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ORIGINAL PAPER

A selective determination of norepinephrine on the glassy carbon electrode modified with poly (ethylenedioxypyrrole dicarboxylic acid) nanofibers

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Abstract A glassy carbon electrode modified with poly (3,4-ethylenedioxypyrrole-2,5-dicarboxylic acid) nanofibers (PEDOPA-NFs) was prepared for the determination of norepinephrine (NE) in phosphate buffer saline. The modified electrode demonstrated an improved sensitivity and selectivity toward the electrochemical detection of NE and could detect separately ascorbic acid (AA), uric acid (UA), and NE in their mixture. The separations of the oxidation peak potentials of NE–AA and NE–UA were 160 and 150 mV, respectively. Meanwhile, the modified electrode showed higher sensitivity and selectivity toward NE than dopamine and epinephrine. Using differential pulse voltammetry, the oxidation peak current of NE was found to be linearly dependent on its concentration within the range of 0.3–10 μM, and the detection limit of the NE oxidation current was 0.05 μM at a signal-to-noise ratio of 3. The PEDOPA-NFs promoted the electron transfer reaction of NE, while the PEDOPA-NFs, acting as a negatively charged linker, combined with the positively charged NE to induce NE accumulation in the NFs at pH under 7.4. However, the PEDOPA-NFs restrained the electrochemical response of the negatively charged AA and UA due to the electrostatic repulsion. The result indicates that the modified electrode can be used to determine NE without interference from AA and UA and selectively in the mixture of catecholamines.

Keywords Electropolymerization · 3,4-Ethylenedioxypyrrole-2,5-dicarboxylic acid · Glassy carbon electrode · Norepinephrine · Template synthesis · Nanofibers

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Introduction

The electrochemical determination of biomolecules has been intensively investigated over the past two decades. Among these biomolecules, norepinephrine (NE) is one of the most important catecholamine neurotransmitters in the central nervous system. Many diseases are related to changes of its concentration, and the determination of NE concentrations in biological systems provides important information on its physiological functions. NE determination is usually executed by high-performance liquid chromatography, gas chromatography, and spectrophotometry [1–3]. Meanwhile, NE is an electroactive species and can be detected with electrochemical oxidation at various modified electrodes [4–10]. NE determination can be interfered by the coexistence of ascorbic acid (AA) and uric acid (UA) with low NE level in biological samples. The separate detection of NE in the presence of AA and UA at bare electrode is difficult due to the overlaid oxidation peak potentials of these species. To overcome this problem, chemically modified electrodes have been utilized to determine NE in the presence of AA and UA for electrochemical oxidation of NE without their interference. The many chemically modified electrodes include carbon-based electrodes [11–13], self-assembled monolayers gold electrodes [14], lead–ruthenium oxide electrodes [15],

carbon nanotubes [16], and electropolymerized films [17–26]. The separate detection of NE in the presence of dopamine (DA) and epinephrine (EP) is difficult due to their overlaid oxidation peak potentials at the bare and even the modified electrodes. A noble modified electrode is required for selective NE determination in the mixture of catecholamines that have similar oxidation potentials.

Recently, nanostructured materials have become attractive due to their unusual optical, electrical, and catalytic properties [27–31]. Various nanowires and nanotubes have been prepared using an ordered, porous, anodic aluminum oxide (AAO) template due to its remarkable hardness, uniform pore size, and high pore density [32–39]. The porous AAO template with cyclic voltammetric electrodeposition is a very effective and powerful method for obtaining highly ordered nanowires by controlling the electrochemical parameters [40–44]. Nanowires might offer a high surface area for interaction with the analyte due to their high surface area-to-volume ratios that would enable a higher retention of the analyte.

In this work, poly(3,4-ethylenedioxypyrrole-2,5-dicarboxylic acid) nanofibers (PEDOPA-NFs) were synthesized for the first time by cyclic voltammetric electrodeposition with porous AAO template. The electrochemical properties of the synthesized PEDOPA-NFs were determined and their application to the determination of biomolecules such as NE, DA, EP, AA, and UA investigated. The PEDOPA-NFs-modified glassy carbon electrode (GCE) was demonstrated to be an excellent amperometric sensor for NE in the presence of DA, EP, AA, and UA, thereby overcoming the first challenge for the selective detection of NE by amperometric method in a mixture of catecholamines.

Experimental

Reagents and electrochemical apparatus

EDOPA, NE, DA, EP, UA, AA, and Nafion were purchased from Aldrich. All other reagents used were of analytical grade. AAO films (d=200 nm, Whatman) were used as a template for the PEDOPA-NFs. The pH of the phosphate buffer saline (PBS) solutions was adjusted with 0.1 M H_3PO_4 and 0.1 M NaOH. High-purity argon was used for deaeration. All experiments were carried out at room temperature. Doubly distilled water with resistibility over 18 MΩ cm in a quartz apparatus was used to prepare all aqueous electrolyte solutions.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a three-electrode potentiostat [Bioanalytical Systems (BAS) 100B/W] in a ground Faraday cage. A platinum wire electrode was used as an auxiliary electrode. A Ag/AgCl electrode supplied by BAS was used as the reference electrode. The PEDOPA-NFs/GCE-modified electrode was used as the working electrode. GCE (3 mm in diameter) was purchased from BAS. All potentials were reported with respect to the Ag/AgCl electrode at room temperature under argon atmosphere. The pH measurements were performed with a JENCO meter. A field emission scanning electron microscope image of the modified electrode was obtained on a JSM-7500F field emission scanning electron microanalyzer (JEOL, Japan).

Preparation of PEDOPA-NFs

A thin, 30-nm-thick platinum film was sputtered onto one side of the porous AAO template (Anodisc, Whatman) with a nominal pore diameter of 200 nm to make the template conductive. The prepared AAO template served as the working electrode in a three-electrode configuration for electrodeposition of PEDOPA-NFs. The EDOPA solution of the mixture solvent (acetonitrile/methanol=10:3) was absorbed into the pores of the Pt-coated AAO membrane slowly, and then PEDOPA-NFs were prepared under CV conditions by sweeping the potential from 1.2 to −1.2 V versus Ag/AgCl with a scan rate of 20 mV/s for 40 cycles at room temperature in PBS solutions (pH 7.4; see Fig. 1). The AAO membrane was treated with 1.0 M NaOH solution to dissolve the AAO template and then washed several times with distilled water and methanol carefully to achieve neutral pH. PEDOPA-NFs were obtained.

Preparation of the GCE-modified electrode with PEDOPA-NFs

The PEDOPA-NFs were dispersed in deionized water at a concentration of 1 mg/mL by ultrasonic agitation for

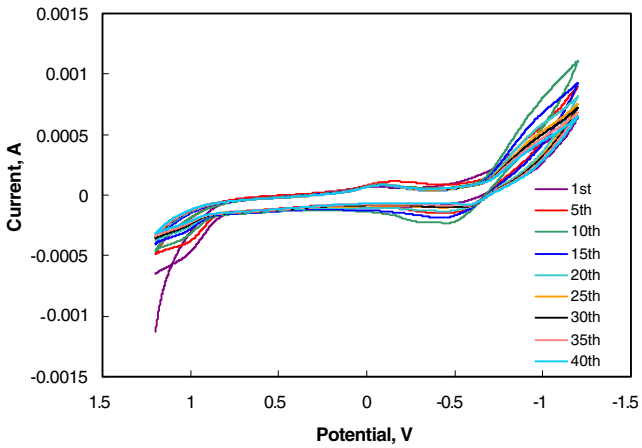


Fig. 1 Cyclic voltammograms recorded during electrochemical deposition of PEDOPA-NFs on an AAO template in PBS solutions of pH 7.4. Scan rate was 20 mV/s, 40 cycles

10 min. The GCE surface was highly polished with alumina paste, sonicated with ultrasonic agitation for 5 min, washed with 1.0 M HCl solution, and then rinsed with distilled water several times and finally with methanol. After being cleaned thoroughly, the GCE was coated with 5 μL of PEDOPA-NFs solution containing 0.1% Nafion, and then the solvents were evaporated in the air at room temperature. The PEDOPA-NFs/GCE-modified electrode was used as the working electrode for NE determination. Before and after each experiment, the PEDOPA-NFs/GCE-modified electrode was washed with distilled water. All experiments were carried out in a 15 mL electrolytic cell with 5 mL PBS solutions, with dioxygen being continuously removed by purging with high-purity argon.

Formation and morphology of the PEDOPA-NFs

Firstly, the EDOPA monomer solution was absorbed into the narrow pores of the Pt-coated AAO membrane by diffusion. After 40 cycles of electrodeposition, the deposition was completed. Figure 1 shows cyclic voltammograms recorded during the electrochemical deposition of PEDOPA-NFs at an AAO template in PBS solutions (pH 7.4). During the electrodeposition, the redox peak currents were largely increased to fifth or tenth after they were decreased and then saturated around −0.2, −0.5, −1.0 V, respectively, but the oxidation peak current was continuously decreased around 1.0 V. The AAO template was immersed in a 1.0 M NaOH solution for 1 h in order to liberate the nanofibers by dissolving the template membrane. After dissolution of the template, the synthesized PEDOPA-NFs were freed and then characterized using scanning electron microscopy (SEM). Figure 2 shows the SEM image of the synthesized PEDOPA-NFs. The well-aligned PEDOPA-NFs present a comparatively even distribution of diameters (200 nm) that correspond to the diameters of the nanopores in the blank AAO membrane. The prepared PEDOPA-NFs are smooth and straight, implying that PEDOPA-NFs can be prepared in a very uniform and controlled method.

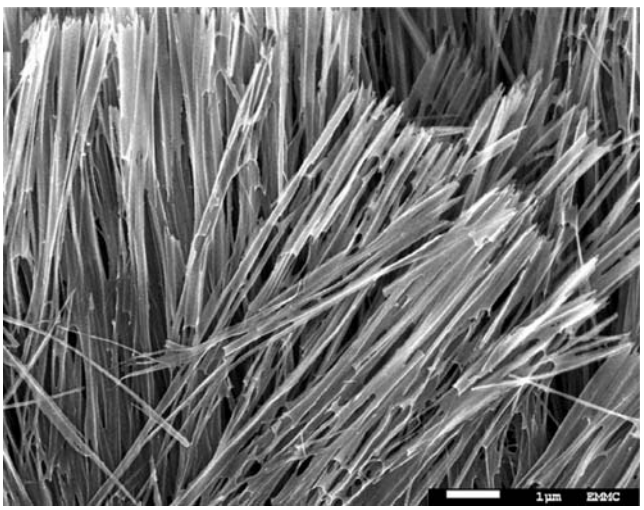


Fig. 2 SEM image of the synthesized PEDOPA-NFs

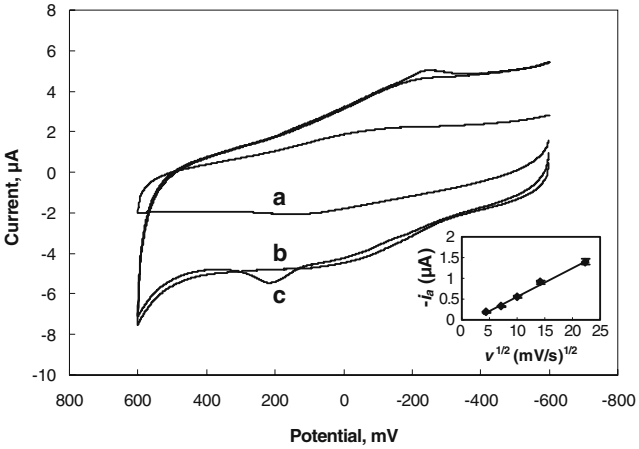


Fig. 3 CVs of the 1.0 μM NE at the bare GCE and PEDOPA-NFs/GCE-modified electrodes in the PBS solution of pH 7.4. *Inset* Plot of peak currents for NE oxidation vs. square root of scan rate at the modified electrode. Scan rate was 100 mV/s

Results and discussion

Electrochemical behavior of NE at the PEDOPA-NFs/GCE-modified electrode

The PEDOPA-NFs/GCE-modified electrode was used as the working electrode for NE determination. Figure 3 illustrates the NE CVs at the bare GCE and PEDOPA-NFs/GCE-modified electrodes in the PBS solution at pH 7.4. Figure 3a shows the voltammetric response of 1.0 μM NE at the bare GCE, and a very small anodic NE peak current was observed in the PBS solution. No voltammetric response was evident on the PEDOPA-NFs/GCE-modified electrode in the blank PBS solution, as shown in Fig. 3b. The cyclic voltammogram of 1.0 μM NE at the PEDOPA-NFs/GCE-modified electrode in the PBS solution is presented in Fig. 3c. NE is easily oxidized to norepinephrinequinone and subsequently transformed to leuconorephinechrome. The anodic peak potential (0.20 V) and current of NE (0.55 μA) are represented. The NE oxidative current density at the modified electrode was markedly increased relative to that at the bare GCE, demonstrating the NE electrocatalytic ability of the PEDOPA-NFs/GCE-modified electrode, which was attributed to the excellent electrical characteristics of the

PEDOPA-NFs and the strong interaction tendency of the PEDOPA-NFs to NE.

The effect of scan rate (25–900 mV/s) on the NE oxidation at the PEDOPA-NFs/GCE-modified electrode in the PBS solution of pH 7.4 was tested (see the inset of Fig. 3). The anodic peak current of the modified electrode in the NE solution increased linearly with the square root of the scan rate (correlation coefficient, 0.9952), implying that direct electron transfer between NE and the modified electrode occurred on the modified electrode surface and indicating that the NE electrode reaction at the modified electrode is a diffusion-controlled process.

Figure 4 shows that the pH of the PBS solution significantly affected the NE oxidation at the PEDOPA-NFs/GCE-modified electrode by influencing both the peak current and peak potential. The anodic peak current increased to a peak and then decreased with increasing solution pH. The pH effect on the NE peak current may have been caused by the electrostatic interaction of NE on the PEDOPA-NFs/GCE-modified electrode.

NE is known to be a catecholamine molecule that can be deprotonated or protonated as a function of solution pH. In addition, PEDOPA-NFs have a COO^- functional group that may become protonated or deprotonated. Electrostatic repulsion between NE and PEDOPA-NFs on the electrode decreases the detection of NE on the modified electrode at higher or lower pH, and NE interaction with PEDOPA-NFs was maximized in the PBS solution at about pH 7.4. NE could be a cation in pH 7.4 PBS, and the increased anodic current of NE can be attributed to enhanced oxidation of NE by electrochemical and chemical processes (EC mechanism) in PEDOPA-NFs. The peak current of 1.0 μM NE was maintained at the same level after several continuous scanning cycles. After the modified GCE was immersed into the solution of 1.0 mM NE for 1 h and then rinsed with distilled water, no NE response was observed in the PBS not containing NE. These results indicated that NE

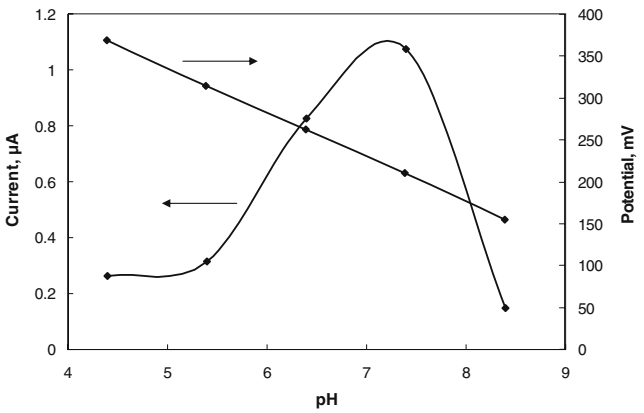


Fig. 4 Effect of the buffer solution pH on the peak current and peak potential for NE oxidation

was not adsorbed onto the modified GCE surface. The pH effect on the peak potential for the NE oxidation on the PEDOPA-NFs/GCE-modified electrode was also investigated. The NE peak potential decreased with increasing pH, showing a high linearity with a correlation coefficient of 0.999($y = -53.3x + 603.3$) on the peak potential. The potential shift vs. pH (53.3 mV/pH) was close to the corresponding figure of 59.2 mV/pH for NE, indicating that the number of protons and electrons involved in the NE oxidation was the same. The effect of accumulation time on the NE peak current was investigated. The peak currents quickly increased with increasing accumulation time and then plateaued after 2 s, thus demonstrating the rapid interaction of NE on the surface of the PEDOPA-NFs/GCE-modified electrode.

The reproducibility and reusability of the PEDOPA-NFs/GCE-modified electrode for NE determination were investigated. Repetitive NE determinations were carried out in a solution of 1.0 μM NE in 0.1 M PBS solution at pH 7.4. The PEDOPA-NFs/GCE-modified electrode was easily regenerated by potential cycling between −0.6 and 0.6 V at a scan rate of 100 mV/s in the blank PBS solution. In the determination/regeneration/cleaning cycle, a relative standard deviation of 2.1% was obtained for NE for five replicate measurements. Meanwhile, the NE currents were monitored for 15 days, and the peak current was measured at 98% of the initial current. This result confirmed the good stability and reproducibility for NE determination using the PEDOPA-NFs/GCE-modified electrode.

Determination of NE at the PEDOPA-NFs/GCE-modified electrode

The negatively charged polymeric nanofibers were expected to promote the selectivity and sensitivity of NE detection, and PEDOPA-NFs can be used as a negatively charged polymer to improve NE determination. NE determination was investigated in the presence of AA, UA, DA, and EP in order to establish the method's selectivity and sensitivity for NE. DPV was used to determine the NE concentration, and Fig. 5 illustrates a series of DPVs obtained for NE at varying concentrations (0.5–10 μM) at the PEDOPA-NFs/GCE-modified electrode in 0.1 M PBS solution of pH 7.4. Figure 6a shows the plot of the peak currents vs. the NE concentration at the oxidation potential of 0.18 V, and the peak currents exhibited a linear relation to the NE concentration over the range from 0.5 to 10 μM with a correlation coefficient of 0.998($y = 1.45x + 0.21$). The detection limit (3:1 signal-to-noise ratio) of NE was estimated to be 0.1 μM. As various possible interferences were evaluated in separate experiments, Fig. 6b also shows the plot of the peak currents vs. the DA concentration at the oxidation potential

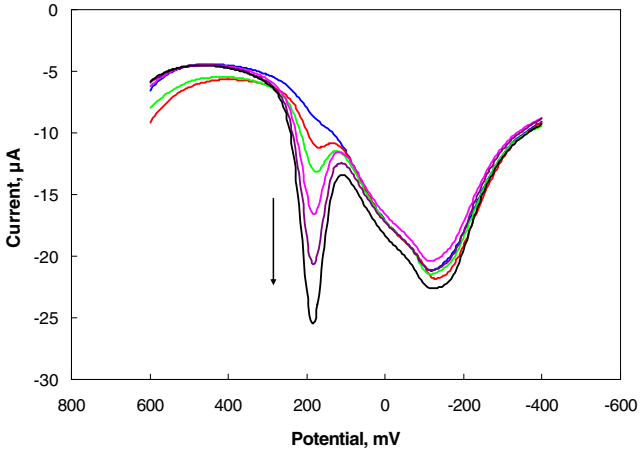


Fig. 5 DPVs obtained for NE at varying concentrations (0.5–10 μM) at the PEDOPA-NFs/GCE-modified electrode in 0.1 M PBS solution of pH 7.4. DPV conditions were 20 mV/s scan rate, 50 mV pulse amplitude, and 50 ms pulse width

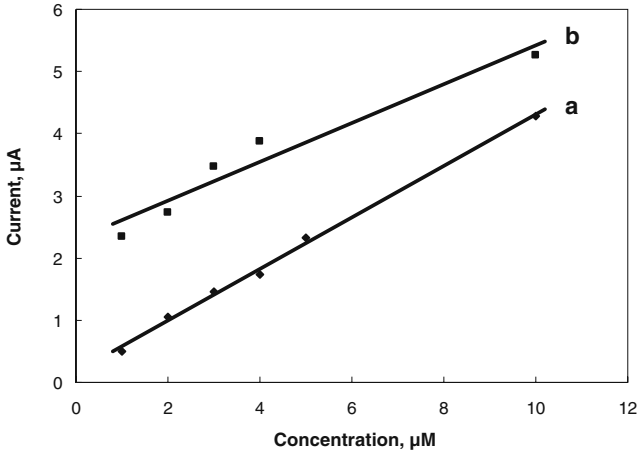


Fig. 7 Plot of the peak currents vs. the DA concentration in the absence (**a**) and presence (**b**) of 1.0 μM NE at the oxidation potential

of 0.25 V, and there is no current in the presence of EP (see Fig. 6c). The peak currents demonstrated a linear relation to the DA concentration over the range from 1.0 to 10 μM with a correlation coefficient of 0.997($y = 0.41x + 0.017$), but a very small voltammetric response was obtained on the PEDOPA-NFs/GCE-modified electrode in the PBS solution of EP (1.0–10 μM). These results indicated that the sensitivity of NE compared with DA and EP was enhanced in the absence (a) and presence (b) of 1.0 μM DA, 1.0 μM EP, and 0.1 mM AA-modified electrode and that NE can be selectively measured in the presence of DA and EP. Figure 7 shows the comparative plots of the peak currents vs. the DA concentration in the absence (a) and presence (b) of 1.0 μM NE at the oxidation potential. The figure confirmed that the detection of DA was interfered by NE because the

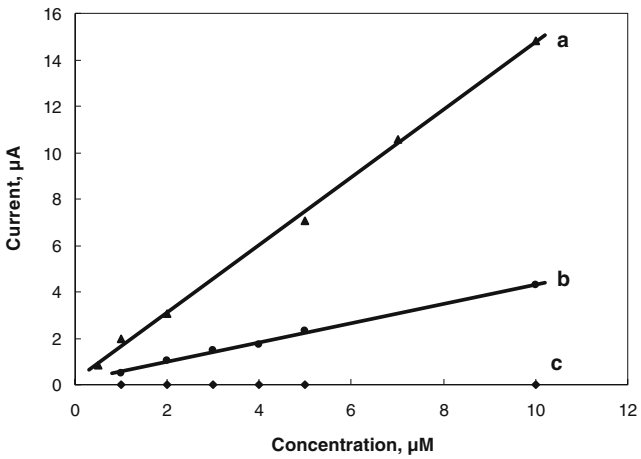
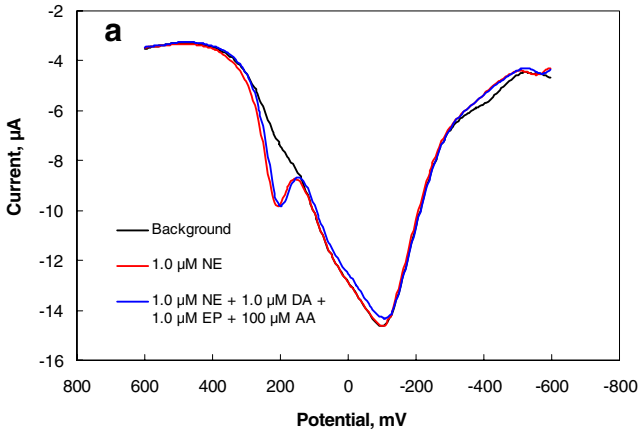


Fig. 6 Plot of the peak currents vs. the NE concentration (**a**) vs. the DA concentration (**b**) vs. the EP concentration (**c**)



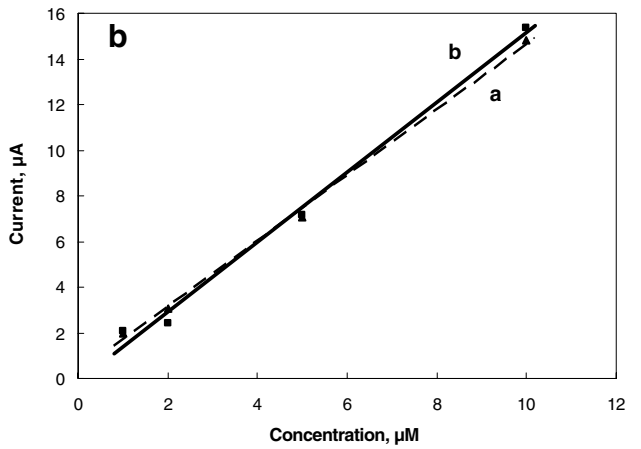


Fig. 8 **a** DPVs of the background (*black line*), the 1.0 μM NE concentration in the absence (*red line*), and presence (*blue line*) of 1.0 μM DA, 1.0 μM EP and 0.1 mM AA in PBS solution. **b** Plot of the peak currents vs. the NE concentration in the absence (**a**) and presence (**b**) of 1.0 μM DA, 1.0 μM EP, and 0.1 mM AA at the oxidation potential. DPV conditions were 20 mV/s scan rate, 50 mV pulse amplitude, and 50 ms pulse width

response of DA is smaller than that of NE. Meanwhile, Fig. 8a shows the DPVs of the NE concentration in the absence and presence of 1.0 μM DA, 1.0 μM EP, and 0.1 mM AA at the oxidation potential. Figure 8b shows the comparative plots of the peak currents vs. the NE concentration in the absence (a) and presence (b) of 1.0 μM DA, 1.0 μM EP, and 0.1 mM AA at the oxidation potential. The peak currents demonstrated a linear relation to the NE concentration over the range from 1.0 to 10 μM in the presence of 1.0 μM DA, 1.0 μM EP, and 0.1 mM AA with a correlation coefficient of 0.998($y = 1.53x + 0.13$), implying that the deviation of peak current was less than 6%. The interference of AA was considered in the NE determination, but its oxidation potential was −0.14 V in comparison with 0.20 V for the oxidation potential of NE. The response of NE vs. AA was separated by a potential difference of 320 mV, which rendered clearly distinguishable the anodic peak potentials of NE and AA at the PEDOPA-NFs/GCE-modified electrode.

Analytical applications for NE determination

The applicability of the PEDOPA-NFs/GCE-modified electrode as an electrochemical sensor was tested for the determination of NE injection in the presence of DA, EP, AA, and UA as interfering substances. The NE concentration in the injection was determined by applying a calibration plot using the procedures proposed in this paper. The NE determination results in the injections (n=5) were as follows: 1.00 μM NE (labeled) in the presence of 1.00 μM DA, 1.00 μM EP, 0.1 mM AA, and 10 μM UA, 1.02 μM NE (found).

Conclusions

The PEDOPA-NFs/GCE-modified electrode was prepared in a rapid and simple procedure and demonstrated to be a useful and effective sensing surface for selective and sensitive NE determination in the presence of DA, EP, UA, and AA. The modified electrode provided excellent selectivity and very efficient electroactivity to NE oxidation in voltammetric measurements of NE in the presence of interferences. The oxidation peak currents were correlated with NE concentrations over the range 0.1–10 μM that was tested in this work. The negatively charged PEDOPA-NFs in the electrode provided an efficient electrochemical sensor for NE measurement with good stability and reproducibility.

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