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Nanomolar and Selective Determination of Epinephrine in the Presence of Norepinephrine Using Carbon Paste Electrode Modified with Carbon Nanotubes and Novel 2-(4-Oxo-3-phenyl-3,4-dihydro-quinazolinyl)-*N'*-phenyl-hydrazinecarbothioamide

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A novel modified carbon nanotube paste electrode of 2-(4-oxo-3-phenyl-3,4-dihydro-quinazolinyl)-*N'*-phenyl-hydrazinecarbothioamide (2PHC) was fabricated, and the electrooxidation of epinephrine (EP), norepinephrine (NE), and their mixture has been studied using electrochemical methods. The modified electrode displayed strong catalytic function for the oxidation of EP and NE and resolved the overlap voltammetric response of EP and NE into two well-defined voltammetric peaks of about 240 mV with square wave voltammetry (SWV). A linear response in the range of $(5 \times 10^{-8})-(5.5 \times 10^{-4})$ M with a detection limit (S/N = 3) of 9.4 nM for EP was obtained.

Carbon nanotubes are a kind of porous nanostructure material with properties such as high electrical conductivity and high mechanical strength.[1,2] The subtle electronic properties of carbon nanotubes suggests that they have the ability to promote electron transfer reaction when used as the electrode material in electrochemical reaction, which provides a new way in the electrode surface modification for designing new electrochemical sensors.[3−5]

EP, often called adrenaline, is an important catecholamine neurotransmitter in the mammalian central nervous system. Many life phenomena are related to the concentration of EP in blood.[6] Medically, EP has been used as a common emergency healthcare medicine.[5] Also, low levels of EP have been found in patients with Parkinson's disease.[7]

Also, NE is one of the derivatives of cathecholamines secreted in the adrenal medulla and plays important physiological roles in the central nervous system. It affects muscle and tissue control, stimulates arteriole contraction, decreases peripheral circulation, and activates lipolysis in adipose tissue.[8] It is also critical in mental disease, heart failure, DNA breaks in cardiac myoblast cells, and diabetes. Recent reports have indicated that NE enhances adhesion of human immunodeficiency virus-1 (HIV-1)-infected leukocytes to cardiac microvascular endothelial cells and also accelerates HIV replication via protein kinase.[9] Therefore, the determinations of EP and NE have attracted much attention of researchers.

In recent years, many methods have been reported for the determination of EP and NE, such as high-performance liquid chromatography (HPLC), fluorometry, chemiluminescence, and spectrophotometry.[10−13] However, most of these methods are complicated because they need derivatization or combination with various detection methods. Also, some of them suffer from low sensitivity and low specificity. Because of the simple procedure and high sensitivity of electroanalysis and the electroactivity of EP and NE, studying and measuring of these two compounds with electrochemical methods were carried out.[14−17]

However, a major problem is that at bare electrodes, the anodic peak potentials for EP and NE are almost the same, which results in overlapped current responses, and makes their discrimination very difficult. There are some promising strategies for the selective determination of EP and NE, such as using fast scan cyclic

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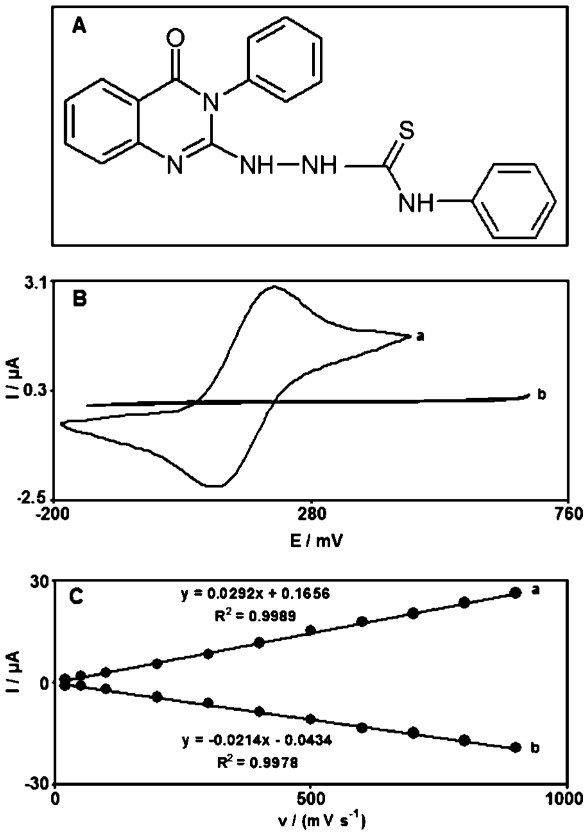

Figure 1. (A) Structure of 2PHC. (B) The cyclic voltammograms of (a) 2PHCMCNPE and (b) bare CPE in 0.1 M PBS (pH 7.0) at a scan rate of 100 mV s^{-1}. (C) The plot of anodic (a) and cathodic (b) peak currents of 2PHCMCNPE vs *v* from the cyclic voltammograms of 2PHCMCNPE in 0.1 M PBS (pH 7.0) at various scan rates (data points from left to right): 20, 50, 100, 200, 300, 400, 500, 600, 700, 800, and 900 mV s^{-1}.

voltammetry (FSCV)[18,19] carbon fiber microelectrodes,[20,21] and chemically modified electrodes (CMEs).[15,22]

To our knowledge, most of the published electrochemical studies have utilized glassy carbon electrodes or other kinds of electrodes[15,18−23] for simultaneous determination of EP and NE, and no study has reported the simultaneous electrocatalytic determination of EP and NE by using carbon nanotube paste electrodes. Also, no paper has reported quinazoline or its derivatives as catalyst for electrocatalysis and specially for electrocatalysis of EP and NE. Thus, in this paper, we described initially the preparation and suitability of a 2-(4-oxo-3-phenyl-3,4-dihydro-quinazolinyl)-*N*′-phenyl-hydrazinecarbothioamide modified carbon nanotube paste electrode (2PHCMCNPE) as a new electrocatalyst in the electrocatalysis and determination of EP in an aqueous buffer solution, then we evaluated the analytical performance of the modified electrode in quantification of EP in the presence of NE.

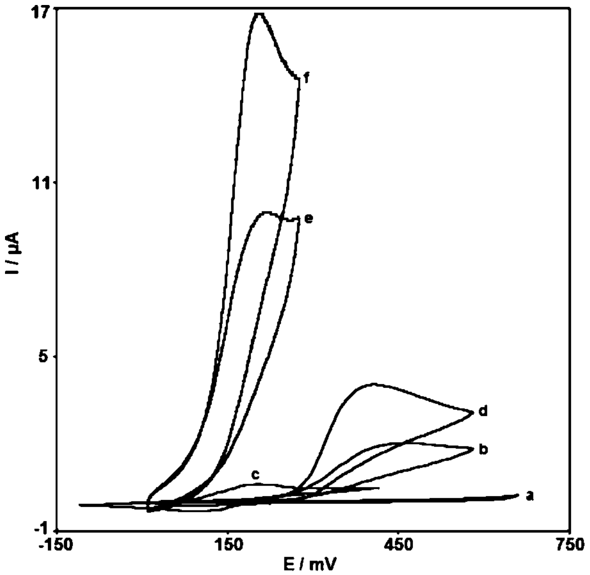

Figure 2. Cyclic voltammograms of (a) CPE in 0.1 M PBS (pH 7.0), (b) CPE in 0.5 mM EP, (c) 2PHCMCNPE in 0.1 M PBS, (d) CNPE in 0.5 mM EP, (e) 2PHCMCPE in 0.5 mM EP, and (f) 2PHCMCNPE in 0.5 mM EP. In all cases the scan rate was 30 mV s^{-1}.

EXPERIMENTAL SECTION

Apparatus and Chemicals. All electrochemical measurements were performed using an Autolab PGSTAT12 potentiostat/galvanostat (Eco Chemie, Utrecht, The Netherlands). An Ag/AgCl/KCl 3 M, a platinum wire, and a 2PHCMCNPE were used as the reference, auxiliary, and working electrodes, respectively. A digital pH/mV meter (Metrohm model 710) was applied for pH measurements. Epinephrine, norepinephrine, graphite fine powder, paraffin oil, and reagents were analytical grade from Merck. Multiwalled carbon nanotubes (purity more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm, and tube length from 0.5 to 200 nm were prepared from Nanostructured & Amorphous Materials, Inc. The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range 2.0−11.0.

Synthesis of 2PHC. Anthranilic acid (10 mmol) was dissolved in 50 mL of acetic acid, and 10 mmol of phenyl isothiocyanate was added. The mixture was refluxed for 3 h. After cooling, the resulted precipitate was filtered and recrystalized from ethanol to give pure 2-mercapto-3-phenyl-3*H*-quinazolin-4-one (MPQ).

To a solution of 10 mmol of (MPQ) in 50 mL of *n*-butanol, 20 mL of hydrazine monohydrate was added. The solution was refluxed for 18 h. Then the reaction mixture was cooled and filtered off. The resulting precipitate recrystalized from ethanol to give pure 2-hydrazino-3-phenyl-3*H*-quinazolin-4-one (HPQ).

A mixture of 10 mmol of (HPQ) and 10 mmol of phenyl isothiocyanate in 40 mL of DMF was heated at 60 °C for 1 h. After completion of the reaction, 30 mL of water was added. The resulting precipitate was recrystalized from acetic acid after filtration to give 2-(4-oxo-3-phenyl-3,4-dihydro-quinazolinyl)-*N*′-phenyl-hydrazinecarbothioamide (2PHC) (Figure 1A).

Preparation of the Electrode. Modified carbon nanotube paste electrodes were prepared by dissolve 0.01 g of 2PHC in CH_3Cl and hand mixing with 89 times its weight of graphite powder and 10 times its weight of carbon nanotube with a mortar and pestle. The solvent was evaporated by stirring. A 70:30 (w/w) mixture of 2PHC spiked carbon nanotube powder and paraffin

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oil was blended by hand mixing for 20 min until a uniformly wetted paste was obtained. The paste was then packed into the end of a Teflon rod with a hole (2 mm diameter, 5 mm deep) bored at one end for the electrode filling. Contact was made with a platinum wire through the center of the rod which screwed to the rotating disk electrode (RDE) device.

RESULTS AND DISCUSSION

Electrochemistry of 2PHCMCNPE. According to our knowledge, there is no report on the study of the electrochemical properties and especially the electrocatalytic activity of 2PHCMCNPE in aqueous media. Because this compound is insoluble in aqueous media, we prepared 2PHCMCNPE and studied the electrochemical behavior of 2PHCMCNPE in pure buffered aqueous solution by cyclic voltammetry. Figure 1B shows the cyclic voltammogram of 2PHCMCNPE in a 0.1 M phosphate buffered solution (PBS).

As Figure 1B shows, the cyclic voltammogram of 2PHCMCNPE exhibits an anodic and cathodic peak at the forward and reverse scans of the potential related to the oxidation and reduction of the 2PHC, respectively. A pair of reversible peaks observed at $E_{pa} = 0.21$ V and $E_{pc} = 0.11$ V. The half-wave potential ($E_{1/2}$) and ΔE_p were 0.16 and 0.10 V, respectively. The peak separation potential, ΔE_p ($= E_{pa} - E_{pc}$), is greater than the ($59/n$) mV expected for a reversible system, which indicates a quasi reversible behavior for the mediator in an aqueous medium. In addition, the effect of the scan rate of the potential on the electrochemical properties of the 2PHCMCNPE was studied in PBS by cyclic voltammetry. The plots of the anodic and cathodic peak currents were linearly dependent on the sweep rate (v) (Figure 1C). This behavior indicates that the nature of the redox process is diffusionless controlled.

Electrocatalytic Oxidation of EP. Figure 2 depicts the cyclic voltammetric responses from the electrochemical oxidation of 0.5 mM EP at 2PHCMCNPE (curve f), 2PHC modified CPE (2PHCMCPE) (curve e), CNPE (curve d), and bare CPE (curve b). As can be seen, the anodic peak potential for the oxidation of EP at 2PHCMCNPE (curve f) and 2PHCMCPE (curve e) is about 210 mV, while at the CNPE (curve d) and bare CPE(curve b), peak potentials are about 410 and 450 mV, respectively. From these results it is concluded that the best electrocatalytic effect for EP oxidation is observed at 2PHCMCNPE (curve f). For example, the results are shown that the peak potential of EP oxidation at 2PHCMCNPE (curve f) shifted by about 200 and 240 mV toward the negative values compared with that at a CNPE (curve d) and bare CPE (curve b), respectively. Similarly, when we compared the oxidation of EP at the 2PHCMCPE (curve e) and 2PHCMCNPE (curve f), there is a dramatic enhancement of the anodic peak current at 2PHCMCNPE relative to the value obtained at the 2PHCMCPE (curve e). In the other words, the data obtained clearly show that the combination of carbon nanotube and mediator (2PHC) definitely improve the characteristics of EP oxidation. The 2PHCMCNPE in 0.1 M PBS, without EP in solution, exhibits a well-behaved redox reaction (curve c) upon the addition of 0.5 mM EP; the anodic peak current of the mediator was greatly increased, while the corresponding cathodic peak disappeared on the reverse scan of the potential (curve f). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes.[24]

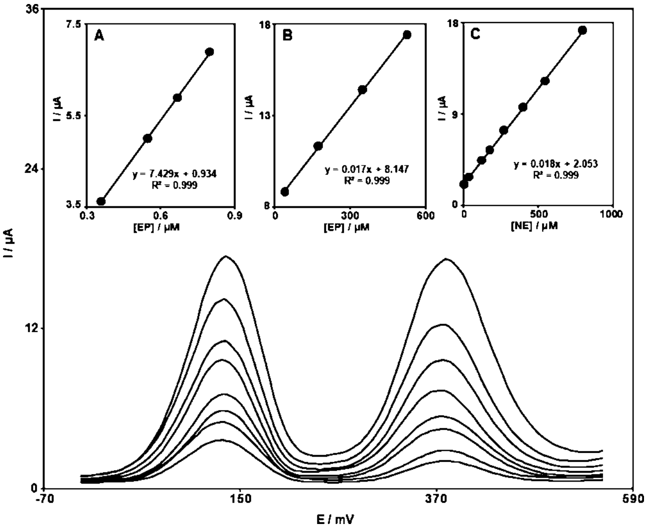

Figure 3. Square wave voltammograms of 2PHCMCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of EP and NE: (from inner to outer) mixed solutions of 0.36 + 10, 0.55 + 40, 0.67 + 125, 0.80 + 175, 40 + 275, 175 + 400, 350 + 550, and 525 + 800, respectively, in which the first value is concentration of EP in micromolar and the second value is concentration of NE in micromolar. (A and B) Plots of the peak currents as a function of EP concentration and (C) plot of the peak currents as a function of NE concentration.

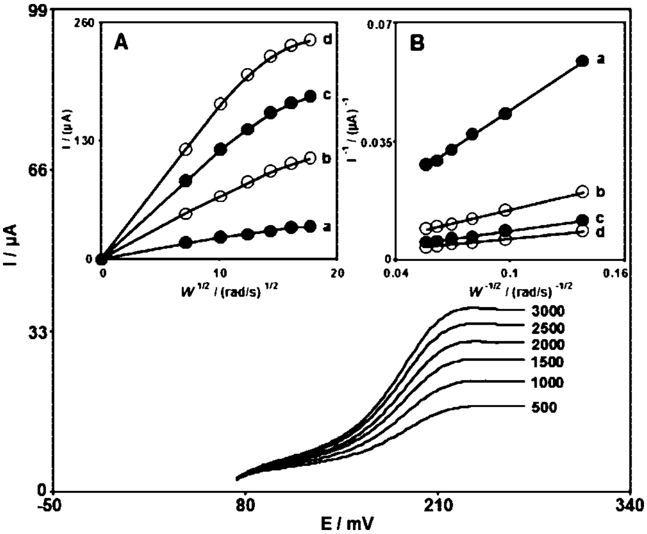

Figure 4. Voltammograms of rotating disk 2PHCMCNPE in 0.1 M PBS (pH 7.0) containing 0.5 mM EP at the various rotation rates indicated for each voltammogram. Scan rate: 30 mV s^{-1}. Insets: (A) Levich plots constructed from the modified RDE voltammograms of solution with (a) 0.5, (b) 1.0, (c) 1.5, and (d) 2.0 mM EP. (B) Koutecky−Levich plots obtained from Levich plots shown in part A.

Calibration Plot and Limit of Detection. Square wave voltammetry was used to determine the concentration of EP. The responses are linear with the EP concentrations ranging from (5.0×10^{-8})−(9.5×10^{-7}) M and (9.5×10^{-7})−(5.5×10^{-4}) M with current sensitivities of 7.7494 and 0.0181 μA/μM. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitations.[14,24] The detection limit (S/N = 3) is 9.6 nM.

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Simultaneous Determination of EP and NE. One of the main objectives of the present study was the development of a modified electrode capable of the electrocatalytic oxidation of EP and separation of the electrochemical responses of EP and NE. The utilization of the 2PHCMCNPE for the simultaneous determination of EP and NE was demonstrated by simultaneously changing the concentrations of EP and NE. The square wave voltammetric results show that the simultaneous determination of EP and NE with two well-distinguished anodic peaks at potentials of about 240 mV is possible at the modified electrode (Figure 3).

It is very interesting to note that the sensitivities of the modified electrode toward EP in the absence and presence of NE are virtually the same, which indicates the fact that the oxidation processes of EP and NE at the 2PHCMCNPE are independent and therefore simultaneous, or independent measurements of the two analytes are possible without any interference. If the EP signal is affected by the NE, the above-mentioned slopes would be different.

Rotating Disk Electrode Voltammetry. To our knowledge, no paper has used the rotating disk electrode voltammetry technique with carbon paste and especially carbon nanotube paste electrodes for the oxidation of EP and in this paper, we used this technique for the first time by this kind of electrodes for the oxidation of EP. Thus, the electrocatalytic activity of 2PHCMCNPE toward oxidation of EP was also evaluated by the RDE voltammetry technique (Figure 4). The plots of catalytic currents (I) measured at 240 mV versus $\omega^{1/2}$ (Levich plots) are shown in inset A of Figure 4. As can be seen, the clear lack of linearity immediately suggests that the reaction is limited by kinetics and not by diffusion.

In this case, the Koutecky−Levich plots (Figure 4, inset B) can be used to determine the value of the catalytic reaction rate constant, k, between the oxidized form of 2PHC and EP. The Koutecky−Levich equation can be formulated as follows:[24]

$$1/I_{\mathrm{cat}} = 1/I_{\mathrm{Lev}} + 1/I_{\mathrm{k}} \quad (1)$$

Here I_{Lev} is the Levich current and I_{k} is the kinetic current and are defined by eqs 2 and 3:

$$I_{\mathrm{Lev}} = 0.620nFAD^{2/3}v^{-1/6}\omega^{1/2}C \quad (2)$$

$$I_{\mathrm{k}} = nFACk\Gamma \quad (3)$$

With the use of these equations, the k value was found to be 5.82×10^3 M^{-1} s^{-1} for EP. Also, the diffusion coefficient of EP, D, may be obtained from the slope of the Koutecky−Levich plots. The mean value of D was found to be 7.87×10^{-6} cm^2 s^{-1}.

CONCLUSION

This work demonstrates the construction of a 2PHCMCNPE and its application in the simultaneous determination of EP and NE. The high current sensitivity, low detection limit, and high selectivity of the 2PHCMCNPE for the detection of EP prove its potential sensing applications.

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