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Full Paper

Voltammetric Determination of Epinephrine using a Thiourea Modified Glassy Carbon Electrode

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Abstract- In the present work, we describe the preparation of a glassy carbon electrode modified with thiourea and investigate its performance for the determination of EP in aqueous solutions. Differential pulse voltammetry (DPV) exhibits a linear dynamic range from 5.0×10^{-8} to 1.1×10^{-5} M and a detection limit of 2.3×10^{-8} M for EP. We also evaluate the analytical performance of the modified electrode for quantification of EP in real samples.

Keywords- Epinephrine, Glassy Carbon Electrode, Modified Electrode

1. INTRODUCTION

Catecholamine is a class of important compounds for message transfer in the mammalian central nervous system. It is released by the adrenal medulla in situations of psychological stress or low blood sugar level [1,2]. Catecholamines are also used as drugs to treat bronchial asthma, hypertension, myocardial infarction and cardiac surgery [3]. Epinephrine (EP), also known as adrenaline, is one of the important catecholamine, plays a central role during physical or mental stress and also stimulates a series of actions of the sympathetic nervous

system (SNS) known as the "flight or fight response" [4]. It prepares the body for action in perceived emergency situations, boosting the supply of oxygen and energy,giving glucose to the brain and muscles [5]. It elevates the blood sugar level by increasing catalysis of glycogen to glucose in the liver, and at the same time begins the breakdown of lipids in fat cells [5]. These important actions of EP also make it a potent doping agent and hence, it is also banned in competitive games by World Anti Doping Agency [6,7]. Clinically, EP has been utilized as a common emergency healthcare medicine such as a drug to treat cardiac arrest, dysrhythmias and as a bronchodilator for asthma [8]. It is also used to treat anaphylaxis and sepsis because of its suppressive effect on the immune system [9]. Studies show that changes of EP concentration in nervous tissues and body fluids are diagnostic symptoms of several diseases [10]. The amount of EP present in blood, plasma or serum is considered as a diagnostic aid to monitor therapeutic administration or to identify the causative agent in potential poisoning victims [11]. The quantitative determination of EP concentration is also quite helpful for developing nerve physiology, clinical diagnosis of some diseases and controlling medicine in pharmacological research [12]. Therefore, it is important to examine its electrochemical behavior and to develop a quantitative method for studying its concentration in body fluids.

Numerous electrochemical methods have been developed to determine EP due to its electro active nature [13–16]. Most of these reported methods have two major problems in EP determination that reduce accuracy and sensitivity of the method. The first is that in natural environment EP often exists together with high concentration of electro active biomolecules like uric acid, dopamine, nor EP, and ascorbic acid that interfere with each other. The second problem of the reported methods is that the product of EP oxidation (epinephrine chrome) can easily transform into polymers, which block its further oxidation on the electrode surface. Hence, despite of considerable investigation, the preparation of a sensitive sensor with satisfactory selectivity and low detection limit with high sensitivity is still of great interest [17-20].

Electrochemical sensors and biosensors for pharmaceutical, food, agricultural and environmental analysis have been growing rapidly due to electrochemical behavior of drugs and biomolecules and partly due to advances in electrochemical measuring systems.

The merger between fast, sensitive, selective, accurate, miniaturizable and low-cost electrochemistry-based sensing and fields like proteomics, biochemistry, molecular biology, nanotechnology and pharmaceutical analysis leads to the evolution of electrochemical sensors [21-46].

In the present work, we describe the preparation of a thiourea-modified glassy carbon electrode (TUMGCE) and investigate its performance for the electrocatalytic determination of EP in aqueous solutions.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

Voltametric experiments were performed using Metohm VA computrace Model 757. The measurements were recorded using VA computrace version 1.2 (Metohm) running under Windows 98. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the (TUMGCE) were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements. All solutions were freshly prepared with double distilled water. EP, thiourea and all other reagents were of analytical grade from Merck. The buffer solutions were prepared from orthophosphoric acid and its salts.

The preparation of thiourea-modified GCE (TUMGCE) was performed by mechanically polishing a glassy carbon electrode with 0.05 μm Al_2O_3 in water slurry and electrochemically activating it in a 0.1 M sodium bicarbonate solution by continuous potential cycling from −1.1 to 1.6 V at a sweep rate of 100 mVs^{-1} and finally it was placed in a 1.0 mM solution of TU (pH 6.0). It was modified by 5 cycles of potential scan rate between -1.5 V and 2.4 V at 100 mVs^{-1} in N_2 atmosphere.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of EP at the surface of TUMGCE

The electrochemical behavior of EP is dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electro catalytic oxidation of EP. Thus the electrochemical behavior of EP was studied in 0.1 M phosphate buffer solutions in different pH values (2.0<pH<9.0) at the surface of TUMGCE by cyclic voltammetry. It was found that the electrocatalytic oxidation of EP at the surface of TUMGCE was more favored at pH 6.0. This appears as a gradual growth in the anodic peak current of EP in the cyclic voltammograms of TUMGCE (Fig. 1). Thus, the pH 6.0 was chosen as the optimum pH for electro catalysis of EP oxidation at the surface of TUMGCE.

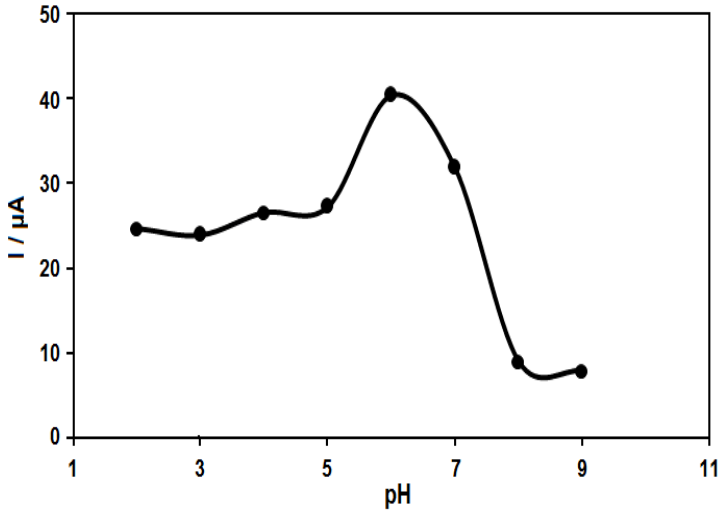

Fig. 1. Plot of peak currents *vs.* pH

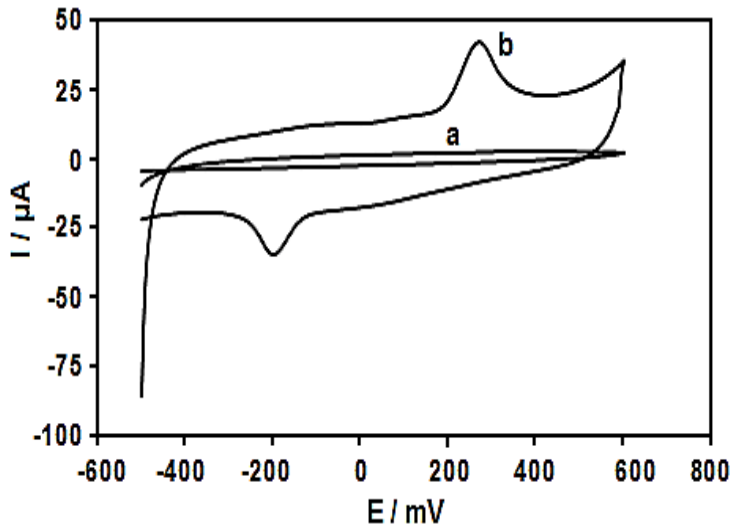

Fig. 2. CVs of (a) unmodified GCE in 0.1 M PBS containing 10.0 µM EP (pH 6.0) and (b) as (a) at the surface of TUMGCE. In all cases the scan rate was 100 mV s^{-1}

3.2. Electrocatalytic determination of EP

Fig. 2. depicts the CV responses for the electrochemical oxidation of 10.0 µmol/L EP at unmodified GCE (curve a), and TUMGCE (curve b). The bare GCE is not reactive under these conditions while the TUMGCE shows an anodic peak at 0.25 V and a cathodic peak at -0.2 V. The results indicating that the modification of bare GCE with TU significantly improved the performance of the electrode toward EP oxidation.

The effect of scan rate on the electrocatalytic oxidation of EP at the TUMGCE was investigated by CV (Fig. 3). Results showed that a plot of peak height (I_p) *vs.* the square root

of scan rate ($\nu^{1/2}$) was found to be linear, suggesting that, at sufficient overpotential, the process is controlled by diffusion (Fig. 4) [47].

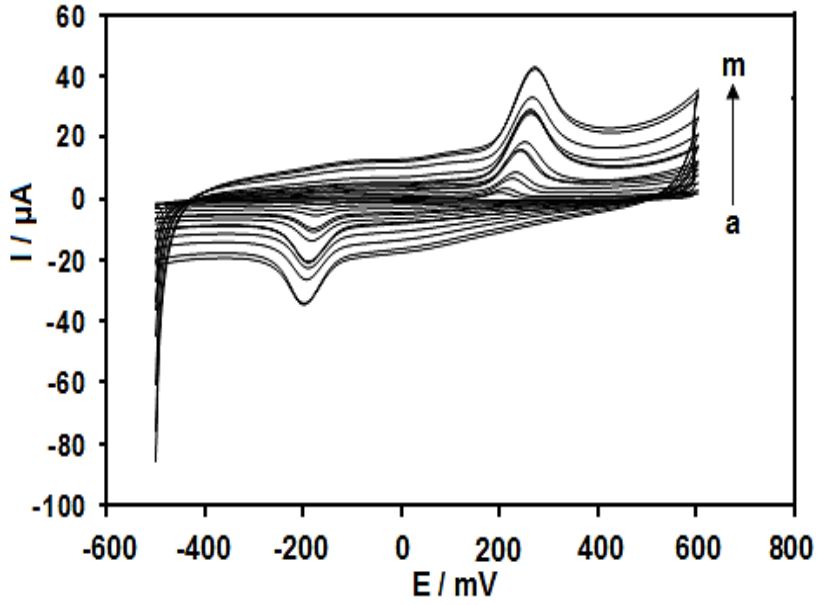

Fig. 3. CVs of TUMGCE in 0.1 M PBS (pH 6.0) containing 10.0 µM EP at various scan rates; a to m correspond to 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120 and 140 mV s^{-1}, respectively

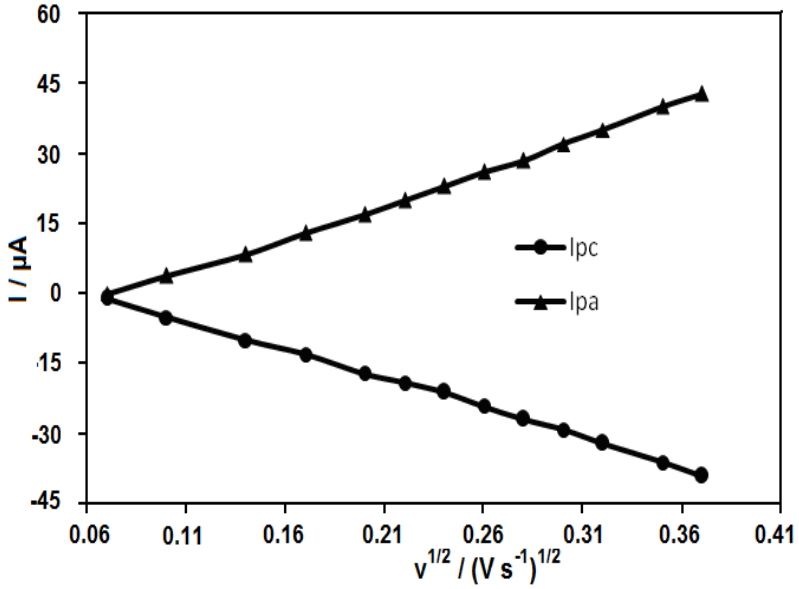

Fig. 4. Variation of anodic and cathodic peak currents *vs*. square root of scan rate

3.3. Chronoamperometric measurements

Chronoamperometry, as well as other electrochemical methods was employed for investigation of electrochemical process at the chemically modified electrodes. Fig. 5A shows the current–time curve of TUMGCE obtained by setting the working electrode potential at 0.4 V for the various concentration of EP in buffered aqueous solutions (pH 6.0). For an electroactive material (EP in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [47]. Experimental plots of I *vs.* $t^{-1/2}$ were employed, with the best fits for different concentrations of EP (Fig. 5B). The slopes of the resulting straight lines were then plotted *vs.* EP concentration (Fig. 5C). From the resulting slope and Cottrell equation the mean value of the D was found to be 3.42×10^{-5} cm^2/s.

Chronoamperometry can also be employed to evaluate the catalytic rate constant, k, for the reaction between EP and the TUMGCE according to the method of Galus [48]:

$$I_C / I_L = \gamma^{1/2}[\pi^{1/2} \text{ erf } (\gamma^{1/2}) + \exp(-\gamma) / \gamma^{1/2}] \quad (1)$$

Where I_C is the catalytic current of EP at the TUMGCE, I_L is the limited current in the absence of E_P and $\gamma=kC_bt$ is the argument of the error function (C_b is the bulk concentration of EP). In cases where γ exceeds the value of 2, the error function is almost equal to 1 and therefore, the above equation can be reduced to:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_bt)^{1/2} \quad (2)$$

Where t is the time elapsed. The above equation can be used to calculate the rate constant, k, of the catalytic process from the slope of I_C/I_L *vs.* $t^{1/2}$ at a given EP concentration. From the values of the slopes, the average value of k was found to be $5.6\times10\ 4\ M^{-1}\ s^{-1}$.

3.4. Calibration plot and limit of detection

Differential pulse voltammetry (DPV) method was used to determine the concentration of EP. The plot of peak current *vs.* EP concentration was linear in the concentration range of 0.05 to 11.5 μM. The data analysis presents the value of lower limit detection of EP to be 23.0 nM. These values are comparable with the values obtained by other research groups (Table 1).

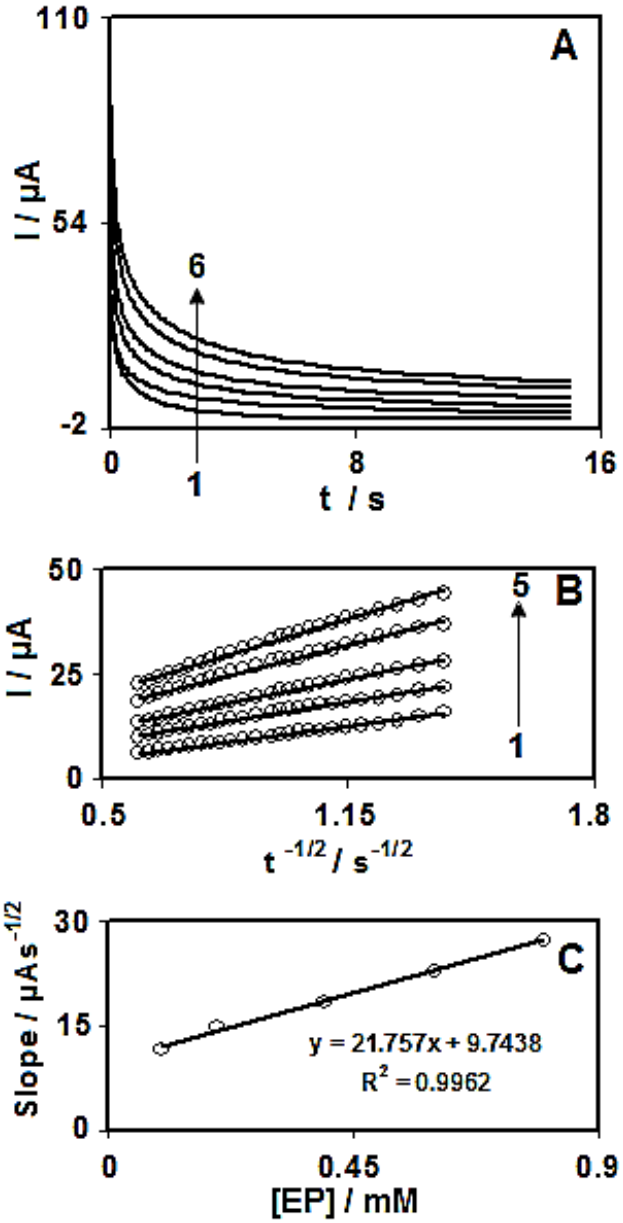

Fig. 5. (A) Chronoamperograms obtained at TUMGCE in 0.1 M phosphate buffer solution (pH 6.0) for different concentration of EP. The numbers 1–6 correspond to 0.0, 0.2, 0.3, 0.45, 0.6 and 0.8 mM of EP, (B) Plots of I *vs.* $t^{-1/2}$ obtained from chronoamperograms 2–6. (C) Plot of the slope of the straight lines against EP concentration

Table 1. The application of TUMGCE for determination of EP in EP ampoule (n=5)

Electrode	Modifier	Dynamic range(M)	Limit of detection (M)	Ref.
Carbon Paste	2,2´-[1,2-ethanediylbis(nitriloethylidyne)]-bis-hydroquinone	7.0×10^{-7} – 1.2×10^{-3}	2.16×10^{-7}	15
Carbon Paste	Ionic liquid	3.0×10^{-7} -4.5×10^{-4}	9.0×10^{-8}	17
Carbon Paste	Molybdenum(VI) complex	9.0×10^{-8} -7.5×10^{-4}	4.9×10^{-8}	23
Glassy carbon electrode	TU	5.0×10^{-8}– 1.15×10^{-5}	2.3×10^{-8}	This work

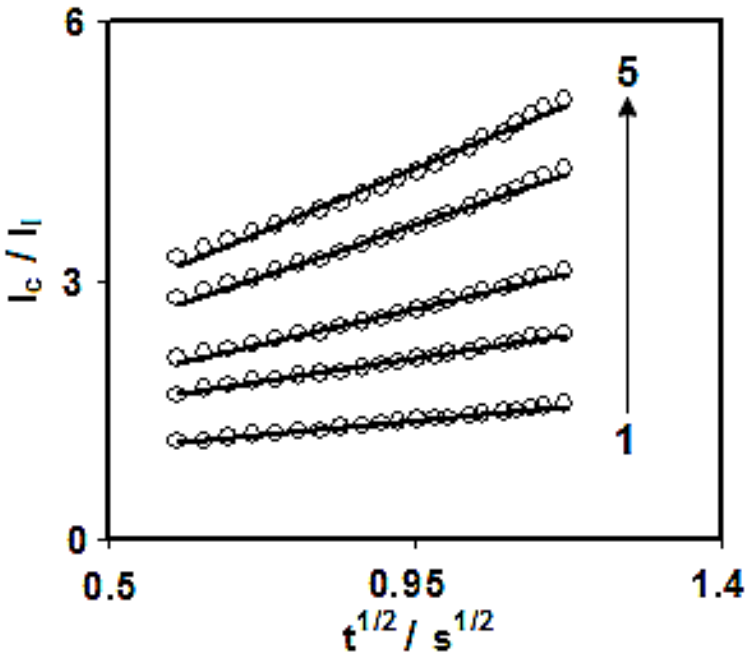

Fig. 6. Dependence of I_{cat}/I_l on $t^{1/2}$ derived from the data of chronoamperograms 1-6 in Fig. 5

3.5. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of EP in EP ampoule. The results are given in Table 1. Satisfactory recovery of the experimental results was found for EP. The average amount of EP in the ampoule was found to be 0.99 mg, a value in well agreement with the value on the ampoule label (1.0 mg).

3.6. Interference study

The influence of various foreign species on the determination of 1.0×10^{-5} M EP was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5% relative error in the determination. The tolerated concentration of foreign substances was 5.0×10^{-5} M for glucose, uric acid, ascorbic acid, citric acid, dopamine, $Na^{+,}$ K^{+}, Cu^{2+}, Mg^{2+}, NO_3^{1-} and SO_4^{2-}.

Table 2. The application of TUMGCE for determination of EP in EP ampoule (n=5)

Original content (μM)	Added (μM)	Found (μM)	Recovery (%)
0.50	0	0.49±0.01	98.0
0.50	0.20	0.71±0.02	101.4
0.50	0.30	0.81+0.02	101.2
0.50	0.50	1.00±0.01	100.0
0.50	0.60	1.08±0.01	98.2
0.50	0.70	1.17±0.02	97.5

4. CONCLUSIONS

In this paper, we have constructed a novel modified glassy carbon electrode for the detection of EP. The results of this study indicated that the electrode exhibited linear response over a wide concentration range (0.05 to 11.5 μM with a detection limit of 23.0 nM). Also, the constructed electrode was used for determination of EP in EP ampoule.

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