

Voltammetric Detection of Clopidogrel in Pharmaceuticals Utilizing Electropolymerized *o*-Aminophenol-MWCNTs Modified Electrode

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Abstract—In the present study, Clopidogrel (CLP), an anti-platelet drug, was analyzed utilizing electropolymerized *o*-aminophenol (EpoAP) and multi-walled carbon nanotubes (MWCNTs) modified graphite electrode (GE). The prepared electrode was characterized by Cyclic Voltammetry (CV) and Scanning Electron Microscopy (SEM) techniques. The dependence of peak intensities on pH, scan rate, and MWCNTs amount were further investigated. Electrochemical determination of CLP was evaluated by Differential Pulse Voltammetry (DPV) and CV analysis under optimized working conditions. The calibration curves of CLP obtained in the range of 2.0–75.0 μM by DPV and 50.0–200.0 μM by CV. The limits of detection (LOD) calculated to be 0.50 and 6.6 μM via DPV and CV, respectively. In addition, surface area and coverage of the modified electrode, electron-transfer coefficient, and the diffusion coefficient of CLP were calculated. Finally, the fabricated electrode was proved successfully for quantification of CLP in pharmaceuticals directly, which means it's not necessary to separate the excipients of the tablets for the analysis, with a mean recovery of 97.49%.

Keywords: clopidogrel, electropolymerized poly *o*-aminophenol, MWCNTs, pharmaceuticals, electrochemical determination

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INTRODUCTION

Clopidogrel hydrogen sulphate, chemically it is methyl (+)-(*S*)- α -(*o*-chlorophenyl)-6,7-dihydrothieno (3,2-*c*) pyridin-5(4*H*)-acetate hydrogen sulphate. As illustrated in Fig. 1, the molecular formula is $\text{C}_{16}\text{H}_{16}\text{ClNO}_2\text{S}\cdot\text{H}_2\text{SO}_4$. Clopidogrel (CLP), a potent antiplatelet agent (thienopyridine class), inhibits adenosine diphosphate-mediated platelet aggregation [1]. Clopidogrel is an inactive pro-drug which needs enzymatic transformation into its active metabolite by various types of cytochromes P450 (CYP) enzymes [2]. It is indicated for the reduction of atherosclerotic events in patients with atherosclerosis documented by recent stroke, myocardial infarction, or cardiovascular disease [2–5]. Hence, various analytical methods such as chromatography with different detectors [6–8], spectroscopy [9], electrochemical sensors [10–12], potentiometry [13, 14] and capillary electrophoresis have been reported for to the determination of CLP [15]. Chromatography methods suffered from methodolog-

ical limitations such as time-consuming, high cost and difficulty of fabricating. On the other hand, electrochemical methods possess beneficial features such as; rapidity, sensitivity, ease of fabricating and affordability [16–18]. Various nano-sized materials have been utilized for development of electrochemical sensors.

Carbon nanotubes (CNTs) have unique properties such as fast electron transfer rate, excellent electrocatalytic effect, tensile strength, high conductivity, biocompatibility, and chemical stability [19, 20]. Depending on their atomic structure, CNTs behave

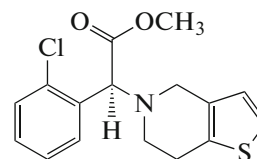


Fig. 1. Chemical structure of Clopidogrel (CLP).

electrically either as a metal or a semiconductor and offer a high electron transfer rate between the electrode surface and analytes, which can improve performances of biosensors and nanosensors [21]. Despite these interesting features, they lack sufficient, which might impair CNTs-based devices. Therefore, we cannot immobilize CNT onto the electrodes surfaces in a stable and uniform because of this limitation manner [18, 22]. In order to overcome this difficulty, in situ electropolymerization is a simple yet powerful procedure for developing of stable CNT modified electrodes in electrochemical sensors [23, 24].

Conducting polymer-CNT modified electrodes are of great interest due to their three-dimensional structures of the polymers that provide the opportunity for incorporating of CNTs into them and high surface areas [25]. In addition, conducting polymer-CNT based devices represent other advantages including stability, reproducibility, homogeneity, many active sites regions and excellent adherence to the electrode surface [26]. In the literature survey, various conducting polymers including poly (phenylene vinylene) [27], polypyrrole [28], polyaniline [29] and polythiophene [30] have been applied. The polymer-CNT modified electrodes were successfully applied for the constructing of electrochemical sensors. Among conducting polymer systems, polyaniline has been of much interest owing to its great electrochemical properties [31]. *o*-Aminophenol with the formula of C_6H_7NO is one of substituted anilines with two oxidizable substituents, exciting stability, easy doping, and its inherent conductivity [18, 20, 32]. These conjugated polymer structures are known to be strongly coupled to carbon nanotubes through π - π stacking interactions [33]. It has been demonstrated that the blending of carbon nanotubes (CNTs) with a polymer network improves the electric conductivity as well as the mechanical property of the pure polymer network [32].

However, to the best of our knowledge, there has been no report focusing on the application of EpoAP-MWCNTs to electrochemical detection of CLP. Herein, we applied an interesting and simple procedure for immobilizing MWCNTs on the surface of (graphite electrode) GE via in situ electropolymerization in the absence and presence of *o*-aminophenol (oAP). The voltammetric detection of CLP based on Electropolymerized *o*-Aminophenol (EpoAP)-MWCNTs nanocomposite by means of Cyclic Voltammetry (CV), differential Pulse Voltammetry (DPV) and Chronoamperometry techniques has been done. The surface properties of the modified electrode has been characterized by Scanning Electron Microscopy (SEM) and CV techniques. Moreover, kinetic, thermodynamic and electrochemical parameters including electron transfer and diffusion constant have been investigated using electrochemical techniques. Under optimum conditions, the modified electrode has been employed

to detect CLP in pharmaceutical products and the assay results compared with the analysis of the same samples (same batch number) by HPLC method reported in the current United States Pharmacopoeia.

EXPERIMENTAL

Chemicals

CLP hydrogen sulfate standard powder (100.20% according to the USP-NF 2016) has received as a gift from the Iranian Quality Control Laboratory of the Ministry of Health and Medical Education Department (Tehran, Iran). MWCNTs (>97% purity, diameter <10 nm, 5–15 μ m length with a special surface area of 180–190 $m^2 g^{-1}$) were obtained from Neutrino Co. (Tehran, Iran). The pharmaceutical samples of CLP have purchased from a local pharmacy. OSVIX tablets are labeled to contain Clopidogrel (as hydrogen sulfate) equivalent to 75 mg of CLP manufactured by OSVAH pharmaceutical Co (Tehran, Iran). All other chemicals in this study were of analytical grade (Merck, Darmstadt, Germany) and used without further purification.

Apparatus

All electrochemical experiments were performed by an electrochemical system comprising of AUTOLAB potentiostat/galvanostat coupled with a NOVA commercial software as well as a conventional three-electrode cell including Ag/AgCl, platinum wire, and a graphite electrode as reference, counter and working electrode (0.0314 cm^2 surface area); purchased from Azar Electrode Co., Urmia, Iran. The surface morphology of the modified electrode was characterized using scanning electron microscopy (SEM, S-4160, Hitachi, Japan). All pH values were determined with a digital pH meter model 827-pH lab (Metrohm, Switzerland).

Preparation of Britton–Robinson Buffer and Stock Solutions

Britton–Robinson Buffer (BRB) solutions were prepared by mixing 0.4 M of three acid (i.e., acetic, sulfuric, and boric acids). Buffer pH has adjusted to the demanded value by 0.2 M NaOH solution and applied as a supporting electrolyte. Stock solutions of CLP (10 mM) were prepared by dissolving the powder in an appropriate volume of methanol and kept at 4°C. Standard solutions were daily prepared by diluting stock solutions using BRB solution.

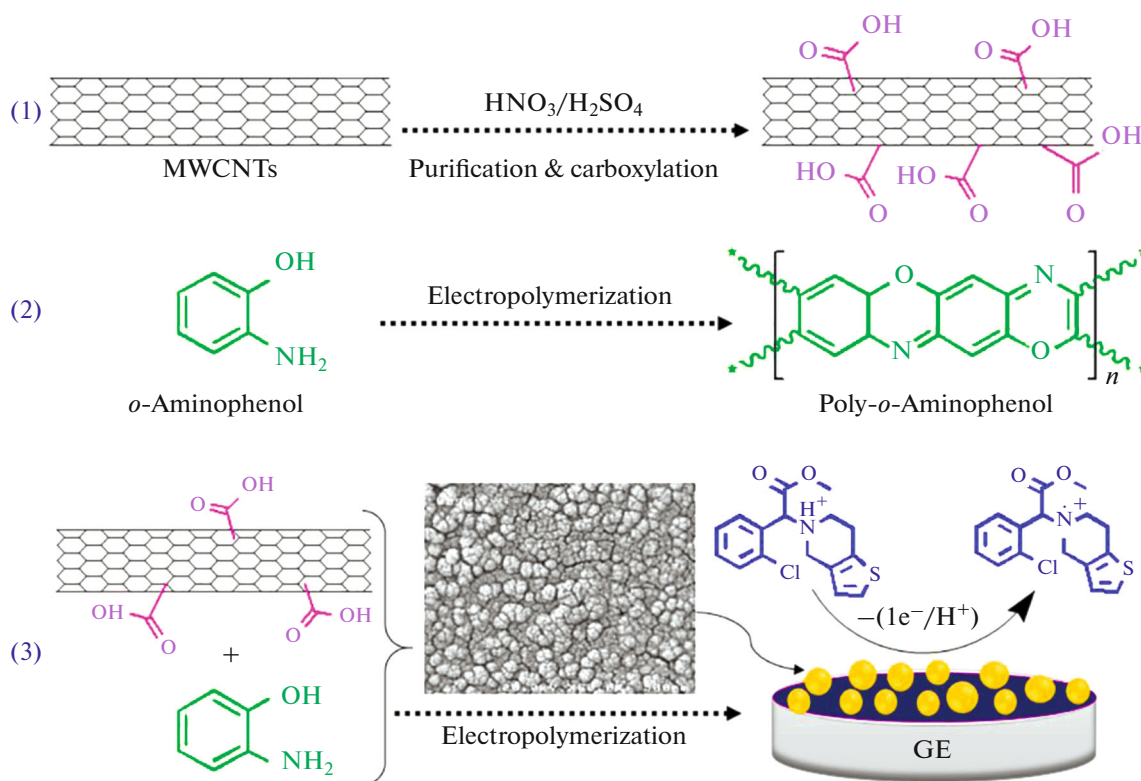
Fabrication of EpoAP-MWCNTs Based Electrode

Due to low solubility of MWCNTs in water, it is necessary to purify them and generate active groups

into the structure of MWCNTs [34]. In order to prepare carboxylic groups in MWCNTs, we have treated them with strong acid to increase the solubility. Herein, MWCNTs have purified by the following procedure: Firstly, 0.2 g MWCNTs added to 10 mL HNO_3 and 30 mL H_2SO_4 for 0.5 h at room temperature. Secondly, the resulted suspension refluxed for 4 h at 65–70°C to avoid the partial destruction. Then, 15 mL H_2O_2 added to the solution drop by drop until brownish NO_2 gas released. Purified MWCNTs were sonicated, after 24 h deionized water added to them. The pH of carboxylated MWCNTs has adjusted to 7.0 with NaOH and stored for 48 h in a dark place. The MWCNTs centrifuged and rinsed with deionized water several times (Scheme 1). Then, GE polished by alumina powder, washed by deionized water carefully, and dried at room temperature. Black homogeneous MWCNTs dispersion (0.8 mg mL^{-1}) in deionized water has achieved by ultrasonication of the dispersion in an ultrasonic for 30 min. Then, 3 μL of the freshly

prepared suspension was dropped on the polished surface of the GE and allowed to dry for 10 min in an oven at 50°C. On the other hand, 0.01 M oAP monomeric solution has been dispersed in 0.5 M HClO_4 to prepare a solution. Thereafter, oAP was electropolymerized on the GE by repetitive voltammogram cycling (50 scans) in a potential window of –0.3 to 1.2 V at a scan rate of 0.1 V s^{-1} . MWCNTs–EpoAP/GE has prepared by the same procedure, while 8% w/w MWCNTs added (Scheme 1). The modified electrodes characterized by CV and SEM techniques.

The resulted sensor was successfully applied for at least 15 measurements without losing its function. After that the response started to decrease. Therefore, we polished the modified electrode by alumina powder, washed with deionized water carefully, and dried at room temperature. Then the bare GE was modified again by MWCNTs–EpoAP as same as described above.



Scheme 1. (1) Purification and carboxylation of MWCNTs, (2) proposed mechanism of the electropolymerization of oAP and (3) fabrication of EpoAP–MWCNTs/GE.

Preparation of Solid Dosage Forms Solutions

Ten tablets (each containing 75 mg CLP) pulverized to a fine powder and mixed well. An aliquot powder with equivalent weight of one tablet was transferred into a

100 mL volumetric flask containing 50 mL methanol. The solution was sonicated for 5 min to ensure complete dissolution of CLP. Then 5 mL of the prepared solution was diluted to 100 mL using BRB (pH 3.7).

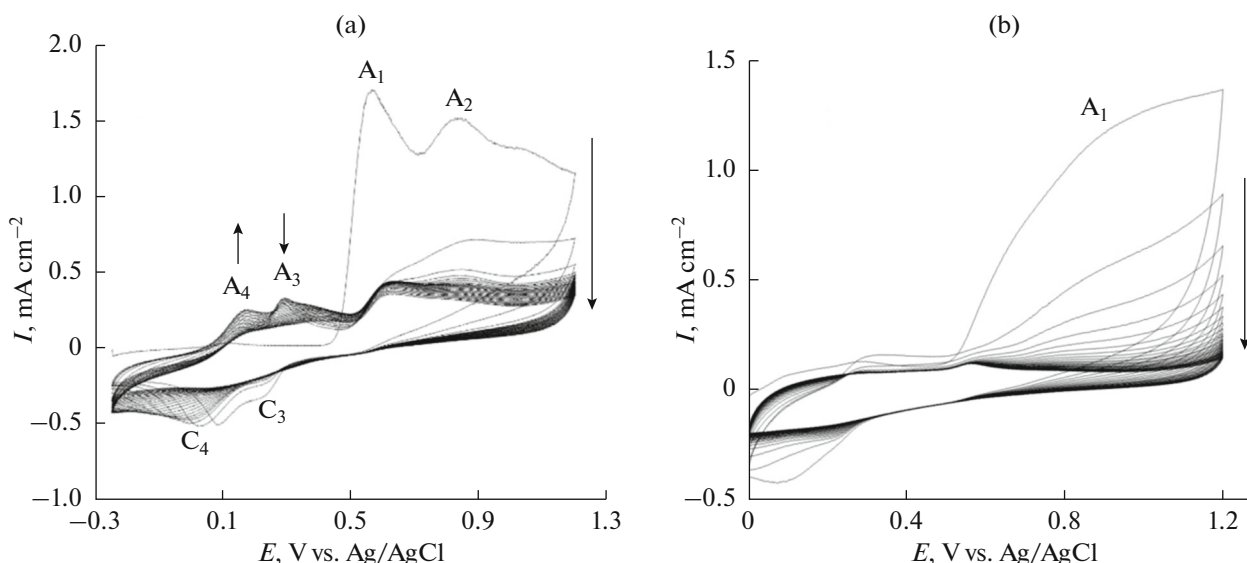


Fig. 2. The typical multi-sweep CV's during electropolymerization of oAP in the presence of the MWCNTs (a) and in the absence of the MWCNTs (b) into the 0.5 M HClO₄ solution at scan rate 0.1 V s⁻¹.

RESULTS AND DISCUSSION

Cyclic Voltammetry Characterization of the Modified Electrodes

The oAP monomer in the presence of the 8% w/w MWCNTs was electropolymerized on the GE surface by repetitive voltammogram cycling (50 scans) in a potential window of -0.3 to 1.2 V at a scan rate of 0.1 V s⁻¹. As seen in Fig. 2a, at the first scan two peaks are defined: one peak (A₁) at 0.60 V is attributed to the oxidation of oAP to monocation radical (oAP⁺) and another peak (A₂) around 0.85 V probably due to the oxidation of the oAP⁺ to dication [35]. On the reverse scan none of these peaks shows complementary peaks, indicating irreversibly oxidation of the monomers [18]. At the second cycle, a pair of redox peaks appeared at 0.3 and 0.31 V (A₃–C₃) attributed to the formation of an intermediate during the oxidation process of the oAP and another pair at 0.1 and 0.23 V (A₄–C₄) is related to the redox process of the phenoxazine units. When the number of CV cycles is increased, the current of the redox peaks at around A₃–C₃ gradually declined because of oAP monomer absence, implying generation of the polymer [18, 32, 35]. Also, EpoAP was fabricated at the bare graphite electrode in the same way. As illustrated in Fig. 2b, a board anodic peak around 0.8 V, at the first scan, was observed which is related to the irreversibly oxidation of the $-OH$ and NH_2 groups of oAP, resulting in the formation of mono-cation radicals on the surface of GE [18, 32]. However, the anodic peak around 0.8 V dropped slowly after continuous cycling indicating that the oxidation process cannot significantly occurred after multiple cycles [36].

Characterization of the Modified Electrodes by SEM Technique

The surface morphology of the EpoAP/GE and EpoAP–MWCNTs/GE nanocomposites were characterized using SEM technique. The SEM micrographs of EpoAP/GE and EpoAP–MWCNTs/GE are represented in Figs. 3a and 3b. Figure 3a micrograph indicates that the EpoAP/GE surface has a globular (spherical) structure that was in compact and uniform state on the surface of electrode. A large number of polymer chains were generated these globular particles. Figure 3c shows the EpoAP/GE in the higher magnification (500 nm). A very high magnification of SEM micrograph of EpoAP shows the coex-

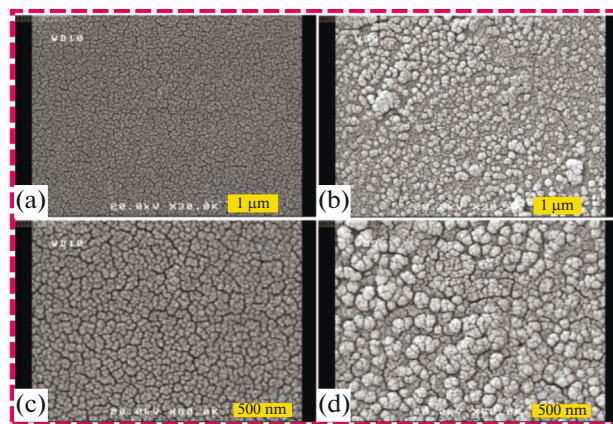


Fig. 3. SEM micrographs of (a) EpoAP/GE (b) EpoAP–MWCNTs/GE, (c) EpoAP/GE, and (d) EpoAP–MWCNTs/GE in the different magnifications (a and d: 1 μm, c and d: 500 nm).

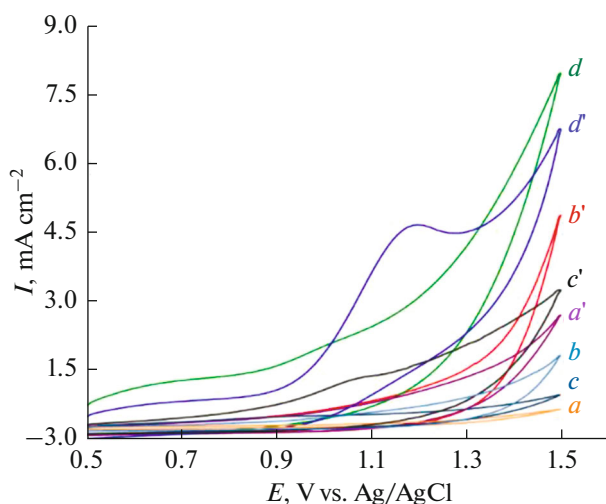


Fig. 4. Recorded CVs at the bare, MWCNTs, EpoAP and EpoAP–MWCNTs modified GEs in the absence (*a*, *b*, *c* and *d*) and presence (*a'*, *b'*, *c'* and *d'*) of CLP (100 μ M) in BRB solution (pH 3.7) at scan rate of 0.1 V s⁻¹.

istence of particle agglomerates, resulting in formation of a coarse surface. It could be seen from Fig. 3b that MWCNTs is well blended into the 3D globular structure of EpoAP. No distinct MWCNTs can be observed on the micrograph, implying that the MWCNTs are probably wrapped by EpoAP [37]. A strong interconnected network was generated between MWCNTs and EpoAP matrix, which enhanced electron transfer properties of the nanocomposite. This observation is well similar to the other reported studies [37–39]. In the previous reports, the prepared EpoAP structure has been characterized via spectroscopy, FT-IR, and the other techniques [40–42].

Electrochemical Behaviors of the Modified Electrodes

Figure S1 (Supporting Materials) shows the cyclic voltammogram recorded with the bare GE in a 0.5 M HClO₄ solution (curve 1). The voltammogram shows no peaks in the potential window of experiment. Figure S1 (curve 2 and 3) display the cyclic voltammograms recorded with the EpoAP/GE and EpoAP–MWCNTs/GE in a 0.5 M HClO₄ solution, respectively. Curve 2 and 3 represented a pair of redox peaks at around 0.1 and –0.1 V are attributed to the redox process of the phenoxazine units. In order to evaluate of the effect of MWCNTs on the property of EpoAP film, electrochemical performance of the EpoAP–MWCNTs/GE was examined by carrying out cyclic voltammogram experiment at the same condition (0.5 M HClO₄ solution). As seen in Fig. S1 (curve 3), the recorded cyclic voltammogram of the EpoAP–MWCNTs/GE shows a pair of redox peaks at around 0.1 and –0.1 V. The results revealed that the presence

of the EpoAP–MWCNTs/GE could increase the peaks in comparison to EpoAP/GE.

Effect of MWCNTs Amount

The thickness of the polymeric film affect the electrochemical response, hence, this thickness is controlled by changing the MWCNTs amount during the electrochemical polymerization. This was performed by measuring CV results, obtained by adding various amount of the MWCNTs (1–15% w/w) to the constant amount of oAP. As shown in Fig. S1 inset, the current of CVs dramatically increased with the increase of the MWCNTs amount from 1 to 8% w/w, while starts to slightly decrease when MWCNTs amount continues to increase from 8 to 15% w/w. This behavior is due to a saturation limit near 8%w/w. Subsequently, using higher amount of MWCNTs may adversely affect the sensitivity of the CLP detection. Thus, the optimal value of MWCNTs was selected to be 8%w/w for whole electrochemical experiments.

Electrochemical Behavior of CLP at the Surface of the EpoAP–MWCNTs/GE

Cyclic voltammograms in the Fig. 4 represented the electrochemical oxidation of CLP at the bare, MWCNTs, EpoAP, EpoAP–MWCNTs modified GEs in BRB solution (pH 3.7) containing 100 μ M CLP. CLP exhibits an anodic peak around 1.15 V, and in the reverse scan, no cathodic peak was observed. The anodic peak current of CLP at modified electrodes is higher than that of unmodified, which can be in this order; EpoAP–MWCNTs > EpoAP > MWCNTs > bare. These results have evidenced for enhanced electrochemical performances of these nanocomposites towards CLP oxidation, suggesting an interesting electrical construction on the electrode surface and accelerating the electron transfer through it. It is assumed that hydroxyl and amine bonds were formed between polymeric film at the surface of the electrode and a carboxylic group of CLP molecule, resulting in improvement of charge transfer kinetics of CLP at the surface of EpoAP–MWCNTs/GE.

Effect of pH

The pH of solution plays an important role in the electrochemical behavior of CLP during voltammetric experiments at EpoAP–MWCNTs/GE. To investigate of pH effect, electrochemical response of 180 μ M CLP in BRB solution over a pH range (from 1.0 to 6.0) was studied. According to the Fig. S2, anodic peak current of CLP increases with increasing pH till 3.7. Afterward, the peak current decreases with increasing pH, possibly because of decreasing solubility of CLP at neutral and basic media [43]. In order to get the highest oxidation current peak, the electrochemical

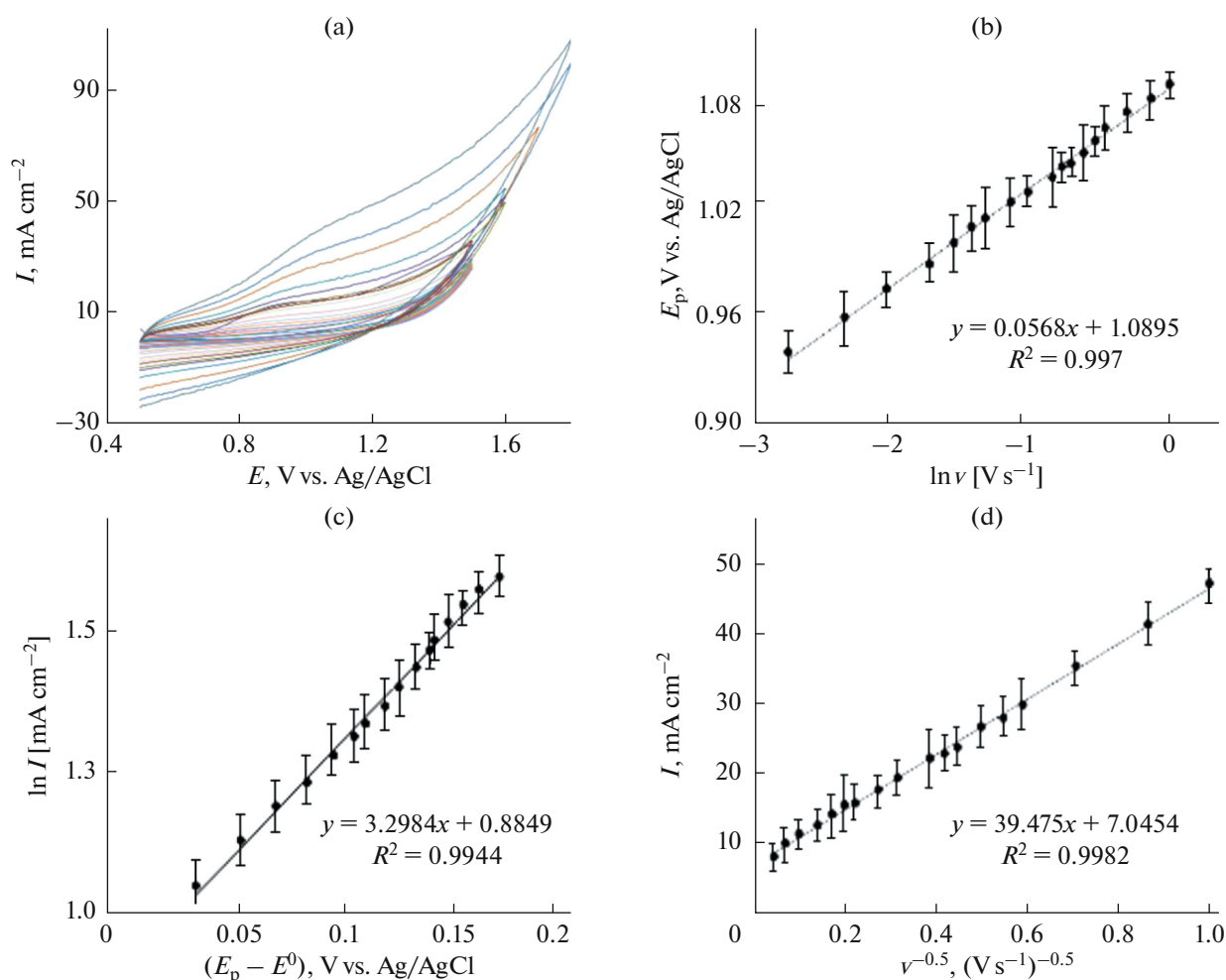


Fig. 5. (a) Recorded CVs of the EpoAP–MWCNTs/GE in BRB solution (pH 3.7) containing 100 μM CLP with different scan rates (from inner to outer correspond to $v = 0.002, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.075, 0.1, 0.15, 0.25, 0.4,$ and 0.9 V s^{-1}). (b) The relationship between the peak potentials and the natural logarithm of scan rate. (c) The dependency of the natural logarithm of peak current on $(E_p - E^0)$, which E_p is the peak potential and E^0 is the formal potential. (d) Linear relationship between the anodic peak currents of CVs and the square root of scan rates using 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KCl solution. Error bars indicate the standard deviation (SD) of three times measurements ($n = 3$).

determination of CLP was performed at pH 3.7 (BRB solution).

Effect of Scan Rate

In Fig. 5a, the influence of the potential scan rate with CLP electrochemical reaction at EpoAP–MWCNTs/GE was examined by cyclic voltammetry. Gradual increasing of the oxidation peak current were observed with increasing scan rate, and the oxidation peak current versus square root of scan rate display an excellent linear proportionality over the range of 20 to 1000 mV s^{-1} (Fig. 5d). This behavior imply that the electrochemical oxidation of CLP at the EpoAP–MWCNTs/GE is a diffusion-controlled process. In addition, dependence of peak on the logarithm of scan

rate was also plotted for EpoAP–MWCNTs/GE in Fig. 5b. It can be realized from Fig. 5b that, with increasing scan rate, the oxidation peak potential shifts to more positive potentials. The relationship between peak potential and logarithm of scan rate corresponded to the equation as follow: $E_{\text{pa}} (\text{V}) = 0.0568 \log v (\text{V/s}) + 1.0895$ ($R^2 = 0.997$). The electron transfer coefficient (αn) for the reaction can be achieved from the following equation, which is suitable for totally irreversible diffusion-controlled process [44, 45]:

$$E_p = \frac{b \log v}{2} + \text{constant}. \quad (1)$$

On the basis of this equation, the evaluated slope of the E_p vs. $\log v$ is $b/2 = 56.8 \text{ mV}$, therefore b (Tafel slope) = 113.6. The value of the electron transfer coef-

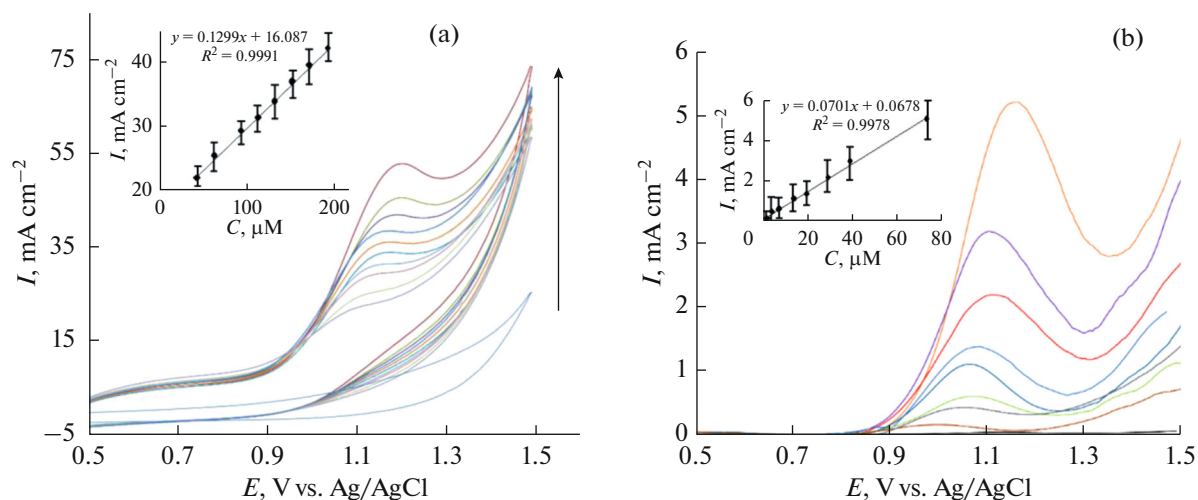


Fig. 6. (a) CVs of the EpoAP–MWCNTs/GE in the solutions containing different concentrations (from bottom to top, 0, 40, 50, 70, 100, 120, 140, 160, 180, and 200 μM) of CLP. Conditions: in BRB buffer (pH 3.7); scan rate: 0.1 V s^{-1} . Inset: the corresponding linear relationship between the CV currents and CLP concentration. (b) Dependence of the DPV response at the EpoAP–MWCNTs/GE surface on the CLP concentration in BRB (pH 3.7): Concentrations from bottom to top are 0, 2, 5, 7.5, 15, 20, 30, 40, and 75 μM . Insets show the corresponding calibration graphs. Error bars indicate the standard deviation (SD) of three times measurements ($n = 3$).

ficient for CLP oxidation on EpoAP–MWCNTs/GE was calculated to be 0.48. These results indicated that the number of electrons involved in the electrochemical oxidation of CLP is one.

The surface area of the EpoAP–MWCNTs/GE was determined using 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KCl solution by cyclic voltammetry technique (Fig. 5d). For a reversible process, the Randles–Sevcik equation was reported as follow [46]:

$$I_{\text{pa}} = (2.69 \times 10^5) n^{3/2} A D_0^{1/2} v^{1/2} C_0 \quad (2)$$

Where I_{pa} , n , A , D_0 , v , and C_0 are the anodic peak current, the number of electron transfer ($n = 1$), the surface area of the electrode (cm^2), the diffusion coefficient ($7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), the scan rate and the concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ($10 \times 10^{-6} \text{ mol cm}^{-3}$). Therefore, from the slope of I_{pa} versus square root of scan rate ($v^{1/2}$), the active surface area of the EpoAP–MWCNTs/GE was found to be 0.53 cm^2 , indicating that the surface area of the modified electrode was increased by deposition of EpoAP-MWCNTs nanocomposite.

Calculation of Surface Coverage on the Surface of the Modified Electrode

The surface coverage of the GE was determined in 0.5 M HClO_4 at a scan rate of 0.1 V s^{-1} by CV according to the following equation [47, 48]:

$$I_p = n^2 F^2 A \Gamma v / 4RT \quad (4)$$

Considering this equation, the peak current (I_p) is related to the surface concentration of the electroactive species (Γ). Where n represents the number of electrons involved in the reaction, A is the surface area of the electrode, Γ (mol cm^{-2}) is the surface concentration, and the other symbols have their common meanings. Subsequently, from the slope of plotting I_p against the scan rate (v), the surface concentration calculated to be $3.44 \times 10^{-6} \text{ mol cm}^{-2}$.

Electrochemical Determination of CLP

CV and DPV techniques were applied to determine the sensitivity of EpoAP–MWCNTs/GE in terms of concentration range and the quantification and detection limits for the CLP. Figure 6a represents the recorded CVs of BRB solution (pH 3.7) containing various concentrations of CLP over the range of 50–200 μM using EpoAP–MWCNTs/GE under the optimized condition. The response of the nanosensor was increased linearly with increasing CLP concentration at the range of 50–200 μM (Fig. 6a, inset). The regression equation was derived as; $I_{\text{pa}} (\text{mA cm}^{-2}) = 0.1299[\text{CLP}] \mu\text{M} + 16.087$, $R^2 = 0.9991$. LOQ and LOD values were obtained using the equations as follows:

$$\text{LOD} = 3.3 \sigma / S, \quad (5)$$

$$\text{LOQ} = 10 \sigma / S. \quad (6)$$

Where σ is the standard deviation of the y-intercept and S is slope of the calibration curve. Consequently, using CV technique, the LOD and LOQ were determined to be 20.1 and 6.6 μM , respectively. DPV tech-

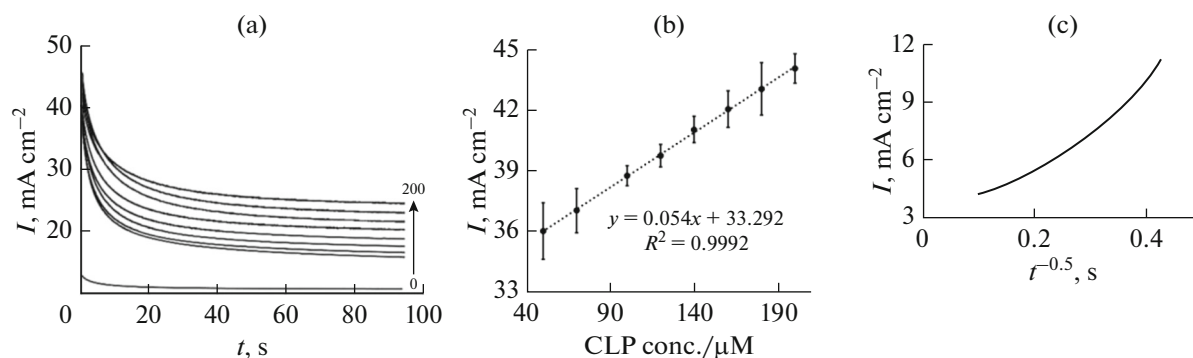


Fig. 7. (a) Chronoamperograms of different concentrations of CLP: 0, 50, 70, 100, 120, 140, 160, 180, and 200 μM on the surface of EpoAP–MWCNTs/GE in BRB. The potential step was 0.9 V. (b) DPV responses as a function of concentrations of CLP from 0 to 200 μM ($n = 3$), and (c) the plot of transient current versus $t^{-0.5}$.

nique was also utilized to achieve a lower linear range of concentrations using EpoAP–MWCNTs/GE. Figure 6b displays the recorded DPVs of BRB solution (pH 3.7) CLP at various concentration. The corresponding calibration curve (Fig. 6b, inset) shows a linear concentration range of 2.0–75.0 μM . The regression equation was I_{pa} (mA cm^{-2}) = 0.0701[CLP] μM + 0.0678 and correlation coefficient $R^2 = 0.9978$ in this range. According above equations, the LOD and LOQ were calculated to be 0.5 and 2.0 μM , respectively.

The results obtained from these techniques were summarized in the Table 1.

Chronoamperometric Studies

Chronoamperometry was employed for the investigation of the electrode process as well. Figure 7a shows the current-time profiles obtained by setting the EpoAP–MWCNTs/GE at 0.9 V for the various concentrations of CLP in the range of 80–200 μM in BRB solution (pH 3.7). The useful net current signals were linearly dependent on the concentration of CLP which is recorded at 90 seconds (Fig. 7b). Herein, a diffusion-controlled mechanism is dominant in electro-oxidation process, which obtained in the previous section by CV technique. Diffusion coefficient of the CLP using the slopes of these lines can be obtained according to the Cottrell equation [47, 49]:

$$I_p = nFAD^{1/2}C^* \pi^{-1/2} t^{-1/2}. \quad (7)$$

Where D , C^* , n , F , and A are the diffusion coefficient, the bulk concentration, the number of electrons, Faraday's constant and electrode area, respectively. The plot of net currents against the minus square roots is linear and according to the Cottrell equation (Fig. 7c). From the slope, the diffusion coefficient of CLP was calculated to be $2.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

Analytical Application of EpoAP–MWCNTs/GE

To assess the applicability of the proposed method, the EpoAP–MWCNTs/GE and a reliable HPLC method [50] were used to determine the content of CLP in “Clopidogrel” tablets as a real pharmaceutical sample. The developed nanosensor has been assessed for practical applicability. To this purpose, pharmaceutical samples of CLP were evaluated by using EpoAP–MWCNTs/GE and compared with HPLC method. The result yielded 97.49 and 100.5% of label claim using the proposed nanosensor and HPLC, respectively ($n = 3$ for each method). In the fabricated nanosensor, the recovery was calculated by comparing the CLP response in the tablet solution and that of in standard solution with CLP direct addition. The recoveries were 95.4–98.2% ($n = 3$), with a RSD lower than 3%. The findings indicated that the developed nanosensor is well suitable for the micro-molar detection of CLP in the presence of excipients in tablets which may have potential application in bio-clinical. Finally, the performance of the prepared device for

Table 1. Regression data of linear range for quantitative determination of CLP by two different techniques (CV and DPV)

Parameter	CLP analysis	
	CV	DPV
Technique	CV	DPV
Linearity range, μM	50.0–200.0	2.0–75.0
Correlation coefficient	0.9991	0.9978
Intercept	16.087	0.0678
Slope	0.1299	0.0701
LOD, μM	6.6	0.5
LOQ, μM	20.1	2.0

Table 2. Comparison conducted nanosensor with other reports for CLP quantification

Analyte	Analytical techniques	LOD	LOQ	Linear range	Ref.
CLP	SWV ^a	0.6 μM	Not reported	0.6–60.0 μM	12
CLP	SWV	117.5 $\mu\text{g cm}^{-3}$	391.6 $\mu\text{g cm}^{-3}$	317.8–935.1 $\mu\text{g cm}^{-3}$	51
CLP	DPV	1.9×10^{-5} M	6.3×10^{-5} M	0.08–1.0 mM	52
CLP	DPV	7.5×10^{-8} M	2.5×10^{-7} M	2.0×10^{-6} – 4.0×10^{-5} M	53
CLP	DPV	Not reported	2.5×10^{-6} M	3×10^{-6} – 1×10^{-3} M	11
CLP	CV	6.6 μM	20.1 μM	50.0–200.0 μM	This study
CLP	DPV	0.5 μM	2.0 μM	2.0–75.0 μM	This study

^a Square Wave Voltammetry.

CLP determination is compared with results reported by other methods [11, 12, 51–53] in the Table 2.

CONCLUSIONS

In this paper, we designed a nano-device utilizing EpoAP–MWCNTs nanocomposite for sensitive detection of CLP by electrochemical techniques. The results showed that combination of EpoAP and MWCNTs in the fabricated electrode can lead to extraordinarily sensitivity for determination of CLP. This behavior is most probably due to three dimensional structure of the nanocomposite, which cause excellent electrochemical performance and higher electroactive surface area for sensing purpose. Application of the fabricated sensor for the determination of CLP in pharmaceuticals exhibited satisfactory results, even in the presence of excipients. In the Table 2, the obtained results were compared with the other studies according to linearity range, LOD and LOQ values. The proposed sensor displayed LOD value of 0.5 μM which is comparable or lower than other published methods. In additionally, EpoAP–MWCNTs/GE exhibited a fast response, high sensitivity, good precision and accuracy. Considering all these advantages, the present nanosensor can also be utilized for sensitive quantification of other molecules of biomedical interest.

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CONFLICT OF INTERESTS

Authors announce that there is no conflict of interest.

SUPPLEMENTARY INFORMATION

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