Simultaneous Voltammetric Determination of Ascorbic Acid, Dopamine and Uric Acid Using Polybromothymol Blue Film-Modified Glassy Carbon Electrode

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A sensitive and selective electrochemical method for simultaneous determination of ascorbic acid (AA), dopamine (DA), and uric acid (UA) using an electropolymerized bromothymol blue (BTB)-modified glassy carbon electrode (GCE) was developed. The electrochemically synthesized film was investigated using electrochemical impedance spectroscopy and voltammetric methods. The electrochemical behavior of the polymer-modified electrode depends on film thickness, *i.e.*, the electropolymyerization time. The poly-BTB-modified GCE shows excellent electrocatalytic activity toward the oxidation of AA, DA, and UA in phosphate buffer solution (pH 5.0). The voltametric peak separations of AA/DA, DA/UA, and AA/UA on this modified electrode are 118 mV, 298 mV, and 455 mV, respectively. Therefore the voltammetric responses of these three compounds can be resolved well on the polymer-modified electrode, and simultaneous determination of these three compounds can be achieved. In addition, this modified electrode can be successfully applied to determine AA and DA in injection and UA in urine samples without interference.

Key words polybromothymol blue-modified electrode; electrocatalysis; ascorbic acid; dopamine; uric acid

Ascorbic acid (AA), dopamine (DA), and uric acid (UA) are compounds of great biomedical interest and play determining roles in human metabolism. AA is a vital vitamin in the diet of humans and is present in the mammalian brain along with several neurotransmitter amines. AA has been used for the prevention and treatment of the common cold, mental illness, infertility, cancer, and AIDS.[1)] 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 neurologic disorders such as Parkinson's disease and schizophrenia[2,3)] and to HIV infection.[4,5)] UA is the primary end-product of purine metabolism. It has been shown that the extreme abnormalities of UA levels in the body are symptoms of several diseases, such as gout, hyperuricemia, and Lesch–Nyan disease.[6)] AA, DA, and UA usually coexist in physiologic samples, but there is an overlapping oxidation potential on solid electrodes. Therefore it is essential to develop simple, rapid methods for their determination in routine analysis. Among many methods for the determination of AA, DA, and UA in biological samples, the voltammetric method is a powerful tool.

It is generally believed that direct redox reactions of these species at conventional electrodes are irreversible, and high overpotentials are usually required for their amperometric detections.[7)] Moreover, these redox reactions take place at similar potential and often show 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 research.[8)] Various approaches have been attempted to solve the problems encountered in the simultaneous determination of AA, DA, and UA.[9—16)] For example, Multi-Wall Carbon Nanotubes (MWNT)s-ionic liquid gel-modified electrodes have been used to detect DA in the presence of AA and UA with satisfactory results. The voltametric peaks corresponding to these three species were separated by *ca.* 200 and 150 mV, respectively.[13)] Gao and Huang reported a remarkable improvement in the square-wave voltammetric responses of AA, DA, and UA at the polypyrrole-tetradecyl sulfate (PPy-TDS) film-modified gold electrode. In this case, voltammetric peaks were separated by about 150 mV.[16)]

Polymer-modified electrodes prepared by electropolymerization have received extensive interest in the detection of analytes because of their high selectivity, sensitivity, and homogeneity in electrochemical deposition, strong adherence to the electrode surface, and chemical stability of the films.[17,18)] Roy *et al.*[19)] reported simultaneous electroanalysis of DA and AA using a poly-(*N*,*N*-dimethylaniline)-modified electrode. Milczarek and Ciszewski[20)] reported an electrode modified with polymeric film of 2,2-bis(3-amino-4-hydroxyphenyl) hexafluoropropane and studied the electrocatalytic activities in the oxidation of DA, UA, and AA. We reported electrodes modified with poly (cresol red),[21)] poly (calconcarboxylic acid),[22)] poly (chromotrope 2B),[23)] poly (eriochrome black T),[24)] and poly (4-(2-pyridylazo)-resorcinol)[25)] and their application in the electroanalysis of neurotransimitters. All theses reports have their advantages and limitations. Thus there is an expanding demand for the development of facile, rapid, efficient electrochemical sensors with improved performance for effective sensing of AA, DA, and UA individually or simultaneously.

The triphenylemothane derivative-modifed glassy carbon electrode showed (GCE) high electrocatalytic activity on AA and DA.[26)] Its polymerization product on the electrode suface forms a dendrimer, a tree-like dendritic structure.[27)] Bromothymol blue (BTB; Chart 1) is also a triphenylmethane, and its structure is similar to that of pyrocatechol sulfonephthalein. The electropolymerization of cresol red has not been reported so far. In this paper, we report for the first time a

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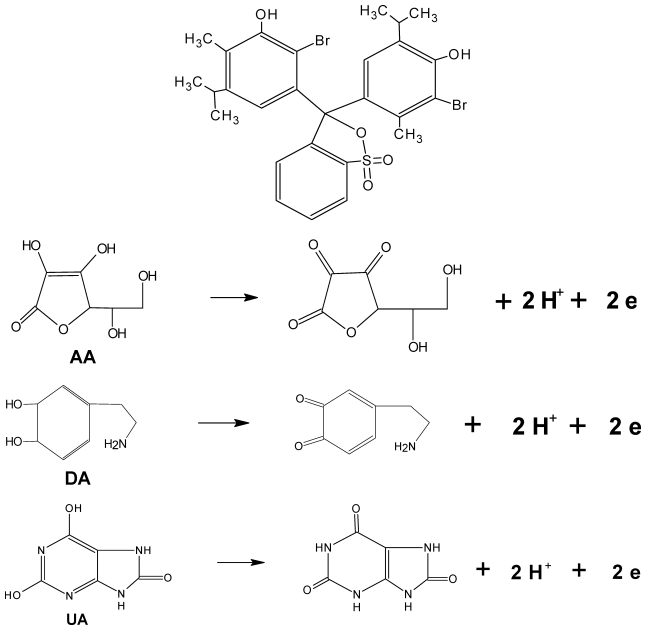

Chart 1. Chemical Structure of BTB and Proposed Electrocatalytic Reaction of AA, DA, and UA at a Poly-BTB-Modified GCE

polymer film of a BTB- modified GCE that can individually and simultaneously analyze AA, DA and UA. The electrochemical behavior of AA, DA, and UA on this poly-BTB-modified GCE was studied.

Experimental

Reagents and Apparatus BTB was purchased from Shanghai Chemical Reagents Company (China). AA, DA, and UA were obtained from Fluka (Switzerland). All reagents were of analytical grade and used without any further purification. Phosphate buffer solutions (PBS) were prepared by mixing stock solutions of NaCl 0.05 M and NaH_2PO_4–Na_2HPO_4 0.05 M, and then adjusting the pH with H_3PO_4 0.05 M or NaOH 0.05 M. All solutions were prepared with double-distilled water. Freshly prepared AA, DA, and UA solutions were used for the measurements.

Electrochemical measurements were performed on a CHI 660C electrochemical workstation (CH Instruments, U.S.A.). A conventional three-electrode system was used throughout the experiments. The working electrode was a bare, a pretreated, or poly-BTB modified GCE (3.0 mm in diameter); the auxiliary electrode was a platinum wire and an Ag/AgCl electrode as the reference. All potentials in this paper refer to this reference electrode. All experiments were carried out in PBS (0.05 M, pH 6.0) at room temperature (25±1 °C). Cyclic voltammetric experiments were carried out with a scan rate of 100 mV s^{-1} unless otherwise stated.

Preparation Procedure of Poly-BTB-Modified GCE A bare GCE was polished successively with 0.3 μm and 0.05 μm of Al_2O_3 slurry on silk. Then, it was rinsed with double distilled water and sonicated in a 1 : 1 aqueous HNO_3 solution, ethanol, and double distilled water in turn for 10 min. After being cleaned, the electrode was immersed in an H_2SO_4 0.05 M solution and was pretreated with cyclic potential scanning from −0.7 to 1.8 V at 100 mV s^{-1} for 40 cycles. Then, electrochemical modification of the pretreated GCE was performed using cyclic voltammetry in pH 5.0 PBS containing 0.2 mM BTB at a scan rate of 100 mV s^{-1} for 15 cycles. After the electropolymerization, the electrode was rinsed thoroughly with distilled water for further application.

Results and Discussion

Preparation of Poly-BTB-Modified GCE The potential scan range, especially the positive potential, markedly affects the formation of polymerization film. It was found that the electropolymerization of BTB was difficult to initiate when the positive limited potential was less than +1.5 V. Figure 1 displays the continuous cyclic voltammograms (CVs) for the

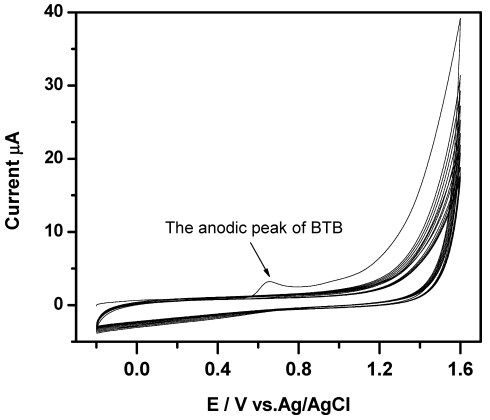

Fig. 1. CVs of Bromothymol Blue (0.2 mM) in PBS 0.05 M (pH 5.0) at a GCE in the Potential Range from −0.2 to 1.6 V at a Scan Rate 100 mV s^{-1} for 15 Cycles

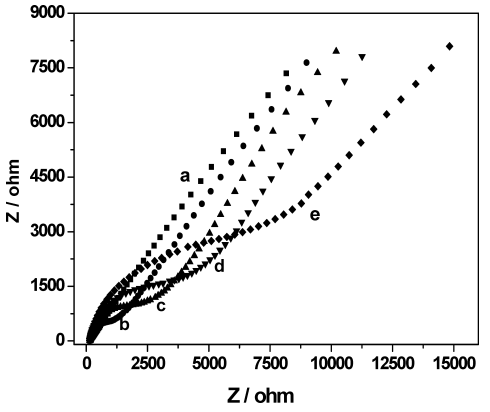

Fig. 2. Nyquist-Diagram (Imaginary Part Z_{Im} *vs.* Real Part Z_{Re}) for the Electrochemical Impedance Measurements of Electrodes in a Solution of KCl 0.1 M Containing 10 mM $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ 10 mM

(a) Bare GCE; (b—e) the poly-BTB film modified GCE with different electropolymerization cycles. Cyclic times: (b) 1; (c) 10; (d) 15; and (e) 20. The electrochemical impedance spectra were recorded in the frequency range from 0.1 to 1.0×10^6 Hz at the formal potential of $Fe(CN)_6^{3-/4-}$ redox couple and with a perturbation potential of 5 mV.

electrochemical polymerization of BTB in the potential window of −0.2 to 1.6 V at 100 mV s^{-1} for 15 cycles. It is clear that the anodic peak at about 0.65 V corresponding to the oxidation of BTB monomer decreased sharply for the first two cycles (Fig. 1). Upon further potential cycling, it decreased gradually (Fig. 1). This phenomenon implies the formation of a poly-BTB membrane on the GCE surface. After electropolymerization, the modified electrode was thoroughly rinsed with double distilled water and then stored PBS at pH 5.0. This modified electrode was used within 4 weeks.

Impedance Characterization of Poly-BTB Film Impedance spectra of the modified electrodes with different electropolymerization cycles in a solution of KCl 0.1 M containing $[Fe(CN)_6]^{3-}$ 10 mM and $[Fe(CN)_6]^{4-}$ 10 mM were collected at a potential of 0.22 V *vs.* Ag/AgCl in the frequency range from 0.1 to 10^6 Hz. As shown in Fig. 2, the redox process of the $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ probe showed an electron transfer resistance of about 63 Ω at the bare GCE (Fig. 2, curve a). When BTB was electropolymerized on the electrode surface, the electron transfer resistance increased significantly for the first cycle (R_{ct}=1132 Ω), then did increase with the electropolymerization time (Fig. 2, curves

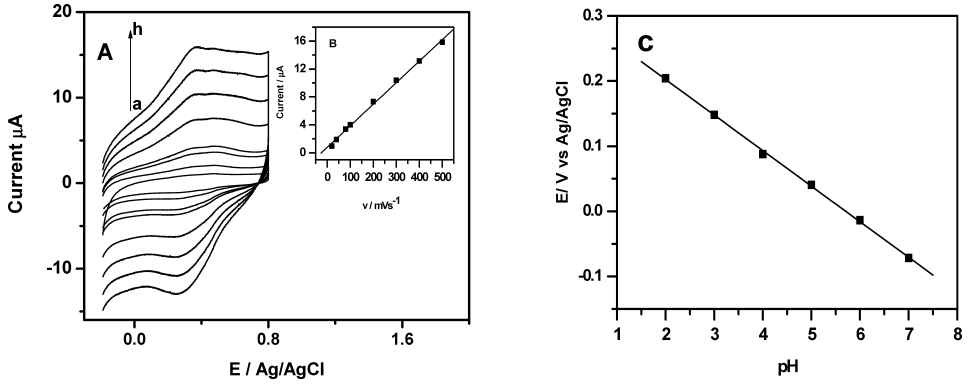

Fig. 3. (A) CVs of the Poly-BTB-Modified GCE in H_2SO_4 0.05 M at Various Scan Rates: (a) 20, (b) 40, (c) 80, (d) 100, (e) 200, (f) 300, (g) 400, and (h) 500 mV s^{-1}, (B) Plot of Peak Current *vs.* Scan Rate and (C) the Effect of pH on the Electrochemical Behavior of the Poly-BTB-Modified GCE in PBS 0.05 M with Different pH Values

b—e). It is evident that a poly-BTB film on the bare GCE surface was already formed after 1 cyclic of electropolymerization.

Electrochemical Properties of the Poly-BTB Film-Modified GCE The CVs of a poly-BTB film-modified GCE in an H_2SO_4 0.05 M solution at different scan rates are shown in Fig. 3. There appears to be a well-defined redox couple. The peak current increases with increasing scan rate. As shown in Fig. 3B, the anodic peak current (I_{pa}) is directly proportional to the scan rate (v) in the range from 20 to 500 mV s^{-1} ($I_{pa}=1.541+8.0442v$, $r=0.9973$). The ratio of the anodic peak current to cathodic peak current (I_{pc}) is almost equal to unity. These results demonstrate that the electrochemistry of the poly-BTB-modified GCE corresponding to a surface-controlled process is reversible. The potential separation of the anodic and cathodic peaks is 75 mV, which implies that one electron is involved in the electrochemical process.[28] The transfer coefficient (α) is estimated to be 0.762 from the peak width at half-height in terms of the method developed by Laviron.[29]

Using the method suggested by Sharp *et al.*,[30] the amount of the poly-BTB on the GCE surface can be estimated. The surface concentration of the electroactive species, Γ (mol cm^{-2}), is proportional to the peak current according to the following equation:

$$I_p=n^2F^2A\Gamma v/4RT \quad (1)$$

where n is the number of electrons involved in the reaction; A is the geometric surface area of the electrode (0.0314 cm^2); v is the scan rate; and R, F, and T have their normal meanings. From the slope of the anodic peak current *versus* scan rate (Fig. 3B), the surface concentration of poly-BTB was determined to be 1.04×10^{-9} mol cm^{-2}.

The influence of the pH value on the electrochemistry of the poly-BTB film-modified GCE was then studied. As shown in Fig. 3C, the anodic peak potential decreased linearly with the increase in solution pH. The slope is −54.4 mV/pH in the pH range from 2 to 7, which follows the Nernst equation. It can therefore be concluded that one electron and one proton are involved in the electrode reaction of the poly-BTB-modified GCE.

Electrochemical Oxidation of AA, DA, or UA at the Poly-BTB-Modified GCE The poly-BTB-modified GCE shows excellent electrocatalytic activity in the oxidation of

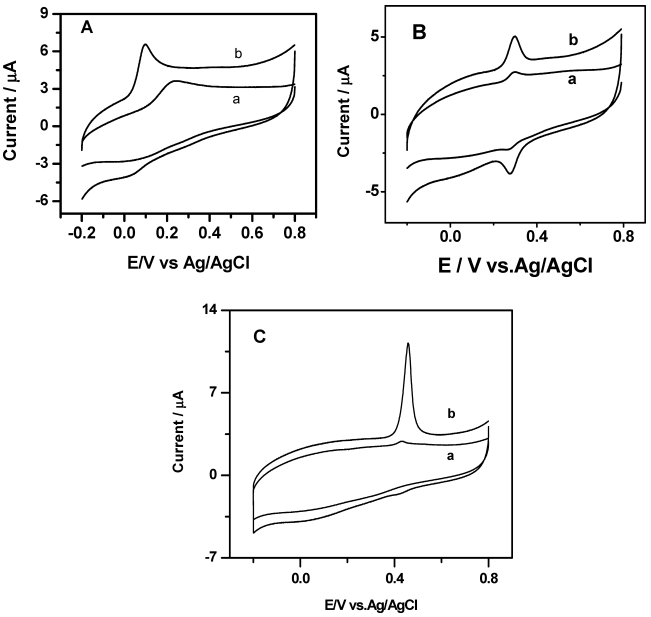

Fig. 4. CVs of (A) AA 20 μM, (B) DA 10 μM, and (C) UA 10 μM at a pretreated GCE (a) and a Poly-BTB-Modified GCE (b) in PBS pH 5.0

Scan rate: 100 mV s^{-1}.

AA, DA, or UA. Figure 4A shows the CV of AA at a pretreated GCE (curve a) and a poly-BTB-modified GCE (curve b) in PBS, pH 5.0. At the pretreated GCE, the peak was rather broad, indicating a slow electron transfer kinetic. The modification of poly-BTB leads to a sharp oxidation peak at 0.096 V. Furthermore, the enhanced current of the anodic peak indicates a strong electrocatalytic effect of poly-BTB on AA oxidation. It was found that the anodic peak current was proportional to the square root of the scan rate in the range from 20 to 500 mV s^{-1} [$I_{pa}(\mu A)=0.03145v(\text{mV s}^{-1})+0.3423$, $r=0.9991$], showing a surface-controlled process.

The CVs of DA at a pretreated GCE (curve a) and a poly-BTB-modified GCE (curve b) in a PBS 0.05 M (pH 5.0) are shown in Fig. 4B. At the bare GCE, DA shows a sluggish and much smaller CV peak response. Substantial increases in peak currents of DA can be observed at the poly-BTB-modified GCE. This suggests an efficient oxidation reaction of DA at the poly BTB-modified GCE. At the poly-BTB modified GCE, the potentials of anodic and cathodic peaks are 0.298 V

and 0.274 V, respectively. The separation of the redox peak potentials (ΔE_p) is 24 mV. In addition, the anodic peak current was directly proportional to the scan rate in the range from 20 to 500 mVs^{-1} [$I_{pa}(\mu A)=0.04641v(\text{mV s}^{-1})+2.091$, $r=0.9991$]. These phenomena suggest that the oxidation reaction of DA at the poly-BTB modified electrode is controlled by a surface process.

Figure 4C shows the CVs of UA at a pretreated GCE (curve a) and a poly-BTB-modified GCE (curve b). The electrochemical response of UA on the pretreated GCE only showed a small oxidation peak at about 431 mV. The larger peak separation and broad reduction peak demonstrated that the electrochemical behavior of UA is irreversible at the pretreated GCE. At the poly-BTB-modified GCE, a sharp anodic peak at 458 mV was observed, indicating that the poly-BTB film showed good electrocatalytic activity in the oxidation of UA. The anodic peak current was directly proportional to the scan rate in the range from 20 to 500 mV s^{-1} [$I_{pa}(\mu A)=0.06238v(\text{mV s}^{-1})+3.723$, $r=0.9982$], which demonstrates that the electrode process is controlled by a surface process.

The amount of the poly-BTB film on the GCE can be increased by continuous potential scans. It was found that the electrochemistry of AA, DA, or UA on the poly-BTB-modified GCE showed strong dependence on the thickness of poly-BTB film deposited on the GCE surface. The anodic peak current of AA, DA, or UA increased almost linearly with the thickness of the polymer film in the first 15 potential scans of electrochemical polymerization (Fig. 5). Then, their oxidation currents decreased significantly. This decrease in peak currents could be due to the low conductivity of the polymer film. Therefore, in the following measurements, the poly-BTB-modified electrode was prepared with 15 cycles of potential scans.

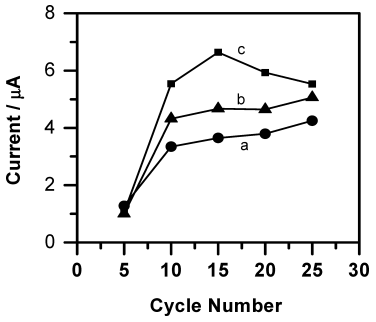

Fig. 5. Influence of Electropolymerization Cyclic Times on the Electrochemical Responses in DPV of the Poly-BTB-Modified GCE in the Single Determination of AA 20 μM (a), DA 10 μM (b), or UA 10 μM (c)

Cyclic times: (a) 5, (b) 10, (c) 15, (d) 20, and (e) 25. Scan rate: 100 mV s^{-1}.

Electrochemical Oxidation of AA, DA and UA on the Poly-BTB-Modified GCE The results in Fig. 4 clearly show that the electrochemical responses of AA, DA, and UA on the poly-BTB-modified electrode occurred at different potential windows, which means that the electrochemistry of these three compounds can be well resolved from their mixed solutions. Figure 6A shows the electrochemistry of these three compounds on a bare GCE (curve a) and a poly-BTB-modified GCE (curve b) in PBS 0.05 M (pH 5.0) containing the mixture of AA 20 μM, DA 10 μM, and UA 10 μM. At the bare GCE, three anodic peaks corresponding to the oxidation of these compounds could be observed. The anodic peaks for DA and UA overlapped each other. If the bare GCE was modified with poly-BTB, the electrochemical responses of the mixture solution clearly showed three well-resolved anodic peaks at 112 mV, 304 mV, and 452 mV for the electrooxidation of AA, DA and UA, respectively. If differential pulse voltammetry was used to characterize the system, three sharp and well-resolved anodic peaks at 94 mV, 296 mV, and 438 mV for AA, DA, and UA, respectively appeared (Fig. 6B). The large peak separation of the anodic peaks for these three compounds allows then to be simultaneously determined in mixted solutions.

The Influence of pH on the Oxidation of AA, DA, and UA at the Poly-BTB-Modified GCE The effect of the solution pH on the electrochemical responses of AA, DA, and UA at the poly-BTB-modified GCE was examined (Fig. 7). It is clear that the anodic peak current of DA increased slightly with the increase in solution pH. At pH 5.0, it reached the maximum. As the solution pH was higher than 5.0, the peak current decreased with the increase in solution pH. The dependence of the peak current of AA showed the same trend, and the peak current continuously increased with the increase in solution pH 2 to 4. At pH>4, the peak current of AA decreased. However, the influence of the solution pH on the peak current of UA showed a different trend. When the pH value was lower than 5.0, the peak current did not change with increasing pH, and then decreased rapidly with further the increase in solution pH. In addition, the solution pH affected the anodic current peak potentials of AA, DA, and UA. For all three compounds, their oxidation peak potentials shifted to negative values as the solution pH increased, showing that protons take part in their electrode reactions. By taking the physiologic conditions into consideration, the solu-

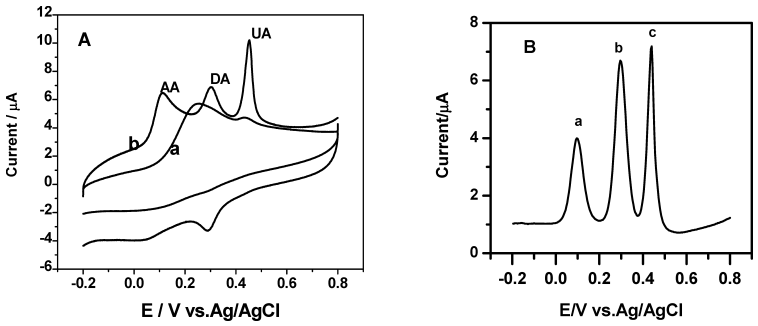

Fig. 6. (A) CVs of AA 20 μM, DA 10 μM, and UA 10 μM at a Bare GCE (a) and a Poly-BTB-Modified GCE (b) in PBS (pH 5.0) and (B) DPV of AA 20 μM, DA 10 μM, and UA 10 μM at the Poly-BTB-Modified GCE in PBS (pH 5.0)

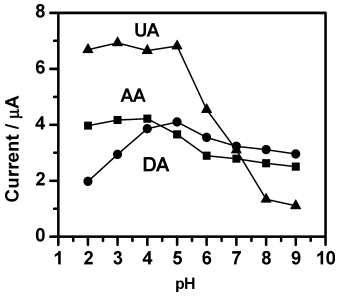

Fig. 7. Effects of pH on the Anodic Peak Current of AA, DA, or UA
Concentrations: AA, 20 μM; DA, 10 μM; and UA, 10 μM. Scan rate: 100 mV s^{-1}.

tion pH of 5.0 was chosen for the following simultaneous determination of these three compounds.

Simultaneous Determination of AA, DA, and UA The influence of the concentration of the analytes on the electrochemical responses of AA, DA, and UA on the poly-BTB-modified GCE was studied. In these measurements, only the concentration of one compound was varied, while the concentrations of the other two compounds remained constant. In the first case, the concentration of AA was changed, while the concentrations of DA and UA remained constant. As shown in Fig. 8A, the electrochemical response of AA increased linearly with the increase in the AA concentration. However, the change of AA concentration did not have a significant influence on the peak currents and peak potentials of the other two compounds. Similarly, as shown in Figs. 8B and C, the oxidation peak currents of DA or UA increased linearly with the increase in the concentration of DA or UA by keeping the concentration of other two compounds constant.

Based on the above results, it is clear that the electrooxidation peaks for AA, DA, and UA oxidation at the poly-BTB-modified GCE are well separated from each other, although they coexist in PBS (pH 5.0). It is therefore possible individually or simultaneously to determine AA, DA, and UA in mixted samples at a poly-BTB-modified GCE without any cross interference. By using the differential pulse voltammetric (DPV) mode, the oxidation current peaks were linearly proportional to the AA concentration in the ranges of 1.0×10^{-6}—8.0×10^{-4} M [$I_{pa}(\mu A)=0.16065C(\mu M)+5.50271$, $r=0.9989$], the peak current increases linearly with the concentration of DA in the range of 5.0×10^{-8}—5.0×10^{-6} M [$I_{pa}(\mu A)=3.8711C(\mu M)+1.1$, $r=0.99878$] and 8.0×10^{-6}—1.5×10^{-4} M [$I_{pa}(\mu A)=1.14127C(\mu M)+22.47206$, $r=0.99821$], and the oxidation current peaks of UA were linearly proportional to concentration in the ranges of 1.0×10^{-5}—5×10^{-4} M [$I_{pa}(\mu A)=0.415C(\mu M)+32.1487$, $r=0.99904$], respectively. The detection limit of AA, DA, and UA is 1 μM, 0.01 μM, and 1 μM, respectively. These results are better than those obtained on the polymerized film-modified electrodes.[25,28)]

Interference from other compounds were also investigated. If the tolerance limit was taken as the maximum concentration of the foreign substances (approximately +5% relative error), Ca^{2+} (200 μM), Mg^{2+} (200 μM), citric acid (100 μM), lysine (50 μM), glucose (50 μM), and cysteine (50 μM) did not affect the determination of AA 20 μM, DA 10 μM, and UA 10 μM.

Sample Analysis. Determination of AA in Ascorbic Acid Injection An AA hydrochloride injection solution

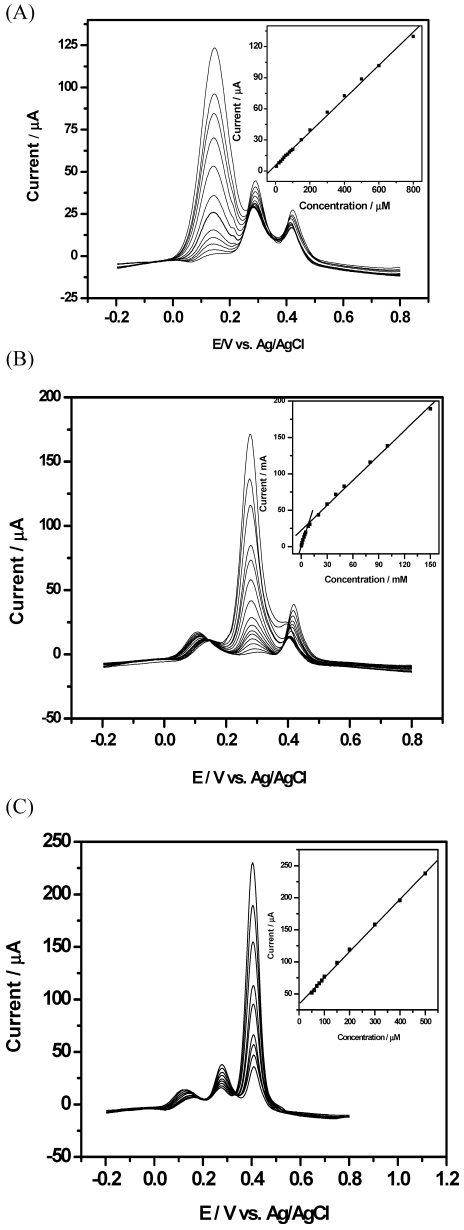

Fig. 8. (A) DPVs of AA at a Poly-BTB-Modified GCE in the Presence of DA 10 μM and UA 20 μM in PBS (pH 5.0)

AA concentrations (from a—e): 5, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 800 μM.

(B) DPVs of DA at a Poly-BTB Modified GCE in the Presence of AA 100 μM and UA 20 μM in PBS (pH 5.0)

DA concentrations (from a—f): 0.05, 0.1, 0.5, 1, 2, 4, 8, 10, 20, 30, 40, 50, 80, 100, and 150 μM.

(C) DPVs of UA at a Poly-BTB Modified GCE in the Presence of AA 100 μM and DA 10 μM in PBS (pH 5.0)

UA concentrations (from a—f): 50, 60, 80, 90, 100, 200, 300, 400, and 500 μM. Scan rate: 100 mV s^{-1}.

(standard concentration of AA 0.25 g ml^{-1}, 2 ml per injection) was diluted to 10 ml with water. One hundred microliters of this diluted solution was injected into each of a series of 10 ml volume flasks and made up to volume with PBS 0.05 M (pH 5.0). Then this test solution was placed in an electrochemical cell for the determination of AA using the above DPV method. The results are listed in Table 1. The recovery rate and RSD were acceptable, showing that the proposed methods could be used efficiently for the determination of

Table 1. Determination of AA in Hydrochloride Injection Solutions ($n=6$)

Analyte	Labeled (μM)	Added (μM)	Found (μM)	R.S.D (%)	Recovery
AA	130	0	128	2.3	
	130	50	182	2.4	108
	130	100	227	2.5	99

Table 2. Determination of DA in Hydrochloride Injection Solutions ($n=6$)

Analyte	Labeled (μM)	Added (μM)	Found (μM)	R.S.D (%)	Recovery
DA	3.15	0	3.12	2.5	
	3.15	2	5.14	2.4	101
	3.15	4	7.23	3.1	103

Table 3. Determination of UA in Human Urine Sample ($n=6$)

Analyte	Labeled (μM)	Added (μM)	Found (μM)	R.S.D (%)	Recovery
UA	Heath adult	0	15.55	3.4	
	A (20 μl)	5	20.15	2.9	112
		10	25.80	3.2	102.5
	Patient B	0	19.32	3.5	
	(20 μl)	5	24.50	2.8	104
		10	30.10	3.2	108
	Patient C	0	24.30	2.6	
	(20 μl)	5	29.55	2.9	105
		10	34.85	3.2	106

AA in injections.

Determination of DA in Dopamine Hydrochloride Injection A DA hydrochloride injection solution (standard concentration of 10 mg ml^{-1}, 2 ml per injection) was diluted to 10 ml with water. Two hundred microliters of this diluted solution was injected into each of a series of 10 ml volume flasks and made up to volume with PBS 0.05 M (pH 5.0). An aliquot of 2.0 ml of this test solution was placed in an electrochemical cell for the determination of DA using the DPV method. The results are listed in Table 2. The recovery rate and RSD were acceptable, showing that the proposed methods could be used efficiently for the determination of DA in injections.

Determination of UA in Human Urine Samples Three human urine samples (A—C) were selected to analyze the contents of UA using the proposed method with the standard addition. Sample A was from a healthy adult and samples B and C were from two gout patients. Then 10 μl of each diluted solution was injected into each of a series of 10 ml volume flasks and made up to volume with PBS 0.05 M (pH 5.0). Then this test solution was placed in an electrochemical cell for the determination of UA using the DPV method. The results are listed in Table 3.

Conclusion

We reported a novel poly-BTB-modified GCE prepared using the electropolymerization method. This modified electrode not only improved the electrochemical catalytic activities towards the oxidation of AA, DA, and UA but also resolves the overlapped oxidation peaks of AA, DA and UA into three well-defined peaks at potentials of 94 mV, 296 mV, and 438 mV in the DPV, method respectively. In DPV determination, the detection limit of AA, DA, and UA was estimated to be on the order of 1.0×10^{-6} M, 1.0×10^{-8} M and 1.0×10^{-6} M, respectively. Thus, the poly-BTB-modified GCE can be used to analyze AA, DA, and UA individually and simultaneously with high sensitivity, good selectivity, and low detection limits. In addition, this modified electrode shows a stable response without fouling of the electrode surface by the adsorption of the oxidized product of AA and UA. The presence of cations and anions does not affect the redox properties of the poly-BTB-modified electrode. Therefore this modified electrode can be used to determine AA, DA, and UA in real samples without interference. These results are of great significance from the viewpoint of practical applications.

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