Speciation analysis of some organic selenium compounds. A review

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Speciation Analysis of Some Organic Selenium Compounds

A Review

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Summary of Contents

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Introduction

Selenium has been recognized as an essential nutrient for humans, based on its presence at the active sites of glutathione peroxidase. This enzyme protects membranes from damage caused by the peroxidation of lipids.[1] Selenium compounds also catalyse the reactions of intermediate metabolism and inhibit the toxic effects of heavy metals such as arsenic, cadmium and mercury.[2–4] For human health, selenium is an essential trace element[5] at concentrations ranging from 0.8 to 1.7 μmol l^{-1}. At higher concentrations, selenium becomes toxic for man, animals and marine organisms. The toxic dose of selenium is very much dependent on its chemical form, with different toxicity for organic and inorganic compounds.[6,7]

In environmental and biological samples, selenium can exist in inorganic forms (as elemental selenium, metal selenides, and selenite and selenate ions) and as organic species with direct Se–C bonds (methylated compounds, selenoamino acids, selenoproteins and their derivatives). Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) appear to be the predominant species in natural waters. Hydrogen selenide is easily oxidized to non-toxic elemental red selenium, which is insoluble in water.

Over the years, several organic selenium compounds have been identified in biological samples (Table 1). Selenoamino acids are the principal dietary forms of selenium; selenomethionine is derived from plants[8] and selenocysteine from animals.[9] Dietary selenium in the form of its amino acids is absorbed to a greater extent than inorganic species. However, selenium from selenite may be more rapidly incorporated into the enzyme glutathione peroxidase.[10,11] In the aquatic environment, such as in marine bacteria and plankton, selenium is predominantly found in proteins as selenoamino acids.[12–14] Selenomethionine has been isolated from the hydrophilic fulvate fraction of soil[15] and from proteins of marine algae.[16] A number of bacteria are able to transform inorganic selenium species into volatile compounds such as dimethylselenide (DMSe) and dimethyldiselenide (DMDSe), which are exhaled and excreted through the skin. The trimethylselenonium ion ($TMSe^+$), the major product of selenium metabolism, leaves the body of humans in urine. The biomethylation processes are considered to be detoxification steps, because DMSe and $TMSe^+$ are less toxic than other selenium compounds.

Several schematic biogeochemical cycles which link selenate and selenite with simple methylated and more complex organic selenium compounds have been proposed.[17–20] Compared with the extensive investigations on total selenium or selenite and selenate determination, very little work has been carried out on organic selenium compounds. The organically bound selenium is an essential part of the natural selenium cycle and seems to be the most readily available for humans; hence, the study of organoselenium species is important.

Sampling and Storage

Various factors affect the losses of selenium or interconversion of one species into another during sampling and sample storage.

The collection of samples for the determination of the alkylselenium species, such as DMSe and DMDSe, is a significant problem because of the volatility of these compounds. The common sampling procedure is based on scryogenic trapping. The volatile selenides are removed from the sample by sucking with a pump (from air) or by helium gas stripping (water, soil and sediment) and swept into a cold trap. Various types of solid adsorbents such as activated carbon,[21–23] glass wool[24,25] and GC stationary phases[19,21,22,26,27] were found to be successful for trapping selenium species. The pre-column adsorption technique allows an accumulation of the selenides to levels suitable for detection. Selenium species are then thermally desorbed[19,27] or extracted from the adsorbent with

Table 1 Organoselenium compounds found in living organisms

Compound	Formula
Selenocysteine	$HSe–CH_2CH(NH_2)–COOH$
Se-methylselenocysteine	$CH_3Se–CH_2CH(NH_2)–COOH$
Selenomethionine	$CH_3Se–CH_2CH_2CH(NH_2)–COOH$
Se-methylselenomethionine	$(CH_3)_2Se^+–CH_2–CH_2–CH(NH_2)–COOH$
Selenocystine	$HOOC–CH(NH_2)CH_2–Se–Se–CH_2–CH(NH_2)–COOH$
Selenourea	$Se=C(NH_2)_2$
Selenoniocholine	$(CH_3)_2Se^+CH_2CH_2–COOH$
Selenobetaine	$(CH_3)_2Se^+CH_2–COOH$
Dimethylselenide	$CH_3–Se–CH_3$
Dimethyldiselenide	$CH_3–Se–Se–CH_3$
Trimethylselenonium	$(CH_3)_3Se^+$

organic solvents[23] prior to analysis by GC or AAS. However, when a high volume of air sample is used some losses of selenium may occur.[28] Cutter[24] stated that the volatile methylated selenium compounds should be frozen (with liquid nitrogen) because even in airtight containers they are completely lost within a day.

Several papers concerning the stability of inorganic selenium species have been published. During the storage of selenium solutions, adsorption, desorption, volatilization processes, *etc.*, may occur, altering the original concentration. These processes depend on pH, storage medium, temperature, selenium concentration, container material and the ratio of surface area per unit volume.[2,29–34] Significant Se^{IV} losses were observed at pH 6 in PTFE containers.[34] Selenium(VI) is more stable than Se^{IV} in aqueous solutions and less dependent on the acidic conditions of the sample. The optimum temperature at which there is no significant risk of inorganic selenium losses at 10 and 50 μg l^{-1} concentration levels over the 12 months tested was −20 °C.[34] Samples stored at this temperature need not be acidified, which is an advantage. Acidification can cause changes in selenium speciation, as well as causing hydride generation difficulties during analysis.

The literature indicated that only a few storage experiments had been conducted with selenoamino acids.[35,36] Campanella *et al.*[35] investigated selenium losses from samples containing 100 μg l^{-1} of selenourea stored in PTFE containers at 4 °C and at room temperature. In both instances, no loss of selenium was observed. There was also no significant influence of temperature, selenium concentration and container material on the stability of selenomethionine in a high ionic strength matrix over a 120 d period.[36] A significant loss of this compound was found in low concentration (10 μg l^{-1}) solutions stored in borosilicate glass and polyethylene containers in a low ionic strength matrix.

Sample Preparation

Total selenium determination requires that its organic forms must be transformed into inorganic selenium. The decomposition will also destroy organic matter that may interfere with the detection method.

The most popular methods involve digestion with strong acids (HNO_3, $HClO_4$, H_2SO_4)[37–40] or UV irradiation after addition of hydrogen peroxide.[35,41] Concentrated HCl is avoided, since selenium forms volatile chloride adducts. The main difficulty with wet oxidation is preventing selenium from being volatilized. Lowering the digestion temperature is effective; however, it prolongs the time of digestion. Microwave heating was more effective for the drying and wet ashing (with HNO_3) of fish tissues when applied to selenium determination.[42] Compared with thermal heating in an open vessel, much shorter digestion times and smaller amounts of chemicals were used. A comparison between six decomposition methods for various organoselenium compounds followed by their determination by HGAAS was presented by Ornemark *et al.*[43] They found that the use of peroxodisulfate at pH ≥2 was the most efficient method. It decomposed all the species tested, including the very resistant $TMSe^+$. This substance was not decomposed by permanganate or by oxidative UV irradiation. Selenomethionine from biological samples can only be converted into inorganic selenium after digestion with an HNO_3–H_2SO_4–$HClO_4$ mixture at 310 °C.[44] The efficiency of the mineralization of selenourea solutions was higher using UV photodegradation than with wet acid digestion.[35] Moreover, the former method requires shorter operating times, *viz.*, 2 h against 8–9 h.

However, the best digestion method for a particular purpose is not easy to define and no single procedure is suitable in all instances. The choice of acids and other reagents used for oxidation should also take into consideration the instrumental technique used for detection.

Some of the methods for the determination of selenium require the conversion of its species into Se^{IV} because Se^{VI} does not react with most of the complex-forming agents used for spectrophotometric detection. Moreover, the hydride generation process is most efficient when selenium occurs as Se^{IV}. Hence, after decomposition of organoselenium species, HCl reduction to Se^{IV} is mostly applied. It is recommended that this process be performed at high temperature (90–100 °C).[24,45,46] However, if the sample is boiled for too long, reduction to elemental selenium may occur[24] or selenium may be lost owing to the formation of volatile compounds.[3] Recently, an on-line reduction in a closed system at 140 °C[47] and a microwave energy source with continuous sampling flow[48] have been proposed. Automation of these systems has resulted in a shortening of the analysis time and has minimized the risk of losses.

Despite the very sensitive analytical methods available for selenium, it is seldom possible to perform direct determinations at the concentration levels present in natural samples. The total selenium content in environmental samples ranges from 0.1 to 400 μg l^{-1} in waters to about 1 ng l^{-1} in atmospheric aerosols and 1–80 μg g^{-1} in soils, but, depending on geological factors, groundwater may contain much higher concentrations up to 6 mg l^{-1}.[33,34,49] This means that the analyte has to be preconcentrated. Moreover, this could also serve as a convenient method for the separation of selenium from the matrix. Several preconcentration–separation procedures for selenium have been described in the literature, including coprecipitation,[50–52] derivatization followed by extraction into an organic solvent[53–55] or application of solid sorbents.[3,56–60]

Supercritical fluid extraction (SFE) is fast becoming an alternative technique to conventional extraction methods for solid samples. This technique is more effective, removing the need for toxic solvents and is very suitable for on-line automation.[61] Although the growing list of SFE applications to environmental samples has been discussed,[61,62] this technique does not yet appear to have been fully used for the speciation of selenium in soil and sediment samples.[63]

Hydride generation, coupled to AAS and ICP detectors, is the technique most commonly used for the separation of selenium from non-volatile matrix components. The enrichment of the hydrogen selenide generated can be achieved using a liquid nitrogen cold trap.[24,44,64,65] Also, commercial graphite furnaces (in AAS methods) can be used as both the trapping medium and as the atomization cell.[66,67] Ethylation, using $NaBEt_4$ as reductant, is an alternative to the hydride generation method.[68] This reaction does not allow the speciation of inorganic selenium species because a single derivative (Et_2Se) is generated from both Se^{IV} and Se^{VI}. It has not yet been explored for organic selenium compounds.

Chromatographic and electrophoretic techniques coupled with different detection systems have been extensively used for the separation and determination of all selenium species. The application of these methods to the speciation of organoselenium compounds and the sample characteristics required for a given separation technique will be discussed later.

Analytical Methods for Selenium Determination

Total Selenium

Several analytical techniques have been applied to the determination of total selenium content at trace levels in environmental and biological samples (Table 2). The determination is usually carried out after mineralization of the organic matrix and an appropriate derivatization process (hydride generation, ethylation, complexation with an aromatic *o*-diamine). Methods for selenium determination have recently been reviewed.[18,33,79,80] The literature data also reviewed the quantification of selenium

in different matrices: water,[33,49,81] urine,[29,82] blood,[83,84] and environmental[68,85] and biological samples.[24,85,86]

Selenium Speciation

A sequential extraction procedure termed 'phase speciation'[87] only provides information about the partitioning of selenium between exchangeable, carbonate, iron and manganese oxide, organic and resistant mineral fractions from the suspended particles and bottom sediments of natural waters. This information helps in the understanding of the processes of removal of selenium from the dissolved to the particulate state and the potential for its bioavailability. However, the problem with all sequential extraction procedures is that they cannot preserve the chemical forms of selenium because of the reagents and conditions employed.[88] The identification and determination of definite selenium compounds requires more refined separation techniques.

Most procedures involving selenium speciation distinguish between two main categories of species: (1) non-volatile, which includes inorganic species such as Se^{IV} and Se^{VI}, elemental selenium and matrix-bound organic compounds; and (2) volatile organic selenides such as DMSe and DMDSe. Volatile selenium species are purged from the sample matrix and trapped either on a solid adsorbent or in a liquid nitrogen trap, followed by thermal desorption prior to GC analysis.[19,24,56,89]

Non-volatile selenium species in four oxidation states (−II, 0, IV and VI) have been determined in natural waters[32,35,90–96] by analysing three separate sample aliquots: (1) with no further chemical treatment—determination of Se^{IV} using fluorimetry, HGAAS, HG-ICP or electrochemical methods; (2) after oxidation by UV irradiation or wet acid digestion—the sum of Se^{-II} + Se^{0} + Se^{IV}; and (3) after reduction to selenite with hydrochloric or hydrobromic acid—all selenium species. The difference between total selenium and the sum of Se^{IV} and Se^{VI} is attributed to organic selenium compounds and elemental selenium. The error for the species determined by difference is always much higher than for the species determined directly. Usually, a preconcentration step (solvent extraction, coprecipitation, sorption on solid sorbents) is required to achieve a sufficient concentration level for detection. The analytical methods proposed differ mainly in the way in which inorganic selenium species are converted into Se^{IV} and in the methods used for preconcentration of selenium. Some workers have also studied a colloidal selenium fraction.[35,88,92,96]

Selenium speciation in plant and biological samples has focused on two fractions: (1) organic selenium and Se^{-II}; and (2) inorganic species, including Se^{IV} and Se^{VI}. The scheme determines the sum of the Se^{-II} and Se^{IV} content after wet oxidation of the matrix. Total selenium is determined after boiling the digested samples with concentrated HCl. Selenium(IV) is found by difference. This procedure has been used, with minor modifications to the oxidation step, for the speciation analysis of selenium in milk products,[97] body fluids[98] and fish.[99,100]

The separation and determination of the dissolved organic selenide fraction can be achieved by using LC methods at atmospheric pressure.[56,101–105] Organoselenium species (other than $TMSe^{+}$) were separated from natural water samples by adsorption on Amberlite XAD-2 resin at pH 8 and 3, respectively.[56] The species adsorbed at pH 8 were classified as neutral and basic forms, while those adsorbed at pH 3 as acidic. As the acidic forms dominated in lake water samples, it was concluded that most of these compounds were selenium-containing humic substances. Blocky *et al.*[102] developed a procedure for the determination of total selenoamino acids in urine. These compounds were derivatized with *o*-phthalaldehyde and 2-mercaptoethanol, followed by their retention on an anion-exchange resin, Bio-Rad AG2-X8. Lithium hydroxide solution was used as the eluent, which, in addition to its elution characteristics, facilitated the continuous denaturating of urine. Dissolved organoselenium species (mainly the selenoamino acid fraction) were separated from inorganic selenium species[103,104] or from their sulfur analogue[105] using Amberlite XAD-8 resin. Sep-Pak C_{18} cartridges[19] and copper-treated Chelex-100 resin[101] were also applied for this purpose.

Table 2 Analytical methods for the determination of selenium

Technique	Matrix	Detection limit	Ref.
Spectroscopy—			
UV–visible	Plants	80 mg l^{-1}	69
Fluorimetry	Sediment	0.28 μg l^{-1}	70
X-ray fluorescence	Waters	60 ng l^{-1}	71
HGAAS	Tissues	20 ng l^{-1}	72
ETAAS	Urine	20 ng l^{-1}	73
ICP-AES	Sediment	0.40 μg l^{-1}	70
Mass spectrometry	Waters	10 ng l^{-1}	74
Electrochemical—			
Anodic stripping voltammetry	Waters	80 mg l^{-1}	75
Cathodic stripping voltammetry	Waters	2 ng l^{-1}	76
Differential-pulse polarography	Fish	5 ng	77
Neutron activation analysis	Human diets	0.5 μg g^{-1}	78

Chromatographic Separation and Determination of Organoselenium Species

Chromatographic methods are based on dynamic partitioning of analytes carried by a mobile phase through a stationary phase between these two phases. These techniques are at present virtually the only ones used in speciation analysis. The elution mode, apart from producing good separations, leaves the column in its original condition, ready for another sample.

Gas chromatography

Gas chromatography is the most frequently used technique for the determination of volatile selenium species, such as DMSe and DMDSe. These naturally occurring compounds are sufficiently volatile to be separated by packed[19,23,106,107] or capillary[27,106,108,109] columns. The packed columns can be conveniently cleaned and have a large sample capacity. In recent years there has been a trend towards capillary or open-tubular megabore columns with thin polymethoxysilane coatings, which provide higher resolution and sharper bands. However, their limited capacity is a disadvantage.

The chromatographic columns are usually coupled to AAS detection methods which use flame atomization, a quartz tube (QT) or a graphite furance (GF). The sensitivity of GC–AAS is affected by the temperatures of the injector, detector and chromatograph. At low temperatures, the species tend to adsorb onto the injector. On the other hand, excessive temperatures lead to thermal decomposition of the compounds. Temperatures of 100 and 160 °C for the injector and detector, respectively, are satisfactory.[110] The detection limits of GC–AAS approach 0.1 ng of selenium using a QT[24] and 5 pg of selenium using a GF.[107]

The successful speciation of volatile alkylselenides has also been carried out with other detection techniques, such as microwave-induced plasma atomic emission spectrometry (MIP-AES),[111] atomic fluorescence spectrometry (AFS),[112] and MS[106] and with a sulfur chemiluminescence detector (SCD).[27] Selected GC applications for selenium speciation are presented in Table 3.

Gas chromatography has also been applied to study the transformation of inorganic selenium species into methylated

compounds in laboratory experiments with animals.[23,82] After administration of selenite and selenocystine in the drinking water of mice, DMSe was exhaled as the predominant species. When selenomethionine was administered, both DMSe and DMDSe were detected.[110] These results represent a step forward in understanding the metabolism of these compounds. Similar experiments were performed to study the biomethylation of inorganic selenium administered to fungal cultures.[27] Both DMSe and DMDSe were separated and detected in the low picogram range by GC–SCD.

Liquid chromatography

The separation of selenium species by LC offers a number of potential benefits. These include minimal preparation of liquid samples and separation at ambient temperature, thus avoiding the risk of thermal decomposition of unstable compounds. Another advantage is that both the stationary and mobile phases can be varied simultaneously to achieve better separation.[18] This technique is mainly applied as HPLC, in which the size of the particles used for the stationary phase is very small to ensure good separation. HPLC provides, depending on the type of detector, low detection limits (in the μg l^{-1} range) with fairly short chromatographic run-times.

Current HPLC methodology for selenium employs conductometric,[79,113,114] UV[115] and fluorimetric[22] detection. Numerous interferences, mainly ionic, from the sample and reagents constitute the major drawback of these detectors. The application of selenium-specific detectors, such as ETAAS,[116] ICP-AES[117] or ICP-MS,[56,118] is very helpful for elimination of these interferences.

Table 3 Speciation analysis of volatile alkylselenides

Species*	Matrix	Detection†	Ref.
DMSe, DMDSe	Surface water, groundwater	AAS	19
DMSe	Air exhaled by mice	FID	23
DMSe, DMDSe	Gases from biological samples	SCD	27
DMSe, DMDSe	Gas evolved from soil cultures	MS	106
DMSe, DMDSe, DESe	Gas evolved from soil	ETAAS	107
DMSe, DMDSe	Gas evolved from sediments	FID	108
DMSe, DMDSe, DESe	Gases evolved from sewage sludge and soils	MIP	111

* DMSe = Dimethylselenide; DMDSe = dimethyldiselenide; DESe = diethylselenide. † FID = Flame ionization detector; SCD = sulfur chemiluminescence detector; MIP = microwave-induced plasma.

A major concern in the development of HPLC coupled with spectroscopic detection has been the interface. Organic solvents can impair the performance because of the instability of the plasma to organic vapours and deposition of carbon on the sampling cone and torch. Improvements to the HPLC–ICP interface have involved a direct injection nebulizer[118] and a thermospray vaporizer,[117] which allow the use of a higher concentration of organic modifiers in the mobile phase. The addition of oxygen to the nebulizer gas flow[119] or nebulization of 2% nitric acid between chromatographic runs[120] can reduce some of these problems. Hill *et al.*[121] have reviewed the coupling of various chromatographic methods to both ICP-AES and ICP-MS.

Blais *et al.*[122] developed a post-column thermochemical hydride generator (THG) as an interface for HPLC–AAS and optimized the determination of low nanogram amounts of selenoniocholine and $TMSe^+$ in urine. The THG interface was also used for the determination of selenomethionine in complex matrices such as nutritional supplements and mixtures of free amino acids.[123] Selenomethionine was first derivatized with 1-fluoro-2,4-dinitrobenzene and, after acidification, was extracted with diethyl ether.

The coupling of HPLC with ETAAS detection has the disadvantage of using discrete sample volumes and does not allow the continuous monitoring of the effluent from the chromatograph. A computerized system for the collection and treatment of data improves the quantification of the analytes.[124]

Several types of chromatographic mode, including normal- and reversed-phase partitioning, ion pairing and ion exchange, have been applied to the separation and determination of selenium species. The stationary phase is usually silica, bonded or coated. The mobile phase is aqueous, but contains a polar organic solvent (usually methanol or acetonitrile) in various proportions to overcome the instability of the analyte complexes in water.

Trimethylselenonium, as a major metabolite of selenium, is mainly determined in urine and water samples (Table 4). Its concentration level in the urine of normal subjects is in the range 10–60 ng ml^{-1}.[102] Two studies have demonstrated the occurrence of $TMSe^+$ in lake water samples with a mean concentration of 12 ng l^{-1}.[53,56] The information concerning the $TMSe^+$ content is useful in predicting the intake of excess of selenium and the detoxification mechanism in living organisms.[130] However, the chemical forms of selenium in urine have not yet been completely characterized. Other potential metabolites, such as selenoniocholine, may constitute a significant portion of total selenium.[29,102,122]

The high resolution obtained by HPLC permits a clear separation of selenoamino acids from other selenium species

Table 4 Speciation of trimethylselenonium ion ($TMSe^+$)

Species	Matrix	Column and mobile phase	Detection*	Ref.
$TMSe^+$, Se^{IV}, Se^{VI}	Water	Dowex 50W-X8; 4 mol l^{-1} HCl	ETAAS	53
$TMSe^+$, Se^{IV}, Se^{VI}, total organic selenium	Water	Dowex 50W-X8; 5 mol l^{-1} HCl	IDMS	56
$TMSe^+$, Se^{IV}, total selenoamino acids	Urine, serum	Bio-Rad AG2-X8; 0.5 mol l^{-1} LiOH	NAA	102, 127
$TMSe^+$, Se^{IV}, Se^{VI}	Water	Waters IC-PAK; ammonium citrate, pH 3.3	ICP-AES	117
$TMSe^+$, selenoniocholine	Urine	Cyanopropyl-bonded silica; methanol with CH_3COOH and triethylamine	THGAAS	121
$TMSe^+$, selenocysteine, selenomethionine	Urine	Nucleosil; gradient, 0.003–0.5 mol l^{-1} $(NH_4)_2PO_4$	Radiometric	125
$TMSe^+$, Se^{IV}, Se^{VI}	Urine	Nucleosil 100-SB; ammonium citrate, pH 3 and 7	ETAAS	126
$TMSe^+$, Se^{IV}, Se^{VI}	Urine	Hamilton PRP-1; methanol with tetrabutylammonium	ICP-MS	128
$TMSe^+$, Se^{IV}, Se^{VI}	Water	Hamilton PRP-X-100; phosphate buffer, pH 6.8	HGAAS	129

* IDMS = Isotope dilution mass spectrometry; THGAAS = thermochemical hydride generation atomic absorption spectrometry.

Table 5 Speciation of selenoamino acids

Species	Matrix	Column and mobile phase	Detection*	Ref.
Selenomethionine	Food supplements	Nucleosil; aqueous methanol with trietylamine	THGAAS	123
Selenocysteine	Water	μBondapak C_{18}; methanol–H_2O (30 + 70)	UV	134
Selenomethionine	Soil extract	Amberlite XAD-2; pH gradient	MS	135
Selenocystine, selenomethionine	Extract of white clover	Hamilton PRP-1; aqueous acetonitrile with Et_4NBr	ETAAS	136
Selenocysteine, selenomethionine	Extract from the liver of marine mammals	AminoPac PA1; gradient elution with NaOH, sodium borate and sodium acetate	IPAD	137
Selenocysteine	Spiked plasma	C_{18}; methanol–H_2O (20 + 80)	Fluorimetry	138

* THGAAS = Thermochemical hydride generation atomic absorption spectrometry; IPAD = integrated pulsed amperometric detection.

and from other amino acids.[102,121,123,131–138] Several selenoamino acids and related selenium compounds have been identified in biological tissues or protein fractions. The selenoamino acids isolated probably originate from several enzymic systems which require the participation of selenium-containing proteins. Selected analytical procedures for the determination of selenoamino acids are presented in Table 5.

Several other organic selenium compounds, such as selenocarbohydrates,[139] selenoproteins[140,141] and selenonucleosides[142] have been separated using LC. They were further characterized by gel chromatography or electrophoresis.

Electrophoretic techniques

These techniques are based on differences in the electrophoretic mobilities of ions and are realized in three basic modes: zone, isotachophoresis and isoelectric focusing. The electrophoretic mobility of an ion is largely determined by its mass-to-charge ratio, physical dimensions and interactions with buffer components. CZE is a relatively new technique that is potentially applicable to the separation of a range of species from small molecules and ions to large biomolecules. The wider use of this technique is hampered by the lack of a sensitive and specific detection system in the on-line mode. Work on interfacing CZE and ICP-MS is in progress,[143] but this coupling has not yet been applied in selenium speciation analysis. Usually, UV detection has been used.[5,144]

CZE was applied to the separation of inorganic selenium forms from selenomethionine and selenocystine[5] and dialkylselenium compounds,[144] but applications to real samples were not reported. The electrokinetic potential was modified by the addition of a cationic surfactant.

The identification of the analyte peak when complex matrices are analysed plays a major role, because the migration times of molecules present at low concentrations are influenced by closely migrating substances present at high concentrations.[145] As a consequence, the migration time for a specific analyte in a real sample is different from that in a model or standard solution. Michalke[146] proposed a method for the identification and quantification of selenocystine, selenocystamine and selenomethionine in human milk samples despite the shift of migration times caused by the different ionic composition.

Conclusion

Selenium speciation studies have become increasingly important in recent years as more information is sought in order to gain a better understanding of the role of selenium in the environment and human health.

The measurement of total selenium content at trace levels in water and biological samples can be reliably performed by several established techniques. The accurate determination of different selenium species, particularly organic compounds, is still a major challenge for the analyst. Many of the speciation procedures rely on the separation technique and there are still limitations with sample preparation. Various factors affect the sorption or loss of selenium, and the interconversion of one species into another during sampling, sample storage and analysis.

The most efficient way for the quality control of analytical results is through the analysis of certified reference materials. Although several certified reference materials having different matrices are available,[33] they are only certified for total selenium content. The methods developed for selenium speciation should be validated by using spiked samples. However, the compounds that are present naturally, particularly in solid samples, are generally more strongly bound to the matrices than the spiking compounds.[147] The best conditions under which spiking experiments should be carried out are still the subject of controversy and further studies are needed.

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