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Simultaneous determination of dopamine, ascorbic acid and uric acid at poly (Evans Blue) modified glassy carbon electrode

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Abstract

A sensitive and selective electrochemical method for the determination of dopamine using an Evans Blue polymer film modified on glassy carbon electrode was developed. The Evans blue polymer film modified electrode shows excellent electrocatalytic activity toward the oxidation of dopamine in phosphate buffer solution (pH 4.5). The linear range of 1.0×10^{-6}–3.0×10^{-5} M and detection limit of 2.5×10^{-7} M were observed in pH 4.5 phosphate buffer solutions. The interference studies showed that the modified electrode exhibits excellent selectivity in the presence of large excess of ascorbic acid and uric acid. The separation of the oxidation peak potentials for dopamine–ascorbic acid and dopamine–uric acid were about 182 mV and 180 mV, respectively. The differences are large enough to determine AA, DA and UA individually and simultaneously. This work provides a simple and easy approach to selectively detect dopamine in the presence of ascorbic acid and uric acid in physiological samples.

Keywords: Poly (Evans blue) modified electrode; Electrocatalysis; Dopamine; Uric acid; Ascorbic acid

1. Introduction

Dopamine (DA) is an important neurotransmitter molecule of catecholamines which is widely distributed in the mammalian central nervous system for message transfer. Low levels of DA are related to neurological disorders such as Parkinson's disease, schizophrenia [1–3] and to HIV infection [2,4]. 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 [5]. AA is the agent which prevents scurvy and is known to take part in several biological reactions. DA, UA and AA usually coexist in physiological samples, but there has an overlapping oxidation potential on the solid electrodes. Therefore it is essential to develop simple and rapid methods for their determination in routine analysis. Among many methods for determination of UA, DA and AA in biological samples, voltammetric method has shown to be a powerful tool.

It is generally believed that direct redox reactions of these species at bare electrodes are irreversible and high overpotentials are usually required for their amperometric detections [6]. Moreover, the direct redox reactions of these species at the bare electrodes 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 determine DA, UA and AA selectively has been a major goal of electroanalytical researches[7]. Various approaches have been attempted to solve the problems encountering in simultaneous determination of DA, UA and AA [8–15]. For example, MWNTs-ionic liquid gel modified electrodes have been proposed to detect DA in the presence of ascorbic acid and uric acid with satisfactory results. The current peaks corresponding to these three species were separated by ca. 200 and 150 mV, respectively [12]. Gao and

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Huang reported a remarkable improvement in square wave voltammetric responses of UA, DA and AA and a noticeable enhancement of voltammetric sensitivity was observed at the polypyrrole-tetradecyl sulfate (PPy-TDS) film modified gold electrode. In this case, voltammetric peaks were separated by about 150 mV from each other [15].

Polymer-modified electrodes prepared by electropolymerization have received extensive interest in the detection of analytes because of its high selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the films [16,17]. Protiva Rain Roy et al. [18] has reported Simultaneous electroanalysis of dopamine and ascorbic acid using poly-(*N*,*N*-dimethylaniline) modified electrode. Milczarek and Ciszewski reported an electrode modification with polymeric film of 2,2-bis(3-Amino-4-hydroxyphenyl) hexafluoropropane and studied the electrocatalytic activities toward the oxidation of DA, UA and AA [19].

We have employed poly (cresol red) [20] and poly (Bromophenol Blue) [21] modified electrodes to detect DA and AA. In this work, we report for the first time a polymer film of Evans Blue (Scheme 1) modified glassy carbon electrode and describe electrochemical behavior of DA, AA and UA. Based on the different electrocatalytic activities of the modified electrode toward these species, a sensitive and selective method for simultaneous determination of DA and UA in the presence of AA was set up for routine analysis.

2. Experimental

2.1. Chemicals

Evans Blue was purchased from Shanghai Chemical Reagents Company (China). Dopamine and uric acid 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 the 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 Evans Blue solution, adjusted with 1.0 M NaOH solution, was used for electrochemical polymerization on the glassy carbon electrode.

2.2. 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.

2.3. Preparation of poly-Evans Blue modified glassy carbon electrode

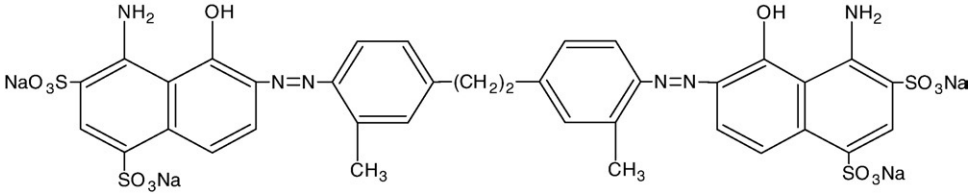
Before modification, the GCE was polished with 0.3 and 0.05 μm alumina slurries, thoroughly rinsed with water and sonicated in distilled water, ethanol and distilled water in turn. Then it was electrochemically activated by using cyclic potential sweep in the range of −0.2 and 1.8 V in PBS (pH 9) at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

3. Results and discussion

3.1. Electrochemical properties of poly-Evans Blue film modified GCE

Before modification, the electrode activated showed a pair of redox peak with a formal potential of 0.26 V (the CV graph is not shown) in 0.1 M H_2SO_4, which resulted from the intrinsic surface chemistry of electrode carbon. After modification with Evans Blue, the electrode still showed a pair of redox peak. But the redox couple shifted positively with a formal potential of 0.34 V and, comparing to the unmodified electrode, the peak currents were also increased drastically, which indicated that poly-Evans blue film was indeed formed on the surface of GCE. In general, the electropolymerization process of Evans Blue film in solution is similar to those of a few azo compounds reported in references [22,23]. According to the reported works, the polymerization reaction could be suggested as follows: (1) $-NH_2$ groups of Evans Blue were first oxidized to −NH&z.rad; free radicals; (2) the two free radicals combined together rapidly to hydrazobenzene sulfonic acid; (3) then hydrazobenzene sulfonic acid was oxidized to azobenzene sulfonic acid, and azobenzene sulfonic acid was reduced to hydrazobenzene sulfonic acid.

Fig. 1A shows that the poly-Evans Blue film on the glassy carbon electrode had a chemically reversible redox couple in 0.1 M H_2SO_4 solution and the peak currents were increased due to the cyclic voltammetric scan rate. As shown in Fig. 1B, I_{pa} was linearly dependent on scan rate. And the ratio of the anodic



Scheme 1. Structure of Evans blue.

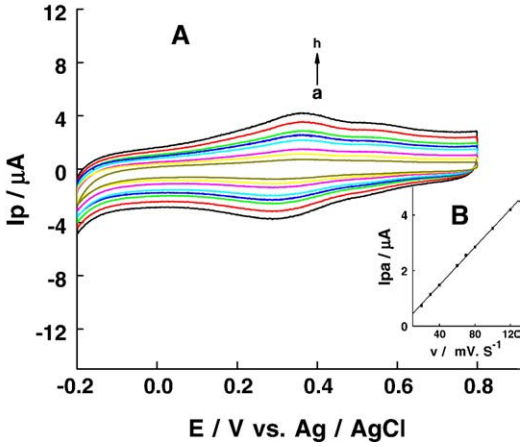


Fig. 1. (A) Cyclic voltammograms of the poly-Evans Blue modified glassy carbon electrode in 0.1 M H_2SO_4 at various scan rates: (a) 20, (b) 30, (c) 50, (d) 60, (e) 70, (f) 80, (g) 100, (h) 120 mV s^{-1}. (B) Plots of peak currents vs. scan rates.

peak current to the cathodic peak current, I_{pa}:I_{pc}, is almost equal to unity. These behaviors are consistent with a diffusionless system, reversible electron transfer process at low scan rates [24]. Literatures have reported the electrochemical reaction mechanisms of some azo compounds film modified electrodes involving in a double electron transfer process [22,23]. So, it suggested that the poly-Evans Blue film modified electrode reaction could be a double electron transfer process (n=2). An approximate estimate of the surface coverage of the electrode was made by adopting the method used by Sharp et al. [25]. According to this method, the peak current is related to the surface concentration of electroactive species, Γ, by the equation:$I_p = n^2F^2A\Gamma v/4RT$.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 (Fig. 1B) the calculated surface concentration of Evans Blue is 1.3×10^{-10} mol cm^{-2} for n=2.

The effect of pH values of the supporting solution on the electrochemical behavior of poly-Evans Blue 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}. These implied that the ratio of the participated protons to the transferred electrons through the poly-Evans Blue film is 1:1.This result indicates that the redox process was confined to the polymer modified surface of the electrode, further confirming the immobilized state of the poly-Evans Blue. According to the previous discussions and references reported [22,23], it can be suggested that the electrode reaction of poly-Evans Blue film is a redox process between azo group and hydroazo groups on the surface of electrode.

3.2. Electrochemistry of single DA, AA and UA at poly-Evans Blue modified GCE

Fig. 2(A) shows the cyclic voltammograms of DA in a pH 4.5 phosphate buffer solution at a bare GCE (curve a) and a poly-Evans Blue modified GCE(curve b). As can be seen, at a bare GCE, DA shows a sluggish and much small CV peak response. The potentials of anodic and cathodic peaks are 0.520 V and 0.198 V, respectively. The separation of redox peak potentials (ΔE_p) is 0.322 V After electropolymerization, the anodic peak potential shifted negatively to 0.423 V and the cathodic peak potential shifted positively to 0.395 V, which results in a well-defined redox wave of DA with a ΔE_p of 28 mV. Further, substantial increases in peak currents were also observed due to the improvements in the reversibility of the electron transfer processes. This suggests an efficient oxidation reaction of DA at the poly-Evans Blue modified GCE.

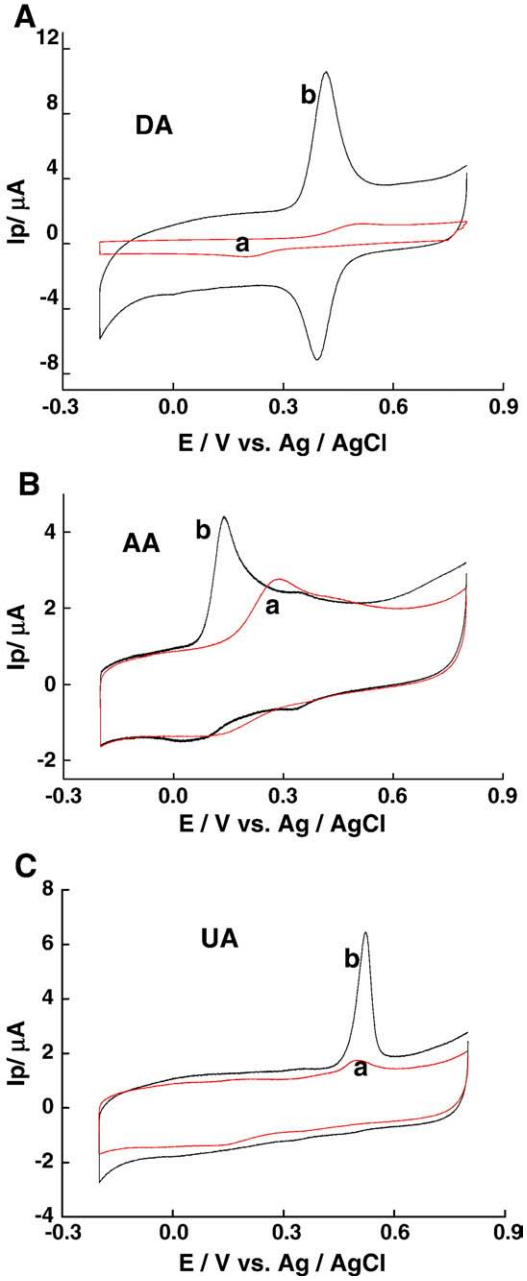
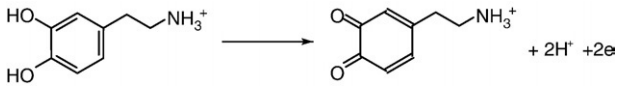


Fig. 2. CVs of 40 μM DA, 20 μM AA and 10 μM UA at (a) bare GCE (b) poly-Evans Blue modified GCE in pH 4.5 PBS. Scan rate: 100 mV s^{-1}.



Scheme 2. Mechanism of DA oxidation at poly-Evans Blue modified GCE.

Fig. 2(B) shows the CVs of AA at a bare (curve a) and the poly-Evans Blue modified GCE (curve b). At the bare GCE, the peak was rather broad, indicating a slow electron transfer kinetic, while at the modified GCE, a sharp oxidation peak at 0.139 V. The 150 mV negative shift and enhanced current of the anodic peak indicates that the poly-Evans Blue modified GCE also plays a strong catalytic effect on the AA oxidation.

Fig. 2(C) depicts the oxidation of UA at the poly-Evans Blue modified GCE (curve b) and bare GCE (curve a). At bare GCE, the oxidation peak potential appeared at 0.502 V. However, at polymer film modified electrode, the oxidation peak potential a little shifted to 0.512 V. It's also obvious to see enhanced peak current at the poly-Evans Blue modified GCE, indicating a strong catalytic effect of the poly-Evans Blue modified layer.

4. Effect of scan rate on the peak current of DA

The effect of scan rate on the anodic peak current of DA was studied. As the scan rate increased, the oxidation peak current (I_{pa}) increased. The I_{pa} was proportional to the square root of scan rate over the range of 20 to 140 mV s^{-1}, which suggested a diffusion-controlled process in solution. The linear regression equation was I_{pa} (μA)=0.770 $v^{1/2}$ (mV s^{-1})−1.421, with a correlation coefficient of 0.9993.

4.1. Effect of pH on the oxidation of DA

The effect of pH on the formal potential and anodic peak current was investigated by cyclic voltammetry in the solution containing 40 μM DA. The values of $E^{o'}$, which was dependent on the pH value of the buffer solution, show that the redox couple of the DA includes some proton transfer in the redox 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 reaction is 1:1. As DA oxidation is a two-electron process, the number of protons involved is also predicted to be two. Therefore, a mechanism for the DA oxidation can be proposed in Scheme 2.

The effect of pH on the electrode signal and oxidation potential was investigated by cyclic voltammetry in the solution containing 40 μM DA (shown in Fig. 3). The E_{pa} vs. pH graph clearly indicates that the catalytic peak shifts to a more negative potential with increasing pH. From Fig. 3A, it could also be seen that the current reached a maximum at pH 4.5. The reduction in currents observed at higher values may correspond to the instability of DA in less acidic conditions.

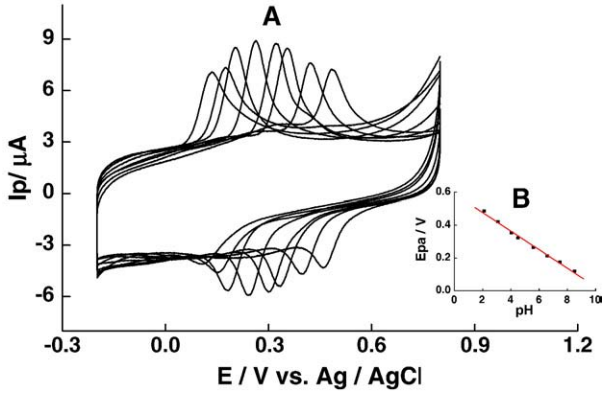


Fig. 3. (A) Cyclic voltammograms of 40 μM DA at the poly-Evans Blue modified GCE in pH: (a) 2.10; (b) 3.08; (c) 4.03; (d) 4.50; (e) 5.60; (f) 6.56; (g) 7.46; (h) 8.50 (B) Plots of peak potentials vs. pH.

4.2. Determination of DA,AA and UA

Since differential pulse voltammetry has a much higher current sensitivity and better resolution than cyclic voltammetry, it was used in determination of DA, AA and UA concentration at

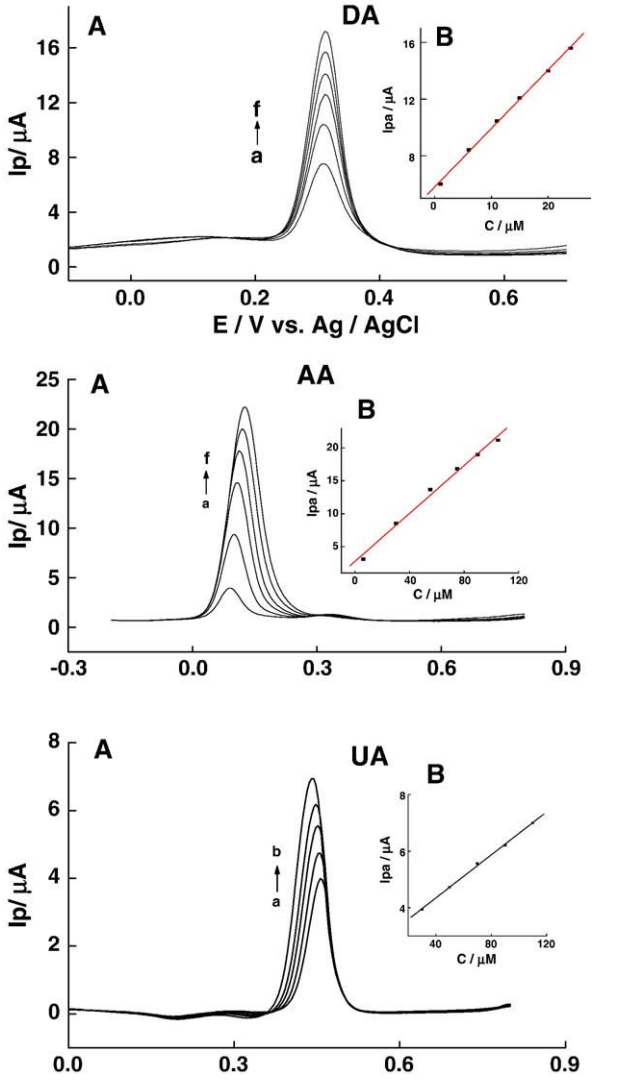


Fig. 4. (A) Differential pulse voltammograms of DA, AA and UA at modified electrode in pH 4.5 PBS. DA concentration (μM): (a) 1; (b) 9; (c) 15; (d) 20; (e) 25; (f) 30. AA concentration (μM): (a) 5; (b) 30; (c) 55; (d) 75; (e) 90; (f) 105. UA concentration (μM): (a) 30; (b) 50; (c) 70; (d) 90; (e) 25; (f) 110. (B) The plot of I_{pa} vs. C.

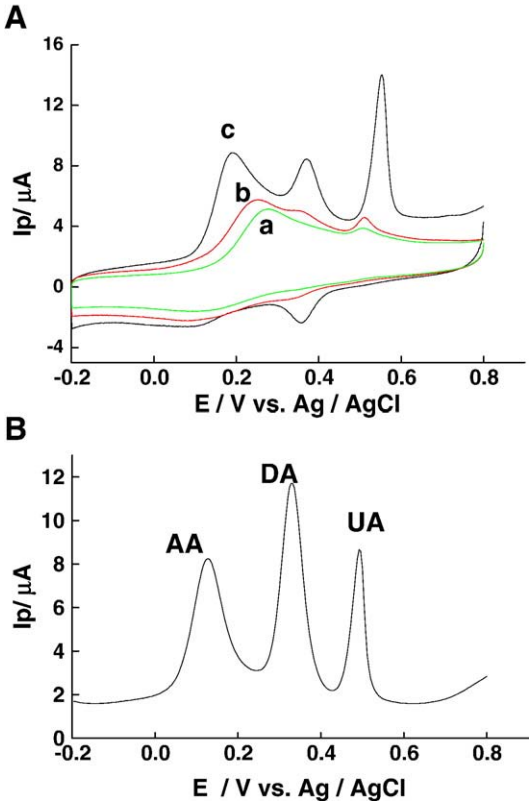


Fig. 5. (A) Cyclic voltammograms at bare (a), pretreated (b) and poly-Evans Blue (c) modified GCE (B) Differential pulse voltammograms of poly-Evans Blue modified GCE. Concentration: 200 μM AA,60 μM DA and 20 μM UA. Scan rate: 100 mV s^{-1}.

the poly -Evans Blue modified electrode and estimating the lower limit of detection. The oxidation peak currents of DA, AA and UA were measured in 0.05 M pH 4.5 PBS, and plotted against the bulk concentration of DA, AA and UA (Fig. 4). The dependence of peak currents on the concentration of DA, AA and UA is a linear relationship in the range of 1–10 μM, 5–105 μM and 30–110 μM. The linear regression equations of DA, AA and UA can be expressed as I_p (μA)=0.3305C (μM)+5.710 (r=0.9995), I_p (μA)=0.1817C (μM)+2.774(r=0.9953),

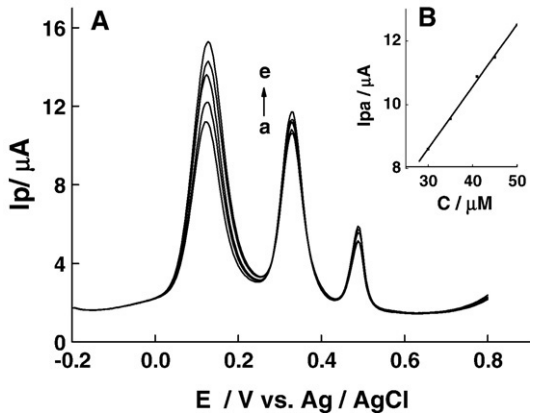


Fig. 6. Differential pulse voltammograms of poly-Evans Blue modified GCE in pH 4.5 PBS containing 60 μM DA and 15 μM UA in the presence of different concentrations of AA. AA concentration (μM): (a) 300; (b) 350; (c) 410; (d) 450; (e) 500.

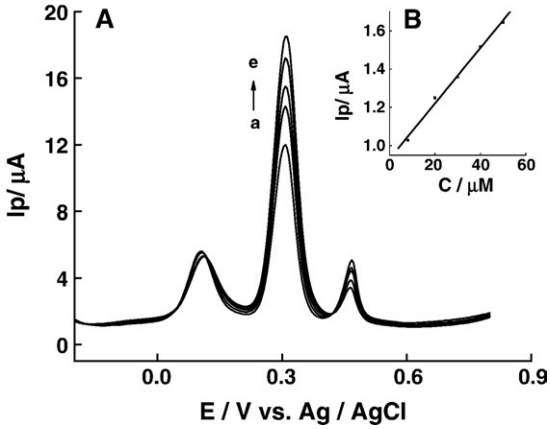


Fig. 7. Differential pulse voltammograms of poly-Evans Blue modified GCE in pH 4.5 PBS containing 150 μM AA and 12 μM UA in the presence of different concentrations of DA. DA concentration (μM): (a) 8; (b) 20; (c) 30; (d) 40; (e) 50.

I_p (μA)=0.038C (μM)+2.825(r=0.9993) respectively. The detection limit (S/N=3) is 0.25 μM, 0.3 μM and 2.0 μM. The relative standard deviations of 10 successive scans are 1.4%, 1.5% and 1.3% for 10 μM DA, 90 μM AA and 200 μM UA. The reproducibility of five different electrodes has been completed. The relative standard deviations are 4.6%, 3.5% and 5.1% for 10 μM DA, 90 μM AA and 200 μM, respectively, which indicate that the poly-Evans Blue modified electrode had an excellent reproducibility.

4.3. Separation of the electrochemical responses of DA, AA and UA at the poly-Evans Blue modified electrode

It is well known that the electrochemical detection of DA 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 DA. The ability of the modified electrode to promote the voltammetric resolution of DA, UA and AA was investigated. The cyclic voltammetric responses of a mixture of 200 μM AA, 60 μM DA and 20 μM UA at a bare

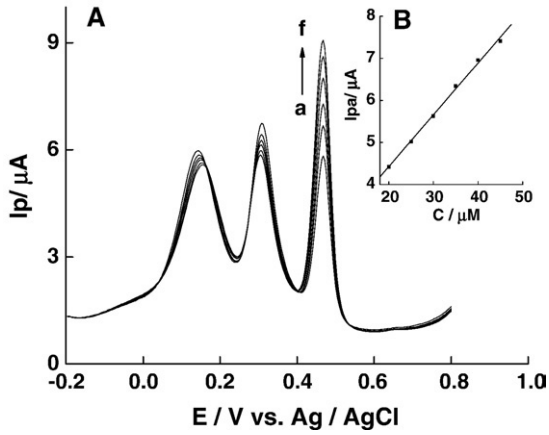


Fig. 8. Differential pulse voltammograms at the poly-Evans Blue modified GCE in pH 4.5 PBS containing 100 μM AA and 10 μM DA in the presence of different concentrations of UA. UA concentration (μM): (a) 20; (b) 25; (c) 30; (d) 35; (e) 40; (f) 45.

Table 1
Interferences of some foreign substances for 10 μM DA, 100 μM AA, and 10 μM UA

Foreign substances	Tolerance level (μM)
Starch	400
K^+ Mg^{2+} Ca^{2+} Zn^{2+}	200
Citric acid	100
Cysteine, lysine, glucose	50

GCE, a pretreated GCE and a poly-Evans Blue modified GCE in pH 4.5 PBS are shown in Fig. 5. Fig. 5A (a) shows the cyclic voltammogram obtained at the bare GCE. A rather broad oxidation peak was obtained and the peak potentials of DA and AA were indistinguishable. It is thus impossible to determine the individual concentrations of these compound from the merged voltammetric peak. Fig. 5A (b) shows that only the signal of AA was clearly observed from a mixture of AA and DA at the pretreated electrode. But as shown in Fig. 5A (c), modification of GCE surface with poly-Evans Blue film resolved the merged voltammetric peak into three well-defined voltammetric peaks at potentials around 0.373 V and 0.191 V and 0.553 V for DA, AA and UA, respectively. The separation of the oxidation peak potentials for AA–DA, DA–UA and UA–AA are about 182 mV, 180 mV and 362 mV. Fig. 5B shows that there were 204 mV, 160 mV and 364 mV in DPV between DA and AA, DA and UA, and UA and AA respectively. This separation was large enough to achieve the simultaneous determination of these three compounds in a homogeneous solution. The good separation in peak potential for DA, UA and AA could be attributed to the different adsorption affinity of these compounds on the structure. More investigation is undergoing in our laboratory.

We carefully examined the oxidation current of DA and UA at the poly-Evans Blue modified GCE in the presence of increasing concentration of AA (Fig. 6 A). No obvious change in the DA and UA oxidation currents was observed while varying the concentration of AA, and the peak current of AA increased linearly with increasing AA concentration (300 μM to 500 μM) with a correlation coefficient of 0.9989 (Fig. 6B). Thus the homogeneous catalytic oxidation of AA by the oxidized DA is advantageously eliminated at the poly-Evans Blue modified electrode.

As shown in Fig. 7A, various concentrations of DA in the presence of 150 μM AA and 12 μM UA exhibit excellent differential pulse voltammetric responses with the responses to UA and AA keeping almost constant, indicating that the responses to DA, AA and UA at the poly-Evans Blue modified electrode are relatively independent. The peak current of DA increased linearly with increasing DA concentration (8 μM to 50 μM) with a correlation coefficient of 0.9970 (Fig. 7B).

Table 2
Determination of DA in hydrochloride injection solutions ($n=5$)

Batch no	Labeled (μM)	Spiked (μM)	Found (μM)	R. S. D. (%)	Recovery
060706	19.62	10.0	29.59	1.9	97
060711	19.62	10.0	29.63	1.7	101
060720	19.62	10.0	29.60	1.8	98

Table 3
Determination of AA in hydrochloride injection solutions ($n=5$)

Batch no	Labeled (μM)	Spiked (μM)	Found (μM)	R. S. D. (%)	Recovery (%)
060729	355.0	50.0	406.0	1.9	101
060730	355.0	50.0	406.2	1.8	105
060732	355.0	50.0	404.8	1.9	99

Therefore simultaneous or independent measurements of the three analytes are possible.

We also carefully examined the oxidation currents of DA and AA at the poly-Evans Blue modified GCE in the presence of increasing concentration of UA (Fig. 8A). No obvious change in the DA and AA oxidation currents was observed while varying the concentration of UA, and the peak current of UA increased linearly with increasing UA concentration(20 μM to 45 μM) with a correlation coefficient of 0.9985 (Fig. 8B). It is very interesting to note that the oxidation processes of DA, AA and UA at the Evans Blue modified GCE are independent and simultaneous or independent measurements of the three analytes are possible without any interference.

Moreover, this electrochemical response has good stability, as the peaks remain unchanged after consecutive 40 cyclic voltammetric scan. As the electrode fabrication is very easy and low cost, the present modified electrode seems to be of great utility for making voltammetric sensor for the detection of neurotransmitters.

4.4. 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 15 μM DA, 100 μM AA, and 10 μM UA, no interference was observed for the following compounds (μM): K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, starch, citric acid, cysteine, lysine, glucose. The results are listed in Table 1.

4.5. Determination of DA in dopamine hydrochloride injection, AA in hydrochloride injection solutions and UA in human urine samples

3 μL of the dopamine hydrochloride injection solution (10 mg mL^{-1}, 2 mL per injection), 2.5 μL of the AA hydrochloride injection solution (250 mg mL^{-1}, 2 mL per injection) and 20 μL

Table 4
Determination of UA in human urine samples

Analyte	Labeled (μM)	Spiked (μM)	Found (μM)	R. S. D (%)	Recovery (%)
Healthy Adult	15.12	5	20.25	2.0	115
	15.09	5	20.15	1.6	98
	15.76	5	25.80	1.6	113
Patient	21.35	5	26.32	1.9	99
	21.56	5	26.59	1.7	104
	23.67	5	28.70	2.1	103

of the UA in human urine samples (they came from our lab and The First Affiliated Hospital of Fujian Medical University) were injected into a 10 mL volume flask and made up to volume with 0.05 mol L^{-1} PBS (pH 4.5) respectively. Then this test solution was placed in an electrochemical cell for the determination of DA, AA and UA using the above DPV method. The results are listed in Tables 2,3 and 4.

5. Conclusions

This study has indicated that poly-Evans Blue film modified glassy carbon electrode exhibits highly electrocatalytic activity to DA oxidation. The redox response of the modified electrode is that it anticipated for a surface-immobilized redox couple. The electrochemical behavior of the modified electrode is strongly dependent on the solution pH. AA, DA and UA coexist in a homogeneous solution can be simultaneously detected by this modified electrode. And the separation of the oxidation peak potentials for AA–DA and AA–UA are about 182 and 362 mV, respectively. Therefore, simultaneous or independent measurements of the three analytes are possible without any interference. The proposed methods can be applied to the determination of DA, AA and UA in real samples with satisfactory results.

Acknowledgements

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