which was injected along with modifiers: 5 μl of 1000 mg l^{-1} Pd solution (5 μg) and 6 μl of 500 mg l^{-1} $Mg(NO_3)_2$ solution (3 μg) respectively. Since 15 μl or 75% of the sample volume collected in the cup was injected into the furnace, the autosampler had to be carefully adjusted. Water purified with a Milli-Q system (Millipore, Bedford, MA, USA) was added to cover the bottom of the carousel in order to decrease evaporation from the samples. Other operating conditions for ETAAS are given in Table 1.

Table 1 Instrumental conditions for the determination of selenium by ETAAS

Electrothermal atomizer—

Step No.	Temperature/°C	Ramp/s	Hold/s	Int. flow	Read
Dry 1	110	1	20	250	—
Dry 2	130	2	35	250	—
Pyrolysis	1000–1150	7	20	250	—
Cool	20	1	5	250	—
Atomize	1900	0	5–8	0	On
Clean-out	2400	1	4	250	—

Spectrometer—

Wavelength/nm	196
Spectral bandwidth/nm	2
Background correction	On (longitudinal Zeeman effect)
Signal evaluation mode	Integrated absorbance

Reagents and materials

Stock standard solutions of selenite (88 mg l^{-1}) (sodium selenite, *pro analysi* quality, Merck, Darmstadt, Germany), selenate (101 mg l^{-1}) (sodium selenate, AnalaR, BDH, Poole, Dorset, UK), seleno-D,L-methionine (93 mg l^{-1}) (Sigma, St. Louis, MO, USA), seleno-D,L-cystine (94 mg l^{-1}) (Sigma) and trimethylselenonium iodide (107 mg l^{-1}) (Tri Chemical Laboratory, Kanagawa, Japan, purity > 98%) were prepared gravimetrically by dissolving the compounds in Milli-Q Plus 185 quality water obtained from a Milli-Q apparatus (all concentrations are expressed as selenium). All standard solutions were stored in Pyrex glass vessels at 4 °C. The 15 mmol l^{-1} ammonium carbonate eluent (pH 10 in 2% methanol) was prepared as described earlier.[24] Magnesium nitrate and palladium nitrate modifier stock standard solutions were obtained from Perkin-Elmer and were diluted with Milli-Q water to the required concentrations. The certified reference material CRM 402 was used for evaluating the applicability of the method to real samples. CRM 402 is a lyophilized powder from white clover grown on selenium rich soil and was obtained from BCR (IRMM, Geel, Belgium). The chemicals used for extraction using a method denoted EM-2C have also been described previously.[24]

Extraction and clean-up procedures

In previous work, the extraction efficiency of selenium from the certified reference material CRM 402 was found to be highest (*i.e.*, close to 50%) using a method based on two cycles of an ultrasonic bath and methanol–water (1 + 1) containing 4% of ammonia.[24] This method for extraction was therefore used throughout the present work. Approximately 0.5 g of CRM 402 (further ground using a pestle and mortar) was placed in a 100 ml conical glass flask and 10 ml of Milli-Q water–methanol (1 + 1) containing 4% of ammonia used for extraction was added. The extracts from each sonication or the combined extracts from the repeated extractions were then centrifuged at 5000 rpm for 5 min and filtered through a 0.45 μm pore size filter. Prior to clean-up using the method described earlier,[24] the ammonia and methanol were evaporated in a rotary evaporator (IKA Labor Technik, Janke-Kunkel, Vel, Germany) and the sample was diluted to its initial volume with Milli-Q water. When using the IC-ETAAS method, no further reductions of the volume were found to be necessary since the high sensitivity allows the use of more dilute samples, avoiding impaired chromatographic performance. Results for other extraction methods, the exact procedure and the subsequent extraction efficiencies have been reported elsewhere.[24]

Results and discussion

Unless constant heating of the furnace is employed, the ETAAS system must be used in a cyclic discontinuous mode. The nature of the ETAAS detector thus stands in contrast with the constant flow of eluent (or sample) eluting from the column. Two different approaches have been adopted for solving this problem, namely fraction collection (survey mode)[8,13] or well systems (pulse mode).[7–12] Using pulse mode interfaces, the eluent is injected at regular intervals, normally employing fast furnace programs and hot injections to increase the sample throughput (*i.e.*, the chromatographic resolution).[7–12] The complexity of this system is higher than that of the simpler survey mode of collection and analysis of fractions.[8,13] The time for analysis and obtaining a chromatogram using ETAAS becomes longer than that in on-line detection modes owing to cycles of 60–90 s per data point using pulse mode interfaces or the need for a large number of fractions to be analysed. A compromise must be reached between the overall separation time, chromatographic resolution and the sampling frequency of the detector, which is very low for pulse mode interfaces. In the survey mode, a higher sampling frequency is possible, but in that case a large number of fractions needs to be collected.

The flow rates normally employed in normal bore HPLC (4.6 mm id column) are 0.4–1.0 ml min^{-1}, compared with the 10–40 μl sample volumes injected in ETAAS. Since flow rates between 80 and 120 μl min^{-1} (or less) can be accurately delivered using the latest HPLC pump technology, the total volume of eluent can be reduced 10-fold compared with normal bore systems. The microbore columns also have other inherent advantages, namely higher mass sensitivity and resolution, as discussed by Garraud *et al.*[25] Lower sample volumes are also required in microbore chromatography (2–15 μl), making analysis of special (*e.g.*, clinical) samples possible. By employing the survey mode, it is clear that the ratio between the volume of a fraction collected from the chromatographic system and the volume injected into the ETAAS system should be as close to unity as possible for obtaining maximum sensitivity.[8] This situation is much more easily achieved using microbore chromatography at a low flow rate than using a normal bore system. The low flow rate also makes it possible to keep the number of fractions reasonably low, provided that a short and efficient separation of the species of interest is possible. In this work, four selenium species can be chromatographically resolved and determined at 2.8–4.1 ng ml^{-1} by determining selenium in 24 fractions of 20 μl obtained by automated collection of the eluent coming from a short isocratic run of only 6 min.

Pyrolysis curves

It was desired that the ETAAS system should have the same response for similar concentrations of each species eluting from the chromatographic column. In such a case, only one calibration curve for all species can ideally be used and there is a better chance that unknown selenium species identified in sample extracts will be accurately determined. This important detail of using ETAAS for detection in chromatography has not been thoroughly discussed in previous publications.[7–13] It is also desirable to use a regular pyrolysis step since the matrix introduced from the extract and the eluent might influence the thermal stability of different selenium species. The use of various modifiers for different selenium species has been evaluated in a number of studies since it is well known that different selenium species exhibit very differing volatilities during thermal pre-treatment.[26–32] Palladium–magnesium nitrate is undoubtedly one of the more successful modifier combinations for stabilizing selenium and was therefore used in this work.[27–32] The thermal behaviour of trimethylselenonium, selenomethionine, selenite, selenate and selenocystine with and without addition of modifier for pyrolysis temperatures ranging from 130 to 1500 °C was therefore investigated and the results are displayed in Fig. 2(*a*) and 2(*b*), respectively. As can be seen in Fig. 2(*b*), all five selenium species are equally stabilized, giving a close to uniform response upon addition of the palladium–magnesium nitrate modifier.

Study of characteristic mass for different selenium species

All matrix components introduced into the graphite furnace might influence the formation of free selenium atoms from the various species. Since a 15 mmol l^{-1} ammonium carbonate in 2% methanol eluent is used, it is important to verify that the eluent itself does not cause a signal depression, as occurred with high concentrations of the citrate-based eluent used by Laborda *et al.*[8] The results for characteristic mass values for selenomethionine, selenite, selenate and selenocystine in Milli-Q water and the eluent are reported in Table 2. As can be seen, no signal depression is observed in the eluent.

Figures of merit and analytical characteristics of the IC–ETAAS system

Having established that the response for each species was similar by using the palladium–magnesium nitrate modifier and that the eluent did not impair detection, evaluation of injections of mixtures of selenium species in aqueous solutions into the IC–ETAAS system was undertaken. As described earlier,[24] the use of a 50 × 2 mm id PAX 100 column and a flow rate of 80 μl min^{-1} is sufficient for separating selenomethionine, selenite, selenate and selenocystine within 5 min.[24] Whenever the PAX 100 column is used, selenocystine must be eluted using 0.1 mol l^{-1} nitric acid. The reasons for this have been outlined in previous work.[24] If trimethylselenonium is included or expected to be present in a real sample, a similar column packed with AG-10 must be used in order to separate trimethylselenonium from selenomethionine.

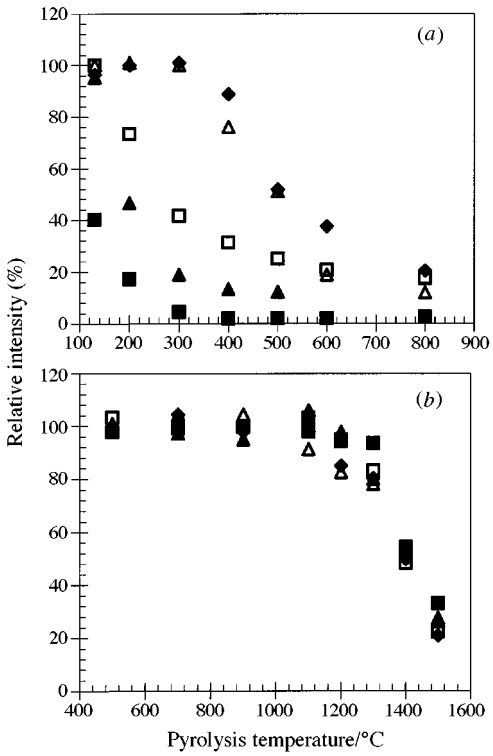

Fig. 2 (*a*) Pyrolysis curves without addition of modifier for selenate (Δ), selenite (◆), selenocystine (□), selenomethionine (▲) and trimethylselenonium (■). (*b*) Pyrolysis curves for the same five selenium species with addition of modifier: Pd (5 μg) and $Mg(NO_3)_2$ (3 μg).

Table 2 Characteristic mass values for different selenium species in Milli-Q water and eluent. Values obtained using Pd–$Mg(NO_3)_2$ modifier and the STPF concept[26]

	Characteristic mass, m_0/pg Se*	
Species	Milli-Q water	Eluent†
Selenomethionine	44	45
Selenite	48	40
Selenate	43	39
Selenocystine	43	44

* Values obtained using ETAAS only. † Eluent composition: 15 mmol l^{-1} ammonium carbonate, pH 10, in 2% methanol.

Calibration curves were obtained for selenomethionine, selenite, selenate and selenocystine, for which data are displayed in Table 3. Since the signal obtained from ETAAS is normally the integrated (peak area) absorbance for selenium in the analysed fraction, the signal intensity for each species and concentration level is equal to the sum of peak areas measured in the set of fractions where each selenium species was detected. The retention order, peak shapes and retention times were known *a priori* since the separation method was developed using on-line detection IC–DIN-ICP-AES as displayed in Fig. 3(*a*).[24] An IC–ETAAS chromatogram [Fig. 3(*b*)] and the volume collected in each fraction [Fig. 3(*c*)], as determined by weighing, are displayed for direct comparison with the IC–DIN-ICP-AES chromatogram. (A density of 1 g ml^{-1} was used for calculating the volume of the eluent.) The average volume per fraction was 19.7 ± 0.3 μl (n = 24), which corresponds to a 1.5% RSD. The fractions in which each selenium species normally was detected are reported in Table 3 based on the highly reproducible appearance of each species in a particular set of fractions as shown in Fig. 4. The calculation of the peak area for each species in an IC–ETAAS chromatogram was performed manually using a column chart such as that displayed in Fig. 3(*b*). By subtracting the average blank signal from the signal obtained in relevant fractions, the peak area signals were then summed. (Note that the abscissa is, ideally, a constant, allowing summing of the integrated absorbances.)

The slopes of the calibration curves, displayed in Table 3, are similar for selenite, selenate and selenocystine, resulting in similar detection limits for these three species, as can be expected from the features of the ash curves with addition of modifier. Selenomethionine had a lower response, which can be explained by deliberately omitting the last fraction containing selenomethionine when summing the integrated absorbances. This was done in order to avoid over-estimation since selenomethionine and selenite elute close together, as can be seen in Fig. 3. The detectability of trimethylselenonium is as high as that of the former three species since the response was characterized using ETAAS and the chromatographic properties are well known from the previous study.[24] The detection limit was calculated as three times the standard deviation of a blank divided by the peak height slope for the response for each

Table 3 Figures of merit for the IC–ETAAS system

Se species	Detection limit (3σ criterion)/ng ml^{-1}	Slope ± 95% confidence intervals, Σ absorbance (1×10^{-3}) s^{-1}/ng ml^{-1}	r*	Retention time/s (fraction nos.†)	Repeatability, peak area RSD (%) (n = 4)‡
Selenomethionine, A	4.1	0.72 ± 0.05*	0.998	75 (4–7)	4.3
Selenite, B	3.1	0.96 ± 0.12*	0.994	150 (9–11)	4.8
Selenate, C	2.8	1.08 ± 0.02*	0.999	270 (16–19)	5.5
Selenocystine, D	3.2, 0.4§	0.94 ± 0.10*	0.995	330 (22, 23)	6.2

* Regression data for the four selenium species are based on six concentration levels in the following intervals: selenomethione, 8.4–88.2 ng ml^{-1}; selenite, 8.5–90.9 ng ml^{-1}; selenate, 9.3–97.7 ng ml^{-1}; and selenocystine, 7.3–76.9 ng ml^{-1}. † Fraction number indicates in which fractions each species were found with fractions ranging from 1 to 24; see also Fig. 4. ‡ Data obtained for standards containing 40–51 ng ml^{-1} of selenium species. § Following on-line enrichment as described in earlier work.[24]

species. The peak height slope is not reported but was essentially identical with the integrated (peak area) response from the ETAAS detector. The standard deviation of the blank was similar for a number of chromatographic runs of blanks, *i.e.*, 0.001–0.0015 absorbance. The detection limits reported here are similar to the best values reported for coupled normal bore chromatography–ETAAS[8,9,13] and approximately 10 times higher than the lowest values reported for chromatography coupled to ICP-MS[18,21] and high-resolution ICP-MS.[23] Other studies involving HPLC coupled to ICP-MS achieved detection limits comparable to or higher than those with this system.[17,19,20,22] Also note the possibility of the on-line enrichment of selenocystine, thereby lowering the possible detection limit below 0.5 ng ml^{-1} for this species.

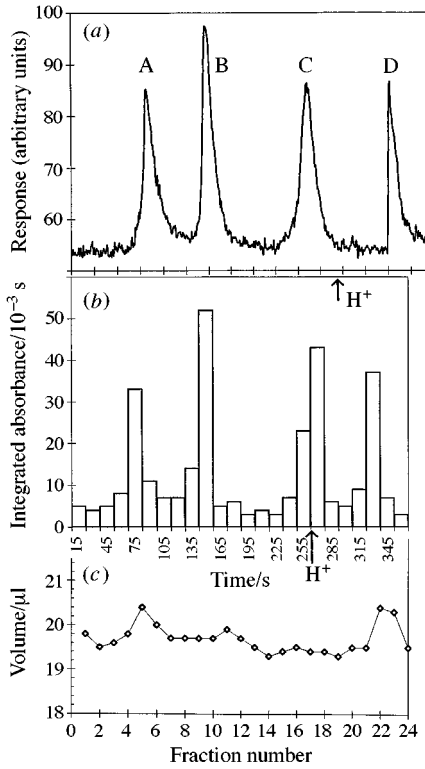

Fig. 3 (*a*) IC–DIN-ICP-AES chromatogram of a 15 μl injection of a mixed standard containing 500 ng ml^{-1} of selenomethionine (A), 600 ng ml^{-1} of selenite (B), 650 ng ml^{-1} of selenate (C) and 510 ng ml^{-1} of selenocystine (D). Selenocystine (D) was eluted by injection of 15 μl of 0.1 mol l^{-1} of HNO_3 after 295 s as indicated by the arrow. (*b*) IC–ETAAS chromatogram based on selenium analysis in 24 fractions of 20 μl following a 15 μl injection into the IC system of a mixed standard containing 46 ng ml^{-1} of A, 47 ng ml^{-1} of B, 54 ng ml^{-1} of C and 42 ng ml^{-1} of D. D was eluted by acid injection after 270 s as indicated by the arrow. (*c*) Volume collected in each fraction as determined by weighing.

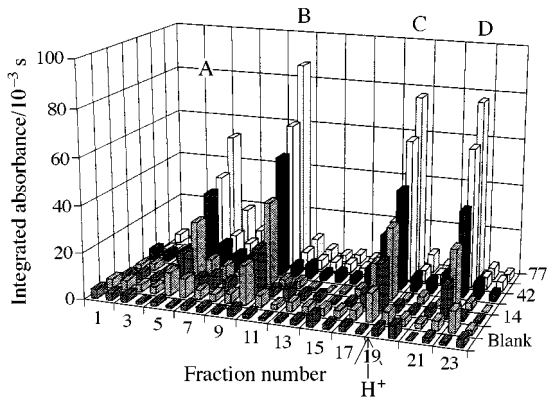

Fig. 4 Seven IC–ETAAS chromatograms obtained by injection of a blank and six mixed standards containing selenomethionine (A), selenite (B), selenate (C) and selenocystine (D), in the concentration intervals given in the footnotes to Table 3. Note that the response as a function of fraction number is displayed where each fraction corresponds to 15 s. The axis to the right indicates the concentrations of selenocystine. Dilute HNO_3 was injected after 270 s for elution of D as indicated by an arrow.

The correlation coefficient is very high for the calibration data obtained for the investigated species and the repeatability for summed peak area measurements was 4–6% (RSD) for concentrations ranging from 40 to 51 ng ml^{-1}, as indicated in the last column of Table 3. Note that this uncertainty reflects the small fluctuations in the volume collected from the IC system [as displayed in Fig. 3(*c*), 1.5% RSD], evaporation losses of eluent from the sampler cups while standing in the carousel, slight drift in the retention times, bias in the way the peak areas have been summed, variations in the sensitivity of the ETAAS and imprecisions in the volume injected. Since the eluted selenium species were reproducibly contained in the same 2–4 sampler cups (in a series from cup 1 to 24) from one chromatogram to another (see Fig. 4), the otherwise rather long detection step (approximately 50 min) can be shortened by analysing only the fractions of interest if desired.

Analytical application of IC–ETAAS

As mentioned previously, an extraction method based on two repeated extractions involving an ultrasonic bath and methanol–water (1 + 1) in 4% ammonia was used for liberating selenium species from a lyophilized white clover material (CRM 402). Note that in this context substantial information has been reported in previous work using IC–DIN-ICP-AES.[24] The IC–ETAAS chromatograms in Fig. 5 are for an extract from CRM 402 and the same material spiked prior to extraction with two concentration levels of selenomethionine, selenite, selenate and selenocystine. A different way of displaying the IC–ETAAS chromatograms was chosen in Fig. 5 compared with those presented in Figs. 3(*b*) and (4), since this representation makes it possible to see differences between overlaid chromatograms more easily, although it might be more correct to display the data in a column or a bar chart.

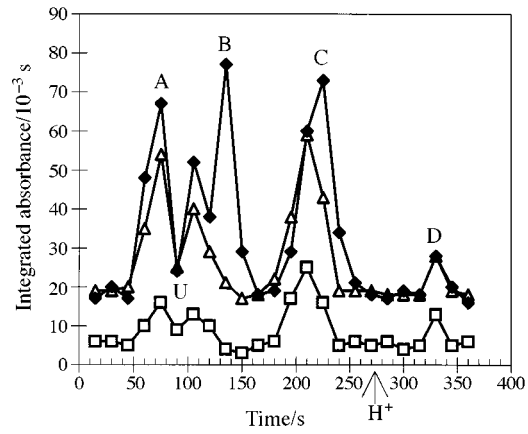

Fig. 5 IC–ETAAS chromatograms of injection of unspiked extract (□) and extracts from CRM 402 spiked with two concentration levels prior to extraction (Δ,◆). The first concentration level (Δ) contained 45 ng ml^{-1} of selenomethionine (A), 46 ng ml^{-1} of selenite (B), 50 ng ml^{-1} of selenate (C) and 40 ng ml^{-1} of selenocystine (D). The second concentration level (◆) contained 88 ng ml^{-1} of A, 90 ng ml^{-1} of B, 97 ng ml^{-1} of C and 76 ng ml^{-1} of D. U denotes unknown selenium species in the unspiked extract. The chromatograms for spiked extracts (Δ,◆) have been displaced by 0.015 absorbance for clarity. See the discussion in earlier work concerning the behaviour of selenocystine (D) in the spiked extracts.[24]

The applicability of the proposed method for determining selenium species in biological extracts is demonstrated by the identification of at least one selenium species in CRM 402 and the discussion will be limited to selenate, peak C. A discussion concerning the other peaks and the indication of breakdown of added selenocystine can be found elsewhere.[24] The presence of selenate (peak C) in the plant extract can clearly be seen in Fig. 5 together with unknown species (U) and a small peak for selenocystine (D). It can also be seen in Fig. 5 that the selenate added to the extract is reproducibly found in the same fractions as for the incipient selenate. It should be mentioned that these chromatograms correspond to injections of extracts from the first cycle of the EM-2C extraction as described in earlier work.[24] No matrix effect could be observed with respect to the detection capability (*i.e.*, no sensitivity loss): a slight shift in retention time was observed, which is in agreement with results obtained using IC–DIN-ICP-AES.[24] The response for selenate was 70% of the slope reported in Table 3. This does not mean that the detection capability was lower in the extract since the remaining 30% of the selenate was recovered in the second cycle of the extraction step, *i.e*, 100% recovery of added selenate. The content of selenate in CRM 402 using EM-2C for extraction was determined as 1.6 μg g^{-1} by standard additions and using IC–ETAAS compared with the certified total concentration of selenium of 6.7 μg g^{-1}.

Other species identified in extracts from CRM 402 and comparisons with earlier studies using this material[10,33] have been discussed extensively elsewhere.[24] It should be mentioned that the presence of selenate as the dominant selenium species extractable from CRM 402 has also been confirmed by Alsing-Pedersen and Larsen.[33]

Conclusion

The proposed microbore IC–ETAAS method exhibits a high chromatographic resolution in a short separation time (6 min) and with very low absolute detection limits (42–61 pg) compared with normal bore methods developed earlier. The relative detection limits are comparable to those with HPLC coupled to ICP-MS. The instrumental system described is, moreover, robust, extremely simple to use and allows extracts from biological matrices to be chromatographed and analysed for their content of selenium species. One drawback with the proposed method is the lower capacity of the microbore separation column than regular columns. Following clean-up of the extracts obtained, no serious matrix effects were observed, however. Owing to the lower chromatographic resolution compared with on-line detection modes, more stringent demands must be put on peak identification for real samples using IC–ETAAS. It is feasible to increase the chromatographic resolution in certain parts of the chromatogram by collecting five eluent droplets, leading to a time resolution of 11 s. (In such a case only 10 μl can be injected into the furnace, consequently leading to a slightly lowered sensitivity.) It is also possible to increase the chromatographic resolution by coupling two 50 mm PAX-100 columns in series if even more complex samples are injected. The first pulse mode interface for chromatography coupled to ETAAS was described 20 years ago. The basic concept has, however, not really been developed further since later work also included normal bore columns. It is likely that some of the future potential for transfer of simple speciation methodology from the research community to possible end-users lies in this field, since the use of microbore columns and ultra low volume fraction collection offers some new possibilities as described here.

Financial support in the form of a research training grant within the training and mobility of researchers programme (TMR/ DG XII) of the European Union is gratefully acknowledged by H.E. The authors also thank Dr. Ulf Örnemark for providing trimethylselenonium iodide.

References

1 *Environmental Health Criteria 58, Selenium*, World Health Organization, Geneva, 1987.
2 Kölbl, G., Kalcher, K., Irgolic, K. J., and Magee, J., *Appl. Organomet. Chem*., 1993, **7**, 443.
3 Cámara, C., Cobo, M. G., Palacios, M. A, Muñoz, R., and Donard, O. F. X., in *Quality Assurance for Environmental Analysis*, ed. Quevauviller, P., Maier, E., and Griepink, B., Elsevier, Amsterdam, 1995, ch. 10, pp. 235–262.
4 Muñoz Olivas, R., Donard, O. F. X., Cámara, C., and Quevauviller, P., *Anal. Chim. Acta*, 1994, **286**, 357.
5 Haraldsson, C., Pollak, M., and Öhman, P., *J. Anal. At. Spectrom*., 1992, **7**, 1183.
6 Nygren, O., Nilsson, C. A., and Frech, W., *Anal. Chem*., 1988, **60**, 2204.
7 Chakraborti, D., Hillman, D. C. J., Irgolic, K. J., and Zingaro, R. A., *J. Chromatogr*., 1982, **249**, 81–92.
8 Laborda, F., Chakraborti, D., Mir, J. M., and Castillo, J. R., *J. Anal. At. Spectrom*., 1993, **8**, 643.
9 Laborda, F., Vicente, M. V., Mir, J. M., and Castillo, J. R., *Fresenius' J. Anal. Chem.*, 1997, **357**, 837.
10 Potin-Gautier, M., Boucharat, C., Astruc, A., and Astruc, M., *Appl. Organomet. Chem.*, 1993, **7**, 593.
11 Gilon, N., Astruc, A., Astruc, M., and Potin-Gautier, M., *Appl. Organomet. Chem.*, 1995, **9**, 623.
12 Gilon, N., Potin-Gautier, M., and Astruc, M., *J. Chromatogr. A*, 1996, **750**, 327.
13 Marchante-Gayón, J. M., González, J. M., Fernández, M. L., Blanco, E., and Sanz-Medel, A., *Fresenius' J. Anal. Chem*., 1996, **355**, 615.
14 González Lafuente, J. M., Fernández Sánchez, M. L., and Sanz-Medel, A., *J. Anal. At. Spectrom*., 1996, **11**, 1163
15 Hagège, A., Niemczyk, S., and Leroy, M. J. F., *Analusis*, 1995, **23**, 476.
16 Laborda, F., de Loos-Vollebregt, M. T. C., and de Galan, L., *Spectrochim. Acta, Part B*, 1991, **46**, 1089.
17 Shum, S. C. K., and Houk, R. S., *Anal. Chem*., 1993, **65**, 2972.
18 Jakubowski, N., Thomas, C., Stuewer, D., Dettlaff, I., and Schram, J., *J. Anal. At. Spectrom*., 1996, **11**, 1023.
19 Quijano, M. A., Gutiérrez, A. M., Pérez-Conde, M. C., and Cámara, C., *J. Anal. At. Spectrom*., 1996, **11**, 407.
20 Crews, H. M., Clarke, P. A., Lewis, D. J., Owen, L. M., Strutt, P. R., and Izquiredo, A., *J. Anal. At. Spectrom*., 1996, **11**, 1177.
21 Muñoz Olivas, R., Donard, O. F. X., Gilon, N., and Potin- Gautier, M., *J. Anal. At. Spectrom*., 1996, **11**, 1171.
22 Kölbl, G., *Mar. Chem*., 1995, **48**, 185.
23 Thomas, C., Jakubowski, N., Stuewer, D., and Klockow, D., Poster 4-7 presented at the European Winter Conference on Plasma Spectrochemistry, Ghent, Belgium, 1997.
24 Emteborg, H., Bordin, G., and Rodriguez, A. R., *Analyst*, 1998, **2**, 245.
25 Garraud, H., Woller, A., Fodor, P., and Donard, O. F. X., *Analusis*, 1997, **25**, 25.
26 Carnrick, G. R., Manning, D. C., and Slavin, W., *Analyst*, 1983, **108**, 1297.
27 Bauslaugh, J., Radziuk, B., Saeed, K., and Thomassen, Y., *Anal. Chim. Acta*, 1984, **165**, 149.
28 Docekalová H., Docekal, B., Komárek, J., and Novotný, I., *J. Anal. At. Spectrom*., 1991, **6**, 661.
29 Radziuk, B., and Thomassen Y., *J. Anal. At. Spectrom*., 1992, **7**, 397.
30 Welz, B., Bozai, G., Sperling, M., and Radziuk, B., *J. Anal. At. Spectrom.*, 1992, **7**, 505.
31 Johannessen, J. K., Gammelgaard, B., Jøns, O., and Hansen, S. H., *J. Anal. At. Spectrom*., 1993, **8**, 999.
32 Volinsky, A. B., and Krivan, V., *J. Anal. At. Spectrom*., 1996, **11**, 159.
33 Alsing-Pedersen, G., and Larsen, E. H., *Fresenius' J. Anal. Chem*., 1997, **358**, 591.

Paper 7/07450F
Received October 16, 1997
Accepted January 22, 1998