ELECTROCHEMICAL SENSOR FOR THE ASSAY OF ZINC IONS IN WHOLE BLOOD SAMPLES

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An electrochemical sensor based on graphite paste modified with 2,6-bis(thiophen-3-yl)-4-(4,6,8-trimethylazulen-1-yl) pyridine was used for the determination of zinc ions in whole blood samples. Differential pulse voltammetry was employed for all measurements. Calcium, magnesium and iron did not interfere in the measurements. The determination limit, sensitivity and recovery test proved that the proposed sensor can be used reliably for the assay of zinc ions in whole blood samples.

Keywords: Zn, electrochemical sensor, differential pulse voltammetry, whole blood samples

1. Introduction

Recent studies have shown that zinc ions had a role in the activity of various organs (liver, kidneys, heart, brain). Low intake of zinc in the body may can cause various symptoms of the central nervous system (neuropathy), heart (low blood pressure), and gastrointestinal tract (diarrhea) [1]. Zinc is involved in cellular respiration, DNA replication, and at the same time, it maintains the integrity of cell membranes and minimizes the effects of free radicals on the body. Zinc ions have an essential role in the reduction of apoptosis [2] and in the structure and activity of more than 300 enzymes in the human body.

Cronje and his team explain in his work how to minimize the carcinogenic activity of chromium consisting of modifying the DNA structure using zinc (II) solutions [3].

Ananda Prasad showed that zinc deficiency influences the activity of the immune system affecting the normal functioning of T and B cells. Therefore, it was induced a mild zinc deficiency by restricting dietary. Because of this deficit,

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the serum thymulin decreased activity in 12 months. After the zinc supplementation all these symptoms vanished [4].

Low levels of zinc ions in the body can affect insulin sensitivity. The organism becomes insensitive in time to insulin action, thus gaining an increased resistance to insulin. Zhou and his team have shown that zinc-bound insulin has beneficial effects on the carbohydrate metabolism [5]. Cyclic voltammetry, DPASV (differential pulse anodic stripping voltammetry) methods were proposed to determine zinc ions in the sweat samples [6].

This paper proposed a new electrochemical sensor based on the modification of the graphite paste with 2,6-bis(thiophen-3-yl)-4-(4,6,8-trimethylazulen-1-yl) pyridine for the assay of zinc ions, using differential pulse voltammetry.

2. Experimental

2.1. Reagents

The calcium acetate, magnesium chloride, iron (II) sulphate and zinc chloride were purchased from Sigma Aldrich. 2,6-bis(thiophen-3-yl)-4-(4,6,8-trimethylazulen-1-yl) pyridine was synthesized in the house [7]. To prepare the buffer solution (PBS pH=7.4) monosodium and disodium phosphate from Sigma Aldrich were used. All Zn^{2+} solutions were prepared in PBS pH=7.4 using serial dilution method.

2.2. Apparatus

The experimental measurements were performed with the AUTOLAB/PGSTAT 302 (Metrohm, Utrecht, Switzerland) which was connected to a computer with a GPES software, used to record the measurements. The electrochemical cell consists of three electrodes: a reference electrode (Ag/AgCl, 0.1mol/L KCl), a working electrode (the new electrochemical sensor) and a counter electrode (platinum wire).

2.3. Electrochemical sensor design

The graphite powder was mixed with paraffin oil to form a homogeneous paste to which was added 2,6-bis(thiophen-3-yl)-4-(4,6,8-trimethylazulen-1-yl) pyridine. The modified paste was inserted into a plastic tube and electrical contact was made of a silver wire. This sensor was kept in a dry place and was washed with deionized water before each measurement. All measurements were made at room temperature (25˚C).

2.4. Differential pulse voltammetry

Differential pulse voltammetry (DPV) was used to determine the concentration of Zn^{2+} in whole blood samples. Voltammograms recorded for the new electrochemical sensor were registered using the following parameters: potential range between 0.5 and 1.3V, scan rate of 50mV/s and amplitude modulation of 50mV. Standard solutions were measured, and the calibration equation was obtained using linear regression method. The unknown concentration of zinc was determined by inserting in the equation of calibration the values obtained for the height of peak.

2.5. Sample preparation for whole blood

To determine zinc ions in whole blood samples, blood samples were collected with the informed consent of the patient in accordance with the Code of Ethics.

Spiked samples. The zinc ion was first determined from the whole blood sample as collected from the patient. Different volumes of zinc solution were added to the whole blood sample and the zinc ions were determined in the resulting solutions.

3. Results and discussion

3.1. Response characteristics of the sensor

DPV was used to obtain all response characteristics of the electrochemical sensor based on graphite paste modified with 2,6-bis(thiophen-3-yl)-4-(4,6,8-trimethylazulen-1-yl) pyridine (**L**). The linear concentration range was between 10^{-5} mol/L and 10^{-3}mol/L, the limit of quantification (LOQ) was $1.00x10^{-5}$mol/L, the limit of detection (LOD) was $1.26x10^{-6}$mol/L and the sensitivity of the electrochemical sensor was $1.74x10^{-3}$A/mol L^{-1}. The calibration curve is shown in Fig. 1. The diagrams used for the calibration of the proposed electrochemical sensor are shown in Fig. 2.

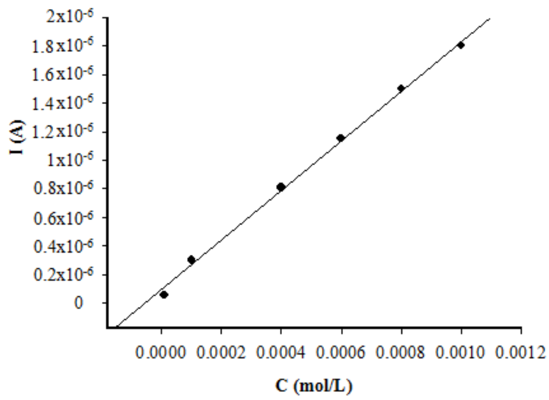

Fig. 1. Calibration curve obtained for an electrochemical sensor based on graphite paste modified with **L** for the assay of Zn^{2+}.

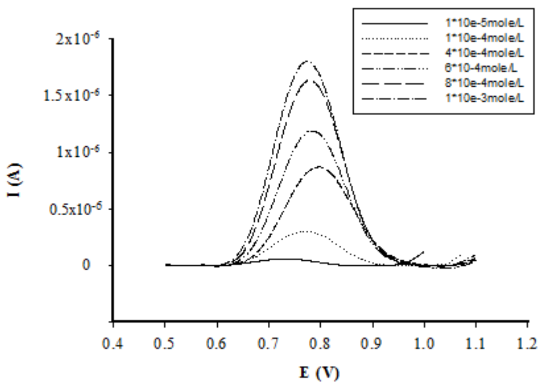

Fig.2. Diagrams used for the calibration of the proposed electrochemical sensor for the assay of Zn^{2+}.

3.2. Selectivity of the proposed microsensor

To determine the selectivity of the proposed electrochemical sensors, the amperometric selectivity coefficients were determined using the mixed solutions (with ratio between interfering ion: Zn ions = 10:1 (mol/mol)). As interferences were considered Ca^{2+}, Mg^{2+} and Fe^{2+} ions. The values shown in Table 1 proved that Ca^{2+}, Mg^{2+} and Fe^{2+} ions did not interfere in the determination of Zn ions.

Table 1

Values obtained for the amperometric selectivity coefficients using a proposed electrochemical sensor used for the assay of Zn ions.

Interferent	$K_{i,j}^{amp}$
Fe^{2+}	$9.4\text{x}10^{-4}$
Mg^{2+}	$5.2\text{x}10^{-4}$
Ca^{2+}	$6.4\text{x}10^{-4}$

Values for the amperometric selectivity coefficients were calculated using the following equation [8]:

$$Ki,j(amp) = \left(\frac{\Delta It}{\Delta Ii} - 1\right) * \frac{ci}{cj}$$

where *Ki,j(amp)* is the amperometric selectivity coefficient, $\Delta I_t=\Delta I_t-\Delta I_b$, where ΔI_t is the total intensity of the current, ΔI_b is the intensity of the current recorded for blank solution, $\Delta I_i=\Delta I_i-\Delta I_b$, where ΔI_i is the intensity of the current registered for main ion, c_i and c_j are the concentrations of the main ion and the interferent ions.

3.3. Analytical applications

The proposed electrochemical sensor was able to detect low levels of zinc ions in whole blood samples due to its high sensitivity, low determination limit and high selectivity. The spiked samples were prepared using different zinc solution volumes added to a certain volume of solution of whole blood sample, as can be seen in Table 2.

Table 2

Recovery of Zn ions in whole blood samples (N=10)

Sample no	**Conc(Zn^{2+}) added (mol/L)**	**Volume ratio Zn^{2+}:sample**	**%Recovery**
1	10^{-3}	0.25:1	90.58±0.15
2		0.50:1	91.25±0.21
3		0.75:1	92.78±0.25
4		1.00:1	91.90±0.19
5		1.25:1	93.05±0.12
6		1.50:1	92.87±0.15
7		1.75:1	93.00±0.20
8		2.00:1	92.91±0.18

The values obtained for the recovery of zinc ions are higher than 90.00%, and the RSD (%) values were lower than 1.00%, proving the precision and accuracy of the proposed electrochemical sensor, when used for the assay of zinc ions in whole blood samples.

Fig. 3 shows the peak obtained for zinc ions, when determined from whole blood samples.

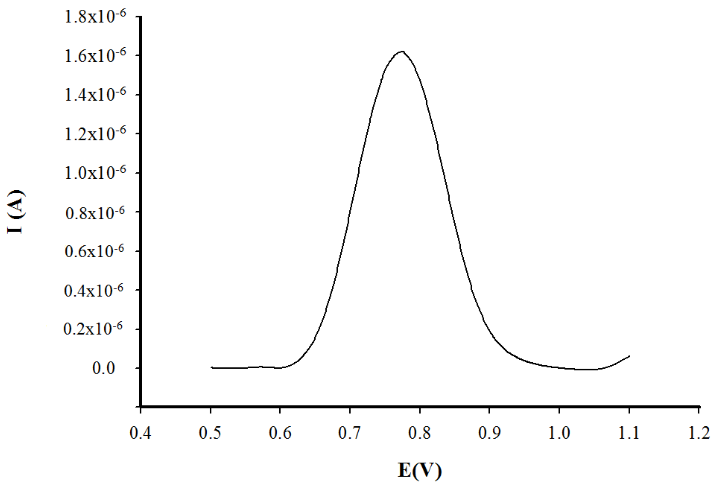

Fig. 3. Example of the diagram obtained for the assay Zn^{2+} in whole blood sample.

4. Conclusions

The proposed electrochemical sensor based on modified graphite paste with 2,6-bis(thiophen-3-yl)-4-(4,6,8-trimethylazulen-1-yl) pyridine has shown a high sensitivity over the linear concentration range: 10^{-5} - 10^{-3}mol/L, with a quantification limit of 1.00×10^{-5}mole/L. The recovery test performed has shown a high reliability of the electrochemical sensor for the assay of zinc ions in whole blood samples.

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R E F E R E N C E S

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