DETERMINATION OF L-TRYPTOPHAN IN WHOLE BLOOD SAMPLES USING A NEW ELECTROCHEMICAL SENSOR

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L-tryptophan is an amino acid used as biomarker for the diagnosis of gastric cancer. A new electrochemical sensor based on graphite paste modified with 2,6-bis((E)-2-(furan-2-yl)vinyl)-4-(4,6,8-trimethylazulen-1-yl)pyridine was proposed for its determination in real whole blood samples. Differential pulse voltammetry was used for all determinations. The limit of detection, sensitivity, and selectivity made possible its determination in whole blood samples with recoveries higher than 90.00% with RSD less than 1.00%.

Keywords: L-tryptophan, electrochemical sensor, clinical analysis

1. Introduction

Amino acids are feasible biomarkers in different diseases because they play an important role as metabolites and metabolic regulators in human body. The amino acids have a huge potential in diagnosis and prognosis of human diseases such as gastric cancer, diabetes, schizophrenia and chronic obstructive pulmonary diseases [1-3].

L-tryptophan (L-Trp) is essential in human body, being a precursor for serotonin and melatonin [4]; however, it must be introduced from the diet because cannot be synthesized in humans. At the same time, L-tryptophan is the amino acid having the most frequently altered concentrations of various cancer types [5]. Up to now, survival of gastric cancer and patient quality of life are only dramatically improved if the disease is diagnosed earlier [6]. Unfortunately, less than 5% of cases of early gastric malignancy are identified and analyzed immediately [7]. Determination of amino acids is essential in medicine, food, soil, biotechnology and in pharmaceutical industry [8-10]. Several methods have been proposed for the determination of L-Tryptophan, such as liquid chromatography [11], capillary electrophoresis [12], spectroscopic methods [13] and electrochemical methods [14].

This paper proposed a new electrochemical sensor based on the modification of graphite paste with 2,6-bis((E)-2-(furan-2-yl)vinyl)-4-(4,6,8-

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trimethylazulen-1-yl)pyridine for the assay of L-tryptophan from whole blood samples. The method used was differential pulse voltammetry.

2. Experimental

2.1Materials and reagents

All chemicals were of analytical grade. 2,6-bis((E)-2-(furan-2-yl)vinyl)-4-(4,6,8-trimethylazulen-1-yl)pyridine was synthesized by the group of Razus [15]. Paraffin oil (d_4^{20}, 0.86 g x cm^{-1}) was purchased from Fluka. L-Tryptophan, graphite powder, uric acid and ascorbic acid were purchased from Sigma Aldrich.

The L-Tryptophan (L-Trp) solutions were made in phosphate buffer solution (PBS, pH = 7.5). For the preparation of L-Tryptophan solutions of different concentrations (10^{-3} g mL^{-1}–10^{-15} g mL^{-1}), serial dilution method was used. When not in use, the L-Tryptophan solutions were stored in the fridge at 2–8°C.

2.2 Apparatus

The experimental measurements were performed with the AUTOLAB/PGSTAT 302 (Metrohm, Utrecht, The Netherland), connected to a computer for data acquisition and processing. The electrochemical cell consists of three electrodes: a reference electrode (Ag/AgCl, 0.1mol/L KCl), a working electrode (the new electrochemical sensor based on graphite paste) and a counter electrode (platinum wire).

2.3 Design of the electrochemical sensor

Graphite powder was mixed with paraffin oil to obtain a homogeneous paste, which was modified with 2,6-bis((E)-2-(furan-2-yl)vinyl)-4-(4,6,8-trimethylazulen-1-yl)pyridine. A plastic tip was filled with the modified paste and the electric contact was made using a silver wire. The surface of the sensor was washed with deionised water and polished with alumina paper before each utilization. If not in use, the electrochemical sensor was kept at room temperature, in a dry place.

2.4 Procedure

The differential pulse voltammetry (DPV) measurements were performed at 25°C for each standard solution (10^{-3} g mL^{-1}–10^{-15} g mL^{-1}). The working parameters were as following: scan rate was 50 mVs^{-1}, potential range 0-1000 mV, and modulation amplitude 50 mV. The peak heights were measured and were plotted against the concentrations of standard solutions of L-Trp (Fig. 1). The unknown concentrations were calculated from the equation of calibration determined statistically.

3. Results and discussion

3.1. Response characteristics of the modified graphite paste sensor

Differential pulse voltammetry (DPV) technique was used to determine the response characteristics of the electrochemical sensor. The response characteristics obtained were: the linear concentration range was between 2ng mL^{-1} and 1mg mL^{-1}, the limit of determination was 2ng mL^{-1}, the limit of detection was 0.74ng mL^{-1} and the sensitivity was $1.89x10^{-3}$A/mol L^{-1}. The equation for the resulting calibration plot was:

$$I= -3x10^{-10} + 1.89x10^{-3}xC,$$

where I (A) is the peak height and C is the concentration of L-Trp. The correlation coefficient was 0.9986. The results showed a good value of the sensitivity and a low limit of determination of L-tryptophan.

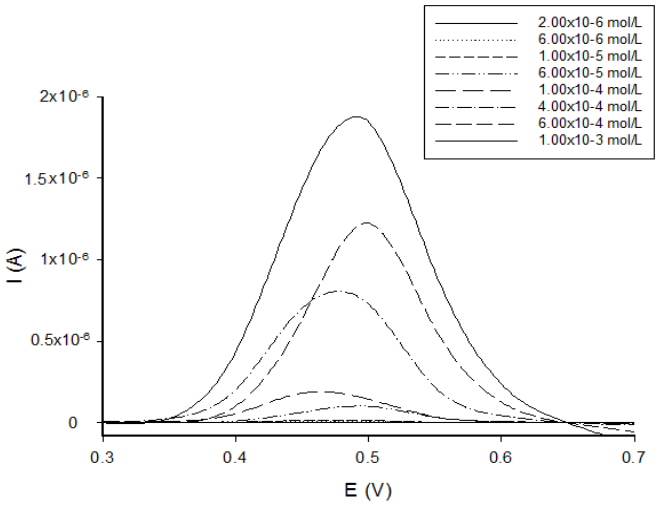

Fig. 1. A) Differential pulse voltammogram obtained for L-trp at different concentrations.

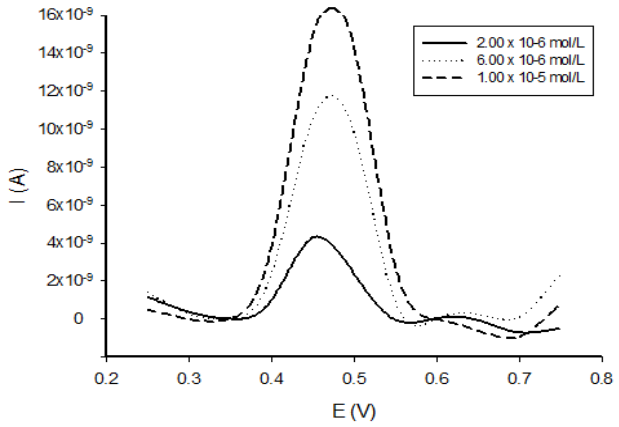

Fig. 1. B) Voltammograms for the lowest concentrations of L-tryptophan**.**

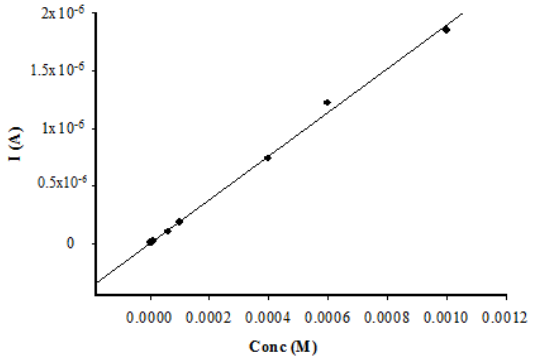

Fig. 2. Calibration graph obtained for L-trp using the modified graphite paste based sensor.

3.2 Interferences

Ascorbic acid (AA) and uric acid (UA) were chosen as possible interferences for the assay of L-Tryptophan. The ratio between the concentrations of the analyte and interferent was 1:10 (mol:mol) in the mixed solution. The amperometric selectivity coefficients were determined using the following equation:

$$Ki,j(amp) = \left(\frac{\Delta It}{\Delta Ii} - 1\right) * \frac{ci}{cj} \quad (16)$$

where $K_{i,j\ (amp)}$ 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 interfering ions.

The amperometric selectivity coefficients obtained vs AA ($3.18x10^{-3}$) and vs UA ($1.75x10^{-5}$) proved that the sensor is selective versus AA and UA.

3.3 Analytical applications

Whole blood samples were obtained from the Universitary Hospital in Bucharest (Ethics committee approval nr. 75/2015). The blood samples were analyzed as obtained directly from patients, without any pretreatment.The DPV technique was used to determine the L-Tryptophan amino acid in whole blood samples. The cell was filled with the whole blood sample and the peak height was measured. The unknown concentrations were determined from the calibration equation as described above. An example of voltammogram obtained using DPV for the assay of L-Tryptophan in whole blood samples is shown in Fig. 3. The average recovery of L-Trp in whole blood samples was 93.72% with RSD of 0.87% (N=5).

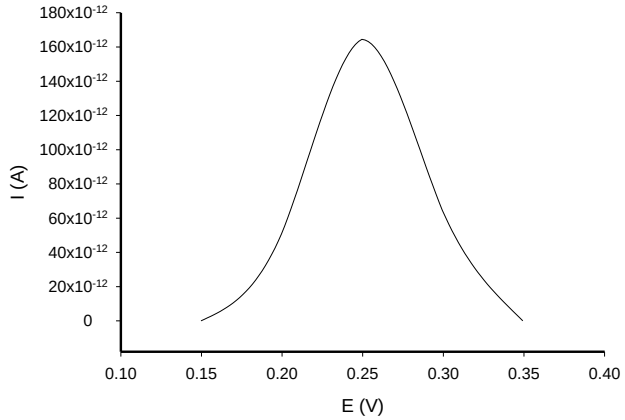

Fig. 3. Example of voltammogram obtained for the assay of L-trp in whole blood samples.

4. Conclusions

An electrochemical sensor based on graphite paste modified with 2,6-bis((E)-2-(furan-2-yl)vinyl)-4-(4,6,8-trimethylazulen-1-yl)pyridine was has been proposed for the assay of tryptophan in whole blood samples. The electrochemical sensor exhibited a low limit of detection and a high sensitivity. Uric and ascorbic acids did not interfere in the measurements. The recovery test proved further that the proposed electrochemical sensor can be used for the assay of L-tryptophan in whole blood samples.

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