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Analytical Methods

Speciation of selenium in vitamin tablets using spectrofluorometry following cloud point extraction

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ABSTRACT

Selenium is one of the trace and essential elements for good health but required only in a very narrow range. Hence, determination of selenium in trace level is important. In this work, cloud point extraction (CPE) with non-ionic surfactant Triton X-114 and the fluorometric ligand, 2,3-diaminonaphtalene (DAN) were used for extraction of trace amounts of organic and inorganic selenium species prior to their determination by spectrofluorometry. CPE parameters affecting complexation and phase separation were optimised. The limit of detection calculated by using nine replicate measurements of 0.020 mg/L Se solution after complexing with DAN and 10-fold CPE preconcentration was 2.1 μg/L. Accuracy of the method was checked using EnviroMat Waste Water, EU-L-2 as CRM and result was found to be in good agreement with the certified value. The suggested method can be used for selenium species of selenite, selenate, and total organic selenium at μg/L level.

1. Introduction

Selenium is a very important trace element in environmental and biological systems. The toxicity and bioavailability of selenium depend on both its chemical form and concentration like other trace metals (Besser, Canfield, & La Point, 1993). In fact, all essential elements may also be toxic for humans if they are taken at high levels for a long period (Aras & Ataman, 2006). Selenium has also been known as an antioxidant in diet (Groff & Gropper, 2000). It is a vital mineral antioxidant that helps prevent oxidative stress by working together with a group of nutrients that prevent oxygen molecules from becoming too reactive. This group of nutrients includes vitamin E, vitamin C, glutathione, selenium, and vitamin B3. Severe selenium deficiency caused by malnutrition has symptoms including weakness or pain in the muscles, discoloration of hair or skin, and whitening of fingernail beds (Pyrzynska, 2001; Wu, Salisbury, Graham, Lyons, & Fenech, 2009).

Selenium supplements contain selenium in different chemical forms. Current evidence favours selenomethionine over the other forms of selenium. Therefore, in the majority of supplements, the selenium is present as selenomethionine. However, in multivitamin preparations, infant formulas, protein mixes, weight-loss products and animal feed, sodium selenite and sodium selenate are predominantly used. In some products on the other hand, the form of selenium is not disclosed or poorly stated or described in label information (Schrauzer, 1998). The quality of some of the presently marketed supplements is thus questionable. Some products, for example, are prepared with inorganic selenium (selenite or selenate) although label information contains selenomethionine or the amount of selenium per tablet is not reported accurately. In recent years, increased research attention has focused on understanding the relationships between the Se species content in foods and supplement tablets and their nutritional benefits (Hymer & Caruso, 2000).

Fluorometric approach is one of the simplest, low cost and most versatile of all the methods to determine selenium. The use of complex forming reagents has been the main requirement for the determination of inorganic selenium by fluorometry. It is based on piazselenol complex formation between the reagent and selenium. The common reagents used are; o-diamines and 3,3-diaminobenzidine (Porcella, Bowie, Sanders, & Cutter, 1991), dithizone and o-phenylenediamine (Campbell & Yahaya, 1980), 2,3-diaminonaphthalene (Lavale & Dave, 1989) and 8-hydroxyquinoline (Bhat & Gupta, 1982). Among them, 2,3-diaminonapthalene (DAN) is the most widely used reagent because it is not toxic and readily available (Watkinson, 1996). The reactions with 2,3-diaminonaphthalene compounds are specific for Se(IV). Hence, Se(VI) must be reduced to Se(IV) before complexing reaction. Se-DAN complex formed in aqueous medium is usually extracted into organic medium in order to improve the sensitivity of Se(IV) determination (Watkinson, 1996).

Determination of trace element is a challenging analytical task, mostly due to the complexity of the sample matrix and the low

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concentrations of the analyte. Hence, determination of trace metals generally requires a preconcentration step. Cloud point extraction is an alternative preconcentration method to traditional extraction systems that use organic solvents because of its efficiency, low cost of commercially available surfactants, rapidity and safety (Lu, Tian, Wu, & Zhao, 2009). The aqueous solutions of nonionic surfactant materials become cloudy when their temperature reaches the cloud point temperature and analyte collapses with a small volume by a surface active material. The cloud point extraction can also be termed as temperature induced phase separation or micelle-mediated extraction (Saitoh, Tan, Watanabe, & Kamidate, 1995). Thus, CPE can lead to a high recovery efficiency and a large preconcentration factor due to a very small volume of micellar phase binding the analyte that was dispersed in the original matrix (Escaleira, Santelli, Oliveira, de Carvalho, & Bezerra, 2009). CPE is not only a preconcentration process but also a separation method, and this approach has been applied for the determination of many elements including Au (Meeravalia & Jiang, 2008) and Se (Ghambariana, Yaminia, Saleha, Shariatib, & Yazdanfar, 2009) in trace levels. Moreover, CPE technique is very suitable for fluorometric determinations. Because the high concentration of the micelles in the surfactant rich phase establishes a rigid and organised medium for the analyte; this, minimizes collisional quenching and leads an enhancement in the fluorescence signal.

In this study, cloud point extraction technique was used for the determination of Se(IV), Se(VI) and total organic selenium species by spectrofluorometry in commercially available selenium supplemented vitamin tablets. In the cloud point extraction process, Se(IV) is taken into a hydrophobic complex with 2,3-diaminonaphthalene (DAN) and then solubilised within the nonionic micelle Triton X-114. The hydrophobic complex, 4,5-benzopiazselenol, preconcentrated in the surfactant rich phase of Triton X-114 was determined spectrofluorometrically. The total selenium contents of the samples were determined utilising microwave nitric acid digestion and ICP-MS detection. This paper is the first study in literature to make speciation of selenium in selenium supplemented tablets using cloud point extraction and spectrofluorometric determination.

2. Experimental

2.1. Chemicals and reagents

2,3-diaminonaphtalene (DAN) was purchased from Sigma Aldrich. Hydrochloric acid, 37% (w/w), was purchased from Merck. DAN solution was prepared by dissolving 0.10 g of DAN and 5.0 g hydroxylamine hydrochloride, $NH_2OH \cdot HCl$ (Carlo Erba), in 100 mL of 0.10 M HCl. In order to effect dissolution, contents were heated at 50 °C for 25 min in a water bath using a magnetic stirrer. All of the working solutions were daily prepared and stored in dark since the reagent is light sensitive. Se(IV) and Se(VI) stock solutions (1000 mg/L) were prepared by dissolving proper amount of sodium selenite and sodium selenate, Na_2SeO_3 (Ventron) and Na_2SeO_4 (Ventron) in 100 mL de-ionised water. Triton X-114, octylphenoxypolyethoxy ethanol (Sigma), has been used as nonionic surfactant. Tris (hydroxymethyl)amino methane hydrochloride (Fluka, FL93363) was taken from Fluka. Protease XIV (Sigma, SIP 5147) was used in extraction studies. All the other reagents used in this study were at least analytical-reagent grade.

2.2. Instrumentation

De-ionised water obtained from a Millipore water purification system was used in all standard solution preparations and dilution studies. AF-2500 FL Hitachi Spectrophotometer equipped with a xenon lamp was used for fluorescence measurements. Instrument excitation and emission slits were adjusted to 20 nm. A glass cell with dimensions of 10 × 10 mm was used. Excitation and emission wavelengths were set to 380 nm and 530 nm, respectively. Spectra were obtained by scanning; PMT voltage was 400 V. Thermo X series ICP-MS was used for the determination of total selenium. Varian AA140 type AAS instrument was used in HGAAS measurements. Elma, Elmasonic S 40 H brand sonication instrument and shaker were used in the extraction studies. Sigma 2-16 (D-37520, Germany) brand ultracentrifuge instrument was used to separate supernatant. Milestone Ethos Plus microwave oven was used for the reduction of selenate to selenite.

2.3. Total selenium determination utilising ICP-MS

Supplement tablets were digested using concentrated nitric acid for the quantification of total selenium. The weight of tablets had a range of 1.0–1.5 g. Each tablet was weighed into a 100 mL PTFE vial and dissolved in 30 mL of concentrated HNO_3. Samples were heated to boiling on a hot-plate for 2.0 h. At the end of digestion, mixtures were filtered using 0.45 μm nylon filters. The final solutions were made up to 100.0 mL with deionised water. Three points standard addition technique was applied to sample solutions to eliminate the possible matrix interferences. In the standard addition technique, selenate, Se(VI), was used because all the selenium species should be converted to highest oxidation state after HNO_3 digestion.

2.4. Cloud point extraction procedure

In this study, Se(IV) reacts with 2,3-diaminonaphthalene (DAN) yielding the Se–DAN complex, 4,5-benzopiazselenol, that allows the determination of selenium in trace amounts either by spectrophotometry or fluorometry (Lott, Cukor, Moriber, & Solga, 1963). At the initial step of the cloud point extraction, pH of the solutions (8.0 mL initially) was adjusted to 1.50 by dropwise addition of 1.0 M NaOH solution and 1.0 M HCl and using a pH meter. In the second step, 0.10% (w/v) of DAN solution was added to the solutions. The molar concentration ratio of DAN to Se(IV) was kept equal to 50 in the final solution. Contents were heated at 70 °C in a water bath for 45 min in order to form Se(IV)–DAN complex. Then, 1.0 mL of 0.25% (w/v) of Triton X-114 was added to the solutions and the contents completed to a total of 10.0 mL and were kept at the refrigerator for 30 min to solubilise the hydrophobic complex in micellar medium. After this step, the mixture was again heated to 50 °C in water and kept there for 10 min in order to form the separated surfactant phase. Resulting mixture was centrifuged for 5 min at 4500 rpm and surfactant rich phase was separated from aqueous phase, then its volume was completed to 1.0 mL with de-ionised water; finally, fluorescence emission was measured.

2.5. Sample preparation for selenite [Se(IV)] containing samples, "Selenite Procedure"

Tablets were removed from the pack and crushed using a mortar and pestle. The resulting powder was accurately weighed into glass round bottom flasks and 30 mL of water was added. The extracts obtained after 25 min of sonication were centrifuged at 4500 rpm for 5.0 min, filtered using 0.45 μm nylon filters and separated into three portions for 3-point standard addition measurement. After spiking of Se(IV) standard solutions, cloud point extraction procedure given above were applied to each portion.