SENSORES ELETROQUÍMICOS À BASE DE FILME POLI (L-FENIL ALANINA) EM MWCNT PARA DETERMINAÇÃO DE TPS

ELECTROCHEMICAL SENSORS BASED ON POLY (L-PHENYL ALANINE) FILM ON MWCNT FOR DETERMINATION OF TPS

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RESUMO

Neste estudo, a voltametria cíclica (CV) é apresentada com uma técnica eletroquímica sensível para a avaliação do medicamento anticâncer Toposar (TPS). O eletrodo foi formado na superfície de um eletrodo de carbono vítreo modificado com MWCNT usando um revestimento de L-fenil alanina para se tornar o novo eletrodo em uma fórmula (PPA-MWCNT/GCE). O PPA foi polimerizado em pH 8,0, o que mostrou vantagens avançadas para o estudo e estimativa do tratamento por eletroanálise por TPS. Um grupo de fatores no eletrodo de carbono vítreo vazio foi estudado para aprimorar o trabalho do eletrodo no estudo do TPS, incluindo esses fatores (influência do pH, curva de calibração, estabilidade do eletrodo, taxa de varredura, potencial de deposição, etapa de voltagem, equilíbrio) tempo, tempo de deposição). Como resultado do desenvolvimento do eletrodo, a estabilidade do eletrodo preparado foi observada com a camada de PPA com a morfologia da camada pela análise SEM, mostrando a morfologia de PPA/GCE e MWCNTs/PPA/GCE por SEM, respectivamente. A largura do filme PPA na resposta TPS tem maior sensibilidade na superfície de MWCNTs/PPA/GCE em comparação com GCE, e reversibilidade redox aumentada com número de 8 ciclos, o CV de 5,0 nM TPS no GCE com taxas de varredura entre 50 e 400 mV·s⁻¹. Essa reação foi prevista para um mecanismo irrevogável de eletrodo. Uma comparação foi feita entre os CVs do TPS nos MWCNTs e o eletrodo modificado pelo PPA com o GCE desencapado a pH 7,0 com uma taxa de varredura de 100 mV·s⁻¹, a corrente de oxidação é 11,18 vezes maior que a comparável no GCE desencapado.

Palavras-chave: *Toposar, poli L-fenil alanina, voltametria cíclica, Sensor, MWCNT*

ABSTRACT

In this study, cyclic voltammetry (CV) is presented with a sensitive electrochemical technique for the assessment of the anti-cancer medicine Toposar (TPS). The electrode was formed on the surface of a modified glassy carbon electrode with MWCNT using a coating of L-phenyl alanine to become the new electrode in a formula (PPA-MWCNT / GCE). PPA was polymerized at pH 8.0, which showed advanced advantages for the study and estimation of TPS electroanalysis treatment. A group of factors on the bare glassy carbon electrode were studied to enhance the electrode 's work to study TPS, including these factors (Influence of pH, calibration curve, the stability of the electrode, scan rate, deposition potential, voltage step, equilibration time, deposition time). As a result of the electrode development, the stability of the prepared electrode was observed with the PPA layer with the Morphology of the layer by the SEM analysis showing the morphology of PPA/GCE and MWCNTs/PPA/GCE by SEM, respectively. The PPA film width on TPS response has higher sensitivity on the surface of MWCNTs/PPA/GCE compared to GCE and increased redox reversibility with 8 cycles number. The CV of 5.0 nM TPS on GCE with scanning rates of between 50 and 400 mV·s⁻¹ This reaction was predicted for an irrevocable electrode mechanism. A comparison was made between the TPS CVs on the MWCNTs, and the PPA modified electrode with the bare GCE at pH 7.0 with a scan rate of 100 mV·s⁻¹, the oxidation current is 11.18 times higher than the comparable one on the bare GCE.

Keywords: *Toposar, poly L-phenyl alanine, cyclic voltammetry, Sensor, MWCNT.*

1. INTRODUCTION:

Toposar (TPS), as a chemotherapy drug, is used for the treatment of testicular cancer, lymphomas, and small cell lung cancer (Toffoli *et al.*, 2004; Sissolak *et al.*, 2010). TPS acts by harming the DNA of the cancer cells. Topoisomerase inhibition, as well as apoptosis, causes DNA damage (Osherooff., 1989; Kovacic and Osuna., 2000).

Podophyllotoxin (PTOX) has been suggested as an anti-cancer drug for the therapy of tumor cells through blocking the tubulin polymerization, which induces the detention of cell cycle at mitosis, and stops cancer cell growth. PTOX products, including TPS, have been proposed for venereal wart and cancer therapies. One of the significant issues with this naturally extracted-plant derivative is its reservoirs (Ardalani *et al.*, 2017).

During electropolymerization, the thickness of polymer film is controlled by the number of cycles. Deposited films on surface electrodes by electropolymerization provide good homogeneity to the electrode surface. Based on the literature review, electrochemical detection of TPS, using a poly (L-phenylalanine) glassy carbon of modified electrode (PPA/GCE), has never been investigated previously. Amino acids have different functional groups, acting as the building blocks of protein structures, and allowing the adsorption mechanism to occur on electrocatalytic surfaces.

The condensation reaction of amino acids occurs when the C-terminus of one comes in proximity of the N-terminus of the other. Initially, oxygen and hydrogen atoms from the associated group of carboxyl and then hydrogen atom from the NH group of amino form a molecule of water (Andrea *et al.*, 2011).

Amino acid adsorption is essential for protein interaction with surfaces (Khan *et al.*, 1996). Generally, the electrochemical determination of medicines, using a poly L-phenylalanine (PPA) film (PPA/GCE) by electrochemical techniques, is a very selective and sensitive method. Voltammetric methods are appropriate for assessing the redox characteristics of functional medicine (Ozkan, 2012; Wang, 1998; Uslu, 2010; Dogan *et al.*, 2011).

Techniques that are applied to examine the medicine communications with DNA are as follows: UV-Visible spectrophotometry (Marky *et al.*, 1983), fluorescence spectroscopy (Jenkins *et*

al., 1998; Song *et al.*, 2006), constant wavelength synchronous fluorescence spectroscopy (CW-SFS) (Garbett *et al.*, 2007), circular dichroism (Garbett *et al.*, 2007; Pasternack., 2003), spectroscopy of Raman resonance (Manfait *et al.*, 1982) and Fourier transform infrared spectroscopy (Nafisi *et al.*, 2013).

2. MATERIALS AND METHODS:

The devices, materials, and arrangement of Poly (L-phenylalanine) on GCE used in this work, are described in the following paragraphs:

2.1. Instrumentation

Voltammetric measurements were done by 797 VA (Computrace Metrohm, Switzerland). A standard three-electrode complex comprised a working electrode, which is modified, a reference electrode, Ag/AgCl with saturated KCl, a bare GCE, and a wire of platinum as an auxiliary, used in all electrochemical tests. All tests were conducted at a temperature of 25.0 ± 0.5 °C. The pH meter (digital HANNA, Portugal) was used to measure the pH (Mohammed *et al.*, 2019).

2.2. Reagents

TPS and the required dosage of pharmaceutical form were acquired by Koçak Farma Inc. (Istanbul, Turkey). L-phenylalanine used in this study was of analytical grade, acquired from Fluka. The supporting electrolytes used here were as follows: Phosphate, Tris-HCl, and Britton Robinson buffers. All solutions were kept in the dark and utilized on a day to prevent decay. All chemicals and reagents were of analytical grade (Fluka, BDH), applied as received with no further purification. Double distilled water (DDW) was utilized during the experimental study.

2.3. Arrangement of Poly (L-phenylalanine) Altered GCE on multi-walled carbon nanotubes (MWCNTs)

Before the electrochemical alteration, the GCE was cleaned with a slurry of alumina of 0.3 and 0.05 μm pore size, to a mirror-like surface, then it was rinsed with DDW. Afterward, the GCE was treated consecutively with 1:1 nitric acid: absolute ethanol, and distilled water in an ultrasonic bath for 5 min, respectively. For the electropolymerization of the L-phenylalanine on the MWCNTs/GCE, after cleaning, the GCE was deposited in 0.01 M phenylalanine solution (0.2 M

PBS, pH 8.0), formerly degassed with high purity nitrogen for 5 min. The electrode was subjected to cyclic scanning between -1.5 and 2.5 V, with a scan rate of 80 mV.s⁻¹, using 8 cycles. A polymer of blue color on the GCE surface was formed and washed with reagent water type IV (ASTM D1193) (Daniel *et al.*, 1982); the electropolymerization mechanism is represented in Figure 1

3. RESULTS AND DISCUSSION:

3.1.1. Voltammetric study of Toposar

The redox mechanism of TPS, a medicine for anti-cancer treatment, was investigated as a further application for bio-detection in 0.2 M PBS at pH 7, by cyclic voltammetry (CV) sweeps from -1.5 to +2.0 V, with a scan rate of 100 mV.s⁻¹, until stable voltammograms were acquired, as presented in Figure 2. The oxidation potential was performed at 0.42 V versus Ag/AgCl/sat. KCl, following the instrument default conditions.

3.1.2. Voltammetric study of Toposar

To optimize the conditions for measurement by CV technique, a cyclic voltammogram of 3.0 nM TPS in PBS at pH 7.0, studied previously (Bozal *et al.*, 2013), used as a supporting electrolyte. Table 1 shows all the parameters optimized for the measurement of TPS.

3.1.3. Voltammetric study of Toposar

The influence of pH of the solution on the feedback of 3.0·10⁻⁹ M TPS was tested in the pH, ranging from 4.0 to 9.0 in PBS, by CV at a scan rate of 100 mV.s⁻¹ (Figure 3-a). As pH was increasing, the peak potential switched to more negative values, at pH 9.0, and turned out unmeasurable. The linear correlation between oxidation potential and pH can be presented as Equation 1.

$$E_{pa} = -58.3 \text{ pH} + 845.8 \quad (\text{Eq. 1})$$

Where E_{pa} is expressed in mV, with $r = 0.997$.

The pH of the solution significantly influenced the oxidation current. The PBS at pH 7.0 was selected for the assessment of TPS with the highest peak current (Figure 3-b).

3.1.4. The calibration curve of the TPS

The relation between concentrations of TPS and oxidation current was examined in serial experiments with different concentrations of TPS in PBS at pH 7.0 (Figure 4). The experiment was performed under optimal conditions, as illustrated in Table 1.

3.1.5. Stability of TPS

The TPS stability was measured in 0.3 nM of TPS, in PBS at pH 7.0, by simulating measurements on the electrode and preparing a periodic assessment of readings for the same cell, as shown in Figure 5.

3.2.1. Electropolymerization of PPA

The PPA film thickness is managed by the number of cycles of voltammetric scans throughout the electrochemical alteration of a GCE surface, as shown in Figure 6 in PBS at pH 8.0.

3.2.2. Morphology characterized by SEM of PPA/GCE & MWCNTs/PPA/GCE

Figure-7 (a-c) and Figure-7 (d-f) show the morphology of PPA/GCE and MWCNTs/PPA/GCE by SEM, respectively.

3.3.1. Impact of the PPA film width on the response of TPS

The diameter of the PPA film on the electrode surface was investigated by CV, concerning the electrochemical response of TPS. The peak currents of TPS were much depended on the thickness of the PPA film. Reduction and oxidation current peak augmented with the thickness elevation of the PPA film. At the same time, the reduction and oxidation potential were negatively and positively shifted, respectively, showing the redox reversibility of TPS was weakened with the thickness elevation of PPA. TPS has higher sensitivity and better redox reversibility; therefore, 8- cycle was chosen to control the PPA film thickness.

3.3.2. The influence of pH on the polymerization of PA

The effect of pH on oxidation currents of PPA on GCE was examined. Figure 8-a shows that by increasing pH, the reduction potential shifted negatively. The increasing pH for PPA was negatively shifted for the potential related to the

current ($I_{pa}/\mu A$), as shown for L-phenylalanine at pH 8.0 (Figure. 8). Therefore, pH 8.0 was selected as an optimal condition for L-phenylalanine.

3.3.3. Effect of pH on PPA for detection of TPS

The impact of the pH of PBS on the reaction of TPS was examined by CV. The TPS was well-behaved in PBS, as pH increased, the anodic peak potential became negative, and a good linear correlation was found in the graph of E_{pa} vs. pH in PBS, with pH ranging from 5.0 to 9.0. Equation 2 is the linear regression equation.

$$E_{pa} V = 0.5631 - 0.422 \text{ pH} \quad (\text{Eq. 2})$$

With an $R^2 = 0.9712$, as shown in Figure 9.

3.3.4. Electrochemical Oxidation of TPS at the PPA Modified Electrode

TPS oxidation potential and current can be determined on bare GCE; oxidation current is more significant than the comparable one, on the naked GCE. This demonstrated that the catalytic response happened between the PPA altered electrode and TPS. The catalytic response simplifies the relocation of an electron between the modified electrode and hydroquinone (TPS). Therefore the redox potential of TPS turned out to be simpler. PPA itself is electroinactive in the potential range from -1.5 to 2.0V (Figure10-a). Because of the high porosity of the PPA, the entire exterior area of the GC electrode is much bigger than that of bare GCE. Therefore, oxidation current elevates more clearly, more detailed in Figure10-b.

3.3.5. Impact of Scan Rate

The impact of the scan rate on oxidation current and the oxidation potential of TPS was examined. For this study, we documented the CV of $5.0 \cdot 10^{-9}$ M TPS on GCE with scan rates, ranging from 50 to 400 $\text{mV} \cdot \text{s}^{-1}$ (Figure 11-a). By elevation of the scan rates, the peak potential (E_{pa}) for the oxidation of TPS was increased. This reaction was predicted for an irrevocable electrode mechanism. The linear graph of I_{pa} as a function of $u^{1/2}$ (Figure 11-b) demonstrates that the oxidation of TPS on the GCE was diffusion-managed. For TPS, the graph of the logarithm of the scan rate as a function of the logarithm of peak current was linear with a slope of 0.91; this amount is near the theoretical value of 1.0, which demonstrates that a standard reaction is a diffusion-managed electrode mechanism (Figure. 11-c). Equation 3 can be presented as

$$\text{Log } I_{pa} (\mu A) = 0.918 \text{ log } u (\text{s})^{-1} + 0.7696 \quad (\text{Eq. 3})$$

With a $R^2 = 0.9506$.

3.3.6. Electrochemical Oxidation of TPS at the MWCNTs-PPA Modified Electrode

A comparison was made between the CVs of TPS on MWCNTs, PPA modified electrode with that of bare GCE. The CVs on bare GCE is demonstrated in Figure 12-curve a, and on MWCNTs PPA modified electrode in Figure 12 - curve b at pH 7.0 with the scan rates of 100 $\text{mV} \cdot \text{s}^{-1}$. The oxidation current is 11.18-fold larger than the comparable one on the bare GCE.

4. CONCLUSIONS:

In this work, a novel approach was presented for the creation of a new voltammetric sensor of TPS depended on MWCNTs PPA coating. Excellent sensitivity, high selectivity, and fast electron transfer were acquired for the oxidation of TPS on the PPA modified electrode. The current GC altered electrode (PPA/GCE) demonstrated a distinguished selectivity, antifouling characteristics, and sensitivity, as well as can isolate oxidation peaks for TPS, which are identical on the naked electrode. Chemically modified electrode, MWCNTs PPA is a promising sensor and has the potential to be used for drug assessment.

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Table 1. Optimized conditions for 3.0 nM TPS in PBS at pH 7.0 resulting in a peak resolution with higher current

Optimum Condition	Values
Scan rate (V/s)	0.1
Deposition potential (V)	0.7
End Potential (V)	2.0
Start Potential (V)	-1.5
Voltage step (V)	0.008
Equilibration time (s)	10
Deposition time (s)	5

Figure 1. The proposed process of the electropolymerization of L-phenylalanine

Figure 2. a) The cyclic voltammetry of TPS in PBS at pH 7.0 with a scan rate of $100 \text{ mV} \cdot \text{s}^{-1}$, b) The square wave voltammetry of TPS in PBS at pH 7.0

Figure 3. a) The influence of pH on TPS peak currents and peak potentials, TPS concentration of $3.0 \cdot 10^{-9} \text{ M}$, b) The cyclic voltammogram of TPS in PBS (pH 4.0-9.0)

Figure 4. The relationship between concentrations of TPS and oxidation current

Figure 5. The stability of TPS / GCE

Figure 6. The cyclic voltammograms for electropolymerization of L-phenylalanine on a GCE

Figure 7. The morphology of PPA/GCE&MWCNTs/PPA/GCE characterized by SEM

Figure 8. a) The correlation between cycle number and the oxidation current of PPA under the effect of concentration (Calibration Curve), b) The correlation between cycle number and the oxidation current of PPA on GCE (blue color), and oxidation current of TPS on PPA/GCE (orange color)

Figure 9. The relation between $I_{pa}/\mu A$ vs. pH

Figure 10. a) The Cyclic voltammograms of TPS on bare GCE (a) and PPA modified electrode (b) with 0.2 M PBS (pH 7.0); scan rate of 100 mV s⁻¹, b) A plot of concentration of TPS and the oxidation current on PPA/GCE

Figure 11. a) The cyclic voltammograms for the oxidation of TPS on a GCE at scan rates of (a) 0.05; (b) 0.1; (c) 0.15; (d) 0.2; (e) 0.25; (f) 0.3; (g) 0.35; (h) 0.4 V s⁻¹, b) The oxidation currents is correlated with the square root of scan rates, c) Logarithm of oxidation current is correlated with the logarithm of scan rates

Figure 12. a) A plot of oxidation current peak of TPS as a function of the concentration of the TPS on MWCNTs-PPA/GC modified electrode, b) The cyclic voltammograms of TPS on (a) bare GCE (b) PPA modified electrode, (c) and MWCNTs PPA modified electrode with 0.2 M PBS (pH 7.0); scan rate of 100 mV.s⁻¹