DETERMINATION OF DOPAMINE IN WHOLE BLOOD SAMPLES USING A NEW ELECTROCHEMICAL SENSOR BASED ON GRAPHENE

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Dopamine is a neurotransmitter used for diagnosis of many neurodegenerative diseases and also of brain cancer. A new electrochemical sensor based on graphene modified with sulphur paste and protoporphyrine IX was proposed for dopamine 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 92.00% andrelative standard deviations less than 1.00%.

Keywords: Dopamine, electrochemical sensor, graphene, clinical analysis

1. Introduction

Dopamine is a neurotransmitter that plays several important roles in the brain. Dopamine homeostasis is an important clinical diagnostic index, because an abnormal level in the human body is closely related to neurodegenerative diseases [1]. Dopamine inhibits tumor growth and angiogenesis [2]; therefore, it can be also considered as a biomarker for brain tumor diagnosis.

There are many methods proposed for the determination of dopamine in different biological samples; almost all analytical techniques were used to date for the assay of dopamine, in order to obtain better sensitivity, selectivity, and lower limits of detection and quantification [3-6]. The utilization of electrochemical sensors for biomedical analysis had many advantages: the method is fast; the sample does not need any pretreatment; the cost of analysis is low.

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This paper proposed a new electrochemical sensor based on the modification of graphene with sulphur and protoporphyrin IX. The method used was differential pulse voltammetry. The sensor was validated using whole blood samples.

2. Experimental

2.1 Materials and reagents

All chemicals were of analytical grade. The graphene modified with sulphur was synthesized in house by the group of Stela Pruneanu from the National Institute for Research and Development of Isotopic and Molecular Technologies. Paraffin oil (d_4^{20}, 0.86 g x cm^{-1}) was purchased from Fluka. Dopamine, uric acid, glutamine, serine, and ascorbic acid were purchased from Sigma Aldrich.

Dopamine solutions were prepared fresh, every day, in phosphate buffer solution (PBS, pH = 7.5).

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 proposed electrochemical sensor) and a counter electrode (platinum wire).

2.3 Design of the electrochemical sensor

The graphene powder doped with sulphur was mixed with paraffin oil to obtain a homogeneous paste, which was further modified with protoporphyrin IX. 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 in the fridge at 2–8°C.

2.4 Procedure

The differential pulse voltammetry (DPV) measurements were performed at 25°C for each standard solution (10^{-3}mol L^{-1}–10^{-12}mol L^{-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 intensities were measured, and the equation of calibration was found using the linear regression method. The unknown concentrations were calculated from the equation of calibration determined statistically.

3. Results and discussion

3.1. Response characteristics of the graphene 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 $1.0x10^{-7}molL^{-1}$ and $1.0x10^{-4}mol\ L^{-1}$, the limit of determination was $1.0x10^{-7}mol\ L^{-1}$, the limit of detection was $5.0x10^{-8}mol\ L^{-1}$ and the sensitivity was $1.0x10^{-3}A/mol\ L^{-1}$. The equation for the resulting calibration plot was:

$$I=3.0x10^{-8}+1.0x10^{-4}xC,$$

where I (A) is the peak height and C is the concentration of dopamine. The correlation coefficient was 0.9994. The results showed a good value of the sensitivity and a low limit of and detection of dopamine. The linear concentration range is wide. The voltammograms used for the calibration of the proposed sensor were shown in Fig. 1.

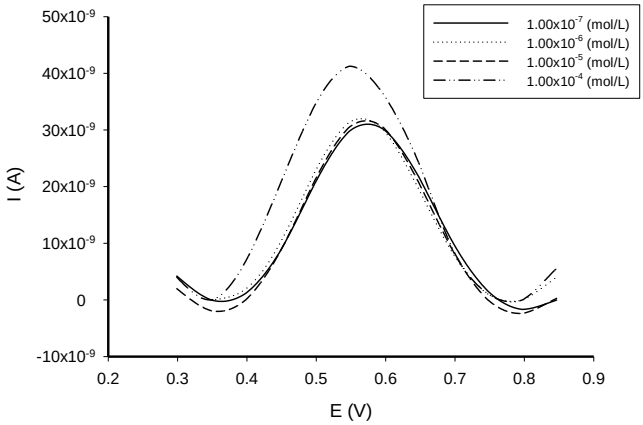

Fig. 1**.** Differential pulse voltammogram obtained for dopamine at different concentrations.

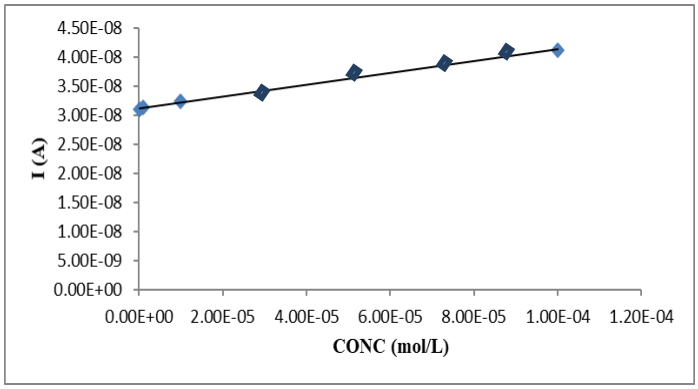

Fig. 2. Calibration graph obtained fordopamine using the modified graphene paste based sensor.

3.2 Interferences

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

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

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 ($2.68x10^{-3}$), vs UA ($1.70x10^{-3}$), vs GLU ($1.29x10^{-3}$), and vs L-SER ($2.78x10^{-5}$), proved that the sensor is selective versus AA, UA, GLU, and L-SER.

3.3 Analytical applications

Whole blood samples were obtained from the Universitary Hospital in Bucharest (Ethics committee approval nr. 65573/14.12.2018). The blood samples were analyzed as obtained directly from patients, without any pretreatment.

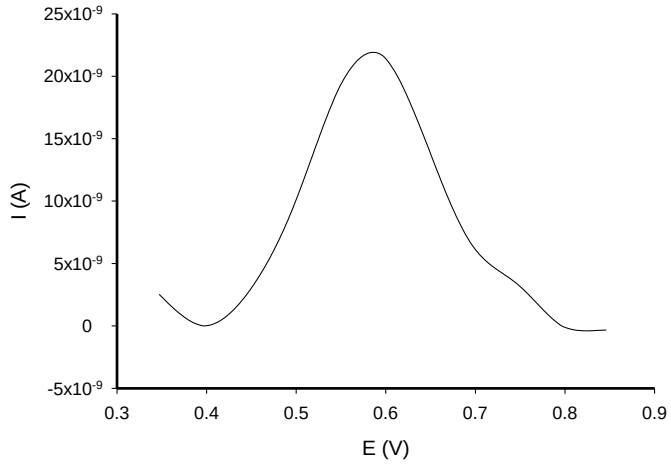

Fig. 3**.** Example of voltammogram obtained for the assay of dopamine in whole blood samples.

The DPV technique was used to determine the dopamine 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 dopamine in whole blood samples is shown in Fig. 3. The average recovery of dopamine in whole blood samples was 95.52% with RSD of 0.34% (N=5).

4. Conclusions

An electrochemical sensor based on graphene paste modified with sulphur and protoporphyrin IX was proposed for the assay of dopamine in whole blood samples. The electrochemical sensor exhibited high selectivity, low limits of detection and determination, and high sensitivity. The sensor was validated using whole blood samples.

R E F E R E N C E S

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