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Food Chemistry 92 (2005) 771–776
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Development of a chronopotentiometric stripping method for the determination of selenium in mixed diets

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Received 28 July 2004; received in revised form 12 October 2004; accepted 21 October 2004

Abstract

Selenium is an element both toxic and essential for humans, animals and microorganisms. The selenium effect depends mostly on its chemical form and concentration. For the determination and monitoring of selenium content in different foodstuffs reliable and selective analytical method should be applied. In this work two modifications of the chronopotentiometric stripping analysis (CSA) using mercury film electrode were investigated and optimised. The first one included deaeration step (CSA-DEA) prior selenium deposition in the electrode, while applying medium-exchange technique (ME-CSA) time consuming deaeration step was avoided. Experimental parameters such as electrolysis potential, electrolysis time, dissolution current, linearity and the concentration of the $CaCl_2$ were optimised in standard solutions of tetravalent selenium. Detection limits obtained for the CSA-DEA and ME-CSA modifications were 0.40 and 1.15 ppb, respectively. Interferences from copper, lead and iron were examined as well. For the methods verification standard reference material, wheat durum flour (RM 8436), was used. Two modifications of the chronopotentiometric stripping technique were applied for selective determination of total, Se^{6+}, and the sum of Se^{0}, Se^{2-} and Se^{4+} in different samples of flour, garlic and sunflower seed. Recovery test as well as the analysis of the reference material confirmed the correctness of the defined methods. Selenium contents determined with two different modifications were in agreement.

Keywords: Selenium; Chronopotentiometric stripping analysis; Diets

1. Introduction

Selenium has been shown to be essential for living organisms and to be toxic at levels little above those required for health. Its biochemical and physiological effect depends mostly on its amount and chemical form. The essentiality of selenium results from its presence in selenol (–SeH) group in many selenoenzymes (Berry, Banu, & Larsen, 1991; Rotruck, Pope, Swarson, Hafeman, & Hoekstra, 1979), positive effect on human immune and cardiovascular system and from powerful cancer chemopreventive effect (Schrauzer, White, & Schneder, 1978). Selenium, usually administrated as inorganic salts, decreases toxic effect of heavy metals (Zingaro & Cooper, 1974).

Selenium bioavailability is rather complex and depends on many factors. Absorption rate is not limiting step for the element bioavailability. After the absorption different chemical forms of the element are differently transformed in biologically active form and these processes are limiting selenium bioavailability. Lowest bioavailability is attributed to elemental selenium. Both selenomethionine and selenite are considered as biologically active forms of the element, though their biochemical pathways are different.

Selenide, selenomethionine, selenocysteine and other organoselenium compounds are efficient in preventing certain types of diseases, while in cases of some other diseases inorganic selenite is recommended. Inorganic

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doi:10.1016/j.foodchem.2004.10.040

selenate has lower biological activity and absorption comparing to selenite.

In order to understand selenium metabolism and its significance in biology, toxicology and nutrition, analytical techniques for selective determination of particular species of the element are of a great importance. Applied instrumental method must be specific to particular selenium species or the species must be separated prior selenium determination. Selenium speciation can be performed by coupling of electrophoretic or chromatographic separation techniques with an atomic spectrometric or other selenium specific technique (Uden, 1992; Meija, Montes-Bayon, Le Due, Terry, & Caruso, 2002). Neutron-activation analysis can be applied for selenium speciation only off-line after compounds have already been separated, while HPLC-ICP-MS offers possibility of direct on-line speciation (Pyrzinska, 1998). Neither ICP AES nor AAS coupled directly to HPLC can offer detection limits sufficient for selenium determination in biological samples. The detection limit can be improved by conversion of selenium into hydride. Coupled techniques still have little use in practice, since they are very complex, expensive, time consuming and often undefined completely. Many problems are associated with their use, such as dependence of the results on the way the sample is prepared, matrix interferences and lack of standards. Measured signals are often very hard to identify. Mentioned coupled techniques are attractive from scientific point of view rather than practical. Most commonly used techniques for selenium determination in practice are fluorimetry, AAS and voltammetric stripping techniques.

Fluorimetry and its many modifications are very sensitive and reliable analytical methods, which can be applied at most of the samples (Lu & Zheng, 1991; Mejuto-Marti, Bollain-Rodrigez, & Bermejo-Martinez, 1982; Pene & De Ment, 1942). They are based on piazselenol formation which is extracted with organic solvent and fluorimetrically determined. Graphite furnace or hydride generation atomic absorption spectrometry is often used for biological samples (Mailer & Paretley, 1983; Masscheleyn, Delaune, & Patric, 1991; Bernhard & Sperling, 1996; Plecko, Nordmann, Rukganer, & Kruse-Jares, 1999).

Electrochemical methods, such as dc stripping voltammetry (Gregori, Pinochet, Pottin-Gautier, & Astruc, 1996; Inam & Somer, 1999; Lange & Van den Berg, 2000; Mattsson, Nyholm, & Olin, 1994a; Mattsson, Nyholm, & Olin, 1994b) and differential pulse stripping voltammetry (Elleout, Quentel, & Made, 1996; Forbes, Bounds, & West, 1979; Rurikova & Tothova, 1999) are also commonly used techniques for selenium determination. Comparing to atomic absorption spectrometry and fluorimetry electrochemical techniques enable selective determination of the particular selenium species since only tetravalent selenium is electrochemically active. The techniques are less time consuming and enable the repetition of the analysis. Chronopotentiometric stripping analysis (CSA) comparing to voltammetric stripping techniques has better selectivity due to lesser influence of the capacity current. Accuracy of time measurement (quantitative characteristic in CSA) is higher comparing to current measurement (quantitative characteristic in stripping voltammetry). Nevertheless the technique has been less frequently used for selenium determination (Eskilsson & Haraldson, 1987; Gozzo et al., 1999). First step of the CSA is similar as in voltammetric techniques and represents the accumulation of the analyte in the electrode medium in the conditions of reproductive and intensive solution stirring. After the rest period which enables diffusive mass transfer in the next step and the homogenisation of the deposit in the electrode medium, the analyte is dissolved. In the CSA the dissolution of the analyte is performed by applying the constant current. Identification of the analyte is performed on the basis of the dissolution potential and quantitative analysis on the basis of the dissolution time. The aim of this work was to define and optimise the experimental parameters for the chronopotentiometric stripping determination of selenium using mercury film electrode. Mercury film electrode comparing to commonly used mercury drop electrodes shows better sensitivity due to significant surface and volume ratio. Background diffusion is avoided due to the nearness of the inert support. In order to avoid deaeration step, which is time consuming and is potential source of contamination, medium exchange modification of the CSA was also developed. Dissolution of the selenium deposit was performed in the $CaCl_2$ solutions. Developed techniques were used for specific selenium determination in wheat flour, garlic and sunflower seed. Procedure for the sample pretreatment is described.

2. Materials and methods

2.1. Instrumentation

CSA measurements were carried out on an automatic system for potentiometric and chronopotentiometric stripping analysis produced by Faculty of Technology, Novi Sad and "Elektrouniverzal", Leskovac (Suturović, Marjanović, & Jankovits, 1992). A glassy carbon disc working electrode of a total surface area of 7.07 mm^2 was used as an inert support of the mercury film. An Ag/AgCl (3.5 mol/l) electrode was used as the reference, and platinum wire as the counter electrode.

2.2. Reagents

Selenium (IV) stock solution (2 g/l) was prepared by dissolving sodium–selenite bihydrate (p.a., Merck) in

0.1 mol/l hydrochloric acid and was kept in polyethylene bottle in the dark. Working solutions containing 40 and 1 mg/l of Se(IV) were prepared every week and daily, respectively, by diluting selenium stock solution with double distilled water. Standard reference material (SRM 3149) containing 10 mg/ml of H_2SeO_4 in nitric acid was used for the preparation of the working solutions of Se(VI). Working solutions containing 40 mg/l of Se(VI) were prepared every week by diluting the stock solution with double distilled water.

Nitrogen of extra purity was used for the deaeration of the analysed solutions. Calcium-chloride solution (2 mol/l) was prepared by dissolving calcium-chloride bihydrate (p.a., Merck) in 0.05 mol/l hydrochloric acid. All containers and cells were washed with nitric acid (1:1), distilled and double distilled water.

2.3. Samples

Three different samples of wheat flour (type 400) and dried garlic as well as whole, partially and totally dehulled sunflower seed samples were commercial products, randomly collected at Novi Sad (Vojvodina) market.

Reference material (RM 8436), wheat durum flour with certified content of total selenium (1.23 ± 0.09 ppb), was used for the validation of the technique. Flour was prior preparation dried for 4 h at 85 °C in 1 cm layer.

2.4. Pretreatment of the samples

Samples were prepared for analysis by wet acid digestion of organic matter in open system and reduction of selenium (VI) to selenium (IV). Samples (3 g) were transferred to 100 ml quartz long-necked flasks and 20 ml of nitric acid, 10 ml of perchloric acid and 15 boiling pearls were added. After 15 min of heating at 75 °C when evolution of dark nitrogen oxides fumes stopped, heating was continued at 150 °C during 20 min until solution became clear and colourless. After the destruction of organic matter, perchloric acid was completely evaporated using a glass tube connected to vacuum pump. Dry residue was dissolved in 5 mol/l ($V = 5$ ml) hydrochloric acid and heated for 15 min at 75 °C in order to reduce hexavalent selenium to tetravalent one.

2.5. Chronopotentiometric determination

Preconcentration step of the modification with prior deaeration (CSA-DEA) was performed after 10 min of the deaeration at constant electrolysis potential in stirred solution. After 10 s of a rest period, mercury-selenide was stripped by an adequate current. Medium exchange modification of the CSA (ME-CSA) was performed without deaeration step. During last few seconds of the electrolysis step analysed solution was exchanged with acidified calcium-chloride solution.

3. Results and discussion

3.1. Influence of the CSA parameters

The influence of the most important experimental factors of the CSA was investigated in the model solutions of selenium (IV) in 0.2 mol/l hydrochloric acid. Mercury film electrode was formed by constant current electrolysis ($I = 50$ μA; $j = 0.7$ mA/cm^2) from a separate solution containing 100 mg/l of mercury(II) and 0.02 mol/l hydrochloric acid. Prior to each mercury film formation the glassy carbon surface was cleaned mechanically with chromatographic filter paper wetted firstly with acetone and than with double distilled water. Influence of the thickness of mercury film (d) on selenium analytical signal was examined by varying electrolysis time from 60 to 420 s. The mercury film formed at the deposition time of 300 s ($d \approx 163$ nm, assuming 100% electrolysis efficiency) has enabled highest analytical signal and its best reproducibility. About 10–15 analyses were possible to carry out at same mercury film if the medium exchange modification of the CSA was applied, but only 2–3 when the modification with the prior deaeration was used, because the fact that during the deaeration, nitrogen bubbles were damaging the mercury film.

Influence of the electrolysis potential was investigated in the range from −0.05 to −0.3 V. Selenium analytical signal decreased with more negative electrolysis potentials. Considering the height of the analytical signal as well as its reproducibility, potential of −0.1 V was chosen as adequate.

For lower selenium content (10 ppb) selenium analytical signal increased linearly with the longer deposition time. For higher selenium contents (60 ppb) longer electrolysis times caused the saturation of the electrode surface and the curving of the dissolution time (quantitative characteristics)-electrolysis time dependence. For shorter electrolysis times the dependence was linear. Comparing the slopes (the relative sensitivity) of the linear dependences obtained for higher and lower selenium contents, higher relative sensitivity was observed for the higher content.

Investigation of the influence of the dissolution current showed almost exponentional decrease of the sensitivity with higher dissolution currents for both lower (10 ppb) and higher (60 ppb) selenium contents. Dependence of the product of the dissolution current (I) and dissolution time (τ) on dissolution current ($I \cdot \tau = f(I)$) was used for the determination of the optimal range of the dissolution current. Namely, it is well known that the optimal dissolution currents are in the range where the "$I \cdot \tau$" product is approximately constant

(Suturović et al., 1992). For lower selenium content appropriate dissolution currents were in the range from −1.5 to −2.2 μA and for higher from −2.6 to −4.2 μA.

The linearity of the analytical signal was investigated in the range 20–60 and 100–140 ppb. Calibration yielded linear dependence for lower range of selenium content with average value of slope 0.030 s/ppb, intercept of 0.84 s and correlation coefficient (r) of 0.9947 ($n = 5$) for the modification with prior deaeration, and 0.026 s/ppb, 0.70 s and 0.9907 ($n = 5$) for the medium exchange modification, respectively. For higher range of selenium contents (100–140 ppb) for both modifications the polynomial regression gave a good fit to the experimental data with correlation coefficients of 0.9947 and 0.9907, respectively. Considering the application of standard addition method for liner range attention should be paid to the concentration of the standard added. In order to obtain corresponding enlargement of analytical signal much grater concentrations of the standard then expected should be added, due to small slopes. Also, for the application of the standard addition method enlarged results can be expected due to significant "τ" intercepts. For selenium content calculation calibration curve method is recommended.

Influence of the calcium-chloride concentration for the medium exchange modification of the CSA was investigated in the range 1–5.1 mol/l of calcium-chloride solutions, containing 0.05 mol/l hydrochloric acid. Investigation was carried out in solutions containing 60 ppb of selenium (IV). Increasing calcium-chloride concentration, selenium analytical signal decreased. As an adequate concentration was chosen 2 mol/l of calcium-chloride because it has enabled the highest sensitivity of the method with satisfactory reproducibility.

Repeatability (analytical reproducibility) for the CSA-DEA was calculated by analysing solutions containing 10 and 60 ppb of Se(IV). Electrolysis was performed during 90 s. After the solutions were left quiescent for 10 s, selenium was stripped applying currents of −1.5 and −4.2 μA, respectively. Determined repeatability for both contents was very good and expressed as variation coefficient was, for lower selenium content 6.02% ($n = 7$) and for higher 2.99% ($n = 7$).

After the methods optimisation the detection limits of Se(IV) (LOD) and limit of quantitation (LOQ) were determined. LOD and LOQ were calculated on the basis of $\bar{X}_b \pm 3$ SD and $\bar{X}_b \pm 10$ SD, respectively, where $\bar{X}_b$ is average of five selenium contents in blanks (ASC, 1980). The obtained values of LOD for the modification with prior deaeration and for the medium exchange modification were 0.40 ppb with coefficient of variation of 24.5% and 1.12 ppb with coefficient of variation of 20.3%, respectively. Quantitation limits were for CSA-DEA and ME-DEA, respectively, 1 and 1.18 ppb, with reproducibility expressed as variation coefficient, 9.3% and 9.87%, respectively.

3.2. Interferences

Influence of the copper on selenium analytical signal was examined in solutions containing 40 ppb of selenium (IV) by increasing the copper(II) concentration. For higher selenium concentrations, copper concentration that could be present in the solution and not to influence height and sharpness of selenium analytical signal, also increased. When the concentration of the copper(II) reached 2.5 mg/l mercury film electrode was blocked due to significant amount of the copper deposited at the electrode under the experimental conditions. Selenium could not be determined with "copper" electrode.

Influence of the iron(III) on selenium analytical signal was examined in solutions containing 40 ppb of selenium (IV) by varying ratio Fe:Se from 1:1 to 500:1. Iron(III) did not influence the selenium signal until the ratio Fe:Se was 200:1. For higher iron(III) concentrations change of the electrode potential was switched to the positive values, due to the strong oxidative Fe(III) effect.

The experiment concept of the investigation of lead influence was the same as in the case of iron. Under applied experimental conditions chronopotentiometric lead wave has been appearing at the similar potential as selenium. Two waves were observed at close potentials starting from ratio Pb:Se = 1:1. Within the ratio Pb:Se < 50:1 lead did not influence the selenium signal, but at higher concentrations lead signal covered the selenium wave and selenium could not be determined.

3.3. Determination of selenium in diets

Samples of garlic, wheat flour and sunflower seed were analysed in five parallel probes under defined experimental conditions, applying both modifications of the CSA. Blanks were analysed as well. For each sample selenium contents with and without reduction of hexavalent selenium were determined. The first one included total selenium content, and the second, elemental selenium (Se^0), selenide(Se^{2-}) and selenite(Se^{4+}) content. Therefore, subtracting these two contents, the content of electro-inactive hexavalent selenium could be determined. Recovery test regarding only tetravalent selenium was performed by adding standard solution of Se^{4+} into a mixture of the sample and the acids prior the destruction of organic matter. Results of recovery assay were in the range 96–102% and showed that in the conditions of controlled temperature tetravalent selenium do not oxidise significantly to hexavalent even in such aggressive conditions. Efficiency of the reduction of hexavalent selenium was examined in solutions containing 50 and 15 ppb of selenium (VI). Reduction procedure was performed after the same procedure for the

destruction of organic matter was applied. Efficiency of the reduction calculated on the basis of five parallel probes, was for higher selenium content 89.9% and for lower 98.9%. For lower selenium content which corresponds to selenium contents in real samples reduction procedure enables almost complete reduction. In the case of higher selenium contents correction on the basis of the determined reduction efficiency should be made. Selenium content was calculated using the method of calibration curve. At the same mercury film all probes of the sample as well as the standard solutions for calibration curve were analysed. In the case of the modification with the prior deaeration, during deaeration step electrode was held in double distilled water in order to avoid the damage of the mercury film by the bubbles of nitrogen (Švarc-Gajić, 2001). For all samples deaeration time of 10 min and electrolysis potential of −0.1 V was applied. Electrolysis time and dissolution current varied depending on selenium content and chronopotentiogram sharpness. Applying modification with prior deaeration (CSA-DEA), electrolysis times for garlic, wheat flour and sunflower seed samples were 120, 180 and 600 s, respectively and dissolution currents 3, 1.8 and 3.9 μA, respectively. In the case of medium exchange modification (ME-CSA) electrolysis time was 120 s and dissolution current 3.3 μA for all samples.

Total selenium contents (C_{tot}), the contents which represent the sum of Se^0, Se^{2-} and Se^{4+} (C) and the Se^{6+} content (C_{VI}) determined by both of the described modifications of the CSA in the samples of garlic, wheat flour and sunflower seed are shown in Table 1. Recovery was calculated for total selenium content which included reduction step, most critical for selenium losses.

Results of selenium determination show good agreement of the techniques. On the basis of variation coefficients calculated from shown 2SD, modification CSA-DEA has better reproducibility than ME-DEA. Reproducibility decrease is observed for both modifications for lower contents. Good results of recovery assay calculated for total selenium content validated the procedure for the sample pretreatment, procedure for the reduction of hexavalent selenium as well as the electroanalytic technique applied, assuming that the systematic errors were avoided. Since recovery of tetravalent selenium has been confirmed previously (p. 6.) recovery assay showed that at relatively low temperature (75 °C) no losses of volatile selenium species during the reduction step were observed. CSA-DEA modification expressed slightly higher recovery. Even though the CSA-DEA modification has better reproducibility and express higher recoveries, ME-CSA modification is more convenient for routine analysis, since 10–15 analysis can be performed at one mercury film and since time consuming deaeration step is avoided.

Accuracy of the developed modifications was confirmed by analysing reference material of durum wheat flour (RM 8436), with certified total selenium content of 1.23 ± 0.09 ppb. Determined average selenium content applying the CSA-DEA modification was 1.30 ppb ($n = 4$) and 1.27 ppb ($n = 4$) applying the ME-CSA modification.

Results of selenium determination in the samples are showing that garlic is richer natural selenium source comparing to wheat flour and sunflower seed. Hexavalent selenium, which has lower bioavailability comparing to organoselenium compounds and selenite, makes ~23% of total selenium content in garlic and ~20% in whole sunflower seed. Wheat flour contains higher amount of hexavalent selenium. The selenium contents obtained in whole, partially and a totally dehulled

Table 1
Selenium content in garlic, wheat flour and sunflower seed determined with the modification with prior deaeration (CSA-DEA) and medium-exchange modification (ME-CSA) of the chronopotentiometric stripping analysis

	Selenium content [ppb]					
	CSA-DEA			ME-CSA		
	C [a]	C_{tot} [b]	C_{VI} [c]	C	C_{tot}	C_{VI}
Garlic 1	0.62 ± 0.03[d]	0.81 ± 0.07 (100.1)[e]	0.19	0.61 ± 0.06	0.83 ± 0.08 (97.6)	0.22
Garlic 2	0.68 ± 0.04	0.95 ± 0.02 (99.9)	0.27	0.66 ± 0.05	0.94 ± 0.03 (98.6)	0.28
Garlic 3	0.80 ± 0.02	0.99 ± 0.01 (98.0)	0.19	0.78 ± 0.04	0.99 ± 0.02 (97.0)	0.21
Wheat flour 1	0.02 ± 0.01	0.10 ± 0.02 (97.5)	0.08	nd[f]	0.11 ± 0.02 (92.0)	nd
Wheat flour 2	nd	nd	nd	nd	nd	nd
Wheat flour 3	0.08 ± 0.01	0.18 ± 0.01 (99.5)	0.10	0.08 ± 0.02	0.18 ± 0.01 (97.6)	0.10
Whole sunflower seed	0.09 ± 0.01	0.11 ± 0.02 (99.1)	0.02	0.09 ± 0.02	0.12 ± 0.02 (93.2)	0.03
Partially dehulled sunflower seed	0.06 ± 0.02	0.06 ± 0.01 (95.0)	nd	0.06 ± 0.02	0.06 ± 0.02 (98.2)	nd
Totally dehulled sunflower seed	0.04 ± 0.01	0.05 ± 0.01 (91.0)	0.01	0.05 ± 0.02	0.05 ± 0.01 (88.4)	nd

[a] The sum of Se^0, Se^{2-} and Se^{4+}.
[b] Total selenium content.
[c] The content of Se^{6+}.
[d] Uncertainties for the experimental results are calculated as twice the standard deviation ($\bar{X} \pm 2$ SD).
[e] Values in parentheses are results of recovery assays (%).
[f] Not detected.

sunflower seed show that selenium is present in both the seed husk and kernel.

Increased selenium intake can be achieved either by consuming the food enriched with selenium or by taking adequate pharmaceutical products. Consequently, selenium determination and determination of its different chemical forms in foodstuffs, applying accurate and reliable analytical methods, is of a great importance.

4. Conclusions

Developed modifications of the CSA can be applied for the reliable selenium determination in garlic, wheat flour and sunflower seed. Developed techniques offer certain advantages, such as simplicity, selectivity and relatively low price of the instrumentation and methods exploitation comparing to other instrumental methods. Duration of the analysis, especially with ME-CSA is much lower comparing to any other techniques. Applying different procedures for the sample pretreatment and obliged to hexavalent selenium electroinactivity, content of Se^{6+}, content of the sum of Se^{0}, Se^{2-} and Se^{4+}, as well as total selenium content can be determined. Determined total selenium contents in garlic samples were higher than those determined in wheat flour and sunflower seed. Using appropriate sample pretreatment the described methods can be applied for the selenium determination in other similar diets.

Acknowledgements

These results are part of the project 'Development of Methods for identification of Toxic and Carcinogenic Pollutants in Mass Cultures and Procedures of their separation', Financially supported by the Ministry of science, Technology and Development of the Republic of Serbia.

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