DETERMINATION OF D-SERINE FROM WHOLE BLOOD SAMPLES USING AN ELECTROCHEMICAL SENSOR BASED ON ZINC (II) – 5(4-CARBOXYPHENYL)-10,15,20-TRIS(4 PHENOXYPHENYL)PORPHYRINE

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D-Serine is a non-essential amino acid that can be synthesized in the human body for patients with breast cancer. An electrochemical sensor has been proposed for the determination of D-serine in whole blood samples. The electrochemical sensor is based on physical immobilization of Zn(II)-5(4-carboxyphenyl)-10,15,20-tris(4 phenoxyphenyl)porphyrine in a nanographene paste. Differential pulse voltammetry was used for all determinations. The limit of detection was 0.5 pmol/L. The linear concentration range was between 1 pmol/L and 1 nmol/L. The sensor is selective versus D-tryptophane, D-glutamine, D-arginine, L-Serine and D-aspartic acid, and also enantioselective.

Keywords: D-serine, electrochemical sensor, porphyrin, Zn(II)-5(4-carboxyphenyl)-10,15,20-tris(4 phenoxyphenyl)porphyrin

1. Introduction

Breast cancer represents one of the most frequent forms of tumors detected in females, as well as the second leading cause of death form malignant tumor, after lung cancer [1]. This disease is triggered when some cells in the structure of the mammary gland undergo some mutations at the genetic level, multiplying uncontrollably forming a tumor [2]. Further, these cells circulate from breast level to other parts of the body, to lymph nodes and vital organs, therefore the cancer metastasize.

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There are some research studies [3,4] that point out that hormonal and lifestyle factors as well as environmental ones may have a role in developing breast cancer at some point in a woman's life. Consequently, early detection of cancer biomarkers is very important for early diagnosis and effective therapy of cancer [5], due to the fact that in more than half of cancer cases, the patients ignored the first signs and symptoms of the growing cancer or delay the medical visit.

There are many detection methods used for D-serine, many of them belonging to the classical category of methods, such as electrophysiology [6], immunostaining [7] and analytical detection [8]. Though these methods have advantages, they also have insufficient quantitative information beside being time-consuming and having numerous costs. To determine D-serine concentration from human biological samples, including blood, serum or urine, most of the researchers have faith in HPLC (high-pressure liquid chromatography) [9] and LC-MC (liquid chromatography-mass spectrometry) [10-12] techniques. Likewise, to the classical techniques, these too present high costs, but also trained specialists and do not offer measurements that can be performed in real-time. Sensors, in general, represent a good alternative for quantitative measurements due to their numerous advantages compared to the classical methods.

In the last ten years, different sorts of (bio) sensors, such as electrochemical [13-15], magneto-mediated sensors [16], molecularly imprinted sensors [17,18] and biosensors [19,20] were accounted for distinguishing breast cancer biomarkers. Among them, there are some studies that evidenced themselves, due to their unique approach in detection of D-serine, and the fact that the biosensors used allowed the analysis from real samples like tissue [21,22].

A good sensor is based upon the materials used for its design. Especially for sensor materials a very necessary demand is represented by a high sensitivity in detecting the analyte in very low concentrations and also a good selectivity regarding other species that are present in the biological sample. A well-received and extensive used sensor materials are porphyrins [23,24] and graphenes [25] since they have the ability to modify their electrocatalytic and electrochemical properties, as well as a chemical stability. Some advantages of these porphyrin and graphene based sensors compared to other types of sensor materials involve a reduced response time, at ambient temperature the response of the sensor can be reversed. There are some studies that proved the usage of porphyrins and graphene as sensing materials for designing sensors with the ability for cancer biomarkers detection. In the group of Prof Raluca van Staden, very good results were obtained, including low limits of determination, high sensitivities and the sensors covered a wide range of patients and cancer biomarkers [26-28].

Some of the porphyrine properties can be assigned into nanostructured materials based on porphyrin derivatives, namely metalloderivatives, that have

some of the optimum features in electrochemical applications, being used as modifiers.

These metalloderivatives include a porphrine nucleus that has in its structure a supplementary conjugation of electrons, and the introduction of a metal ion, results in intensifying the electrons capacity of movement. Between metal ions, the ones with the most increased electrocatalytic behavior are represented by copper, nickel, iron [29] and cobalt. Sensors that were designed using carbon and gold [30] pastes proved to show their importance in detection of clinical [31] and pharmaceutical analytes, owing to their demonstrated reliability in providing useful analytical information; improvement of the sensors with suitable electroactive materials amplified the response of the sensors [31,32].

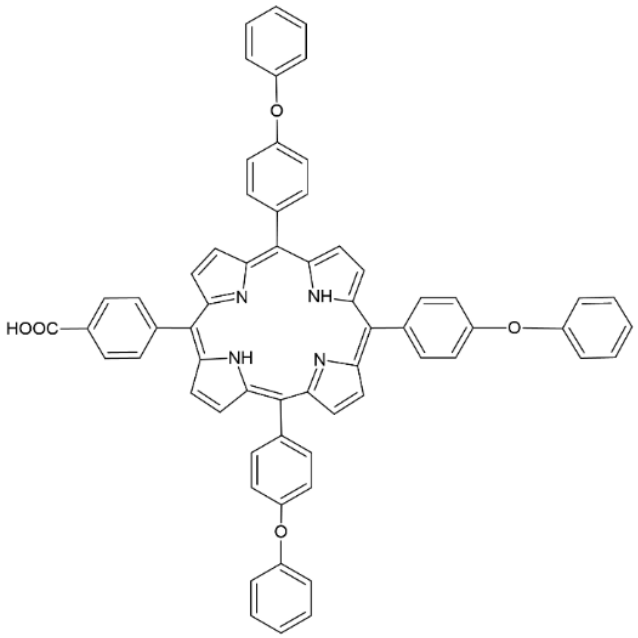

Fig. 1. The chemical structure of 5-(4-carboxyphenyl)- 10,15,20-tris(4-phenoxyphenyl)-porphyrin

The utilization of electrochemical sensors for biomedical analysis [34] has many advantages: the method is fast and reliable, and the sample does not need any pretreatment.

In this paper we proposed a new electrochemical sensor based on physical immobilization of Zn(II)-5(4-carboxyphenyl)-10,15,20-tris(4 phenoxyphenyl) porphyrine in a nanographene paste. The possible mechanism of the electrochemical oxidation of d-serine is illustrated in Fig.2, the detection of D-serine being made through the stoichiometric product of hydrogen peroxide, during the oxidation process of D-serine. The method proposed was differential pulse voltammetry (DPV). The sensor was validated using whole blood samples.

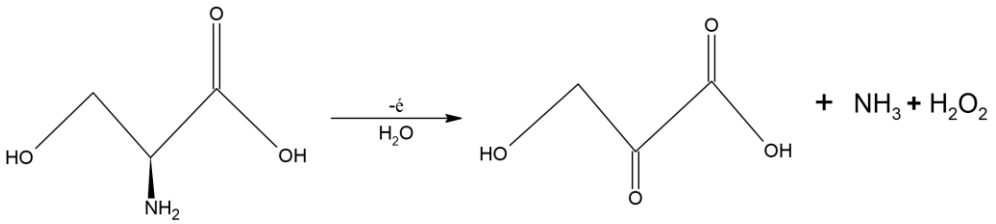

Fig. 2. The possible mechanism of the electrochemical oxidation of D-serine on the surface of the modified nanographene paste.

2. Experimental

2.1 Materials and reagents

L- and D-serine, D-tryptophane, D-arginine, D-aspartic acid, Zn(II)-5(4-carboxyphenyl)-10,15,20-tris(4 phenoxyphenyl) porphyrine, and nanographene powder were purchased from Sigma Aldrich. Paraffin oil was purchased from Merck. D-Serine solutions were prepared fresh, every day, in phosphate buffer solution (PBS, pH = 7.5).

2.2. Apparatus

All experimental measurements were performed using an AUTOLAB/PGSTAT 302 (Metrohm, Utrecht, The Netherland), connected to a computer for data acquisition. The electrochemical cell consists of three electrodes: a reference electrode (Ag/AgCl, 0.1mol/L KCl), a working electrode (proposed electrochemical sensor) and a counter electrode (platinum wire).

2.3. Design of the electrochemical sensor

The nanographene powder was mixed with paraffin oil to obtain a homogeneous paste, which was further modified with Zn(II)-5(4-carboxyphenyl)-10,15,20-tris(4 phenoxyphenyl) porphyrine. An optimized ratio of 1:1 (mg/μL) [35] between nanographene paste and porphyrin solution was used. A plastic tip was filled with the modified nanographene paste. The electrical contact was an Ag wire. The sensor surface was washed with deionized water and polished with paper before each use. When the electrochemical sensor was not used, it was kept in the dark, at room temperature.

2.4. Recommended procedure

The differential pulse voltammetry (DPV) measurements were performed at 25°C for each standard solution (10^{-3}–10^{-13} mol/L). 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.

The working parameters were as following: scan rate was 10 mV s^{-1}, potential range from -0.5 to - 0.1 V, and modulation amplitude 25 mV.

2.5. Samples

Whole blood samples were obtained from the Bucharest University Emergency Hospital (Ethics committee approval nr. 75/2015) from 3 different patients diagnosed with breast cancer. These samples were used for the direct assay of D-serine without any pretreatment.

2.6. Selectivity studies

The study of selectivity of the electrochemical sensor was done versus: D-Trp, D-Glu, D-Arg, L-Ser and D-Aspartic Acid. To determine the selectivity of the proposed electrochemical sensor, the amperometric selectivity coefficients were determined using the mixed solutions method for determining if there are any interference. The solutions were prepared according to the mixed solution method, before the measurements, taking into consideration the ratio of 1:10 (mol/mol) between D-ser and the interferent. The method was used for a better understanding of using the electrode under effective conditions, thus being a recommended procedure to determine amperometric selectivity coefficients.

3. Results and discussions

3.1. Characteristic response of the proposed electrochemical sensor

Differential pulse voltammetry (DPV) technique was used to determine the response characteristics of the electrochemical sensor. The voltammograms used for the calibration of the proposed sensor were shown in Fig. 3.

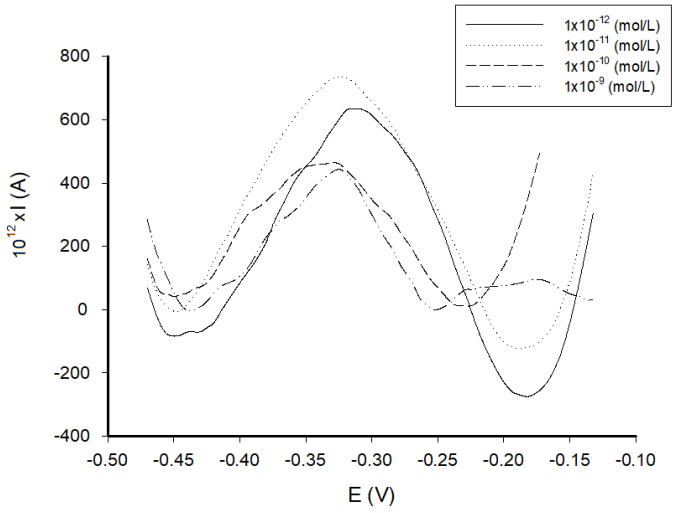

Fig. 3. Differential pulse voltammogram obtained for D-serine at different concentrations. The working parameters were as following: scan rate was 10 mV s^{-1}, potential range from -0.5 to - 0.1 V, and modulation amplitude 25 mV.

The half wave potential was recorded at -0.248 mV for $1x10^{-8}$ mol/L D-serine. Fig. 4 shows the calibration curve. The response characteristics obtained

were: the linear concentration range was between $1x10^{-12}$ mol/L and $1x10^{-9}$ mol/L, the limit of determination was $5x10^{-13}$ mol/L. The equation of calibration was:

$$I = 3.02 \times 10^{-10} + 0.52 \times C_{D-serine} \quad (1)$$

where I is the current in A, $C_{D\text{-serine}}$ is the concentration of D-serine in mol/L. The correlation coefficient, r is 0.9949. The results showed a good value of the sensitivity (0.52 A/mol/L) and a low limit of detection (0.5 pmol/L) for D-serine. The linear concentration range is wide.

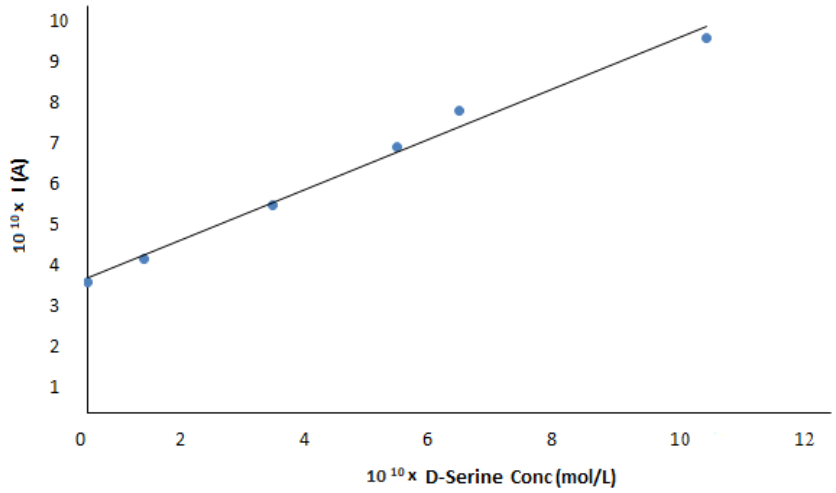

Fig. 4. Calibration graph obtained for D-serine using the modified nanographene paste based sensor.

Compared with the stochastic sensor proposed earlier for the assay of D-serine [36], the proposed electrochemical sensor exhibited better sensitivity; also the determination of D-serine with this electrochemical sensor can be performed faster.

3.2. Selectivity of the electrochemical sensor

The amperometric selectivity coefficients were determined using the following equation [37]:

$$Ki,j(amp) = \left(\frac{\Delta It}{\Delta Ii} - 1\right) * \frac{ci}{cj} \quad (2)$$

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.

Mixed solution method was used for the determination of potential interferences in the assay of D-ser. The values obtained for the coefficients are shown in Table 1.

Table 1

Amperometric selectivity coefficients

Parameters	Analyte	Interferent (10^{-3}M)	K_{sel}^{amp}
scan rate 10 mV s^{-1}	D-Serine (10^{-4}M)	D-Trp	$1.05x10^{-4}$
		D-Glu	$2.09x10^{-3}$
potential range -0.5 ÷ 0.1 V		D-Arg	$1.45x10^{-3}$
		L-Ser	$1.14x10^{-3}$
modulation amplitude 25 mV		D-Asp Acid	$7.37x10^{-4}$

The choice of these aminoacids was due to the fact that they are usually found in the biological samples together with the analyte of interest. The amperometric coefficients were determined using the same potential that was used for the determination of d-serine using the proposed sensor. The results show that there is no interference of D-Trp and D-Asp Acid, and a low interference from D-Glu, D-Arg, and L-ser in the determination of D-serine.

3.3. Determination of D-serine in whole blood samples

DPV was used for the determination of D-ser in three whole blood samples. No pretreatment was done before the measurements. The electrochemical cell containing the three electrodes was immersed in the cell which was filled with whole blood samples. The results of the measurements are presented in Table 2. An example of voltammogram obtained for the whole blood sample is shown in Fig. 5, which is an enlargement of the inset, for better understanding. In order to perform the recovery test, the following mechanism was adopted: D-serine was first determined from whole blood samples, then known amounts of D-serine were added. The recovered amounts were compared to those that were introduced in the biological samples. The recovery test performed showed a high recovery of the enantiomer of serine, with a value of 97.53% with an RSD of 0.10%. A classical method was used to determine D-serine from biological sample. The results obtained using DPV were in good correlation with the result obtained from HPLC, but overall better results were obtained using electrochemistry (Table 2).

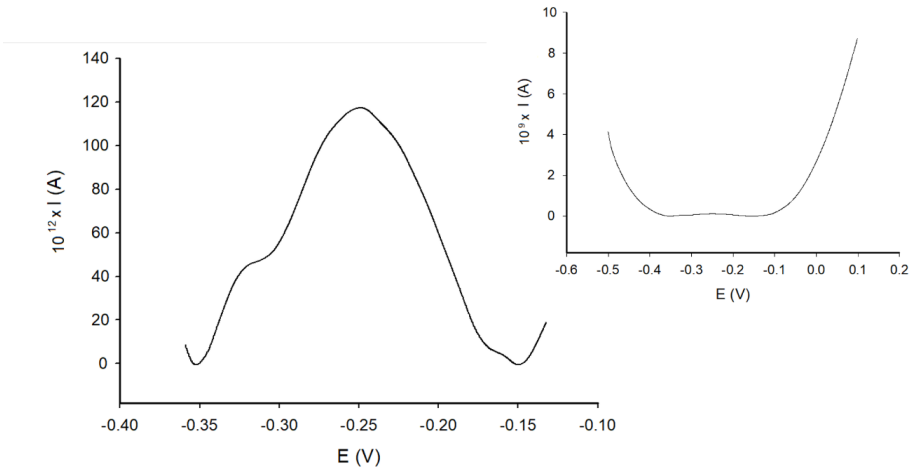

Fig. 5. Example of voltammogram obtained for the assay of D-serine in whole blood sample. The working parameters were as following: scan rate was 10 mV s^{-1}, potential range -0.5 ÷ 0.1 V, and modulation amplitude 25 mV.

Table 2

Determination of D-serine in whole blood samples using the proposed electrochemical sensor (scan rate was 10 mV s^{-1}, potential range -0.5 ÷ 0.1 V, and modulation amplitude 25 mV) and HPLC data

Sample No.	mmol L^{-1}, D-serine	
	DPV Electroanalysis*	HPLC [11]
1	2.57±0.12	2.50±1.14
2	2.86±0.13	2.70±1.20
3	3.80±0.12	3.20±1.40

* Using the proposed electrochemical sensor

4. Conclusions

An electrochemical sensor based on physical immobilization of Zn(II)-5(4-carboxyphenyl)-10,15,20-tris(4 phenoxyphenyl)porphyrine has been proposed for the analysis of D-Serine in whole blood samples. The electrochemical sensor showed high selectivity, as well as low limits of detection and determination, and high sensitivity. The sensor was validated using whole blood samples.

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