

SELENIUM SPECIATION ANALYSIS

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Abstract

The essential nature of selenium as well as its toxicity depend on the concentration and the chemical forms in which this element is present in environmental and biological samples. Dissolved inorganic selenium can be found in natural waters and soils as selenides, selenite and selenate. Organoselenium compounds that may be present in air, soils and plants are volatile methylselenides, trimethylselenonium ion and several selenoamino acids. This review article intends to show some general ideas and important procedures used for speciation analysis of selenium in particular matrices such as water, air, soils and plants.

Introduction

Selenium is the one of the most interesting elements from the point of view of its clinical and environmental effects. This element has been recognised as an essential nutrient for humans based on its presence in the enzyme glutathione peroxidase, which affords cells protection against oxidative damage. It is also important to mention other roles of selenium such as anticarcinogenic activity and preventing heavy metal toxic effects [1,2]. However, the nutritionally required concentration range of selenium is very narrow. A deficiency occurs when its intake is below 50 µg per day and daily doses up to 200 µg are considered as optimum [3]. Chronic selenium dietary deficiency has been associated with various degeneration diseases. Apart from natural sources (mainly metal-sulfur minerals) selenium compounds are widely spread throughout the environment from the combustion of fossil fuels, and uses in the glass and electronic industries as well as in agriculture.

It is generally accepted that selenium reactivity and bioavailability depend not only on its total content. Additionally knowledge of different species of this element present in a particular system is required to understand the biological and environmental impact of selenium. Knowledge of selenium speciation in food, tissues and functional proteins is important for the accurate assessment of selenium status. As the results of this, improved analytical methods for selenium speciation at low concentration levels, including sample preparation step, are increasingly needed. Analytical methods for the determination of selenium were reviewed [4-7]. These works mainly discuss the application of instrumental techniques available for this purpose. The objective of this review is to present some general ideas and important procedures used for speciation analysis of selenium in particular matrices such as waters, air, soils and plants.

In environmental and biological samples, selenium can exist in inorganic (as elemental selenium, metal selenides, selenite and selenate ions) and as organic species (methylated compounds, selenoamino acids, selenoproteins and their derivatives). In the living body, in contrast to metal-protein complexes, selenium is not bound by coordination but forms covalent C-Se bonds. Selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) ions appear to be predominant species in natural waters and soils. Inorganic selenium species can be transformed into volatile compounds such as dimethylselenide ($(\text{CH}_3)_2\text{Se}$) through microbial action of fungi and plants. The reduction of selenium followed by its methylation to $(\text{CH}_3)_2\text{Se}$ and the trimethylselenonium ion ($(\text{CH}_3)_3\text{Se}^+$) is the primary pathway of the metabolism of selenite by animals and man. Some of these methylated species can be excreted in urine. The biomethylation processes are considered to be detoxification steps, because $(\text{CH}_3)_2\text{Se}$ and $(\text{CH}_3)_2\text{Se}^+$ are less toxic than inorganic selenium compounds, especially Se(IV). Organic selenium species are more frequently found in biological systems. They are present as the products of enzymatic reactions leading to selenoamino acids synthesis. Selenoproteins contain selenium in the form of selenocysteinyl residues [8]. Animal and human studies have established that chemical form of selenium in foods and in supplements influences not only the bioavailability of this element but also its distribution in the body [9]. Selenomethionine is

retained in tissue proteins to a greater extent than selenocysteine and inorganic selenium species.

Stability of selenium species

Many problems could occur in selenium speciation analysis. Except very low concentration of each species to be determined in most cases, risk of adsorption, desorption, thermal degradation, light-induced decomposition and environmental contamination could alter the initial composition of the sample. Sampling, storage and sample preparation are the main factors influencing the reliability of results.

The stability of inorganic selenium species depends on pH, storage medium, temperature concentration of the analyte, container material and the ratio of surface area per unit volume [10-14]. Significant Se(IV) losses were observed at pH 6 in PTFE containers [12]. Se(VI) is more stable in aqueous solutions and less depend 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 $\mu\text{g l}^{-1}$ concentration level over the twelve months tested was -20°C . Samples stored at this temperature need not to be acidified, which is an advantage, because acidification can cause changes in selenium speciation [12]. The presence of the excess of chloride ions tended to stabilise both species [13]. However, in acidic and oxygenated medium and in the presence of Cl^{-} ions a 29% oxidation of Se(IV) to Se(VI) in less than one month was observed [14].

The literature indicated that only a few storage experiments had been conducted with selenoamino acids [15-17]. Campanella *et al.* [15] investigated selenium losses from samples containing 100 $\mu\text{g l}^{-1}$ of selenourea stored in PTFE containers at 4°C and at room temperature. In both instances, no loss of selenium was observed. There was no significant influence of temperature, analyte concentration and container material on the stability of selenomethionine in a high ionic strength matrix over a 120 days period [16]. A significant loss of this compound was found in low concentration (10 $\mu\text{g l}^{-1}$) solution stored in borosilicate glass and polyethylene container in a low ionic strength matrix. In the mixed solutions containing 100 $\mu\text{g l}^{-1}$ of selenomethionine, selenocysteine

nad $(\text{CH}_3)_3\text{Se}^+$ in Milli-Q water stored in the dark at 4°C in propylene bottles, all selenium species appear to be stable for the tested period over 6 weeks [17].

Selenium species in waters

In natural water samples inorganic species such as Se(IV) and Se(VI) appear to be predominant and they are most environmentally mobile and biogeochemically important oxidation states of selenium. The ratio of these two forms depends on pH, the presence of complexing agents or dissolved gases (especially oxygen) and suspended matter. In moderately reduced environment, Se(IV) is the major species and its mobility is mainly governed by sorption/desorption processes on different solid surfaces as metallic oxides, clays or organic matter [18]. There is also some evidence that organic forms of selenium exist in aquatic ecosystem [19,20].

In natural water samples selenium species in three oxidation states (-II, IV and VI) have been determined mainly by specific oxido-reduction reactions by analysis three separate aliquots (Fig. 1) : (i) with no further chemical treatment - directly determination of Se(IV) using specific analytical methods for this oxidation state such as voltammetry, fluorimetry, HG-AAS or HG-ICP-MS; (ii) after reduction of Se(VI) to selenite with hot hydrochloric or hydrobromic acid - the sum of Se(IV) and Se(VI); and (iii) after mineralization of organic matrix (by UV irradiation or wet digestion) followed by reduction to Se(IV) - all selenium species. The difference between total selenium content and the sum of Se(IV) and Se(VI) is attributed to organic selenium compounds. The proposed analytical procedures differ mainly in mineralization and reduction processes.

Speciation analysis was also carried out by selective separation of selenium compounds and direct species determination. Solvent extraction after complexation [19,21], coprecipitation [22,23] and solid phase extraction [24-26] are mainly used for this purpose.

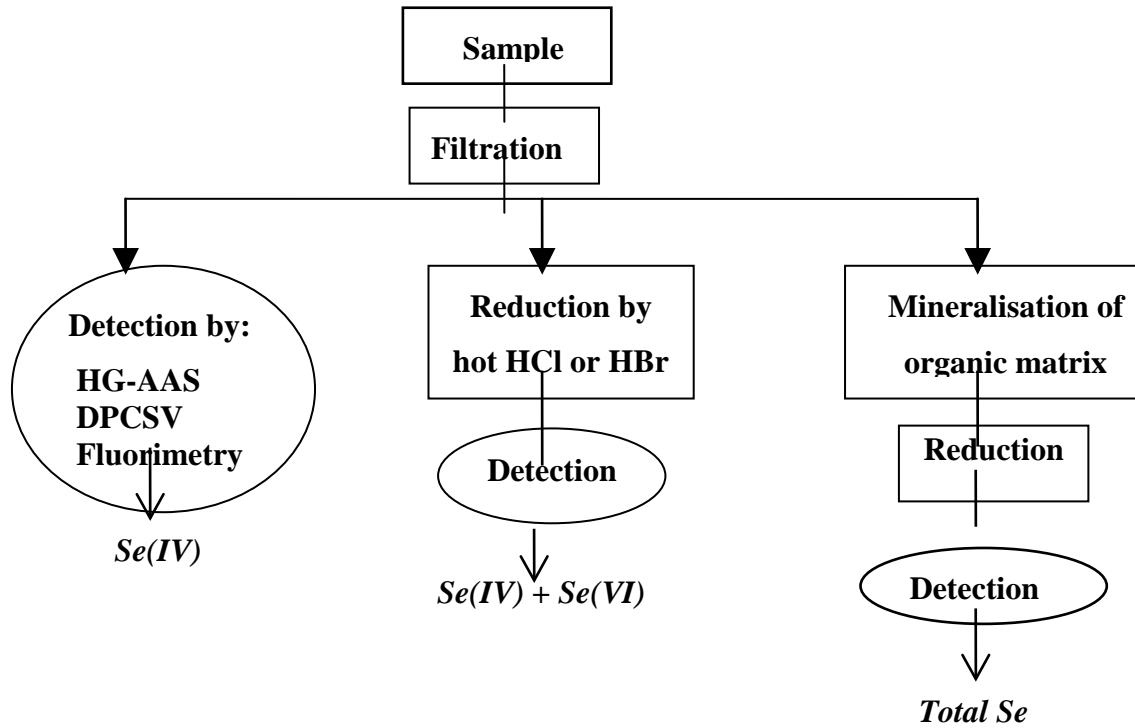


Fig. 1. Scheme for speciation analysis of selenium in natural waters.

The application of so-called hyphenated methods, based on the coupling of a chromatographic separation techniques (used mainly as HPLC) with an atomic spectrometric or other Se-specific detectors offers a number of potential benefits. These include minimal preparation of the sample, possibility of simultaneously variation of both the stationary and mobile phase for better separation and achieve low detection limit for determination [27-30].

Selenium compounds in air

The transformation of inorganic selenium by microbiological action to the more volatile but less toxic methylated species is an important link in the global cycling of this element. Gas chromatography (GC) has been extensively used for the detection and determination of selenium evolved from contaminated soils, sediments and sewage sludges.

The common sampling procedure is based on cryogenic trapping. The alkylselenium species, such as $(\text{CH}_3)_2\text{Se}$ and $(\text{CH}_3)_2\text{Se}_2$, are removed by sucking with a pump (from air) or by helium gas stripping (soil and sediments) and swept into a cold trap. Activate carbon [31], glass wool [32] and GC stationary phases [31] were applied as solid adsorbents. This allows an accumulation of selenides to levels suitable for detection. Selenium species are then thermally desorbed or extracted with organic solvents prior to analysis. The chromatography columns were coupled to electron-capture detection [32], mass spectrometry [31,33], MIP-AES [34] and sulphur chemiluminescence detector [35].

Gas chromatography has been also applied to study the transformation of inorganic species in laboratory experiments with animals. After administration of selenite and selenocystine in drinking water by mice, $(\text{CH}_3)_2\text{Se}$ was exhaled as the predominant species. In the case of selenomethionine administration, both $(\text{CH}_3)_2\text{Se}$ and $(\text{CH}_3)_2\text{Se}_2$ were detected [36]. These results represents a step forward in understanding the metabolism of selenium compounds.

Selenium in Soils and sediments

Total selenium concentration in soil range from $0.1 \mu\text{g g}^{-1}$ (parts of China and Finland) to $100 \mu\text{g g}^{-1}$ of Se (Ireland and some states of the USA) [37]. Generally selenium in soils seems to be present in inorganic forms as selenides, elemental selenium, selenites and selenates. It is also possible to find some organic Se compounds. The distribution of these species depends on soil properties such as acidity, aeration, the mineral and organic-matter contents and the microbiological activity [37-40]. As soil is a complex system affected by many unstable factors, there is no commonly accepted research method suitable for systematic study of selenium speciation.

Hot water extraction was applied to separate non-adsorbed selenium and selenium related to water-soluble organic matter [37,38]. The acid extraction procedures removes selenium associated with calcium compounds and acid-soluble organic matter [37]. The alkaline extraction permit the solubilisation of selenium bound to humic substances and manganese and iron hydroxides [37,39]. It was considered that the non-adsorbed Se, the exchangeable Se and Se associated to organic matter are available to plants.

Considering the speciation analysis, the fraction extracted by hot water (2% of total Se content in soil) mainly consists of selenates (67% and selenites (28%) [37]. Se(VI) is less fixed in soil than Se(IV) and consequently can be directly available to plants. Extraction of soil with 2 mol l^{-1} NaOH solution (50% of total Se) solubilises a large portion of Se(IV) which represents 50% of total extracted selenium. Acidification of the alkaline extract to pH 1.5 allows to separate fulvic acids fraction (acid-soluble species) from the fraction containing humic acids, which is precipitated at this time. 70% of total soluble Se was found in the fulvic acid fraction (about 40% of Se(IV), 15% of Se(VI) and 44% of organic compounds) while 30% was present in the humic acids fraction (60% of Se(IV)) [37].

Application of the sequential extraction procedure for river and estuarine sediments showed that selenium was mainly (92-93%) released from organic fraction [41]. Inorganic selenium species (Se(IV) + Se(VI)) in these sediment samples by weak sodium hydroxide treatment was estimated as 7-8%.

Selenium species in some plants

Consumption of selenium enriched plants or yeast-based nutritional supplements has been found to provide anticarcinogenic benefits which are selenium compound depend. It is almost impossible to increase selenium intake by eating certain types of food due to their low selenium content [42]. However, controlling the intensity and frequency of the crop fertilisation with water-soluble selenite salts it is possible to cultivate Se-garlic enriched with 100-1355 $\mu\text{g g}^{-1}$ of Se [43]. As a point of reference, natural garlic sold in grocery stores contains $< 0.05 \mu\text{g g}^{-1}$ Se. *Allium* group vegetables such as garlic or onion tend to take up inorganic selenium followed by conversion to various organic forms, probably by a route similar to the sulfur assimilatory pathway. In addition to the common selenate and selenite, a number of selenoamino acids have been identified in microorganisms and plants (Fig. 2). Several selenium compounds including selenocystine, selenomethionine, Se-methylselenocysteine and Se-adenosylselenohomo-cysteine appear to be present in Se-enriched yeast [44-48]. Five selenium species and several unknown peaks were observed in Se-enriched garlic, onion and broccoli [49-53].

Speciation analysis of plant and enriched yeast, usually done by HPLC-ICP-MS method, requires that extraction process should no modify the chemical forms of an analyte or disturb the equilibrium between the various species present. Since selenoamino acids are water-soluble, leaching with hot water has been often used. However, the efficiency of water extraction is only about 10% and this part of selenium species are in free, non-protein bound forms [51-54%].

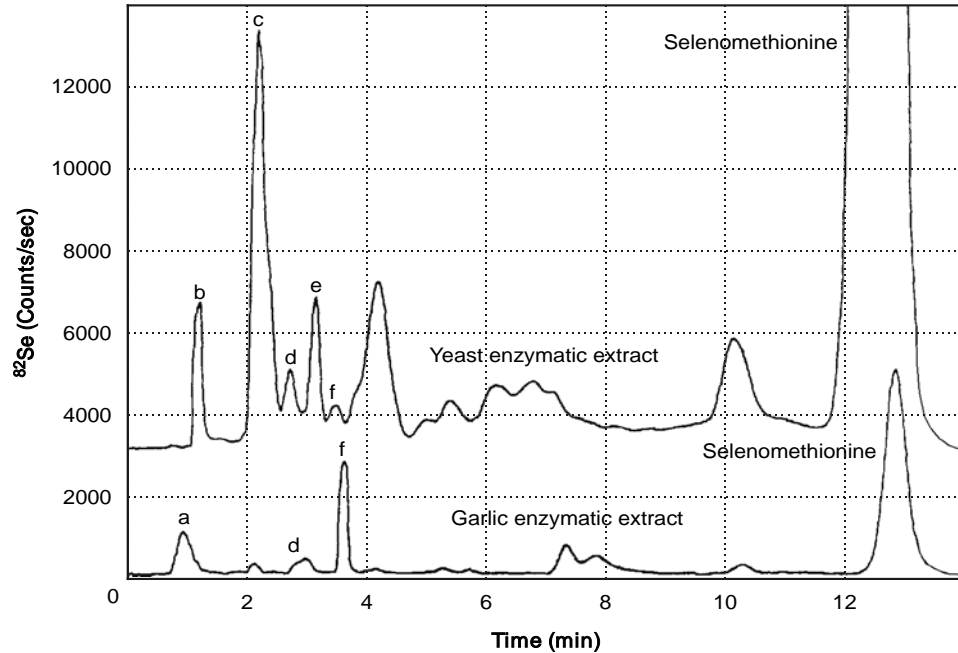


Fig. 2. HPLC-ICP-MS extracts chromatograms of selenium-enriched garlic and yeast containing $296 \mu\text{g g}^{-1}$ and $1922 \mu\text{g g}^{-1}$ total Se, respectively [48]. Peak identification: (a) Se(VI); (b) Se(IV); (c) selenolanthionine; (d) selenocystine; (e) selenocystathione; (f) Se-methylselenocysteine.

To release the covalently bound selenoamino acids, enzymatic or acidic hydrolysis is necessary [45,53-55]. This is especially important for yeast samples in which selenomethionine is found to be the major component after hydrolysis (as a part of larger stable protein), but only a minor component after simple hot water extraction [51]. The enzymatic extraction efficiency from yeast were 80-90% based on the total selenium content [48,51]. The addition of sodium dodecyl sulfate (SDS) increases the yield of selenium by releasing selenoamino acids bound in selenoproteins [45]. Se-adenosylselenohomocysteine and selenomethionine account for approximately 82% of the total selenium eluting from the enzymatic extract of yeast samples. For garlic samples, the efficiency of enzymatic extraction approached 100% with selenomethionine and γ -glutamyl-Se-methylselenocysteine together accounting for ~86% of the total selenium species eluted [48,51]. Casiot *et al.* [45] proposed a sequential leaching procedure for the evaluation of selenium speciation in yeast without the need for a chromatographic separation. This procedure included hot water extraction to release water-soluble fraction, enzymatic hydrolysis by Driselase for extraction of selenium present in the yeast cell walls (probably as a polysaccharide) and finally leaching with SDS to give the protein-soluble fraction.

The direct coupling of chromatographic or electrophoretic separation methods to Se-specific detection such as ICP-MS is an established approach to the determination of selenium species in plants and nutritional supplements [8,55-57]. Reversed-phase chromatography applying perfluorinated carboxylic acids as a cation-pairing reagents offers the highest resolution [51,52]. The identification of the species is based mainly on their retention or migration time. However, except for popular compounds such as selenate, selenite, selenomethionine and selenocysteine, the commercial standards for selenium species are not available. This problem can be solved by the synthesis of Se compounds expected to be found in the analysed samples or by isolation of the purified compound from the sample for its further identification by electrospray mass spectrometry [46,53,54].

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