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Determination of selenium in garlic by cathodic stripping voltammetry

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Abstract

The selenium content in a garlic sample was determined on hanging mercury drop electrode (HMDE) using cathodic stripping voltammetry (CSV). In this method the dried garlic sample was digested in HNO_3:$HClO_4$ (1:1) by wet-digestion procedure. The CSV voltammogram in 0.1 M HCl solution showed a peak for Se at −0.56 V. Effect of deposition potential, deposition time and sweep rate on this peak were tried to determine the optimum experimental conditions. A deposition potential of −0.2 V was applied for 120 s while stirring the solution by passing nitrogen and followed a potential scan of 50 mV/s in a negative direction. The standard addition method was used to determine selenium in the sample. The linear domain range of Se (IV) was 2.0×10^{-8}–6.0×10^{-7} M with a correlation coefficient of 0.9985. This method was used for the first time for the determination of selenium in garlic sample without any separation procedure and preconcentration techniques such as ion exchange, solvent extraction or hydride generation. The amount of selenium determined in three different samples from three different regions were 370 ± 26 ($n=5$), 485 ± 35 ($n=4$) and 365 ± 46 ($n=4$) ng/g (dry-weight) with relative standard deviations of 7.0, 7.2 and 12.6%, respectively.

Keywords: Selenium; Garlic; Cathodic stripping voltammetry

1. Introduction

The physiological effects of selenium have only recently been understood. Although it is an essential nutrient at trace levels, it becomes toxic when in excess (Zingaro & Cooper, 1974). The possibility that increased intakes of selenium might protect against the development of cancer in humans has generated great interest. Moreover, a recent analysis of the relationship between selenium and cancer suggests that "the question of whether selenium protects against cancer is still wide open". Furthermore, an increased intake of selenium appears to stimulate tumorigenesis in some animal models of pancreatic and skin cancer (Birt, Pour, & Pellin, 1989).

Typical values of the selenium content of various foods have been reported as follows (WHO, 1987) (mg/kg wet weight): liver, kidney and sea food 0.4–1.5; muscle meats 0.1–0.4; cereals and cereal products 0.1–0.8; dairy products, <0.1–0.3 and fruits and vegetables <0.1. Despite the regular intake of the element, its levels are generally low in human tissues and body fluids. Selenium concentration ranges of 4.8–46 μg/l in urine, 57–320 μg/l in whole blood, 261–410 ng/g in liver, and 100–630 ng/g in kidney have been reported (Iyengar, Kollmer & Bowen, 1978). These low concentrations of selenium require a highly sensitive and specific method for accurate determination.

The determination of trace levels of selenium in biological material is usually difficult. Analysis by atomic absorption spectroscopy has been shown to be a quick and simple method using the hydride vapour generation technique (Mailer & Paretley, 1983). Although there are several references to this method, there are also numbers of factors generally overlooked such as interferences, matrix effects and losses through volatilization at high temperatures. The limit of detection is also poor compared to voltammetric methods and thus preconcentration is usually needed. Modern voltammetric methods have been used for the determination of trace amounts of selenium. Of these anodic stripping voltammetry (ASV) (Aydin & Somer 1989), cathodic stripping voltammetry (CSV) (Holak & Specihio, 1994), adsorptive stripping voltammetry (AdsSV) (Inam & Somer, 1997; Tanaka, Sugawara & Toga, 1990) and differential pulse polarography (DPP) (Adeloju, Bond & Briggs, 1985) are the most popular.

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As known, garlic has only recently taken an important place in human diet although it is a common food in some parts of the world. Because of its richness of sulphur-containing compounds, it was thought that it would contain selenium compounds also. Only a few spectrophotometric studies (Yuan, 1994; Zhang, Yuan & Tian, 1994) could be found in the literature for the determination of selenium in garlic, however the selenium contents could not be obtained. The aim of this investigation was to develop a direct method for the determination of selenium in garlic and to compare the content of selenium in garlic samples taken from three distinct regions of Turkey using cathodic stripping voltammetry.

2. Materials and methods

2.1. Materials

A polarographic analyzer (PAR 174 A) together with a home-made capillary hanging mercury drop electrode (HMDE), and Linseis LY 1600 Model X-Y recorder was used for all voltammetric measurements. For HMDE (Inam & Somer, 1997), a glass stop-cock was sealed to glass tubing. The lower ending of the tubing was extended as a capillary, and a platinum wire was sealed into the capillary below the stop-cock. The upper section of the stop-cock was used as a mercury reservoir. A platinum wire was used as the counter electrode and SCE as the reference electrode. All the potentials were measured against the SCE.

2.1.1. Reagents

All reagents used were of analytical-reagent grade (Merck). Triply distilled water was used for the preparation of all solutions and at all other stages of analysis. The mercury (pro analysis) used in the HMDE was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 and water columns in the form of fine droplets. The collected mercury was dried between sheets of filter-paper. Before use, a stripping voltammogram of this mercury was recorded in order to confirm the absence of impurities.

A 0.1 M stock solution of Se(IV) was prepared by dissolving SeO_2 in hot water. 1.0×10^{-3}, 1.0×10^{-4} and 1.0×10^{-5} M working solutions of Se(IV) were prepared before every use in order to avoid the aging process of the solution.

2.2. Methods

2.2.1. Preparation and digestion of garlic sample

The bulb of the garlic from three distinct regions of Turkey were collected. After their outer skins were removed, the bulbs were dried for 1 day in oven at 60°C to remove water content and obtain a constant weight.

Five grams of dried garlic sample and 10 ml HNO_3:$HClO_4$ (1:1) was transferred into 100 ml long-necked glass flask. This sample was kept in the acid mixture overnight. A pre-cleaned glass funnel was inserted into the glassware to prevent rapid evaporation, heated directly and gently with a bunsen flame. This mixture was heated until nitrogen oxides fumes were just given off. When the digestive sample turned from yellowish to deep-dark, this indicated a danger of explosion, so three separate additions of 3.0 ml HNO_3 must be added, cooling the flask for about 2 min between each addition. After the final addition of HNO_3, the heating was continued at the same temperature until the nitrogen oxide fumes were completely evolved. The sample solution was still yellowish so three separate additions of 3.0 ml $HClO_4$ was done to obtain complete digestion (clear colorless solution). After each acid addition, it was evaporated nearly to 1.0 ml. Finally, 2.0 ml of 37% HCl was added and heated for at least 20 min to convert all selenium to selenium(IV). The digested sample solution (~1.0 ml) was cooled to room temperature, rinsed the funnel into the flask with water and the contents were transferred into a 10.0 ml calibrated flask, making up to the mark with triply distilled water.

2.2.2. Voltammetric determination

The blank solution was prepared with the addition of 100 μl concentrated HCl into 9.0 ml of the triply distilled water in the 20 ml of pyrex-cell, and nitrogen gas (99.999%) was passed for 5.0 min at a flow rate of 100 ml/min. Then a mercury drop was suspended and the deposition was carried out for 120 s at −0.2 V while stirring the solution by passing nitrogen at a flow rate of 100 ml/min. After a rest period of 15 s, a potential scan of 50 mV/s was applied in a negative direction to obtain cathodic stripping voltammogram (CSV) of the blank solution. After addition of 1.0 mL digested sample solution the same deposition and stripping procedure was repeated and CSV of the garlic sample obtained.

3. Results and discussion

3.1. Effect of deposition potential

In the stripping procedure, the determination of the elements in acidic media is based on the use of a deposition potential. In order to obtain a well defined reduction or oxidation peak, the optimum deposition potential must be obtained. For this purpose, the deposition was carried out on the HMDE for 120 s at several selected deposition potentials. The relation between the peak current and the deposition potential is shown in Fig 1. The selenium could be accumulated

efficiently on the electrode at −0.2 V, and a well-defined peak at −0.56 V which responded well on standard additions of selenium (Fig. 2). At a more negative potential than −0.2 V the peak current decreased sharply and reached to zero at −0.4 V, probably due to the reduction of Se (IV) to Se(−II). The continuous decreasing of the CSV peak for selenium at deposition potentials more positive than −0.2 V may be caused by the progressively incomplete reduction of Se(IV) to Se(0). In practice, the deposition potential was fixed at −0.2 V vs. SCE.

3.2. Effect of deposition time

The accountable electrolysis time in stripping analysis includes deposition time (t_d) and the equilibrium period (t_{eq}). Theoretically the observed peak current should be directly proportional to the t_d, however, this is not often the case in practice. Fig. 3 shows that the height of stripping peak was proportional to the deposition time up to 120 s, and it appeared that an equilibrium surface concentration was reached in the latter case when longer deposition times were used. A 120 s deposition time was suitable for the determination of selenium at the given conditions.

3.3. Effect of the sweep rate

Fig. 4 shows the effect of sweep rate on the CSV peak current between 5 and 50 mV/s. The peak current for selenium was measured as a function of sweep rate

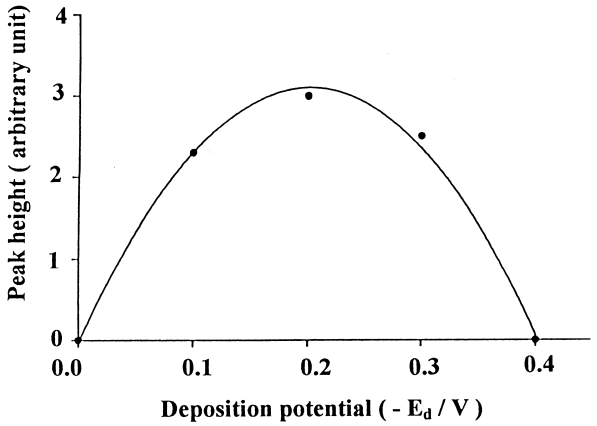

Fig. 1. Effect of varying the deposition potential on the CSV peak for the Se in garlic sample. The standard analytical procedure was used. Deposition time, 120 s.

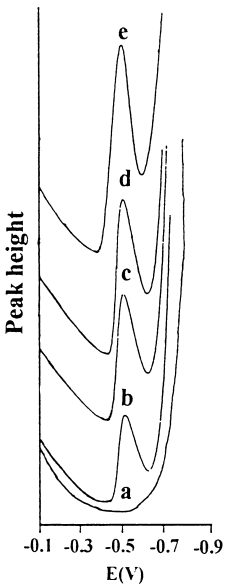

Fig. 2. Response of selenite peak to selenite additions: (a) 10.0 ml 0.1 M HCl; (b) a + 100 μl 1.0×10^{-5} M SeO_3^{2-}; (c) b + 100 μl 1.0×10^{-5} M SeO_3^{2-}; (d) c + 100 μl 1.0×10^{-5} M SeO_3^{2-}; (e) d + 100 μl 1.0×10^{-5} M SeO_3^{2-}.

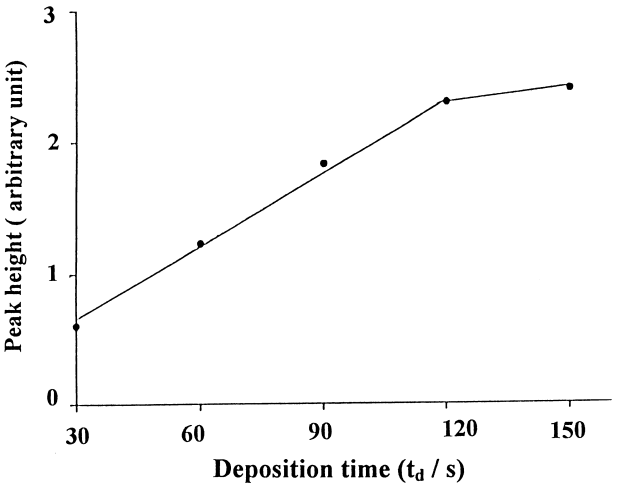

Fig. 3. Effect of varying the deposition time on the CSV peak for the Se in garlic sample. The standard analytical procedure was used. Deposition potential, −0.2 V.

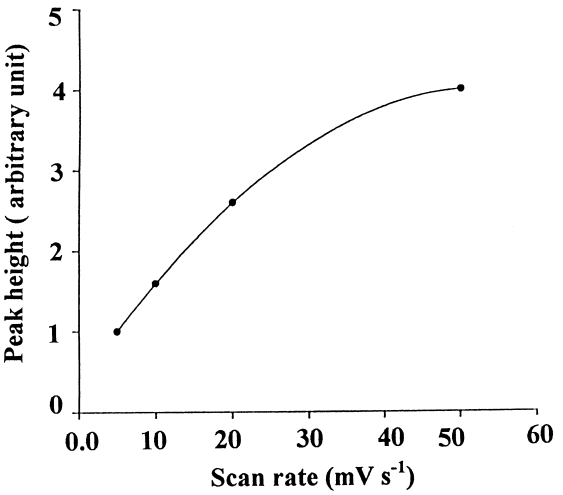

Fig. 4. Effect of varying the scan rate on the CSV peak for the Se in garlic sample. The standard analytical procedure was used. Deposition potential, −0.2 V; deposition time, 120 s.

using the optimized analytical procedure. There was a steady increase in the peak height from 5 to 20 mV/s, whereas the increment of peak height slows down after 20 mV/s. A scan rate 50 mV/s was chosen because it gave a linear response for increasing concentration of selenium and was sufficiently rapid for routine analysis.

3.4. Determination of selenium in garlic sample

Nine ml of triply distilled water and 100 μl concentrated HCl were put into the cell and nitrogen gas (99.999%) was passed through for 5 min at a flow rate of 100 ml/min. Then, a fresh mercury drop was suspended and accumulation carried out for 120 s at −0.2 V while stirring the solution at the given flow rate. After a rest period of 15 s, stripping voltammogram of blank solution were recorded by scanning the potential toward the negative direction at a scan rate of 50 mV/s. After addition of 1.0 ml digested garlic sample solution, the above procedure was repeated and a well-defined selenium peak was observed at −0.56 V vs. SCE (Fig 5). Standard additions of 100 μL 1.0×10^{-5} M SeO_3^{2-} caused increments at the same potential and made the determination of selenium in garlic sample solution possible. The linear domain range of Se (IV) was 2.0×10^{-8}–6.0×10^{-7} M with a correlation coefficient of 0.9985.

According to Lingane (Lingane & Niedrach, 1948) and to our former studies (Inam & Somer, 1998) selenite is reduced to elemental selenium at about −0.2 V in acidic solution:

$$SeO_3^{2-} + 6H^+ + Hg + 4\bar{e}- \rightarrow Se(Hg) + 3H_2O \quad (1)$$

and then selenium is further reduced to selenide during stripping step:

$$Se(Hg) + 2H^+ + 2\bar{e}- \rightarrow H_2Se + Hg \quad (2)$$

Table 1 shows the amount of selenium in three different samples taken from three distinct regions of Turkey. According to the results obtained, samples from cities C and A near the seaboard contain nearly the same amounts (365–370 ng/g) of selenium. Samples from city B, one of the most sulphur rich areas of Turkey, showed higher selenium content (485 ng/g) than the others. The relative standard deviations are 7.0, 7.2 and 12.6% at a concentration of 370 ($n=5$), 485 ($n=4$) and 365 ($n=4$) ng/g selenium in garlic sample, respectively.

The reliable voltammetric determination of trace elements in biological and environmental materials requires careful consideration of choice of decomposition and stripping method. The determination of trace elements in biological samples is usually difficult, since it involves destruction and pre-concentration techniques such as ion exchange, solvent extraction or hydride generation.

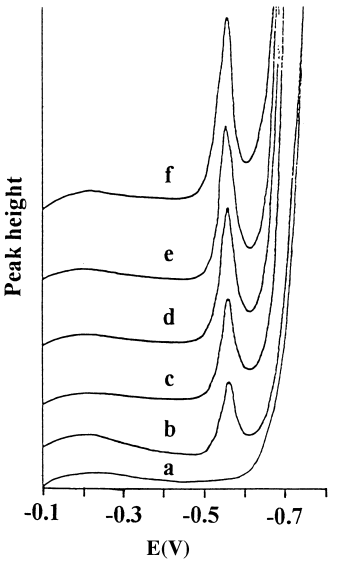

Fig. 5. Determination of Se in garlic using CSV: (a) 9.0 ml triply distelled water and 100 μl HCl; (b) a + 1.0 ml digested garlic sample (c) b + 100 μl 1.0×10^{-5} M SeO_3^{2-}; (d) c + 100 μl 1.0×10^{-5} M SeO_3^{2-}; (e) d + 100 μl 1.0×10^{-5} M SeO_3^{2-}; (f) e + 100 μl 1.0×10^{-5} M SeO_3^{2-}.

Table 1
Determination of Se in garlic by CSV

Garlic sample	Number of experiments	x ± s (Se, ng/g)	x ± (t×s)/$N^{1/2}$ (Se,ng/g)[a]	Relative standard deviation (%)
Mersin(A)	5	370 ± 26	370 ± 25	7.0
Keçiborlu(B)	4	485 ± 35	485 ± 41	7.2
Zonguldak(C)	4	365 ± 46	365 ± 54	12.6

[a] t, Confidence interval (90%); *N*, number of experiment.

Most of these techniques are laborious and time consuming and, as the quantity of selenium in biological samples is small, there is the possibility of loss during the pre-concentration stage. The present method, which was used for the first time for the determination of selenium in garlic, enabled the direct determination of selenium in garlic samples without any separation and pre-concentration techniques. It can be applied to other biological samples containing complex mixtures of metals.

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