DETERMINATION OF SELENIUM IN INFANT FORMULA BY DIFFERENTIAL PULSE CATHODIC STRIPPING VOLTAMMETRY

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ABSTRACT

Selenium as a nonmetallic chemical element has received high attention of biologists because of its dual role as an essential trace nutrient and a toxic element. This interest has created a need for reliable analytical methods for determination of selenium. In this investigation determination of selenium by differential pulse cathodic stripping voltammetry and the influence of various parameters such as deposition potentials, deposition time, Cu^{++} concentration, pH, etc. on selenium peak in voltammogram are described. Determination of selenium was accomplished in mixture of acetic acid, hydrochloric acid and sodium chloride buffer (pH=1) with a scan rate of 60 mv/s and a pulse height of 100 mv by hanging mercury drop electrode (HMDE) as working electrode. The solution was stirred during pre-electrolysis at –350 mv (vs. SCE) for 30 s and the potential was scanned between –350 mv and –800 mv. The determination limit of the method was 0.005 mg/kg for the sample. The calibration curves were linear in the range of 0–30 µg/L (R^2=0.996, $p<0.001$). Repeatability of the method at concentrations of 30 and 0.5 µg/L were 2.5 and 10.5% respectively.

Keywords: Selenium, Infant formula, Differential pulse cathodic stripping voltammetry

INTRODUCTION

Selenium as a nonmetallic element is widely employed in the semiconductor industry and in the production of photosensitive elements, ink, etc (1,2). Interest in analytical methods for determination of selenium was initially concerned with its toxicity but it has been established that selenium is also an essential micronutrient (2-5).

Selenium is an element that exists in at least two and perhaps as many as four oxidation states (1,6). It has received much attention of biologists because of its dual role both as a trace nutrient and a toxic compound (3,7). This in part has demanded for reliable analytical methods for determination of selenium (8,9). Currently, only a few methods could provide the necessary reproducibility and sensitivity required for determination of selenium in biological materials at trace and ultra trace levels (2). The high sensitivity of neutron activation methods has made them appealing, but the special skill, time and cost essential required for these techniques are major drawbacks (7,10). A few studies have employed atomic absorption, ultraviolet or fluorometric spectrophotometry approaches but these techniques often require prior chemical separation of selenium in order to eliminate interferences in analyses (3,4). For example, great care must be taken to avoid interferences from metals such as Fe, which interact with selenium to prevent its volatilization in AAS hydride generation technique (4,11). Electrochemical techniques, especially differential pulse voltammetry provides a simple, relatively cheap and highly sensitive approach for determination of selenium in most matrices (7,12). Many researchers reported interference of copper with selenium peak height in voltammetric methods (8) and we took advantage of this phenomenon in determination of selenium. In this study the influence of various parameters such as selenium potentials, deposition time, and pH on Se peaks in differential pulse cathodic stripping voltammetry methods has been investigated.

MATERIALS AND METHOD

Apparatus

Voltammograms were prepared by use of a Trace Analyzer model No. 746 (Metrohm AG Ltd.,

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Switzerland). The polarographic cell was made of borosilicate glass and had a working volume of 5-50 ml. The cell was a three-electrode system with a saturated calomel electrode, a hanging mercury drop electrode and a platinum electrode, as reference electrode, working electrode and auxiliary electrode, respectively.

Reagents and solutions

The selenium dioxide, acetic acid, sodium acetate, copper nitrate, sodium chloride, hydrochloric acid and nitric acid were pro-analysis grade from Merck Co. (Germany). The metal stock solutions (1 g/L) were prepared in 0.005M nitric acid.

Glassware

All glassware were soaked in 2M nitric acid for at least 7 days, washed three times with distilled deionized water, soaked first in distilled deionized water and then in 0.1M hydrochloric acid until ready for use.

Voltammetric determination

Buffer solution prepared by mixing different parts of sodium acetate–sodium chloride (0.2 M) and acetic acid–hydrochloric acid (0.2 M). Five ml of the buffer (0.2 M) of pH was pipetted into the voltammetric cell, then 0.5 ml of the Cu^{++} solution and 0.5 ml of the selenium solution were added. The solution was deoxygenated for 120 s by high-purified nitrogen, whilst stirring the solution. A fresh mercury drop was extruded as working electrode (HMDE), and electrolyzes was carried out with stirring. The stirrer was stopped and the scan was initiated with a rate of 60 mV/s, 10 s after the solution became quiescent.

RESULTS AND DISCUSSION

Several experiments were performed to determine the optimum conditions for the best sensitivity, robustness and accuracy in working with trace quantities of selenium. All experiments were performed in differential pulse mode using the buffer as a supporting electrolyte.

Effect of Cu^{++} concentration

CSV of Se(IV) without addition of Cu^{++}, produced a peak at –540 mV, due to the formation of the mercury(II) selenide during the deposition step according to the following equation (5-7, 12):

$H_2SeO_3 + 4H^+ + Hg + 4e^- \rightarrow HgSe + 3H_2O$ (1)

This peak became suppressed and a new peak appeared in a more negative potential when Cu^{++} was added. In the presence of Cu^{++} ions an intermetallic compound is formed (5,7):

$H_2SeO_3 + 4H^+ + Cu^{++} + Hg + 6e \rightarrow Cu(Hg)Se + 3H_2O$ (2). The peak potential varied with Cu^{++} concentration and shifted in a negative direction as Cu^{++} concentration increased up to 90 mg/L (Figure 1,2). These changes are perhaps due to the formation of stable insoluble complex Cu_2Se or $Cu_2(Hg)Se$ on the electrode surface (6). A Cu^{++} concentration of 90mg/L was found to be optimum for analytical purposes.

Effect of pH

The supporting electrolyte was acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M). Selenium peak was shown to be strongly pH-dependent. The peak potential shifted in a positive direction by decreasing the pH from 4 to 0 (Figure 3). The peak height increased by decreasing pH (Figure 4) and at higher pH, the sensitivity of the determination was lower. The reaction mechanism (Equation 2) explains that the formation of Cu(Hg)Se is favored at a low pH (6).

Effect of the deposition potential

The conventional approach for the CSV of selenium without copper in acidic media is based on the use of a deposition potential between +50 mV and –50 mV. Determination of selenium at these deposition potentials is quite susceptible to interferences from other metal ions, which often suppress the stripping peak (7).

Nevertheless, in the presence of copper, deposition potentials become more negative than -200 mV and cause an increase in the peak height. The peak height decreased again with deposition potentials more negative than –400 mV (Figure 5). This reduction may be related to the charge on the electrode, which changes from positive to negative at potentials less than ca. –500 mV in media containing chloride ions (6,14). The optimum sensitivity for Se was obtained at deposition potential ca. –350 mV.

Figure 6 shows that the peak potential slowly shifted in a positive direction by increase in deposition potential from –200 mV to -500 mV.

Effect of the time

Depending on selenium concentration, the peak current increased by increased deposition time. For selenium concentrations lower than 10 µg/L a deposition time up to 240 s may be used, though a deposition time of 30s provided more than 90% of the maximum peak current (Figure 7). By the use of a deposition time of 30 s, it was possible to determine selenium within a linear working range of 0-30 µg/L and detection limit of 0.005 mg/Kg.

The peak potential shifted in a negative direction by increasing the deposition time (Figure 8). These results might be caused by saturation of the electrode surface and complex stabilization, respectively.

Influence of pulse height

In particular, the chosen pulse height has a considerable influence on the sensitivity and peak height. Figure 9 shows the peak height increase by increasing the pulse height. The peak potential was then shifted to a positive direction by increasing the pulse height (Figure 10).

Determination of selenium in infant formula

1 g of commercial infant formula samples and 5 ml of nitric acid (65%) were transferred into a clean silica vessel. The samples were incubated at room temperature overnight, and then were heated for 45 min at 125C° to complete the pre-digestion. To digest the samples, they were removed from the block and allowed to cool for 5 min. One ml of perchloric acid was added and the volume was adjusted to 6 ml with nitric acid if necessary. The samples were then placed in the heating block and slowly heated to 130 °C. Once this temperature was reached, the block was further heated to 210 °C, and the samples were kept at this temperature for 15 min. The silica vessel were then removed from the heating block and allowed to cool. To reduce the sample size, 0.5 ml of concentrated HCl was added to the vessels and they were placed back in the heating block and their temperature were raised to 150 °C and held 30 min at this temperature. For the assay, the samples were removed from the block and allowed to cool to room temperature. The volume was adjusted to 10 ml with diluted HCl (1:1). The samples were analyzed by the previously optimized method. Five ml of buffer (0.2 M, pH=1) was pipetted into the voltammetric cell, then 0.5 ml of the Cu^{++} solution (90 mg/L) and 0.5ml of the sample solution were added. The solution was deoxygenated for 120 s by high-purified nitrogen, whilst stirring the solution. A fresh mercury drop was extruded as working electrode (HMDE), then stirring and electrolyzing were carried out for 30 s at –350 mV (vs. SCE). The stirrer was stopped and the potential was scanned from –350 mV to –800 mV with a rate of 60 mV/s and a pulse height of 100 mV, 10 s after the solution became quiescent. Under these conditions, recovery was 96.44% for Se and the detection limit of method was 0.005 mg/Kg for sample. The calibration curve was linear over the range 0-30 µg/L (R^2=0.996, p<0.001). Repeatability and internal reproducibility of method were expressed by relative standard deviation (14). The data related to precision are shown in Table 1. The sensitivity of method is expressed by the angular coefficient of the calibration curve (14). The method validation data are shown in Table 1.

CONCLUSION

Differential pulse cathodic stripping voltammetry (DPCSV) has proved to be a sensitive and accurate method for selenium determination. The proposed method, based on DPCSV of Cu(Hg)Se at a HMDE, provides a simple and sensitive approach for determination of selenium. In this investigation, the experimental conditions for determination of selenium were optimized. Under these conditions, detection limit was 0.005 mg/Kg and repeatability of the method expressed by relative standard deviation for selenium was 2.5% at 30 µg/L and 10.5% at 0.5 µg/L and the calibration curve was linear over the range 0-30 µg/L (R^2=0.996, p<0.001).

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Table 1. Recovery, precision and sensitivity of the method

Recovery			
Sample (n=5, mg/Kg)	Spike added (mg/Kg)	The spiked sample (n=5) (mg/Kg)	Recovery (%)
0.064±0.003	0.01	0.074±0.003	96.4
	0.05	0.112±0.004	96.12
	0.10	0.161±0.006	96.8

Precision (RSD%)		
Sample (0.059 mg/Kg)	Repeatability (n=10)	Internal reproducibility (n=5)
	6.5%	17.3%
Standard (0.5 µg/L)	10.5%	18.2%
Standard (30 µg/L)	2.5%	7.1%

Sensitivity and linearity range			
Calibration range (n=6)	R^2	Slope [nA/(µg/L)]	Intercept (nA)
0-30 µg/L	0.996	7.7413	0.19787

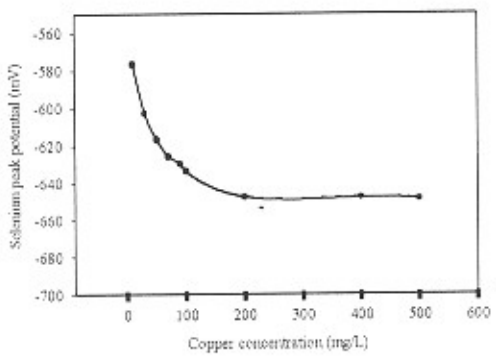

Figure 1. Influence of copper concentration on the selenium peak potential. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2 M); pH, 1; deposition time, 30 s; deposition potential, -350 mV; pulse height, -100 mV.

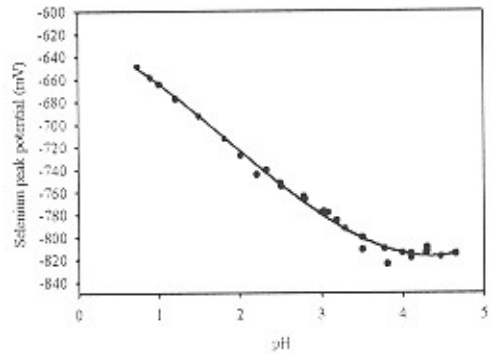

Figure 3. Effect of varying the pH on the selenium peak potential. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); copper concentration, 90 mg/L; deposition time, 30 s; deposition potential, -350 mV; pulse height, -100 mV.

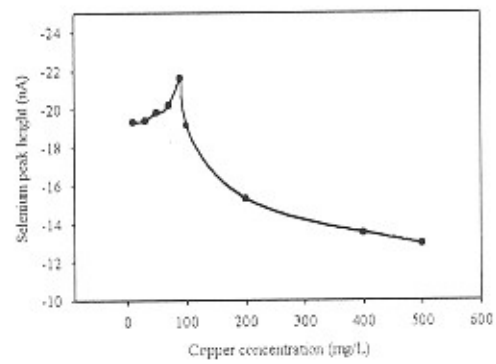

Figure 2. Influence of copper concentration on the selenium peak current. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); pH, 1; deposition time, 30 s; deposition potential, -350 mV; pulse height, -100 mV.

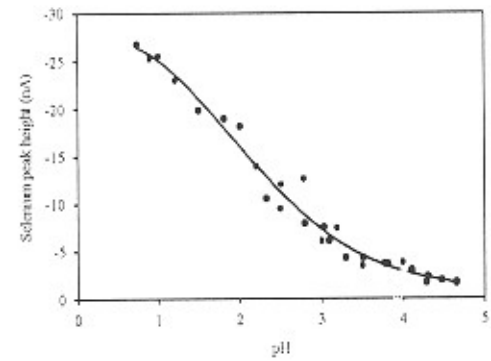

Figure 4. Effect of varying the pH on the selenium peak current. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); copper concentration, 90 mg/L; deposition time, 30 s; deposition potential, -350 mV; pulse height, -100 mV.

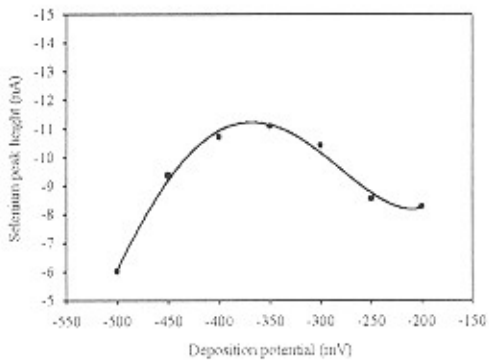

Figure 5. Effect of varying the deposition potential on the selenium peak current. Se concentration, 1.5 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); pH, 1; copper concentration, 90 mg/L; deposition time, 30 s; pulse height, -100 mV.

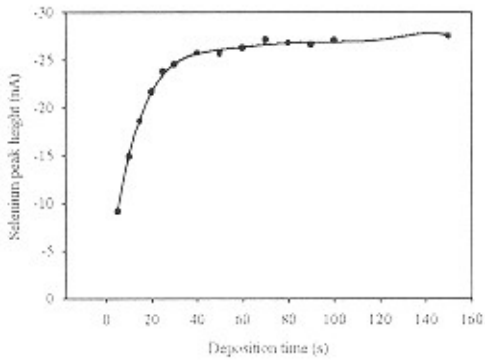

Figure 7. Effect of increasing the deposition time on the selenium peak current. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); pH, 1; copper concentration, 90 mg/L; deposition potential, -350 mV; pulse height, -100 mV.

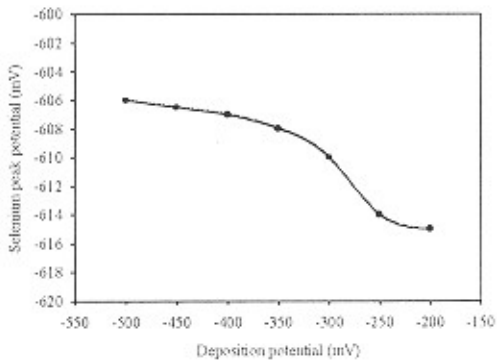

Figure 6. Effect of varying the deposition potential on the selenium peak potential. Se concentration, 1.5 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); pH, 1; copper concentration, 90 mg/L; deposition time, 30 s; pulse height, -100 mV.

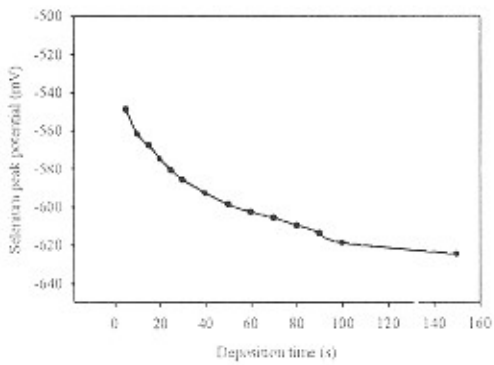

Figure 8. Effect of increasing the deposition time on the selenium peak potential. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); pH, 1; copper concentration, 90 mg/L; deposition potential, -350 mV; pulse height, -100 mV.

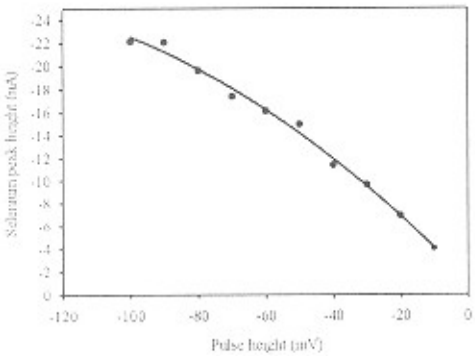

Figure 9. Effect of increasing the pulse height on the selenium peak current. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); pH, 1; copper concentration, 90 mg/L; deposition potential, -350 mV; deposition time, 30 s.

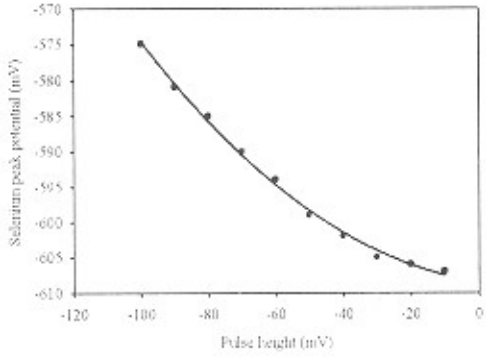

Figure 10. Effect of increasing the pulse height on the selenium peak potential. Se concentration, 3 µg/L; operating mode, DPCSV; electrode, HMDE; supporting electrolyte, acetic acid–sodium acetate–hydrochloric acid–sodium chloride buffer (0.2M); pH, 1; copper concentration, 90 mg/L; deposition potential, -350 mV; deposition time, 30 s.

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