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ARTICLES

Electrocatalytic Oxidation and Determination of Norepinephrine in the Presence of Ascorbic and Uric Acids at a Poly (Evans Blue)—Modified Glassy Carbon Electrode[1]

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Abstract—A sensitive and selective electrochemical method for the determination of norepinephrine using a poly (Evans Blue) film-modified glassy carbon electrode was developed. The polymer film-modified electrode shows excellent electrocatalytic activity toward the oxidation of norepinephrine (**NE**) in phosphate buffer solution (pH 5.0). The linear range of 5.0×10^{-7}–1.8×10^{-5} M and detection limit of 3.5×10^{-8} M were observed for the determination of NE in pH 5.0 phosphate buffer solutions. The interference studies showed that the modified electrode had excellent selectivity for the determination of NE in the presence of large excess of ascorbic acid (**AA**) and uric acid (**UA**). The differences of the oxidation peak potentials for NE–AA and NE–UA were about 175 and 172 mV, respectively. The resolution is large enough to determine AA, NE and UA individually. This work provides a simple and easy approach to selective detection of NE in the presence of AA and UA in physiological samples.

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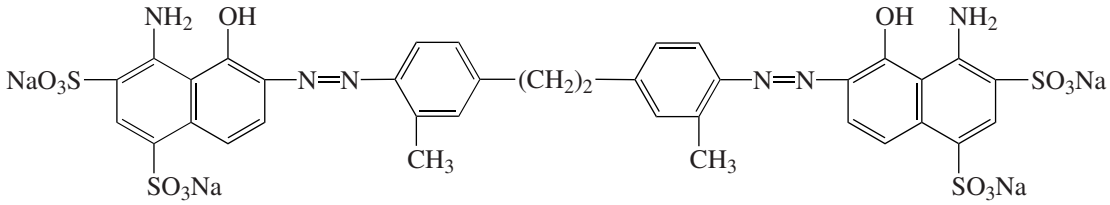
Norepinephrine (NE), an important neurotransmitter of catecholamines, is widely distributed in the mammalian central nervous system for message transfer. The level of NE is important for monitoring and diagnosing diseases [1]. Uric acid (UA) is the primary end product of purine metabolism. Abnormal levels of UA are symptoms of several diseases such as hyperuricaemia, gout and Lesch-Nyan disease [2]. AA is the agent, which prevents scurvy and is known to take part in several biological reactions. NE, UA and AA usually coexist in physiological samples. Therefore, it is essential to develop simple and rapid methods for their selective determination in routine analysis. A variety of techniques have been utilized for the determination of monoamines, such as fluorimetry [3], CE-luminescence [4], etc. However, they have some shortcomings, such as complicated pretreatment, high cost, needs of derivation and time consumption. Electrochemical detection, superior to all these methods, especially in enhancing sensitivity and simplifying operation, is ideal for the analysis of biological samples in which neurotransmitters are at trace, levels.

It is generally believed that direct redox reactions of these species at bare electrodes are irreversible and have overpotentials. The reactions take place at very similar potentials and often suffer from a pronounced fouling effect, which results in rather poor selectivity and reproducibility. The ability to selectively determine NE, UA and AA has been a major goal of electroanalytical researches. Various approaches have been attempted to solve the problems encountering in determination of NE, UA and AA [5–8].



Scheme. Evans blue (EB)

Polymer-modified electrodes prepared by electropolymerization have received extensive interest in the detection of analytes because of high selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the films [9, 10]. In this work, we report for the

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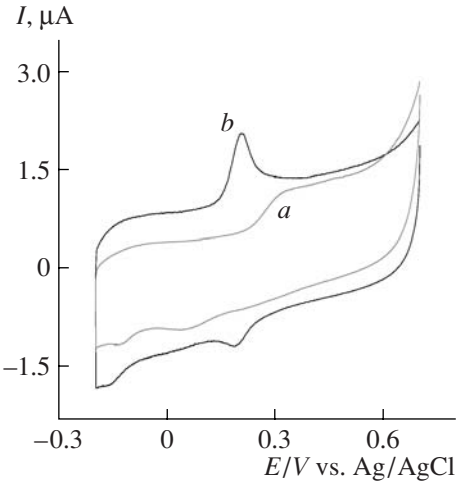

Fig. 1. CVs of 1.0×10^{-5} M NE at (*a*) bare GCE and (*b*) poly EB-modified GCE in pH 5.0 PBS. Scan rate: 100 mVs^{-1}.

first time a glassy carbon electrode (GCE) modified by polymer film of Evans Blue (**EB**) (Scheme) and study on the electrochemical behavior of NE, AA and UA on its surface. Based on the different electrocatalytic activities of the modified electrode toward these species, a sensitive and selective method for selective determination of NE and UA in the presence of AA was set up for routine biomedical research.

EXPERIMENTAL

Reagents and solutions. EB was purchased from Shanghai Chemical Reagents Company (China). NE and UA were obtained from Fluka (Switzerland). L-Ascorbic acid was from Beijing Chemical Factory (China). All reagents were of analytical grade and used without any further purification. Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.05 M NaCl and 0.05 M NaH_2PO_4-Na_2HPO_4, and then adjusting pH with 0.05 M H_3PO_4 or 0.05 M NaOH. All solutions were prepared with double-distilled water. A pH 13 aqueous EB solution, adjusted with 1.0 M NaOH solution, was used for electrochemical polymerization on the GCE.

Apparatus. CHI 660B Electrochemical Workstation (Shanghai CH Instruments, China) was used for electrochemical measurements. A conventional three-electrode system was used throughout the experiments. The working electrode was a bare or a poly (Evans Blue) modified glassy carbon electrode (3.0 mm in diameter, GCE), the auxiliary electrode was a platinum wire and an Ag/AgCl electrode was used as reference. All potentials mentioned in this paper refer to this reference electrode.

Procedure. Before modification, the GCE was polished with 0.3 and 0.05 μm alumina slurries, thoroughly rinsed with water and sonicated in distilled water, ethanol and distilled water again. Then, it was electrochemically activated by using cyclic potential sweep in the range of –0.2–1.8 V in PBS (pH 9) at a scan rate of 100 mVs^{-1} for 40 times. The modified electrode was prepared in 1 mM EB using the same conditions as in the electrode activation procedure. After electropolymerization, the modified electrode was rinsed thoroughly with distilled water.

RESULTS AND DISCUSSION

Electrochemical properties of poly (EB)film-modified GCE. The poly (EB) film on the GCE had a chemically reversible redox couple in 0.1 M H_2SO_4 solution and the peak currents were increased due to the cyclicvoltammetric scan rate. Currents were linearly dependent on the scan rate. The ratio of the anodic peak current to the cathodic peak current, Ipa: Ipc, is almost equal to unity. This behavior is consistent with a diffusionless system, reversible electron transfer process at low scan rates [11]. $\Delta E_p(E_{pa} - E_{pc})$, the difference in peak potentials was 61 mV. ΔE_p is close to 2.3 RT/nF (or 59/n mV at 25°C) [12], so the number of electrons involved in the reaction was 1 ($n \approx 0.97$). An approximate estimate of the surface coverage of the electrode was made by adopting the method used by Sharp et al. [13]. According to this method, the peak current is related to the surface concentration of electroactive species, Γ, by the following equation:

$$I_p = n^2F^2A\Gamma\nu/4RT,$$

where *n* represents the number of electrons involved in the reaction, A is the surface area of the electrode, Γ (mol cm^{-2}) is the surface coverage and other symbols have their usual meanings. From the slope of anodic peak currents versus scan rate the calculated surface concentration of EB is 5.024×10^{-7} mol cm^{-2} for $n = 1$.

The effect of pH values of the supporting solution on the electrochemical behavior of poly (EB)-modified electrode was also studied. Higher pH value made anodic peak potential shift negatively. The plot of peak potential versus pH value showed linearity in the pH value range of 2–8.5 with a slope of –57.6 mV pH^{-1}. This implied that the ratio of the participated protons to the transferred electrons through the poly (EB) film is 1 : 1.

This result indicates that the redox process was confined to the polymer modidied surface of the electrode, confirming the immobilized state of the poly (EB).

Electrochemical behavior of NE at poly (EB)-modified GCE. The cyclic voltammograms of NE in a pH 5.0 PBS at a bare GCE and a poly-EB-modified GCE were recorded (Fig. 1). As can be seen, at a bare GCE, NE shows a sluggish and small CV peak response. After electropolymorization, the anodic peak potential shifted negatively and the cathodic peak potential shifted positively. A well-defined redox wave of NE was observed with $a\Delta E_p$ of 27 mV. Further, substantial increases in peak currents were also observed due to the improvements in the reversibility of the elec-

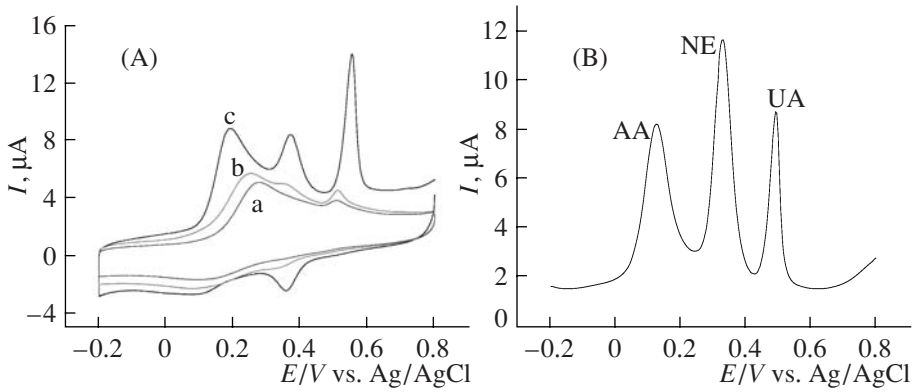

Fig. 2. (A) CVs of 8.0×10^{-5} M AA, 1.2×10^{-5} M NE and 3.0×10^{-6} M UA at (a) bare (b) pretreated (c) poly (EB)-modified GCE in pH 5.0 PBS. (B) DPVs of 8.0×10^{-5} M AA, 1.2×10^{-5} M NE and 3.0×10^{-6} M UA at poly (EB)-modified GCE in pH 5.0 PBS. Scan rate: 100 mVs^{-1}.

tron transfer processes. This suggests an efficient oxidation reaction of NE at the poly-EB-modified GCE.

The effect of scan rate on the anodic peak current of NE was studied. As the scan rate increased, the oxidation peak current (I_{pa}) increased. The I_{pa} was proportional to the scan rate over the range of 20 to 140 mVs^{-1}, which suggested a surface-controlled process in the solution. The linear regression equation was $I_{pa}(\mu A) = 0.0448v\,(mVs^{-1}) + 0.537$, with a correlation coefficient of 0.9992.

The effect of pH on the formal potential ($E^{0'}$) and anodic peak current was investigated by cyclic voltammetry in the solution containing 40 μM NE. The values of $E^{0'}$, which were depended on the pH value of the buffer solution, show that the redox couple of the NE includes some proton transfer in the reduction and oxidation processes. According to the Nernst equation, the slope of –57.6 mV pH^{-1} reveals that the proportion of the electron and proton involved in the reactions is 1 : 1. As NE oxidation is a two-electron process, the number of protons involved is also predicted to be two. Therefore, a mechanism for the NE oxidation could be the following. NE was oxidzied reversibly to the orthobenzoquinone of NE with a two-electron and two-proton transfer process.

The effect of pH on the oxidation potential and current was investigated by cyclic voltammetry in the solution containing 20 μM NE. The E_{pa} vs. pH graph clearly indicates that the catalytic peak shifts to a more negative potential with increasing pH. It could also be seen that the current reached a maximum at pH 5.0. The reduction in currents observed at higher values might correspond to the instability of NE under less acidic conditions.

Determination of NE. Since differential pulse voltammetry has a higher current sensitivity and better resolution than cyclic voltammetry, it was used in determination of NE at the poly (EB)-modified electrode and estimating a lower limit of detection. The oxidation peak currents of NE were measured in 0.05 M pH 5.0 PBS, and plotted against the bulk concentration of NE. The dependence of peak currents on the concentration of NE is a linear relationship in the range of 5.0×10^{-7}–1.8×10^{-5} M. The linear regression equation is expressed as $I_p\,(\mu A) = 0.1694C \times 10^{-6}$ M + 1.9355, r = 0.9956. The detection limit (S/N = 3) is 3.5×10^{-8} M. The relative standard deviation of 10 successive scans is 1.4% for 1×10^{-5} M NE indicating that the poly Evans Blue modified electrode had an excellent reproducibility.

Separation of the electrochemical responses of NE, AA and UA at the poly EB-modified electrode. It is well known that the electrochemical detection of NE in the presence of high levels of AA on untreated carbon-based electrodes or on ordinary electrodes severely struggle due to the catalytic oxidation of AA by NE. The ability of the modified electrode to promote the voltammetric resolution of NE, UA and AA was investigated. The cyclic voltammetric responses of a mixture of 8.0×10^{-5} M AA, 1.2×10^{-5} M NE and 3.0×10^{-6} M UA at a bare GCE, a pretreated GCE and a poly EB-modified GCE in pH 5.0 PBS are recorded. As shown in Fig. 2A (a), a rather broad oxidation peak was obtained and the peak potentials of NE and AA were indistinguishable. It is thus impossible to determine the individual concentrations of these compound from the merged voltammetric peak. Fig. 2A (b) shows that only the signal of AA was clearly observed from a mixture of AA and NE at the pretreated electrode. However, as shown in Fig. 2A (c), the modification of GCE surface with poly-EB film resolved the merged voltammetric peak into three well-defined voltammetric peaks at potentials around 0.306 V, 0.131 V and 0.478 V for NE, AA and UA, respectively. The separation of the oxidation peak potentials for AA–NE, NE–UA and UA–AA are about 175, 172 and 347 mV. Fig. 2B shows that there were 203, 159 and 362 mV in DPV between NE and AA, NE and UA, and UA and AA, respectively. This difference was large enough to achieve the simul-

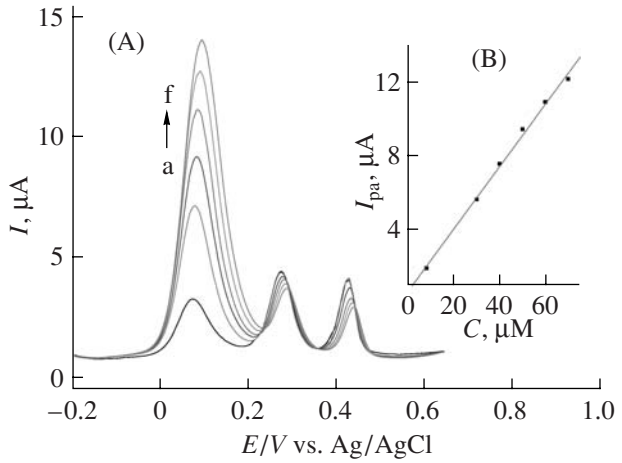

Fig. 3. (A) DPVs of poly (EB)-modified GCE in pH 5.0 PBS containing 1.2×10^{-5} M NE and 3.0×10^{-6} M UA in the presence of AA. AA concentration (M): (a) 8.0×10^{-5}; (b) 3.0×10^{-4}; (c) 4.0×10^{-4}; (d) 5.0×10^{-4}; (e) 6.0×10^{-4}; (f) 7.0×10^{-4}. (B) The relationship between concentrations of AA and the peak current.

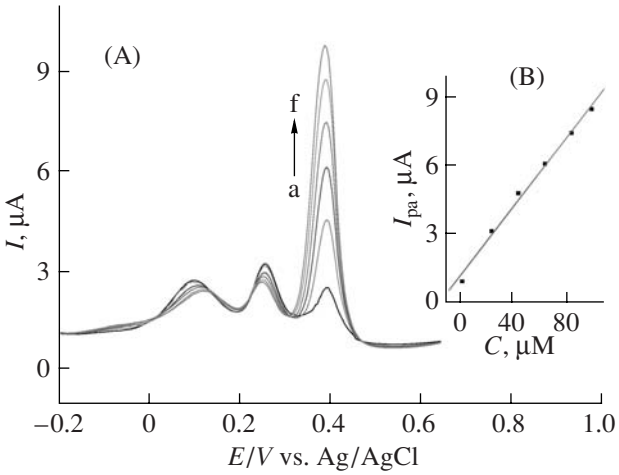

Fig. 5. (A) DPVs at the poly (EB)-modified GCE in pH 5.0 PBS containing 8.0×10^{-5} M AA and 1.2×10^{-5} M NE in the presence of UA. UA concentration (M): (a) 3.0×10^{-6}; (b) 2.5×10^{-5}; (c) 4.5×10^{-5}; (d) 6.5×10^{-5}; (e) 8.5×10^{-5}; (f) 1.0×10^{-4}. (B) The relationship between concentrations of UA and the peak current.

taneous determination of these three compounds in a homogeneous solution. The good separation in peak potential for NE, UA and UA could be attributed to the different adsorption affinity of these compounds on the structure. Further investigation is undergoing in our laboratory.

We carefully examined the oxidation currents of NE and UA at the poly (EB)-modified GCE in the presence of increasing concentration of AA (Fig. 3A). No obvious change in the NE and UA oxidation currents was observed while varying the concentration of AA, and the peak current of AA increased linearly with increasing AA concentration (8.0×10^{-5} to 7.0×10^{-4} M) with a correlation coefficient of 0.9991 (Fig. 3B). Thus, the homogeneous catalytic oxidation of AA by the oxidized NE is advantageously eliminated at the poly (EB)-modified electrode.

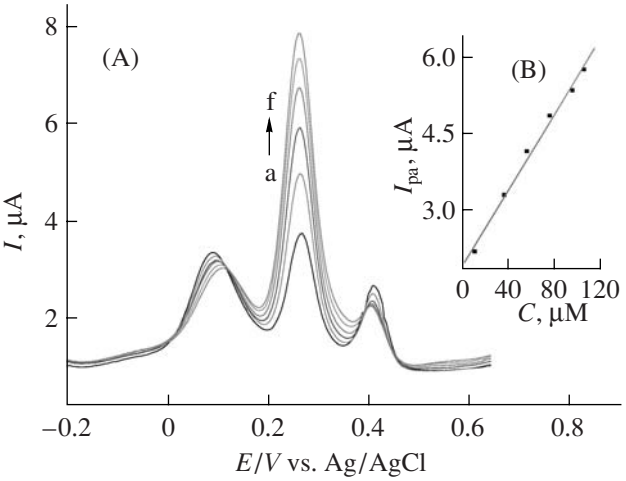

Fig. 4. (A) DPVs of poly (EB)-modified GCE in pH 5.0 PBS containing 8.0×10^{-5} M AA and 3.0×10^{-6} M UA in the presence of NE. NE concentration (M): (a) 1.2×10^{-5}; (b) 4.0×10^{-5}; (c) 6.0×10^{-5}; (d) 8.0×10^{-5}; (e) 1.0×10^{-4}; (f) 1.1×10^{-4}. (B) The relationship between concentrations of NE and the peak current.

As shown in Fig. 4, various concentrations of NE in the presence of 8.0×10^{-5} M AA and 3.0×10^{-6} M UA exhibit excellent differential pulse voltammetric responses while responses to UA and AA are almost constant, indicating that the responses to NE, AA and UA at the poly (EB)-modified electrode are relatively independent. The peak current of NE increased linearly with increasing NE concentration (1.2×10^{-5} to 1.1×10^{-4} M) with a correlation coefficient of 0.9970 (Fig. 4B). Therefore, simultaneous or independent measurements of the three analytes are possible without any interference.

We also carefully examined the oxidation currents of NE and AA at the poly (EB)-modified GCE in the presence of increasing concentration of UA (Fig. 5A). No obvious change in the NE and AA oxidation currents was observed while varying the concentration of UA, and the peak current of UA increased linearly with increasing UA concentration (3.0×10^{-6} M to 1.0×10^{-4} M) with a correlation coefficient of 0.9991 (Fig. 5B). It is very interesting to note that the oxidation processes of NE, AA and UA at the poly (EB)-modified GCE are independent and simultaneous. Hence, independent measurements of the three analytes are possible with a little interference.

Moreover, the electrochemical response has good stability, as the peaks remain unchanged after consecutive 40 cyclic voltammetric scan. As the electrode fabrication is very easy and cheap, the present modified

Table 1. Determination of NE in hydrochloride injection solutions ($n = 5$; $P = 0.95$)

Analyte	Labeled, M	Added, M	Found, M	RSD, %	Recovery, %
NE	3.13×10^{-6}	0	$(2.90 \pm 0.060) \times 10^{-6}$	2.0	–
	3.13×10^{-6}	6.30×10^{-7}	$(3.71 \pm 0.067) \times 10^{-6}$	1.8	92.1
	3.13×10^{-6}	1.26×10^{-6}	$(4.42 \pm 0.84) \times 10^{-6}$	1.9	102.4

Table 2. Determination of AA in hydrochloride injection solutions ($n = 5$; $P = 0.95$)

Analyte	Labeled, M	Added, M	Found, M	RSD, %	Recovery, %
AA	3.55×10^{-4}	0	$(3.56 \pm 0.068) \times 10^{-4}$	1.9	–
	3.55×10^{-4}	5.00×10^{-5}	$(4.06 \pm 0.071) \times 10^{-4}$	1.8	102.4
	3.55×10^{-4}	1.00×10^{-4}	$(4.55 \pm 0.088) \times 10^{-4}$	1.9	99.8

Table 3. Determination of UA in human urine samples ($n = 5$; $P = 0.95$)

Analyte	Volume, μL	Added, M	Found, M	RSD, %	Recovery
urine sample1		0	$(1.52 \pm 0.030) \times 10^{-5}$	2.0	–
	20	5.00×10^{-6}	$(2.02 \pm 0.031) \times 10^{-5}$	1.5	98.0
		1.00×10^{-5}	$(2.58 \pm 0.063) \times 10^{-5}$	2.4	105.5
urine sample2		0	$(2.13 \pm 0.041) \times 10^{-5}$	1.9	–
	20	5.00×10^{-6}	$(2.55 \pm 0.046) \times 10^{-5}$	1.8	83.6
		1.00×10^{-5}	$(2.99 \pm 0.069) \times 10^{-5}$	2.3	85.8

electrode seems to be of great utility for making voltammetric sensor for the detection of neurotransmitters.

Interferences. For investigating the interference, several compounds were selected. If the tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5% relative error, for 1.0×10^{-5} M NE, 1.0×10^{-4} M AA, and 1.0×10^{-2} UA, no interference was observed for the following compounds (10^{-6} M): Na^+ (400), Ca^{2+} (200), Mg^{2+} (200), citric acid (100), cysteine (50), lysine (50), glucose (50).

Determination of NE, AA in injections and UA in human urine samples. 50 μL of the NE hydrochloride injection solution (2 mg/mL, 1 mL per injection), 2.5 μL of the AA hydrochloride injection solution (250 mg/mL, 2 mL per injection) or 20 μL of human urine samples (from two Health Adult) were transferred into a 10 mL volume flask and made up to volume with 0.05 M PBS (pH 5.0), respectively. Then, this test solution was placed in an electrochemical cell for the determination of NE, AA or UA using above DPV method. The results are listed in Tables 1, 2 and 3. Total content was obtained by multiplying the detected value and the diluted factor.

This study has indicated that poly (EB) film-modified GCE exhibits high electrocatalytic activity to NE oxidation. The electrochemical behavior of the modified electrode is strongly dependent on the solution pH. AA, NE and UA coexisting in a homogeneous solution can be simultaneously detected by this modified elec-

trode. The differences in the oxidation peak potentials for AA–NE and AA–UA are about 182 and 362 mV, respectively. Therefore, simultaneous or independent measurements of the three analytes are possible with a little interference. The proposed methods can be applied to the determination of NE, AA and UA in real samples with satisfactory results.

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