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Determination of optimal conditions for the dosage of selenium in whole human blood by differential pulse cathodic stripping voltammetry

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ABSTRACT

The objective is the determination of the conditions operating optimal to determine the concentration of the selenium dissolved in the human blood. An electroanalytical method has been developed for the determination of selenium in whole blood by differential pulse cathodic stripping voltammetry (DPASV) on a hanging mercury drop electrode (HMDE). The best conditions were found to be electrolyte support 200 $\mu\text{g mL}^{-1}$ of copper in HCl 0.3M, the accumulation potential is -325 mV and the accumulation time is 300 s. The optimum value of stirring rate was determined to be 400 rpm. Calibration curve is linear in the range $9.75 \cdot 10^{-3}$ to $3.25 \mu\text{g L}^{-1}$ with a detection limit of $29 \mu\text{g L}^{-1}$. The correlation coefficient and relative standard deviation were 0.9999 and 1.62%. The developed method was validated by the analysis of reference materials certified by total blood.

Key words: Selenium, whole blood, Differential pulse cathodic stripping voltammetry.

INTRODUCTION

The analysis of trace and ultra-trace elements is continuously stimulating the progress in analytical chemistry. Environmental chemistry, radiochemistry, biology, health, agri-food are prescriptors of trace analyses, with continuously increasing exigencies: lowering detection limits, lowering costs and analysis time [1]. Selenium is a trace element associated with the activity of the antioxidant enzyme glutathione peroxidase. It is considered to be a protective agent against free radicals through enhanced enzyme activity [2]. Keshan disease is a dilated cardiomyopathy closely related with a diet deficient in the mineral selenium. It is named for the northeastern Chinese county Keshan, where the disease prevalence is high because of selenium-deficient soil [3]. Selenium deficiency results in microangiopathy, muscular dystrophy, edema, hemorrhage and necrosis of the liver, impaired immune response, impaired reproduction and sudden death [4,5]. Furthermore, selenium status decreases with old age [6]. Therefore, marginal or deficient selenium status may be a risk factor for a decline of cognitive functions. Selenium and cognition changes could also both reflect the ageing process [7]. Selenium toxicity can also develop chronically with signs of weight loss, hair loss and hoof damage with resulting lameness [8]. If

selenium deficiency is suspected, it is prudent to undertake testing to confirm the diagnosis rather than leaping straight to supplementation as unwarranted selenium supplementation could have dire consequences.

The electrochemical methods have many advantages compared with the spectrometric methods, the equipment being less expensive and measurements being able to be realized in difficult mediums of access [9]. These methods occupy a particular place because its applications can be extremely varied, as well in the field of the mineral analysis as in that of the organic analysis [10]. Its principle is usually based on the accumulation of metals per electrochemical reduction on electrode of mercury, followed by their qualitative and quantitative analysis. Among the electrochemical methods most recent, the stripping voltammetry is a precise, significant and selective method electrochemical [11].

In this work we will study the influence of the principal experimental parameters on the current of stripping, namely the potential of accumulation, the time of deposit and the stirring rate of the solution. The effects of scan rate, drop size, duration pulse and equilibrium time on the voltammetric response were examined in developing a suitable analytical procedure for the determination of selenium. The chosen working conditions were: drop size of 1, scan rate of 20 mV s^{-1} , duration pulse 20 ms and equilibrium time of 5 sec. The objective is the determination of the optimal operating conditions for each one as of these parameters in order to determine the concentration of the selenium dissolved in whole blood.

MATERIALS AND METHODS

2. 1. Equipment

The measurement of the total concentration of selenium in blood was carried out on a stand MDE150. It is made up by Polarographic Analyzer POL150; the unit is controlled by a microcomputer, using the software Trace Master degree 5. The Polarographic cell is made up of a mercury electrode with hanging drop (HMDE), of an electrode of reference (Ag/AgCl/KCl 3M) and of an auxiliary platinum electrode. The solution is agitated using a magnetic stirrer, the cell of electrolysis to double wall, allows the temperature control by the circulation of the fluid. The sample is purged by the argon saturated beforehand with water. Equipment is installed under a hood to minimize the contaminations.

2. 2. Reagents

The selenium solution which was used for the voltametric analyses prepares every day by dilution of the solution 1g/L (Pro Analysis Sigma-Aldrich). Acids used for the analysis, the nitric acid (high purity than 69.5%, Fluka), the perchloric acid (70-72%, Merck) and hydrochloric acid (37%, Merck). Each proportioning was preceded by a thorough cleaning of the electrodes and electrolytic cell with the nitric acid 10% and by a rinsing by ultrapure water (MilliQ Gradient A10).

2. 3. Sample digestion

A 1.0 ml of whole blood in a long-necked 50 ml flask, 4.0 ml of acid mixture (3 ml HNO_3 and 1 ml HClO_4) was added in flask. The temperature is maintained with 150°C during 4 hours and then the temperature is fixed at 200°C until quasi total evaporation of the contents. After cooling, we add the same mixture of acids to the residue, then we let evaporate until obtaining a dry residue. Digestion was completed when the solution was clear. Finally 1 mL of HCl was added in the residue until obtaining a dry residue. This residue is taken again by 5 ml of nitric acid with 0.25% and preserved in polyethylene tubes [12,13]. In this case, the mixtures of strong acids

used allow, in combination with high temperatures, to destroy the organic matter and to transform it into by-products of gas reaction, which are then eliminated under the hood. It is in fact necessary, to reduce the contaminations, to use quantities as weak as possible reagents. The hydrochloric acid addition plays a very important part for selenium (reduction of selenite a selenate) [14,15]. This method of digestion was selected among so many others because it gave the best results.

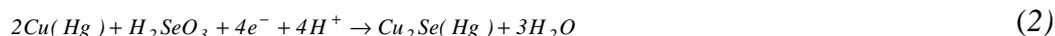
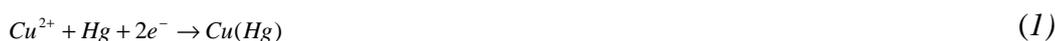
RESULTS AND DISCUSSION

3.1. Effect of operational parameters

Taking into account that the differential pulse voltammetry technique was used to develop the method for quantitative determination of selenium, and in order to choose the optimum conditions, some analytical and instrumental parameters were studied.

3.2. Effect of the electrolyte support

The surface of the mercury electrode with hanging drop is put in contact with a solution containing of copper (II) and of the selenium because from an electrochemical point of view, selenium alone cannot deposit directly on the mercury electrode. For watch the effect of copper on the electrolyte support we proceeded, by means of the method of the proportioned additions, with the layout of polarograms (Fig. 1) of 2 groups of solutions containing or not copper (groups A and S respectively). The results obtained proved the effect of copper on the linearity of the answers obtained. In the cathodic stripping, it there thus not interferences between copper and selenium, copper make it possible moreover to increase the sensitivity of the peak of selenium like obtaining a better linearity. The Copper concentration was fixed, thereafter, with $200\mu\text{g mL}^{-1}$ (in acid HCl 0.3M); this optimal value indeed gave us best reproducibility and a good linearity. In this preconcentration step, elemental copper as soon as deposited onto the mercury surface is chemically oxidized by selenite to yield copper (II) selenide, according to the reaction pathway (1,2). Subsequently, by applying a differential pulse voltammetric scan towards negative potentials a cathodic peak is detected at -620 mV for the reduction of copper (II) 'reaction (3)'. Copper is necessary to form copper selenite which is the base of selenium determination. The reactions can be written as [16]:



3.3. Effect of the potential of accumulation

Accumulation potential is an important parameter for stripping techniques that influences the sensitivity of the determination. The effect of varying deposition potential on the peak height for selenium is shown in (Fig. 2). The effect of the deposition potential on the stripping signals was studied in the potential range from -300 to -400 mV versus reference electrode. The peak current was found to increase with decreasing deposition potential up to -325 mV. The peak currents decreased in more negative potentials thereafter. Thus, a deposition potential of -325 mV was chosen.

3.4. Effect of the time of accumulation

The deposition time is always important factor in stripping voltammetric analysis because of its prevailing influence on sensitivity and detection limit of the method. A pre-electrolysis was studied between 120 and 360 seconds (Fig. 3). The height of the peak increases considerably

with the time of deposit and decreases from 300 S, we thus regarded as optimum time of deposit for the next analyses that of 300 S.

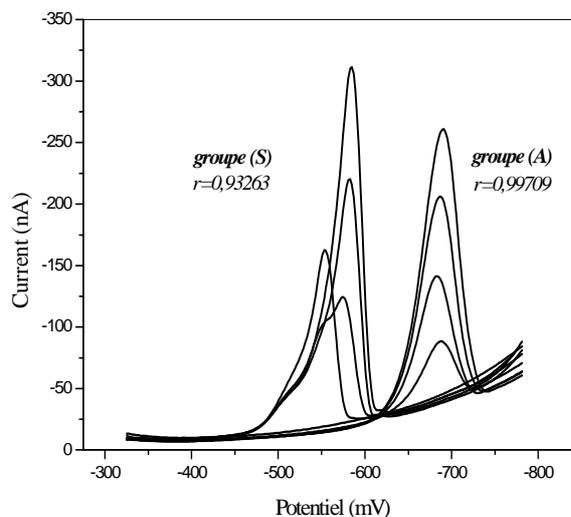


Fig. 1. Effect of copper in support electrolyte. $E_{acc} = -325$ mV, $u = 400$ rpm, $t_{acc} = 300$ s.

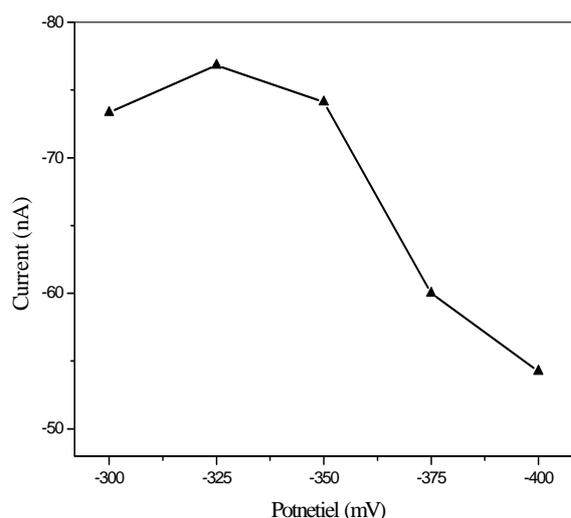


Fig. 2. Effect of deposition potential on peak current. $u = 400$ rpm, $t_{acc} = 300$ s.

3. 5. Effect of stirring rate

Stirring intensity is one of the important parameters that affect the accumulation efficiency. Agitation of the sample solution facilitated the mass transfer process. In present work, the samples were agitated at various stirring rates (150, 225, 300, 400, and 525 rpm). The result showed that the current intensity efficiency was first improved with increased agitation rate before 400 rpm and then decreases starting after this agitation rate (Fig. 4). Hence, a stirring rate of 400 rpm was chosen for further studies.

3. 6. Calibration data and Analytical application

A linear response over the concentration range of $9.75 \cdot 10^{-3}$ to $3.25 \mu\text{g L}^{-1}$ selenium was observed under optimum conditions, with correlation coefficient of 0.9999. The relative standard deviation is 1.69%. The certified value of selenium is of $79.8 \mu\text{g L}^{-1}$, with a range of acceptable values located between 74.4 and $85.2 \mu\text{g L}^{-1}$, Seronorm TM Trace Element Whole blood (level 1,

MR4206). The percentage recovery of the selenium determination was tested by measurement of a standard reference material is of 97.17%, with a measured exactitude is of 98.01% (n=06). As an application the figure below represents a voltammograms relating to the proportioning of selenium in the blood demineralized starting from the conditions which were optimized previously (Fig. 5). The selenium concentration of this sample is equal $70.125 \mu\text{g L}^{-1}$.

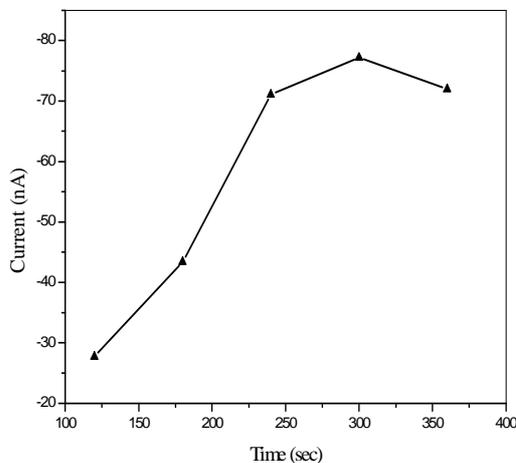


Fig. 3. Effect of deposition time on peak current. $E_{acc} = -325 \text{ mV}$, $u = 400 \text{ rpm}$.

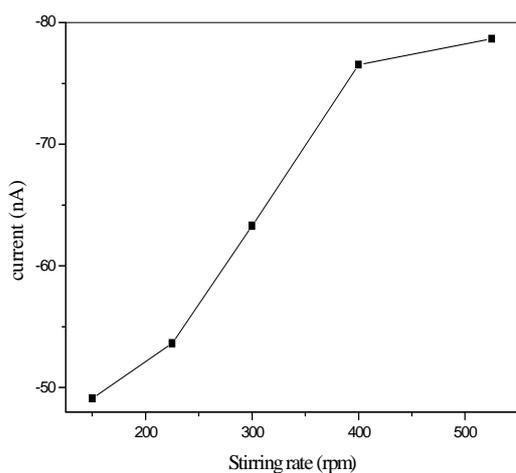


Fig. 4. Effect of stirring rate on peak current. $E_{acc} = -325 \text{ mV}$, $t_{acc} = 300$

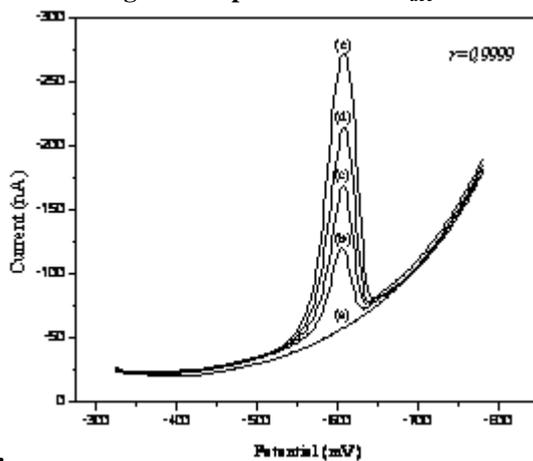


Fig. 5. Determination Effect of selenium in whole blood. (a): solution electrolyte support, (b):(a)+ demineralised blood, (c):(b)+(X= $0.20 \mu\text{g L}^{-1}$), (d):(c)+X, (e):(d)+X.

CONCLUSION

The experimental results and those obtained by the analytical calculation of validation, enable us to conclude that the cathodic stripping in mode pulsed on electrode with hanging mercury drop has the advantage of being fast, reproducible, specific and does not ask a special installation for the proportioning of selenium. Indeed, the limit of detection obtained is better than that reported in the literature in same the conditions [17]. The DPCSV also has the advantage of being not very expensive compared with other methods of analysis such as the ICP-MS; it is in addition precise and selective. From where interest of its use for speciation of the elements traces.

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