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A direct method for the determination of selenium and lead in cow's milk by differential pulse stripping voltammetry

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Abstract

The selenium and lead contents in milk samples from Turkey were determined using a hanging mercury drop electrode (HMDE) and differential pulse cathodic stripping voltammetry (DPCSV) and differential pulse anodic stripping voltammetry (DPASV), respectively. In this method, the milk samples were digested in HNO_3 : $HClO_4$ (1:1) mixture by a wet digestion procedure. The DPCSV of milk samples in 0.1 M HCl solution showed a peak for selenium at −0.56 V , and DPASV for lead showed a peak at -0.35 V. A deposition potential of -0.2 V for selenium, and −0.5 V for lead were suitable. The standard addition method was used to determine selenium and lead in the sample. The linear domain range was 75–1.2 μg/l for selenium with a correlation coefficient of 0.9981 and 185–8.7 μg/l for lead with a correlation coefficient of 0.9945. The proposed method provides a simple and suitable procedure for the determination of trace amounts of selenium and lead. In this method, there is no need for sophisticated instruments and tedious separation procedure. Selenium and lead contents of milk samples from three distinct regions of Turkey were obtained between 21.5–69.4 and 22.1–59.2 μg/l ($n = 4$–5), with the relative standard deviations of 10.3–10.7 and 6.8–9.9% , respectively.

Keywords: Selenium; Lead; Milk; Differential pulse stripping voltammetry

1. Introduction

Milk and milk products are one of the most important foods due to their protein and mineral contents. Minerals are single inorganic elements widely distributed in foods and living bodies. In their ionized form they build body tissue ; activate , regulate and control metabolic process. Selenium was first recognized to be important in normal metabolism in 1957 by Schwarz and Foltz, who found that it prevented necrotic degeneration of the liver in the vitamin-E deficient rat (Combs & Combs 1984). Selenium functions as an essential part of an antioxidant enzyme that protects cells and their lipid membranes against oxidative damage (Zingaro & Cooper, 1974). The role of selenium in fatty acid metabolism explains in part the nutritional interrelationship between the actions of selenium and vitamin-E since vitamin-E is also involved in the oxidation of fatty acids. Selenium is an essential trace element for humans. There is a small difference between the concentration range at which it is toxic and at which it is considered essential (Burk 1977). For example, selenium is a highly toxic agent and excess intake leads to pronounced toxic symptoms (Roekens, Deelstara & Robberecht, 1985). Despite the regular intake of the element, its level is 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, 260–410 ng/g in liver and 100–630 ng/g in kidney have been reported (Iyengar, et al. 1978).

Meat, sea foods, milk, eggs and other high protein foods are good sources of selenium (Chaney, Ross & Witsch, 1979). The amount of selenium in foods is influenced by the selenium content of the soil. The minerals move from the soil to the plants that grow on it, then to the animals that eat plants. The concentration of selenium in human milk samples obtained from vegetarian women was significantly greater (22.2 ng/ml) than from nonvegetarian women (16.8 ng/ml) (Debski, Finley, Piciano, Lonnerdal & Milner, 1989). Selenium concentration in cow milk was determined by several workers as 10.9 ± 0.3 μg/l in Belgium (Dael, Vlaemynck, Renterghem & Deelstara, 1991) , 17.0 ± 5.0 μg/l in Greece (Bratakos, Zafiropoulos, & Ioannou, 1987), 25.73 ± 5.25 μg/l in India (Vivekanandan, Krishnasamy, Ayyudurai & Swaminathan, 1993), 22.4 μg/l in United State (Lean, Troutt, Boermans, Moller, Webster & Tracey, 1990) , 5.4–11.7 μg/l

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in Hungary (Lassu-Merenyi, & Nagy, 1989), 11–17 μg/kg in the Netherlands (Koops, Klomp & Westerbeek, 1989). The selenium content of milk is mostly determined by fluorimetric analysis. In this method the selenium is to be extracted by complexone III (sodium ethylenediaminetetraacetate) or 3,3 diaminonapthalene after digestion with acids. This procedure is time consuming and obtaining these reagents is quite difficult. For this reason alternative procedures have been evaluated. Lead poisoning has been a major public health problem for centuries. Jeng, Lee and Lin, 1994 determined lead in raw milk by graphite furnace atomic absorption spectrophotometer (GFAAS), the mean Pb content was 2.03 μg/l. Barreira and Arribas (1990), determined Pb in cows milk by differential pulse striping voltammetry (DPSV) ; milk from Spain contained 4–46 μg/kg Pb. Zorica & Liljana, (1989), determined Pb in milk by DPSV , it was between 12.5 and 100 μg/kg milk. The large differences of Pb content of milk samples may be due to the nutrition of cows. Although the neutron activation technique has a high sensitivity , it is not frequently used because of the specialized techniques, skills, time and costs involved (Conrad & Kenna 1967). Other accessible technique capable of multi-element determination such as X-ray fluorescence (XRF), atomic emission spectrometry with inductively coupled plasma excitation (AES–ICP) and ICP–MS are very expensive for the determination of most elements at the trace to ultra-trace concentrations usually encountered in biological and environmental samples. Voltammetric techniques such as anodic stripping voltammetry (ASV) (İnam & Aydin, 1996; Karacan, Somer & Kalaycı, 1997), cathodic stripping voltammetry (CSV) (Ahmad, Hill & Magee, 1983; Nimmo & Fones, 1994) , differential pulse polarography (DPP) (İnam and Somer 1998) and the more recent approach of adsorption voltammetry (AV) (Kalvoda, 1984 ; İnam & Somer, 1997) require relatively inexpensive instrumentation, are capable of determining elements accurately at trace to ultra-trace levels and have demonstrated ability for multi-element determination.

In the present work, we propose a method for the determination of selenium and lead in cow milk using DPSV without any separation, pre-concentration and limitations. For this purpose, selenium and lead concentrations in cow milk collected from different cities in Turkey are determined and the data obtained are compared with the published values.

2. Materials and methods

2.1. Materials

A polarographic analyzer (PAR 174 A, Princeton Applied Research Company) together with a home-made capillary hanging mercury drop electrode (HMDE), and Lenseis LY 1600 Model X–Y recorder were used for all voltammetric measurements. For HMDE (İnam & 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 in to 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 a counter electrode and saturated calomel electrode(SCE) as the reference electrode. All the potentials were measured against SCE.

2.1.1. Reagents

All the reagents used were of analytical grade. Nitric acid (65%), perchloric acid (70%), hydrochloric acid (37%) were used in digestion procedure. The stock solution of Pb(II) (0.1 M) was prepared by dissolving $Pb(NO_3)_2$ in water. A 0.1 M stock solution of Se(IV) was prepared by dissolving SeO_2 in hot water. Working solutions of Pb(II) and Se(IV), 1.0×10^{-3}, 1.0×10^{-4} and 1.0×10^{-5} M were prepared before use in order to avoid aging of the solutions. Triply distilled water was used for the preparation of all solutions and at all stages of analysis. The mercury (pro analisis) used in the hanging mercury drop electrode (HMDE) was obtained from Merck (Darmstat, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) 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.

2.2. Methods

2.2.1. Digestion of samples

The cow milks were collected from three regions of Turkey. Fifty milliters of milk, 10.0 ml of nitric acid and 10.0 ml of perchloric acid were transferred into a 300 ml Erlenmeyer flask and kept overnight. It was evaporated gently by using a Bunsen flame until its volume reached approximately one-third of the original volume. This mixture was transferred into a pre-cleaned 250 ml Kjeldahl digestion flask. A glass funnel was inserted into the digestion flask to prevent rapid evaporation. The flask was heated directly and gently with a Bunsen flame. Heating was continued until the evolution of brown fumes of nitrogen oxides ceased. When the digestive sample turned from yellowish to deep dark, there was a danger of explosion, so 5.0 ml of nitric acid and 5.0 ml of perchloric acid were added, cooling the flask for about 2.0 min before addition. Heating was continued until nitrogen oxide fumes were completely given off. The digestion was completed with the appearance of white fumes of perchloric acid when approximately 1.0 ml solution remained. Finally, 2.0 ml of hydrochloric

acid was added and heated for at least 20 min to convert all selenium to selenium (IV). During the digestion period, care was taken to ensure that no charring of the sample occurred, since it can cause loss of selenium as volatile $SeCl_4$ and SeO_2. After completely performing the above procedure for another two separate 50.0 ml of milk samples, the three samples were combined to concentrate the contents. The final solution was evaporated to approximately 1.0 ml, cooled to room temperature, the funnel rinsed into the flask with water and the contents transferred into a 25.0 ml calibrated flask which was made up to the mark with triply distilled water.

2.2.2. Voltammetric determination

Nine milliliters of supporting electrolyte solution containing 100 μl hydrochloric acid (37%) was put into the voltammetric cell and deoxygenated with high-purity nitrogen (99.999%) for 5 min. A deposition potential of −0.2 V was applied to a fresh mercury drop while the solution was stirred with nitrogen at a flow rate of 100 ml/min for 2 min. Following deposition, the solution was left to stand for 15 s. The DPCS voltamogram was recorded during the potential sweep from −0.2 to −0.7 V at a scan rate of 20 mV/s and pulse amplitude of 50 mV (pulse duration 50 ms). After obtaining the background voltammogram, 1.0 ml of digested milk sample solution was introduced into the cell while maintaining a nitrogen atmosphere over the solution. The same procedure was applied for the milk sample solution and DPCS voltammogram was recorded to obtain the peak for selenite. The above procedure was repeated for the same sample solution to obtain DPAS voltammogram for lead, but at a deposition potential of −0.5 and scanning of potential from −0.5 V to 0.0 V.

3. Results and discussion

3.1. Effect of deposition potential

In the stripping procedure, accurate determination of the elements in acidic media is based on the use of deposition potential. In order to obtain a well defined reduction or oxidation peak, the optimum deposition potential must be obtained. For this purpose, during the DPCSV procedure, deposition was carried out on the HMDE for 120 s at several selected potentials between −0.1 and −0.4 V (in 0.1 M HCl). The relationship between the peak current of selenite in the milk sample and deposition potential is shown in Fig.1. The selenium (0.15 μM) could be deposited efficiently on the electrode at −0.2 V , and gave a well defined peak at −0.56 V which responded well on standard additions of selenite ion. At a more negative potential than −0.2 V the peak current decreased sharply and reached zero at −0.4 V, probably due to the reduction of Se(IV) to Se(−II) during the deposition step. The continuous decrease of DPCSV 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.

On the other hand, for the determination of lead in the milk sample, the DPASV procedure was applied to the sample in 0.1 M HCl. Deposition was carried out for 120 s by applying potentials between −0.7 and -0.4 V (Fig. 2). Lead (0.25 μM) in the milk sample could be deposited efficiently on the electrode at −0.5 V, and a well-defined peak at −0.35 V which responded well on

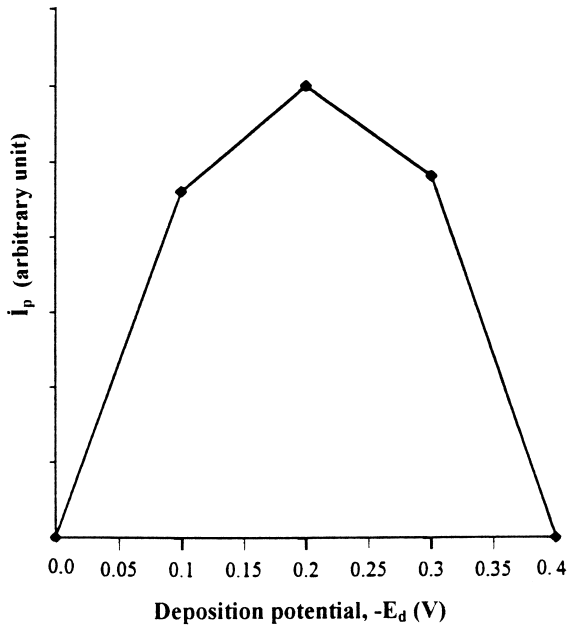

Fig. 1. Effect of varying the deposition potential on the DPCSV peak for the Se (0.15 μM) in milk sample. Deposition time = 120 s (the standard analytical procedure was used).

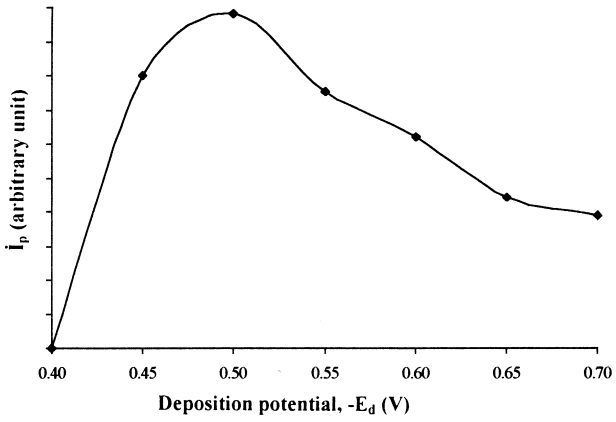

Fig. 2. Effect of varying the deposition potential on the DPASV peak for the Pb(II) (0.25 μM) in milk sample. Deposition time = 120 s (the standard analytical procedure was used).

standard additions of lead ion, was obtained. At a more positive deposition potential than −0.5 V, peak current decreased sharply and it reached to zero at −0.4 V, probably due to the incomplete reduction of lead to form an amalgam with the mercury electrode. At a more negative potential than −0.5 V peak current decreased continuously. The deposition at −0.5 V was suitable for obtaining a reproducible peak current.

3.2. Effect of deposition time

The peak current for selenium (0.15 μM) in the milk sample was measured using DPCSV as a function of deposition time using the optimized analytical procedure. 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 in the case in practice. It was found for selenium that the peak current increased with increasing deposition time when $t_d \leqslant 120$ s and it was nearly constant at deposition times larger than 120 s (Fig. 3a). This observation at long deposition times is caused by the saturation of the HMDE surface.

Fig. 3b shows the change of DPASV peak current of lead (0.25 μM) in the milk sample with deposition time. As can be seen, the stripping peak was proportional to the deposition time and increased up to 120 s. 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 lead and selenium under the optimized conditions.

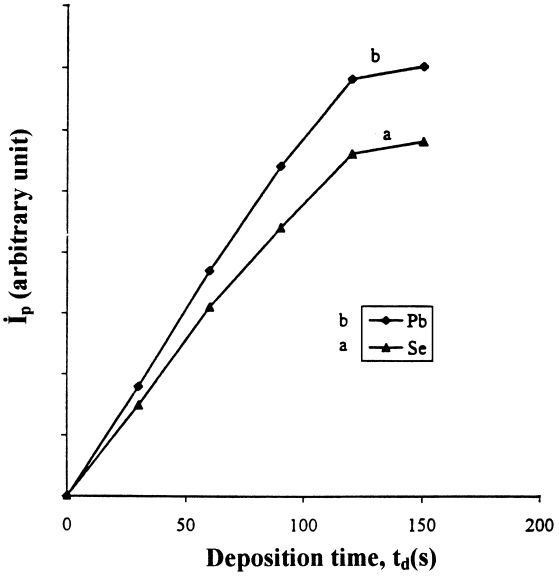

Fig. 3. (a)Effect of varying the deposition time on the DPCSV peak for the Se (0.15 μM), (b) DPASV peak for the Pb(II) (0.25 μM) in milk sample. Deposition potential = −0.5 V (the standard analytical procedure was used).

3.3. Effect of sweep rate

The peak current for selenium and lead were measured as a function of sweep rate (between 5 and 50 mV/s) using the optimized analytical procedure. There was a steady increase in the peak height between 5 and 20 mV/s, whereas the increment of peak height slows after 20 mV/s. A scan rate of 20 mV/s for both selenium and lead was chosen because it showed a linear response for increasing concentrations and was sufficiently rapid for routine analysis.

3.4. Determination of selenium and lead in the milk sample

Nine milliliters of 0.1 M hydrochloric acid solution in the voltammetric cell was deaerated by passing pure nitrogen (99.999%) for about 5 min at a flow rate of 100 ml/min. Then a fresh mercury drop was suspended and deposition was carried out for 120 s at −0.2 V while stirring the solution at the given flow rate. After the solution was left in quiescence for 15 s, cathodic stripping voltammogram of the blank solution was recorded by scanning the potential toward the negative direction at a scan rate of 20 mV/s (pulse amplitude, 50 mV and pulse duration, 50 ms). After transferring of an aliquot (1.0 ml) of the digested sample solution to the voltammetric cell, the above procedure was repeated and a well-defined selenium peak was observed at −0.56 V vs SCE (Fig.4). Standard additions of 100 μl 1.0×10^{-5} M

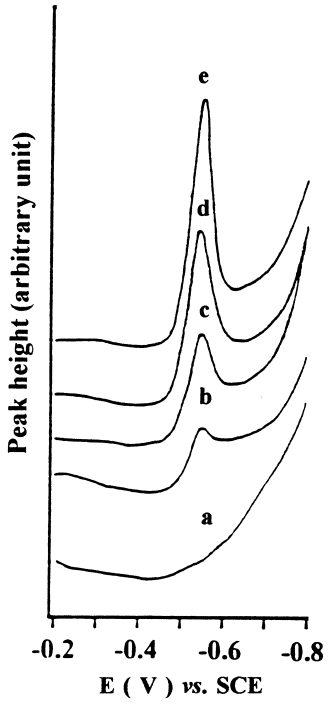

Fig. 4. Determination of Se by DPCSV, (a) 9.0 ml 0.1 M HCl (b) 1 ml milk sample (c) b + 100 μl 1×10^{-5} M SeO_3^{2-} (d) c + 100 μl 1×10^{-5} M SeO_3^{2-} (e) d + 100 μl 1×10^{-5} M SeO_3^{2-}. Deposition potential = × −0.2 V, deposition time = 120 s, pulse amplitude = 50 mV, pulse duration = 50 ms.

Se(IV) caused increments at the same potential and made the determination of Se in the sample possible. The linear domain range of Se(IV) was 75–1.2 μg/l with a correlation coefficient of 0.9981.

The cathodic stripping voltammetric determination of Se(IV) involves two main reduction process on the HMDE (İnam & Somer, 1997; Lingane & Niedrach, 1948). First Se(IV) is reduced to Se(0) and accumulated on the HMDE during the deposition step,

$$Se(IV) + 4e^- + Hg \rightleftarrows Hg(Se) \quad (1)$$

then Se(0) is further reduced to hydrogen selenide during the cathodic stripping step.

$$Se + 2H^+ + 2e^- \rightleftarrows H_2Se \quad (2)$$

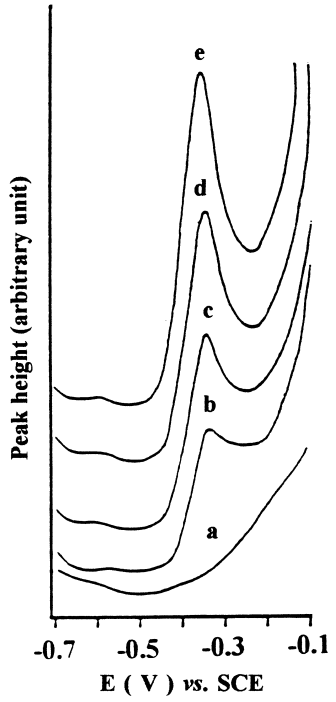

Fig. 5. Determination of Pb by DPASV, (a) 9.0 ml 0.1 M HCl (b) 1 ml milk sample (c) b + 100 μl 1×10^{-5} M Pb(II) (d) c + 100 μl 1×10^{-5} M Pb(II) e) d + 100 μl 1×10^{-5} M Pb(II). Deposition potential = −0.5 V, deposition time = 120 s, pulse amplitude = 50 mV, pulse duration = 50

The anodic stripping voltammetric determination of lead ion is based on the formation of amalgam between the mercury electrode and the lead ion during the deposition step, then its oxidation during the stripping step. Fig. 5 shows the DPASV determination of lead carried out under the same conditions mentioned for selenium, but a deposition potential of −0.5 V was applied following potential scan in the positive direction to obtain the lead oxidation peak. After standard additions of 100 μl 1.0×10^{-5} M Pb(II) into the voltammetric cell, lead determination in the milk sample was made from the increments of the peak at about −0.35 V. The linear domain range of Pb(II) was 185–8.7 μg/l with a correlation coefficient of 0.9945.

This method was applied to milk samples obtained from three distinct region of Turkey (Table 1). The results obtained were between 21.5 and 69.4 μg Se/l and 22.1 and 59.2 μg Pb/l, with the relative standard deviations of 10.3–10.7 and 6.8–9.9%, respectively. According to the results obtained, the highest selenium values were found in Samsun city (near sea board of Black sea) and the lowest, from the samples collected from various farms in Ankara city (located in the central part of Anatolia). Selenium contents of samples from İzmir city (near sea-board of Aegean sea) has been found close to ones in Ankara city. Values for selenium in cow milk from Ankara and İzmir cities are almost similar to those found for Greece (Bratakos et al., 1987), USA (Lean et al.,1990) and India (Vivekanandan et al., 1993). Selenium contents of milk from Samsun city are relatively higher than the published ones.

While lead contents of milk samples from Ankara and Izmir cities do not show large differences and are similar to those found for Spain (Barreira & Arribas, 1990), samples from Samsun city had relatively high lead contents. This milk was taken from a farm which was near to a road of heavy traffic. In summary, the proposed method based on differential pulse stripping voltammetry at a hanging mercury drop electrode has been applied to milk samples and it provides a simple and suitable procedure for determining trace amounts of selenium and lead after wet-digestion of milk samples with HNO_3 and $HClO_4$ (1/1) mixture. This method provides the detection of both elements simultaneously.

Table 1
Determination of Se and Pb in cow milk by differential pulse stripping voltammetry

	DPCSV, Se, μg/l		DPASV, Pb, μg/l		RSD, %[c]	
Milk sample	$x \pm s(n)$	$x \pm ts/n^{1/2a}$	$x \pm s(n)^b$	$x \pm ts/n^{1/2}$	Se	Pb
Ankara city	21.5 ± 2.3(4)	21.5 ± 2.7	32.4 ± 2.2(4)	32.4 ± 2.6	10.7	6.8
Samsun city	69.4 ± 7.4(4)	69.4 ± 8.7	59.2 ± 4.7(4)	59.2 ± 5.5	10.7	7.9
İzmir city	28.2 ± 2.9(5)	28.2 ± 2.8	22.1 ± 2.2(5)	22.1 ± 2.1	10.3	9.9

[a] t: Confidence interval (90%).
[b] *n*: Number of experiment.
[c] RSD: Relative standard deviation.

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