ELECTROCATALYTIC RESPONSE AND DETERMINATION OF NORADRENALINE IN THE PRESENCE OF L-ASCORBIC AND URIC ACIDS WITH POLY(ERIOCHROME BLACK T)-MODIFIED ELECTRODE

Xinhua LIN[1,*], Wei LI[2], Hong YAO[3], Yuanyuan SUN[4], Liying HUANG[5] and Yanjie ZHENG[6]

Department of Pharmaceutical Analysis, Faculty of Pharmacy, Fujian Medical University, Fuzhou 350004, P.R. China; e-mail: [1] yaosyy @sina.com, [2] jojo0738@sina.com, [3] hongyao@mail.fjmu.edu.cn, [4] moonlight_s@163.com, [5] fjmuhly88@sina.com, [6] gillzheng@163.com

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A poly(Eriochrome Black T) chemically modified glassy carbon electrode modified with Eriochrome Black T was prepared by cyclic voltammetry. The modified electrode showed an excellent electrocatalytic activity in oxidation of noradrenaline (NA) and could separate its electrochemical responses from those of L-ascorbic acid (AA) and uric acid (UA). Differences of the oxidation peak potentials for NA–AA and UA–NA were about 150 mV. The responses to NA, AA and UA of the modified electrode are relatively independent. Using differential pulse voltammetry, the peak currents of NA at modified glassy carbon electrode increased linearly with the concentration of NA from 0.5 to 100 μmol l^{-1}. The detection limit was 0.2 μmol l^{-1}. With the modified electrode, UA could be selectively determined in the presence of AA. The method showing a wide linear dynamic range and excellent sensitivity was successfully applied to the determination of NA in pharmaceutical injections and various samples.

Keywords: Eriochrome Black T; Modified electrodes; Electrocatalysis; Noradrenaline; L-Ascorbic acid; Uric acid; Cyclic voltammetry; Electrochemistry.

Noradrenaline (NA) is one of the most important catecholamine neurotransmitters in the mammalian central nervous system existing in the nervous tissue and biological body fluids. It was found that NA, as an excitomotor of α-adrenoceptor agonists, could regulate neural interactions by changing the permeability of gap junctions between adjacent neurons. Many diseases[1,2] are related to changes of its levels in human body. Consequently, it is important to determine the concentration of NA in biological fluids for investigating its physiological functions and for diagnosis of some diseases in clinical medicine. Generally, the determination of NA could be carried out by various methods including high performance liquid chroma-

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tography[3], gas chromatography[4], ion chromatography[5] and spectrophotometry[6,7], etc.

Electrochemical methods have been extensively studied for determination of bioactive molecules over the past two decades due to their simplicity, low costs and convenience. As an electroactive compound, NA has attracted much interest of electroanalytical chemists[8,9]. However, the irreversibility of its electrochemical properties at common electrodes results in a large overpotential[10] so that the voltammetric methods using conventional bare electrodes show poor electrochemical characteristics. Furthermore, the biogenic amines often coexist with L-ascorbic acid (AA) and uric acid (UA) in vivo. For these reasons, practical determination of NA usually suffers from inferior selectivity and accuracy. To solve these problems, there has been extensive interest in polymer-modified electrodes[11–17]. The modified electrodes, in contrast to bare electrodes, showed the great advantages of a large number of reactive sites and offering the possibility of being designed with particular redox-active sites. Such polymers are promising for improving the selectivity and sensitivity of voltammetric measurements of electroactive materials in real samples[18–26].

In this work, a novel polymeric film of Eriochrome Black T (EBT) was fabricated by electropolymerization in 0.01 M NaOH on the surface of a glassy carbon electrode (GCE). The poly(EBT) membrane at the GCE could electrocatalytically oxidize NA and detect it in the presence of AA and UA. The poly(EBT)-film-coated electrode provides a possibility of the selective determination of NA in routine analysis.

EXPERIMENTAL

Instrumentation

CHI 660B Electrochemical Workstation model (Shanghai CH Instruments, China) equipped with a PC was used for electrochemical measurements and treatment of data. A conventional three-electrode system was used throughout. The working electrode was a bare GCE or modified with poly(EBT) film, 3.0 mm in diameter. The auxiliary electrode was a platinum-wire and a saturated calomel electrode (SCE) was used as reference electrode. All electrode potentials were reported with respect to SCE.

Materials

Uric acid was purchased from Fluka (Switzerland). NA and L-ascorbic acid were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). EBT was purchased from Shanghai Chemical Reagents Company (China). All reagents were of analytical grade and were used without further purification. Phosphate buffer solutions (PBS) were prepared by mixing 0.02 M NaCl and 0.05 M NaH_2PO_4–Na_2HPO_4, and

their pH were adjusted with 0.05 M H_3PO_4 and 0.1 M NaOH. All solutions were prepared with twice-distilled water. All experiments were performed at room temperature.

Preparation of Pretreated and Poly(EBT)-Modified GCE

A bare GCE was prepared by polishing first with fine wet emery papers and then with 0.3 and 0.05 μm alumina paste for 2 min each. After polishing, the bare GCE was sonicated with ultrasonic agitation, and then rinsed with twice-distilled water and ethanol for 2 min each. After cleaning, the electrode was checked by cyclic voltammetry using 1 mM potassium ferricyanide solution (by evaluating the oxidation peak current and peak potential). The voltammograms were obtained between –0.2 and 0.8 V at 100 mV s^{-1}. The electrode was then electrochemically pretreated by cycling the potential between –0.4 and 1.5 V in 0.1 M H_2SO_4 at a scan rate of 100 mV s^{-1} 20 times. On this way, the pretreated GCE was obtained. The polymer-coated electrode was fabricated under the same conditions with the pretreated electrode but in the presence of 0.01 M NaOH containing 0.5 mM EBT. After polymerization, the poly(EBT) film was washed with water and cycled (6 cycles) in PBS (pH 4.0) between –0.2 and 0.8 V to eliminate unreacted EBT, at 100 mV s^{-1}.

RESULTS AND DISCUSSION

Electrochemical Polymerization of EBT Film on GCE

Poly(EBT) film was electrochemically deposited on the GCE using 0.5 mM EBT in 0.01 M NaOH. During this process, the anodic peaks at ca. 0.17 and 0.37 V corresponding to the oxidation of EBT decreased with increasing sweep number (Fig. 1), indicating the formation of poly(EBT) film.

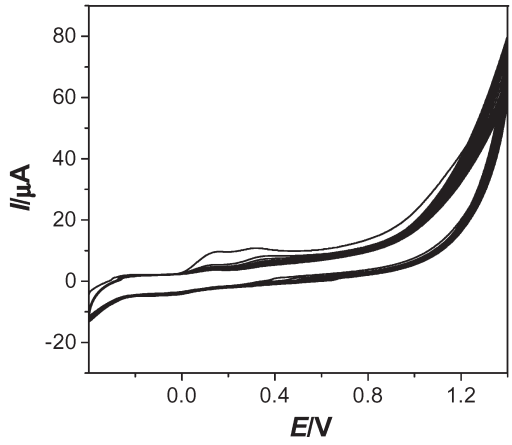


FIG. 1
Voltammetric visualization of successive polymerization

Electrocatalytic Oxidation of NA at Poly(EBT)-Film-Coated GCE

Figure 2 shows the cyclic voltammograms of NA in a phosphate buffer solution (pH 3.5) using a bare GCE, a pretreated GCE and a poly(EBT)-modified

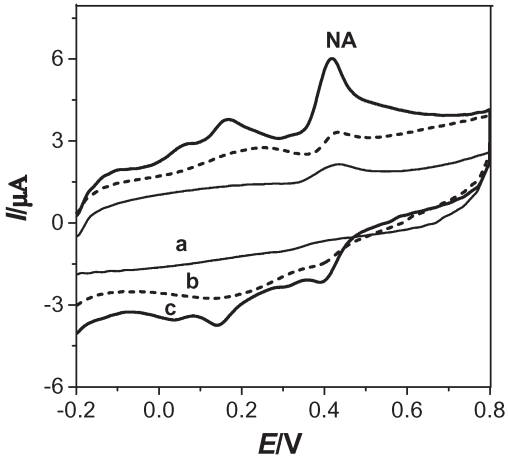


FIG. 2
Cyclic voltammograms of 60 μM NA at bare (a), pretreated (b) and poly(EBT)-modified GCE (c) in PBS (pH 3.5) with a scan rate of 100 mV s^{-1}

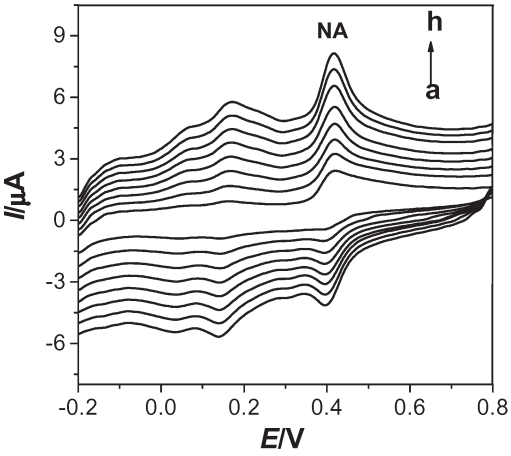
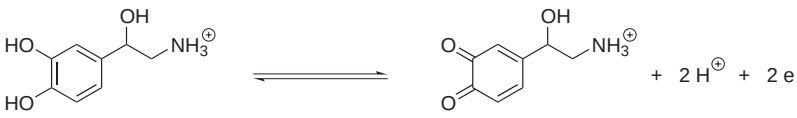


FIG. 3
Cyclic voltammograms of 60 μM NA at the poly(EBT)-modified GCE in PBS (pH 3.5) at scan rate of 20 (a), 40 (b), 60 (c), 80 (d), 100 (e), 120 (f), 140 (g), and 160 (h) mV s^{-1}

GCE. With the bare GCE, NA exhibited only a low and small anodic peak at 0.42 V but no cathodic peak. After the electrochemical pretreatment, a small cathodic peak of NA appeared at 0.4 V. Compared with the bare GCE, the pretreated GCE could improve the electron transfer rate in the electrochemical reaction of NA. However, using the GCE modified with poly(EBT) film, the peak currents increased significantly. A well-defined redox wave of NA was observed with a difference of two peak potentials ΔE_p of 25 mV, which was close to 2.303*RT*/*nF* (or 59/*n* mV) at 25 °C, demonstrating that NA undergoes a two-electron oxidation process at the modified electrode. Substantial increases in peak currents were also observed owing to a dramatic improvement in the reversibility of the electron transfer process and a larger real surface area of polymer film. Moreover, this electrochemical response has good stability, as the peaks remain unchanged after subsequent ten CV scans.

The effect of the scan rate on the anodic peak current of NA was investigated in this work. The oxidation peak current (I_{pa}) increased gradually along with increasing scan rate (Fig. 3). The I_{pa} was directly proportional to the scan rate in the range of 20–160 mV s^{-1}. A linear regression equation was obtained as I_p (μA) = 0.042 *v* (mV s^{-1}) + 1.3432 with a correlation coefficient of 0.9977. This result indicates that the oxidation of NA is a surface-controlled process occurring on the modified electrode surface and that NA possesses a good affinity to the poly(EBT)-film structure.

To investigate the electrochemical reaction mechanism of NA at the poly(EBT)-modified GCE, the effects of pH on the formal potential and anodic peak current were also examined by cyclic voltammetry in 50 mM PBS (pH 3.5) containing 40 μM NA. With pH increasing from 2 to 8, the peak potentials shift towards more negative values. It was also found that the anodic peak potential depends linearly on pH. The regression equation was E_{pa} (V) = –0.0569 *v* (V pH^{-1}) + 0.6143, with a correlation coefficient of 0.9943, suggesting that the proportion of electrons and protons in the redox reaction was 1:1. As the NA oxidation is a two-electron process, the number of protons involved is also predicted to be 2. In accord with the discussion above, the mechanism of the NA oxidation can be proposed (Scheme 1).



SCHEME 1
Oxidation of NA at the polymer-modified GCE

Electrochemical Separation of NA, AA and UA in Solution at Poly(EBT)-Modified GCE

Since NA frequently coexists with AA or UA in biological body fluids and electrochemical response of NA and AA or UA overlap when using the bare electrode, it is very difficult to determine simultaneously NA in the presence of UA and AA by electrochemical methods. The poly(EBT) film shows excellent electrocatalytical activity in the oxidation of NA, which inspired us to attempt at selective detection of NA in a mixture containing NA, AA and UA with the poly(EBT)-film-modified electrode.

The cyclic voltammetric responses of a mixture of 60 μM NA, 200 μM AA and 20 μM UA at a bare GCE, pretreated GCE and a poly(EBT)-modified GCE in 50 mM PBS (pH 3.5) are shown in Fig. 4. At the bare GCE, the graph shows a rather broad oxidation peak and the indistinguishable peak potentials of NA, AA and UA. Using the pretreated GCE, the oxidation peaks of NA, AA and UA occur a little separated, but the NA peak current is very low, which lowers the sensitivity of its detection. However, at the polymer-modified electrode, three well-defined oxidation peaks can be observed at ca. 0.25, 0.40 and 0.55 V for AA, UA and NA, respectively. The separations of the oxidation peak potentials for UA–NA and NA–AA are about 150 mV, which is large enough to permit the simultaneous determination of NA in solutions containing the three compounds.

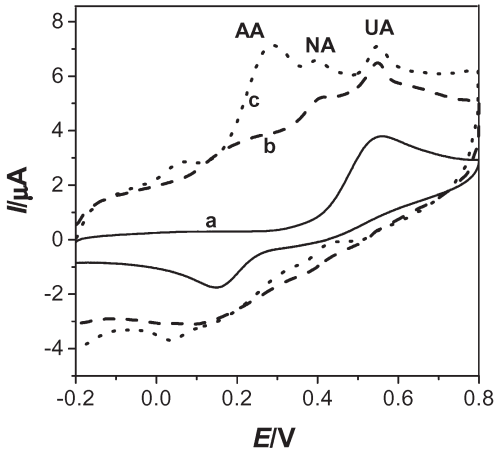


FIG. 4

Cyclic voltammograms of 60 μM NA, 100 μM AA and 20 μM UA at bare (a), pretreated (b) and poly(EBT)-modified GCE (c) in PBS (pH 3.5) with a scan rate of 100 mV s^{-1}

Calibration Curves for Determination of NA

Compared with cyclic voltammetry (CV), the differential pulse voltammetry (DPV) technique shows a higher current sensitivity and better resolution. Hence, the determination of NA concentration in the absence of AA and UA at the poly(EBT)-modified GCE was performed using the DPV method. It was observed that the oxidation peak currents of NA detected by DPV were closely related to pH of the supporting solution. With pH increasing from 2 to 8, the oxidation peak current of NA also gradually increased, which is in line with the results shown in Fig. 5. However, only at pH 3.5, did the separations of oxidation peak potentials for both AA–NA and UA–NA reach a maximum. Therefore, pH 3.5 was selected as the test pH value to gain excellent selectivity.

Of the selected pH, the oxidation peak current of NA was measured in the absence of AA and UA by DPV with the polymer-coated GCE (Fig. 5). The current was dependent on NA concentration. The calibration curve I_p (μA) = 0.0728 C (μmol l^{-1}) + 0.7933 (r = 0.9997) in the range of 0.5–100 μmol l^{-1}, with a detection limit (S/N = 3) of 0.2 μmol l^{-1}, was found. The relative standard deviation (RSD) of ten successive scans was 2.8% for 0.5 μM NA, which shows excellent reproducibility of the poly(EBT)-modified electrode for the determination of NA.

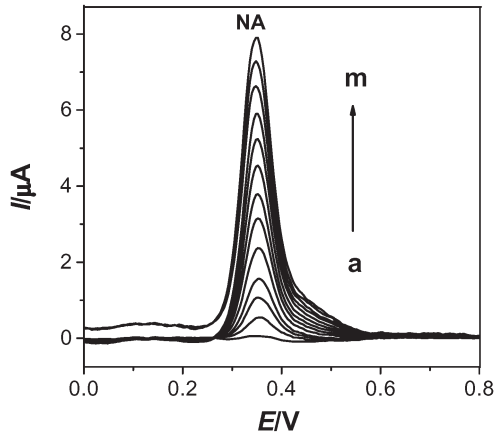


FIG. 5
DPV at the poly(EBT)-film-modified GCE of 0.5 (a), 1.0 (b), 5.0 (c), 10 (d), 20 (e), 30 (f), 40 (g), 50 (h), 60 (i), 70 (j), 80 (k), 90 (l), and 100 (*m*) μM NA in 50 mM PBS (pH 3.5) in the absence of AA and UA

Using the poly(EBT)-modified GCE, the standard curve for the determination of NA in the presence of AA and UA was also examined by DPV from –0.2 to 0.8 V in 50 mM PBS (pH 3.5). The found regression equation was I_p (μA) = 0.0704 C (μmol l^{-1}) + 0.8985 (r = 0.9988) over the range of 1–100 μM NA, which is very similar to the regression equation of NA (see Fig. 6B, ○) in the absence of AA and UA. These results implied that the present method could be used for the determination of NA with a good robustness in a solution containing NA, AA and UA. With the concentration of NA increasing from 1 to 100 μmol l^{-1}, no changes occur in the peak currents and potentials of 80 μM AA and 20 μM UA in DPV at the polymer-modified GCE. The polymer-modified electrode may be used for the selective determination of UA in the presence of AA and NA.

Interference of AA and UA in the Determination of NA in the Presence of AA and UA

The effect of the presence of AA on the determination of NA was investigated by DPV. In the study, the oxidation currents of 50 μM NA at the poly(EBT)-modified GCE were recorded in the presence of 40 μM UA and at increasing concentration of AA (Fig. 7). Little change in the oxidation cur-

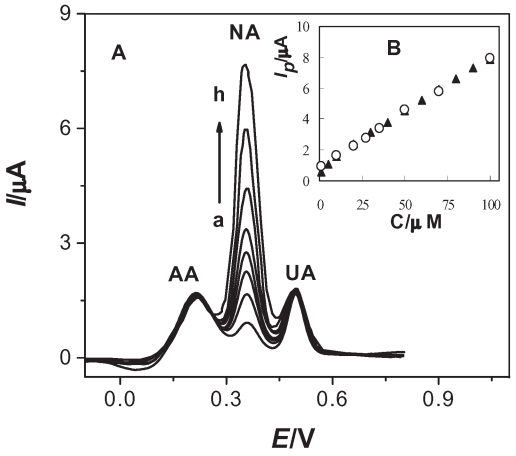


FIG. 6

A DPV at the poly(EBT)-film-modified GCE of 1 (a), 10 (b), 20 (c), 25 (d), 30 (e), 50 (f), 70 (g), and 100 (h) μM NA in 50 mM PBS (pH 3.5) in the presence of 80 μM AA and 20 μM UA. B Plots of the peak current of NA against NA concentration in the absence of AA and UA (▲) and in the presence of 20 μM UA and 80 μM AA (○)

rents of NA and UA was observed while the concentration of AA was lower than 150 mmol l^{-1}.

The effect of UA on the determination of NA was also studied by DPV. As shown in Fig. 8, with UA concentration increasing from 5 to 30 μmol l^{-1}, the peak shape and height of 20 μM NA and 80 μM AA changed only little.

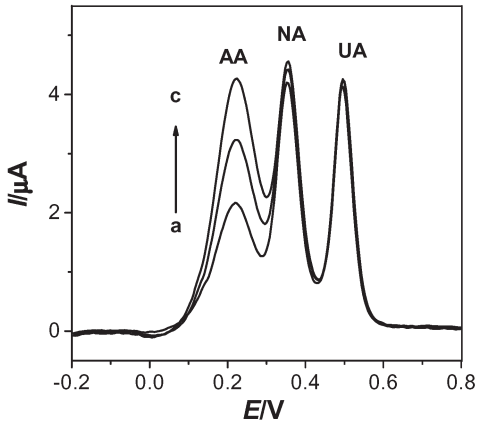


FIG. 7
DPV at the poly(EBT)-film-modified GCE of 100 (a), 150 (b), and 200 (c) μM AA in 50 mM PBS (pH 3.5) in the presence of 50 μM NA and 40 μM UA

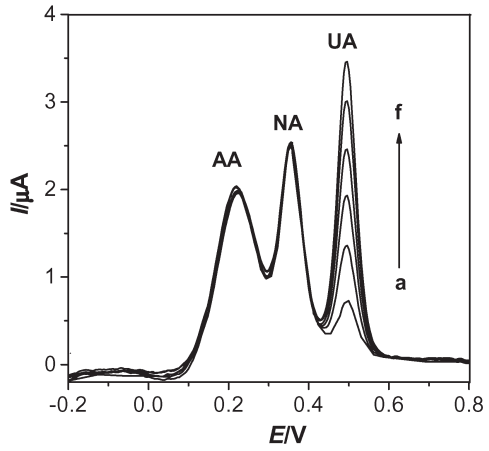


FIG. 8
DPV at the poly(EBT)-film-modified GCE of 5 (a), 10 (b), 15 (c), 20 (d), 25 (e), and 30 (f) μM UA in 50 mM PBS (pH 3.5) in the presence of 80 μM AA and 20 μM NA

All these results suggest that UA and AA to interfere only little with determination of NA by DPV. Interestingly, with increasing concentration of UA, its peak currents correlate linearly with the UA concentration in the range of 5–30 μmol l^{-1}. The respective equation is I_p (μA) = 0.109 C (μmol l^{-1}) + 0.2538 (r = 0.9917). It further suggests that the poly(EBT)-modified electrode can be used for determination of UA in the solutions containing also AA and NA.

Interference of Other Present Materials

The effects of other substances (excipients and common ions) that often accompany NA in various pharmaceutical preparations were studied by analyzing a standard solution of NA (1 μmol l^{-1}). No interference was considered if a foreign species caused a relative error of less than ±5% in the determination of 1 μM NA. No interference has been found in presence of 1000 μM K^+, Zn^{2+}, Ca^{2+}, CO_3^{2-}, NO_3^-, or SO_4^{2-}, and 100 μM sodium citrate, sodium disulfite, starch, lactose, urea, gelatin. The use of 200 μM EDTA or 100 μM AA could eliminate the effect of 50 μM Fe^{3+} in the determination of 1 μM NA.

Applications

The present method was used in determination of NA in pharmaceutical injection (Harvest Pharmaceutical Co. Ltd., Shanghai, China). 20 μM NA injection (2 mg ml^{-1}) was diluted with 10 ml of 50 mM PBS (pH 3.5). The diluted solution was determined by DPV at the poly(EBT)-film-modified GCE. The results are listed in Table I and compared with those obtained by

TABLE I
Determination of noradrenaline in injections

Noradrenaline bitartrate injections	Nominal mg ml^{-1}	Proposed methods[a]		Official methods[a]	
		found, %	RSD, %	found, %	RSD, %
No. 0508271	2	94.6	1.0	98.9	0.8
No. 0506221	2	97.9	2.3	97.5	1.1
No. 0606211	2	95.7	2.1	99.1	0.7

[a] Five determinations for each sample.

an official method. (*The Pharmacopoeia of People's Republic of China*, Part II, 2005.)

To evaluate practicability of the intended method for the determination of NA in a solution containing AA and UA, we prepared three samples by dissolving approximate amounts of NA, AA and UA in a solution. The solution was then diluted with 50 mM PBS (pH 3.5) to fix the concentration of NA in its linear range. NA was determined by using the present method. The results were calculated as 96.5, 95.8, and 97.7% of NA with RSD lower than 3% for five determinations of each sample. The results indicate that the method provides a potential tool for the determination of NA in the presence of AA and UA in their mixture.

CONCLUSIONS

The poly(EBT)-film-modified glassy carbon electrode exhibited high electrocatalytic activities, in the oxidation of NA, AA and UA. The modified electrode provides good selectivity in voltammetric measurements of NA in the presence of AA and UA in aqueous solution. The separations of the oxidation peak potentials for UA–NA and NA–AA are ca. 150 mV. This work proves that EBT film can act as a promoter in the electron transfer in NA oxidation. The method exhibiting good stability, lowed linear range and excellent sensitivity, was used for the determination of NA in real samples with satisfactory results.

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