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Fabrication of an Electrochemical Sensor for Determination of Epinephrine Using a Glassy Carbon Electrode Modified with Catechol

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The electrochemical behavior of epinephrine (EP) at the catechol-modified glassy carbon electrode (catechol/GCE) in phosphate buffer solution (PBS, pH = 7.0) was examined using cyclic voltammetry (CV), chronoamperometry (CA) and differential pulse voltammetry (DPV). The results of electrochemical studies confirmed the ability of catechol to accelerate the electron transfer process and reduce overvoltage for the oxidation of EP. DPV studies showed two dynamic ranges, of which in the low concentration range of EP, the detection limit was reported to be 1.6 µM. Moreover, the reproducibility, repeatability, and selectivity of the designed sensor were investigated using the DPV method. The selectivity of this sensor was studied in the presence of interfering substances such as glucose, fructose, ascorbic acid, sodium chloride, potassium chloride, norepinephrine, and dopamine. Catechol/GCE was used for quantitative measurements of EP in human blood serum samples.

Keywords: Electrochemical sensor, Glassy carbon electrode, Catechol, Epinephrine

INTRODUCTION

Epinephrine (EP), also known as adrenaline, is an important neurotransmitter in the mammalian central nervous system. The electrochemical characteristics of this substance have been widely studied due to the importance of this substance and its derivatives in redox processes in biological media [1]. Many biological phenomena are related to the concentration of EP in blood. Also, it serves as a chemical mediator for conveying the nerve pulse to efferent organs. Medically, EP has been used as a common emergency medicine [2].

There are numerous analysis methods for EP determination in different samples such as high-performance liquid chromatography [3,4], capillary electrophoresis [5], fluorimetry [6], electrochemiluminescence [7], and spectrophotometry [8]. Also, due to the inherent electroactive property of EP, this substance can be determined by electrochemical methods [9-13]. Electrochemical methods with better selectivity, more convenience, less cost, and meeting the demand of practical application have widely attracted the attention of scientists [14-16]. However, there are two challenges in the electrochemical determination of EP. One is its low concentration level, while another challenge is the interference arising from other electroactive compounds such as norepinephrine, dopamine, ascorbic acid, and uric acid. Modified electrodes are used to resolve these problems. The targeted improvements of this modification include increasing the sensitivity and minimizing the interferences for the determination of EP [17].

Catechol is a toxic organic compound with the molecular formula $C_6H_6O_2$. It can be produced by the reaction of salicylaldehyde with base and hydrogen peroxide [18]. Also, it is produced industrially by the hydroxylation of phenol

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using hydrogen peroxide [19]. Catechol and its derivatives including caffeic acid, chlorogenic acid, catechin hydrate, hematoxylin, sophorin, coumestan, and protocatechuic aldehyde have been used as mediators of electron transfer in electrochemical processes. The characteristics of these compounds include high electron transfer, high efficiency, low cost, and excellent redox reversibility [20-24]. These compounds were stabilized on the electrode surface by methods such as adsorption [25], a sol-gel technique [26], mixing with carbon paste [11], and electropolymerization [27].

In the current study, a simple one-step method was used to modify the glassy carbon electrode with catechol. The modified electrode was used for the electrocatalytic oxidation of EP in a phosphate buffer solution (PBS, pH = 7.0). Electrocatalytic activities, electroanalytical applications, selectivity, repeatability, and reproducibility of the modified electrode in the presence of EP were evaluated using various electrochemical techniques.

EXPERIMENTAL

Apparatus and Chemicals

Electrochemical measurements were carried out at standard laboratory temperature (25 ± 1 °C) using an Autolab potentiostat/galvanostat (PGSTAT-302N, Eco Chemie, The Netherlands) connected to a computer with NOVA software version 2.1. A conventional three-electrode system was used consisting of a GCE (A = 0.0314 cm^2, Azar Electrode, Urmia, Iran) as the working electrode, saturated calomel electrode (SCE) as the reference electrode, and platinum wire as the counter electrode. All the potentials were quoted *vs*. SCE. All pH values were adjusted by a pH electrode connected to a Metrohm 691 pH meter. A Sartorius laboratory balance (Goettingen, Germany) with a readability of 0.1 mg was used to weigh chemicals. A Millipore Direct-Q®3-R water purification system was used to prepare distilled water.

Epinephrine, catechol, sodium hydroxide, phosphoric acid (85%), sulfuric acid (98%), glucose, fructose, ascorbic acid, norepinephrine, dopamine, alumina powder, potassium chloride, and sodium chloride were purchased from Merck (Darmstadt, Germany) with analytical grade. These chemicals were used without further purification. All solutions were freshly prepared with double distilled water. A 0.1 M PBS (pH = 7.0) was used as the supporting electrolyte.

Preparation of the Electrode

To prepare the modified glassy carbon electrode with catechol, first, the electrode was polished several times with alumina powder on the polishing cloth. Then, it was rinsed with distilled water to remove the contamination from the surface. The electrode was immersed in 1.0 M sulfuric acid solution for 2 min to remove the adsorbed particles on the surface. Having rinsed the electrode with distilled water, it was immersed in 10.0 mM (optimal concentration) catechol solution for 5 minutes to modify the surface. The modified electrodes were immediately placed in the electrochemical cell for electrochemical measurements.

RESULTS AND DISCUSSION

Electrochemical Properties of Catechol/GCE

To investigate the redox behavior of catechol, cyclic voltammetry (CV) studies were conducted in 0.1 M PBS (pH = 7.0). The anodic and cathodic peaks in Fig. 1, are attributed to the oxidation of catechol to 1,2-benzoquinone and its reduction to catechol, respectively (Scheme 1) [28]. Experimental results demonstrated reproducible and well-defined anodic and cathodic peaks with E_{pa} and E_{pc} of 100 and 44 mV *vs*. SCE, respectively. The peak-to-peak separation value (ΔE_p) was found to be 56 mV. These observations showed a quasi-reversible system.

The effect of the potential scan rate (ν) on the electrochemical behavior of catechol/GCE was investigated using the CV method. In Fig. 1A, the linear plots of the variation of anodic and cathodic peak currents vs. the scan rate in the range of 50-900 mV s^{-1} show a diffusion-less electrochemical system and a surface-controlled redox process [29]. Using the slope of the anodic part of this plot (I_{pa} *vs*. ν) and the Sharp Eq. (1), the surface coverage (Γ) of the modified electrode was calculated to be 5.08 × 10^{-10} mol cm^{-2}, where in this equation, n is the total number of electrons involved in the reaction, A denotes the electrode area (cm^2) and other symbols have their usual significance [29].

$$I_p = n^2 F^2 A\Gamma\nu/4RT \quad (1)$$

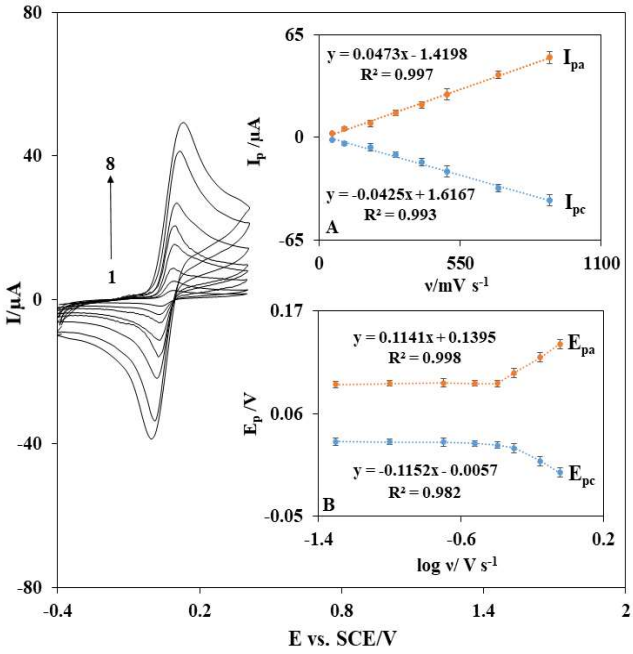
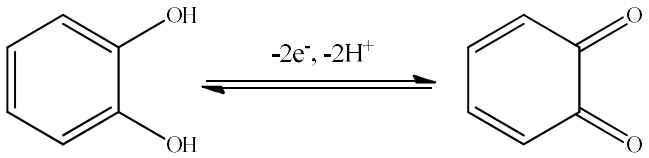


Fig. 1. Cyclic voltammograms of catechol/GCE in 0.1 M PBS (pH = 7.0) for different scan rates. Cycles 1-8 correspond to 50, 100, 200, 300, 400, 500, 700, and 900 mV s^{-1}, respectively. Insets: Variations of (A) I_p *vs.* Scan rate; (B) E_p vs. the logarithm of the scan rate.



Catechol 1,2-Benzoquinone

Scheme 1. Electrochemical conversion of catechol to 1,2-benzoquinone

At scan rates above 500 mV s^{-1} in Fig. 1B, anodic and cathodic peak potential values are proportional to the logarithm of the scan rates, indicating charge-transfer kinetic limitations. The apparent charge transfer rate constant (k_s) and transfer coefficient (α) of a surface-controlled redox couple are calculated by the Laviron procedure [30]. The slopes of the linear segments in Fig. 1B were used to calculate the anodic (α_a) and cathodic (α_c) transfer coefficients. Equations ((2) and (3)) were used to calculate the anodic and cathodic transfer coefficients, respectively.

The evaluated values for α_a and α_c were calculated as 0.49 and 0.51, respectively. The apparent charge transfer rate constant between the electrode and catechol was calculated using the Laviron Eq. (4). The evaluated value of k_s was found to be 9.39 s^{-1}.

$$Slope_a = 2.303RT/(1 - \alpha)n_\alpha F \quad (2)$$

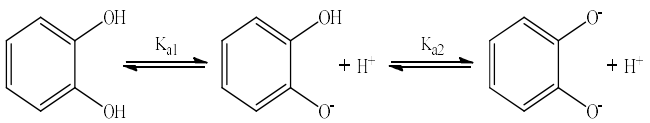
$$Slope_c = -2.303RT/\alpha n_\alpha F \quad (3)$$

$$\log k_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log\alpha - \log(RT/nF\nu) - \alpha (1 - \alpha) \\ n_\alpha F\Delta E_p/2.303\ RT \quad (4)$$

Catechol contains hydroxyl groups, so its electrochemical behavior depends on the pH (Scheme 2) [31]. The electrochemical behavior of catechol/GCE was studied using the CV method at different pH values (Fig. 2). In this figure, the anodic and cathodic peak potentials of catechol shifted toward more negative values by increasing the pH, confirming deprotonation processes in catechol oxidation, and facilitating these processes at higher pH values. Insets of Fig. 2 demonstrate diagrams constructed by plotting the anodic, cathodic, and half-wave potential values as the function of pH. The half-wave potential values were calculated as the average potentials of the anodic and cathodic peaks in the cyclic voltammograms. It can be clearly seen that the three plots are straight lines with slopes close to 54.0 mV/pH. Therefore, the system obeys the Nernst equation for a two-proton-coupled electron transfer (PCET) [32].

Electrocatalytic Oxidation of Epinephrine at a Catechol/GCE

Electrocatalytic oxidation of EP at a catechol/GCE was investigated using the CV method in 0.1 M PBS (pH = 7.0)



Scheme 2. A proposed mechanism for the pH-dependent oxidation of catechol

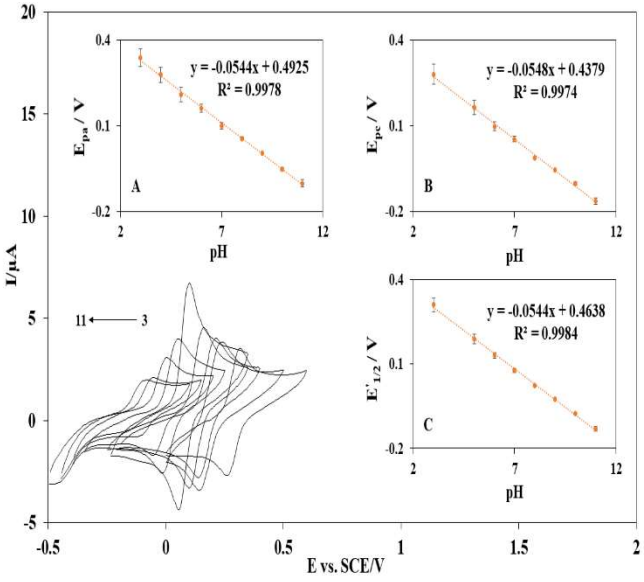


Fig. 2. Cyclic voltammograms of catechol/GCE in 0.1 M PBS with different pH values (3-11), at a scan rate of 100 mV s^{-1}. Insets: Variations of (A) E_{pa} (B) E_{pc} and (C) $E'_{1/2}$ *vs*. pH.

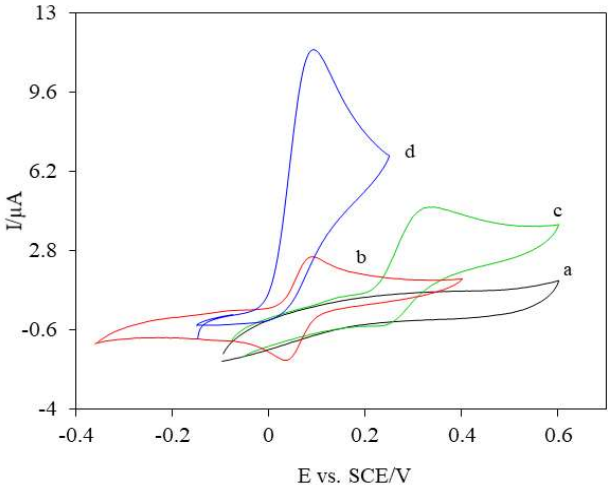
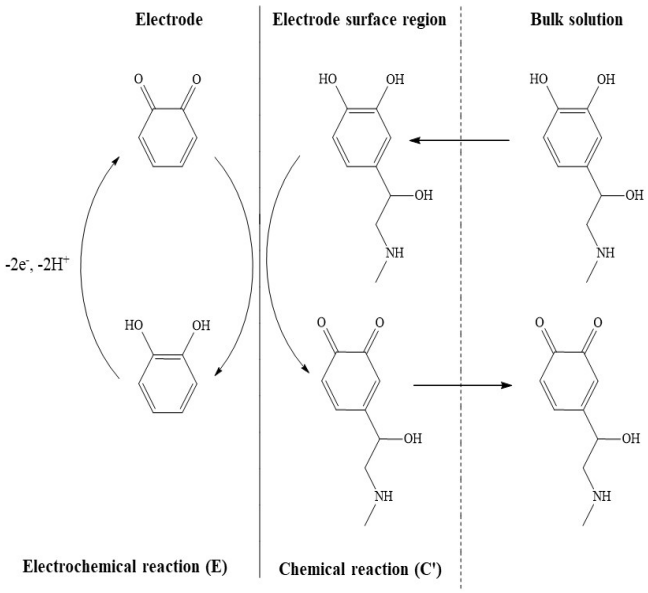


Fig. 3. Cyclic voltammograms of (a) bare GCE in 0.1 M PBS (pH = 7.0); (b) catechol/GCE in 0.1 M PBS (pH = 7.0); (c) as (a) + 1.0 mM epinephrine; (d) as (b) + 1.0 mM epinephrine, at a scan rate of 25 mV s^{-1}.

containing 1.0 mM EP (Fig. 3 curve d). The anodic peak potential for the oxidation of EP at the bare GCE (Fig. 3 curve c) and the corresponding potential at the modified electrode (Fig. 3 curve d) is ~320 mV and ~100 mV, respectively. Also, the corresponding oxidation currents are 4.68 and 11.12 μA, respectively. Therefore, owing to its inherent electrocatalytic ability, the presence of catechol accelerated the electron transfer process, reduced the overvoltage for EP oxidation, and increased the sensitivity of the designed sensor. A comparison of these two curves shows that the peak potential of EP oxidation at the modified electrode shifted negatively by ~ 220 mV compared to the bare electrode. Furthermore, a comparison of cyclic voltammograms for catechol/GCE in the presence (Fig. 3 curve d) and absence (Fig. 3 curve b) of 1.0 mM EP shows that the addition of EP to the supporting electrolyte solution enhanced the anodic peak current of stabilized catechol at the electrode surface, while the cathodic peak disappeared. According to these findings, an electrocatalytic behavior has been observed for EP oxidation at the catechol/GCE *via* an EC' catalytic mechanism (Scheme 3). According to this scheme, EP is oxidized by the oxidized form of catechol (1,2-benzoquinone) *via* a catalytic chemical reaction (C'). The oxidized form of catechol was produced *via* an electrochemical reaction (E). Therefore, catechol and EP have been oxidized at a potential of 100 mV.



Scheme 3. Electrocatalytic reaction mechanism for epinephrine oxidation at the surface of catechol/GCE

The effect of scan rate on the electrocatalytic oxidation of EP at the catechol/GCE was investigated using the CV method in 0.1 M PBS (pH = 7.0) containing 1.0 mM EP (Fig. 4). This figure illustrates that the oxidation peak potential of EP shifted toward more positive values by increasing the scan rate, indicating the kinetic limitations for the electro-oxidation reaction. Also, a linear plot within the range 5-30 mV s^{-1} for the peak height (I_{Pa}) *vs*. the square root of scan rate ($\nu^{1/2}$) shows that at sufficient over-potential, this process has been diffusion-controlled rather than surface-controlled (Fig. 4A). The approximate total number of electrons in the overall oxidation of EP (n) was calculated using the slope of Fig. 4A and the following equation for diffusion-controlled irreversible electrochemical reactions, in which the first electron transfer is rate-determining. In this equation, D = 3.61 × 10^{-5} cm^2 s^{-1} is the diffusion coefficient of EP (obtained using chronoamperometry) and C^* is the bulk concentration of EP (1.0 mM). The resulting value for n was found to be 2 (n = 1.73).

$$I_p = 3.01 \times 10^5 \, n \, [(1 - \alpha) \, n_\alpha]^{1/2} \, A \, C^* \, D^{1/2} \, \nu^{1/2} \qquad (5)$$

In Fig. 4B, a plot of the scan rate-normalized current ($I_P/\nu^{1/2}$) vs. scan rate confirms an EC' catalytic mechanism. Figure 4C shows the Tafel plot (E *vs*. logI) for the rising part of the voltammetric wave at a scan rate of 10 mV s^{-1}. This part of the voltammogram was affected by electron transfer kinetics between EP and catechol. Using the slope of this plot and Eq. (2), the oxidation transfer coefficient of EP at a catechol/GCE was calculated to be 0.36.

Chronoamperometric Measurements

Chronoamperometric measurements were used to study the electrochemical behavior of EP at the catechol/GCE. These measurements were carried out by applying a potential step of 140 mV at the working electrode for various concentrations of EP (Fig. 5). For a substance with a diffusion coefficient of D (*e.g.* EP in this case), the electrochemical current under mass-transfer-limited conditions can be evaluated from the Cottrell Eq. (6). Under these conditions, the plot of I *vs*. $t^{-1/2}$ is linear (Fig. 5A) [32]. Figure 5B displays a plot of the slopes of the resulting straight lines *vs*. EP concentration. The value of D can be calculated by the

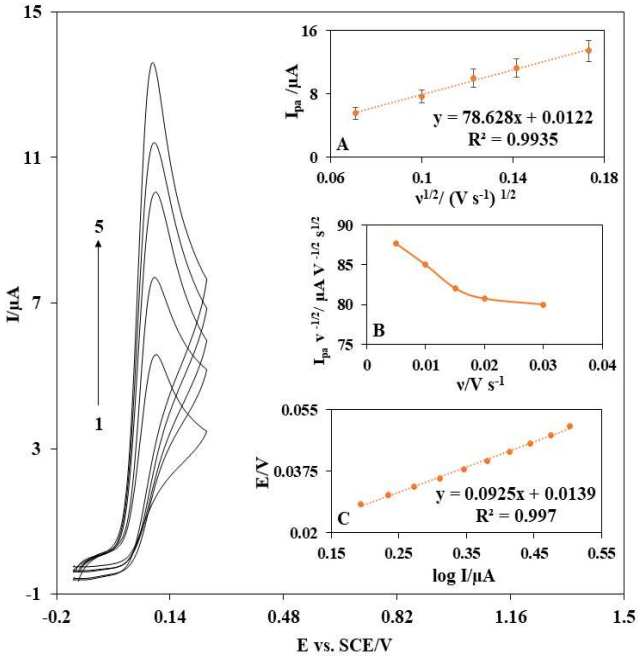


Fig. 4. Cyclic voltammograms of catechol/GCE in 0.1 M PBS (pH = 7.0) containing 1.0 mM epinephrine at different scan rates. Cycles 1-5 correspond to 5, 10, 15, 20, and 30 mV s^{-1}, respectively. Insets: Variations of (A) anodic peak current *vs*. $\nu^{1/2}$; (B) normalized current ($I_{Pa}/\nu^{1/2}$) *vs*. scan rate; (C) the Tafel plot for the rising part of the voltammetric wave at a scan rate of 10 mV s^{-1}.

Cottrell equation and the slope of this plot. The mean value of the D was found to be 3.61 × 10^{-5} cm^2 s^{-1}.

$$I = nFAD_o^{1/2} C_o \, t^{-1/2} \, \pi^{-1/2} \qquad (6)$$

Calibration Plot and Limit of Detection

Differential pulse voltammograms were recorded for the determination of EP (Fig. 6). The plot of peak height *vs*. EP concentration consisted of two linear segments with slopes of 0.0186 and 0.0068 µA μM^{-1} in the concentration ranges of 5.0-80.0 and 80.0-900.0 µM, respectively. The decrease in the sensitivity of the linear segment related to high concentrations of EP is probably due to kinetic limitation. The detection limit ($3S_b/m$) and sensitivity for EP were found to be 1.6 µM and 0.6 µA μM^{-1} cm^{-2}, respectively.

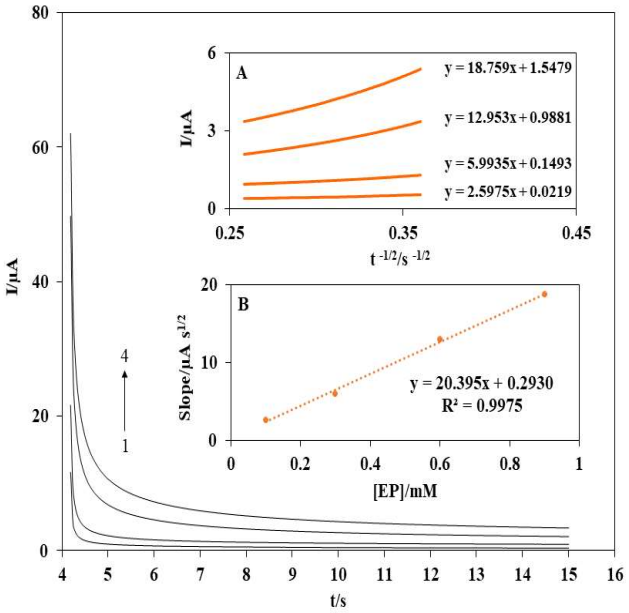


Fig. 5. Chronoamperograms of Catechol/GCE in 0.1 M PBS (pH = 7.0) containing different concentrations of epinephrine. Chronoamperograms 1-4 correspond to 0.1, 0.3, 0.6, and 0.9 mM of epinephrine. Insets: (A) Plots of I *vs*. $t^{-1/2}$ and (B) Plot of the slopes of the straight lines *vs*. the epinephrine concentrations.

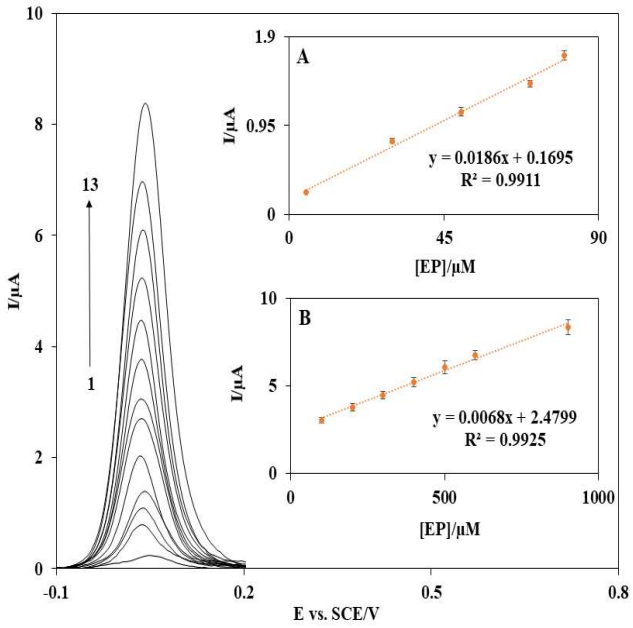


Fig. 6. DPVs of catechol/GCE in 0.1 M PBS (pH = 7.0) containing different concentrations of epinephrine. DPVs 1-13 correspond to 5.0, 30.0, 50.0, 70.0, 80.0, 90.0, 100.0, 200.0, 300.0, 400.0, 500.0, 600.0, and 900.0 µM of epinephrine. Insets: The plot of the electrocatalytic peak current *vs*. epinephrine concentration in the ranges of (A) 5.0-80.0 µM and (B) 80.0-900.0 µM.

The Repeatability and Reproducibility of Catechol/GCE

The repeatability was investigated using the DPV method in 0.1 M PBS (pH = 7.0) containing 0.1 mM EP. Having recorded the first differential pulse voltammogram, four consecutive measurements were immediately recorded for that electrode. A relative standard deviation (RSD) of 9.3% was reported for the oxidation peak current of EP.

The ability to prepare a reproducible surface for the electrode was investigated using the DPV method in 0.1 M PBS (pH = 7.0) containing 1.0 mM EP for three independent modified electrodes on the same day and under optimum conditions. A relative standard deviation (RSD) of 4.2% was found for the oxidation peak current of EP, indicating the satisfactory reproducibility of the modified electrode surface.

Interference Study

The influence of different foreign substances on the determination of 0.5 mM EP was investigated using the DPV method, under optimal conditions (Fig. 7). The tolerance limit was considered as the maximum concentration of the foreign substance, which causes an approximate relative error of ±5% in the determination. The tolerated concentrations of foreign substances were 500.0 mM for glucose and fructose; 62.5 mM for NaCl and KCl; and 50.0 mM for ascorbic acid. Equal molar ratios of norepinephrine and dopamine caused serious interference.

Determination of Epinephrine in Human Blood Serum Sample

In order to evaluate the analytical applicability of catechol/GCE for the determination of EP in biological samples, a human blood serum sample was used. Specific concentrations of EP were injected into this sample and transferred to the electrochemical cell. Then, analytical

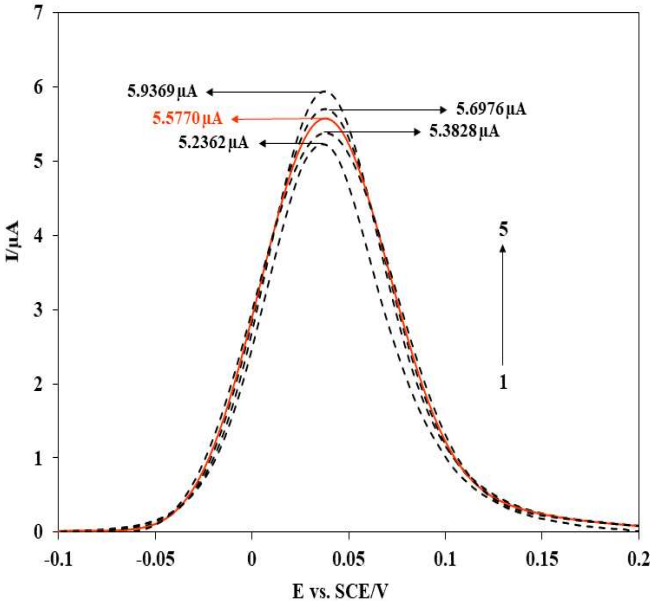


Fig. 7. DPVs of catechol/GCE for investigating the effect of different foreign substances on epinephrine determination in 0.1 M PBS (pH = 7.0) containing 0.5 mM epinephrine and (1) 50.0 mM ascorbic acid; (2) 62.5 mM NaCl and KCl; (4) 500.0 mM fructose; and (5) 500.0 mM glucose. (3) is related to the oxidation of epinephrine in the absence of foreign substances.

concentrations of EP were calculated using the calibration curve obtained from DPV measurements in the potential range of -100 to 200 mV with a scan rate of 10 mV s^{-1}. The reproducibility of this method was reported in the form of relative standard deviation (RSD). In order to reduce the error, the measurements were repeated three times. Analytical results for the determination of EP in a human blood serum sample are shown in Table 1.

CONCLUSION

In the current study, the modified glassy carbon electrode with catechol was used for EP determination. The results of CV studies revealed the ability of catechol as an electron-transfer intermediate, which accelerates the electron transfer process and reduces overvoltage for the oxidation of EP. In CV studies, the peak potential of EP oxidation at the modified electrode shifted negatively by ~220 mV compared to the bare electrode. Also, an electrocatalytic behavior was observed for EP oxidation at the catechol/GCE *via* an EC' catalytic mechanism. DPV studies reported two linear segments with slopes of 0.0186 and 0.0068 µA $µM^{-1}$ in the concentration ranges of 5.0-80.0 and 80.0-900.0 µM, respectively. The detection limit ($3S_b/m$) and sensitivity for the low concentration range of EP were found to be 1.61 µM and 0.6 µA $µM^{-1}$ cm^{-2}, respectively.

Table 1. Analytical Results for Determination of Epinephrine in Human Blood Serum Sample (n = 3)

Added (µM)	Found (µM)	Recovery (%)	RSD (%)
0.0	ND	-	-
20.0	20.7	103.5	2.3
35.0	38.9	111.1	3.7
50.0	48.6	97.2	2.9
65.0	66.6	102.4	3.1
80.0	77.5	96.9	3.5

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