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Simultaneous determination of ascorbic acid, dopamine and uric acid using poly(4-aminobutyric acid) modified glassy carbon electrode

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ABSTRACT

A polymerized film of 4-aminobutyric acid (P-4-ABA) was prepared on the surface of glassy carbon electrode (GCE) by electropolymerization. Cyclic voltammetry (CV), different pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were used to study the electrochemical properties of the modified electrode. The results indicate that the P-4-ABA film has excellent electrocatalytic activity for the oxidation of ascorbic acid (AA), dopamine (DA) and uric acid (UA). The charge transfer coefficient, α, and the charge transfer rate constant, k_s, for electron transfer between 4-ABA and GCE were also calculated as 0.53 and $25.70 \pm 0.59\ s^{-1}$, respectively. Under optimal conditions, the peak separations between AA and DA, DA and UA, AA and UA at modified electrode using DPV method were 208, 136 and 344 mV, respectively. The linear response range for AA, DA and UA were 20.0–800.0 μmol L^{-1}, 5.0–100.0 μmol L^{-1} and 1.0–80.0 μmol L^{-1}, respectively, and the detection limits were 5.0, 1.0 and 0.5 μmol L^{-1} (S/N = 3). The modified electrode was successfully used to simultaneously determine AA, DA and UA in human urine sample.

1. Introduction

Ascorbic acid (AA), dopamine (DA) and uric acid (UA) usually coexist in our body fluids, and play determining roles in human metabolism. AA is very popular for its antioxidant property, and present in the human diet as a vital vitamin. It is also used for the prevention of scurvy, treatment of common cold, mental illness, infertility, cancer and AIDS [1]. DA is an important neurotransmitter molecule of catecholamine which is widely distributed in the mammalian central nervous system for message transfer [2]. Abnormal levels of DA will lead to brain disorders such as Parkinson and schizophrenia diseases [3–5]. UA is a primary end product of purine metabolism. Very low concentration levels of UA will lead to some diseases, such as gout and hyperuricaemia [6]. Therefore, simultaneous determination of AA, DA and UA was important for investigating their physiological functions and diagnosing diseases. Recently, the electrochemical method was widely used for simultaneous determination of AA, DA and UA. However, it is very difficulty to simultaneously determine AA, DA and UA directly at ordinary (carbon and metal) electrodes, because the direct redox reaction of these species at the ordinary electrodes will take place at very similar potentials and often suffer from a pronounced fouling effect, which results in rather poor selectivity and reproducibility [7,8]. To dissolve these problems, chemically modified electrodes have been developed and reported such as using polymer films [2,9–12]. nanoparticles [13,14], self assembled monolayer [15], metal oxide [16], etc. Among above modifiers, electropolymerized layer has many advantages in the detection of bio-molecules because of its selectivity, sensitivity, homogeneity, strong adherence to electrode surface and chemical stability of the film [17,18]. There were various reports using glutamic acid, aminobenzoic acid and p-nitrobenzenazo resorcinol as electropolymerized layer to modify GCE for the detection of AA, DA and UA [19–21]. To the best of our knowledge, the electropolymerization of 4-aminobutyric acid (4-ABA) has not been reported. In this study, poly-4-aminobutyric acid modified glassy carbon electrode (P-4-ABA/GCE) was prepared and employed to determine AA, DA and UA simultaneously. Furthermore, the practical application was investigated using standard addition method and satisfactory results were obtained.

2. Experimental

2.1. Apparatus

CHI660D Electrochemical Workstation (Shanghai Chenhua Instruments, Shanghai, China) was used for electrochemical measurements. The classical three-electrode system consists of a KCl saturated Ag/AgCl electrode (reference electrode), a platinum electrode (auxiliary electrode), and a bare GCE or P-4-ABA/GCE with a diameter of 4.0 mm (working electrode).

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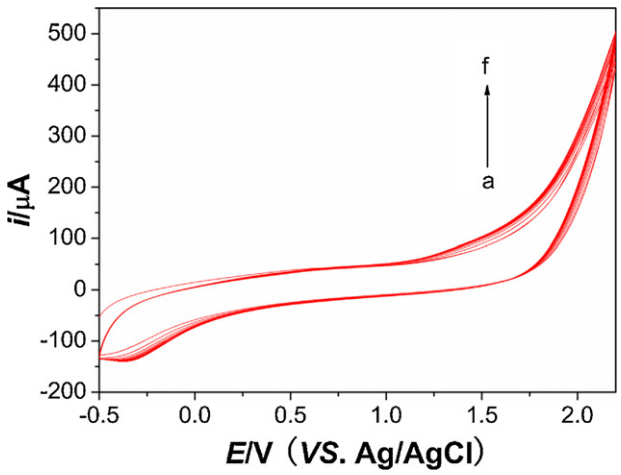


Fig. 1. Cyclic voltammograms for the electropolymerization of 0.25 mol L^{-1} 4-ABA on a GCE in PP buffer solution (pH = 5.0). Scan rate: 100 mV s^{-1}; number of scan: 12; potential range: −0.5–2.2 V.

2.2. Chemicals

4-ABA, AA, DA and UA were obtained from the Chinese Institute of Biological Products Control (Beijing, China). All the reagents involved were bought from local commercial sources with analytical grade. Buffer solution was prepared by mixing 0.2 mol L^{-1} Na_2HPO_4 and 0.2 mol L^{-1} NaH_2PO_4 solutions to form a phosphate (PP) buffer solution (0.2 mol L^{-1}). Distilled water was used for preparation of all solutions and for washing. Both standard solution and buffer solutions were kept in a 4 °C refrigerator.

2.3. Preparation of P-4-ABA/GCE

Prior to the electropolymerization, the surface of GCE was polished by 0.3 μm Al_2O_3, and then sonicated in distilled water for 5 min. The polished electrodes were electrochemically cleaned by cyclic potential sweeps between −0.20 and 1.50 V in 0.5 mol L^{-1} H_2SO_4 until a stable cyclic voltammogram was obtained. Finally, the P-4-ABA/GCE was constructed by electropolymerization of 4-ABA on the surface of GCE in a solution which contains 0.025 mol L^{-1} 4-ABA and 0.2 mol L^{-1} PP buffer (pH = 5.0) by potentiodynamic conditions (12 cycles) in the potential range of −0.5–2.2 V at a scan rate of 100 mV s^{-1}. After electropolymerization, the modified electrode was rinsed thoroughly with distilled water.

3. Results and discussion

3.1. Preparation of P-4-ABA/GCE

Fig. 1 shows a continuous cyclic voltammogram (CV) recorded during the electropolymerization on the surface of GCE in PP buffer (pH = 5.0) solution. The peak current increased with number of scan indicating that an electro-conductive polymer film was formed on the electrode surface [22]. The reaction mechanism may be described as follows: 4-ABA was oxidized to free radical at the surface of GCE and the free radicals then combine with the surface of GCE rapidly resulting in the possible structure of P-4-ABA (Scheme 1).

3.2. Optimization of determination conditions

It is well known that the pH value has a profound effect on the amperometric responses. Fig. 2A shows the effect of pH

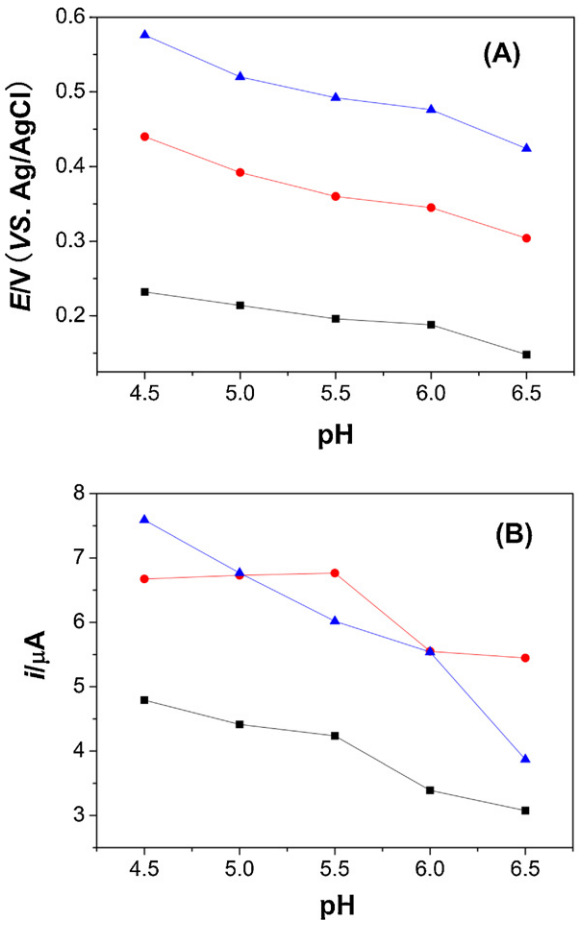
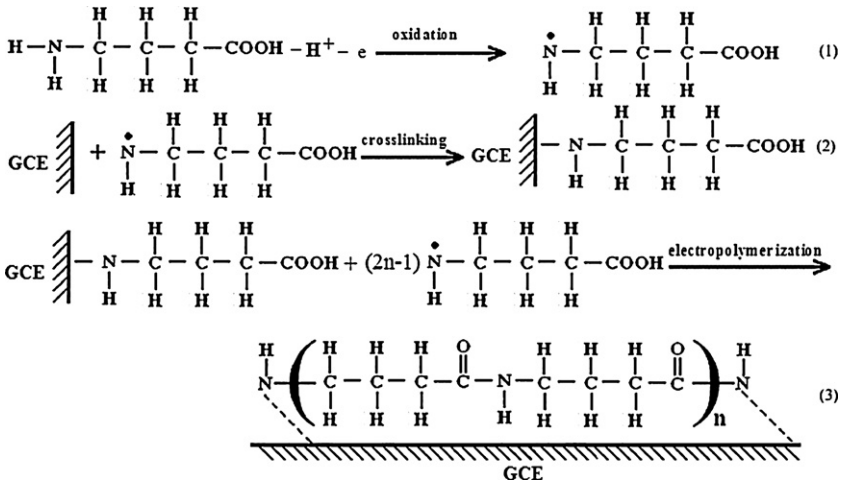


Fig. 2. Effect of pH by DPVs on (A) the peak current and (B) the peak potential for the oxidation of 200 μmol L^{-1} AA, 20 μmol L^{-1} DA and 20 μmol L^{-1} UA in 0.2 mol L^{-1} PP buffer solution (pH 4.5).

values on the peak current and peak potential for 200 μmol L^{-1} AA, 20 μmol L^{-1} DA and 20 μmol L^{-1} UA co-existing in the solution. The current responses of AA and UA decreased from pH 4.5 to 6.5, while the current responses of DA reached the maximum at pH 5.5. However, the peak current of DA at pH 4.5 is almost equal to pH 5.5. Furthermore, at pH 4.5, AA, DA and UA can be completely separated, which make it possible to simultaneously detect them in the mixture. Fig. 2B displays that the DPV peak potential of AA, DA and UA oxidation shifted negatively with a higher pH value, which suggested that protons have taken part in their electrode reaction processes. Thus, solution pH 4.5 was taken for the following experiments.

3.3. AC impedance characterization of the P-4-ABA/GCE

AC impedance spectroscopy is an effective method for studying the interface features of a modified electrode surface. In electrochemical impedance spectroscopy (EIS), the semicircle diameter equals to the electron-transfer resistance (Ret). Fig. 3. shows the results of impedance spectroscopy on different electrodes. A significant decrease in Ret can be observed on P-4-ABA/GCE (Curve "c") when a bare GCE (Curve "a") was electropolymerized with 4-ABA for 12 cyclic potential sweeps. While the Ret of Curve "b" (6 cyclic potential sweeps) was larger than Curve "c" (12 cyclic potential sweeps). This phenomenon indicates that the electron-transfer process of modified electrodes is relatively fast compared to bare GCE. The result of AC impedance supported that the P-4-ABA film had been assembled onto the bare GCE surface.



Scheme 1. Diagram of P-4-ABA/GCE preparation.

3.4. Electrochemical properties of the P-4-ABA/GCE

Fig. 4A exhibits the CVs of the P-4-ABA film modified GCE in 0.1 mol L^{-1} H_2SO_4 solution at different scan rates ranging from 20 to 200 mV s^{-1}. A pair of redox peaks was obtained in each of the CVs, and the peak currents were increased due to the scan rate. As shown in Fig. 4B, the anodic peak current (I_{pa}) is linearly dependent of the scan rate (ν) with the equation: I_{pa} (μA) = 0.0996ν + 1.5955 (r = 0.9974), and the ratio of the anodic peak current to the cathodic peak current, I_{pa}:I_{pc}, is almost equal to unity, the peak potential did not alter with the increase of scan rate. These behaviors are consistent with diffusionless systems or with reversible electron transfer processes at low scan rates. The separation of the peak potentials ($\Delta E_p = E_{pa} - E_{pc}$) is 31 mV at a low scan rate (20 mV s^{-1}). Based on the formula (ΔE_p is close to 2.3 RT/nF or 59/nm V at 25 °C) [23], the number of electrons involved in the electrochemical process is 2 ($n \approx 1.9$). An approximate estimate of the surface coverage of the electrode could be evaluated according to the equation [24,25].

$$i_p = \frac{n^2F^2A\Gamma\nu}{4RT}$$

where n represents the number of electrons involved in the reaction, A is the surface area of the electrode (0.1256 cm^2), i_p is the peak current, Γ represents the surface coverage concentration (mol cm^{-2}), and ν is the scan rate. R, T and F denote as usual for the gas constant, the temperature, and the Faraday constant, respectively. From the slope of the anodic peak currents vs. scan rate, the calculated surface concentration of 4-ABA is 2.11×10^{-10} mol cm^{-2}.

The effect of pH value of the supporting electrolyte solution on the electrochemical behavior of P-4-ABA film modified GCE was also investigated. Higher pH value made the reduction and oxidation peak potential shift negatively. The plot of the anodic peak potential versus pH value showed linearity in the pH value range of 2.0–7.0 with a slope of −60.3 mV pH^{-1}. These implied that the participated protons are equal to the transferred electrons through the P-4-ABA film.

Fig. 4C shows the variations of peak potentials (E_p) as a function of the potential scan rate. Moreover, the peak potential E_p was proportional to the logarithm of scan rate ν for $\nu \geq 0.2$ V s^{-1} (Fig. 4D) and peak separations evidently increased, indicating the limitation of charge transfer kinetics. Based on Laviron's theory [26], the electron transfer coefficient α could be calculated. For cathodic and anodic peaks, the slopes of E_p versus logν were −0.1115 and 0.1251, respectively. The calculated value of α was 0.53. According to the equation

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF\nu} - \frac{\alpha(1-\alpha)nF\Delta E_p}{2.3RT}$$

where k_s is the rate constant of electrode reaction and the other symbols have their conventional meanings, k_s was calculated to be 25.70 ± 0.59 s^{-1}.

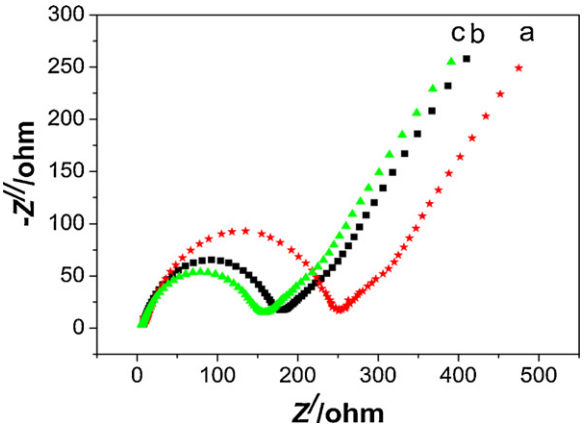


Fig. 3. AC impedance spectroscopy of bare GCE (a), (b) and (c) P-4-ABA/GCE with 6 and 12 cyclic potential sweeps, respectively in 5.0 mmol L^{-1} $K_3Fe(CN)_6/K_4Fe(CN)_6$ containing 0.50 mol L^{-1} KCl. ac voltage: 5 mV; frequency range: 0.1 Hz–10 MHz.

3.5. Electrochemical behavior of AA, DA and UA at bare and P-4-ABA/GCE

Fig. 5A displays the CV of 0.20 mmol L^{-1} AA at bare GCE and P-4-ABA/GCE. As we can see, the peak was rather broad at bare GCE, which indicated a slow electron transfer kinetic. After electropolymerization, the oxidation potential at P-4-ABA/GCE for AA shift to negative about 90 mV, and the current about three-folds higher than that of bare GCE. For DA, a sluggish and much small CV peak response were observed at bare GCE (Fig. 5B), the potentials of oxidation and reduction peaks were 0.566 V and 0.415 V, respectively. The separation of redox peak potentials (ΔE_p) is 151 mV. However, the sharp oxidation and reduction peaks at P-4-ABA/GCE were observed for DA. The separation of redox peak potentials (ΔE_p) is only 40 mV, and the oxidation current is nearly three-folds higher than that of bare GCE, which suggests an efficient oxidation reaction of DA at P-4-ABA/GCE. Similarly UA on modified electrode shows approximately four-fold increase peak current as depicted

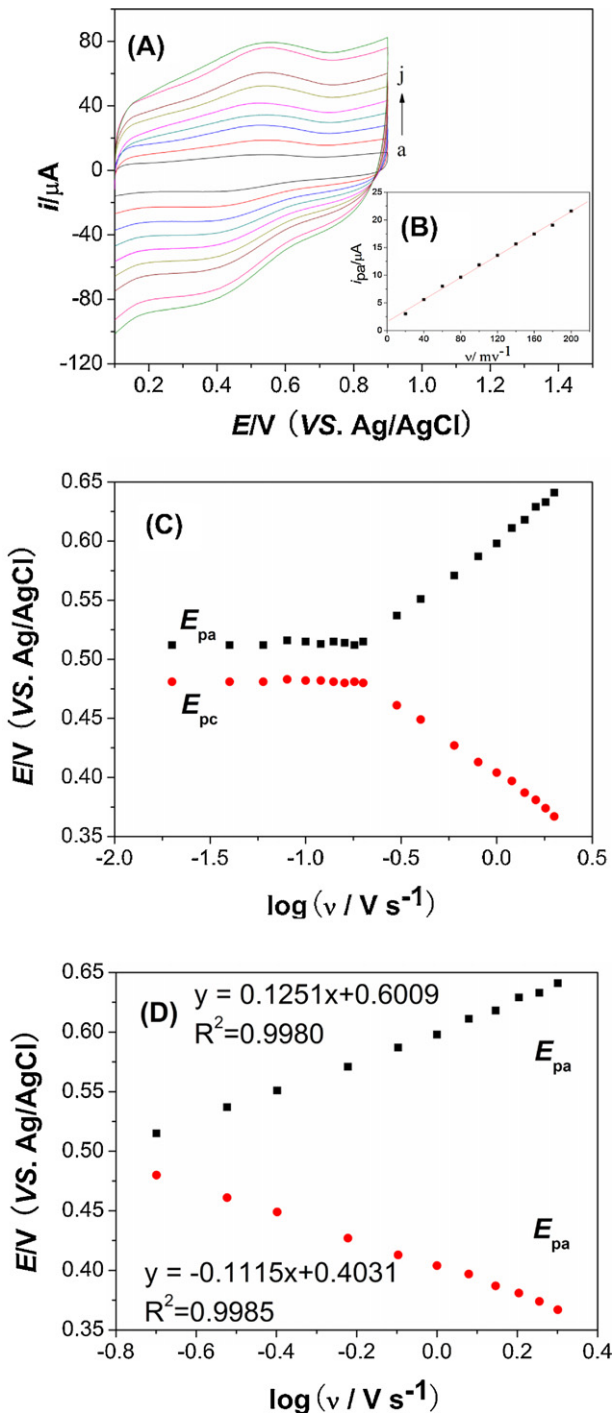


Fig. 4. (A) Cyclic voltammograms of the P-4-ABA/GCE in 0.1 M H_2SO_4 at various scan rates: (a) 20 mV s^{-1}, (b) 40 mV s^{-1}, (c) 60 mV s^{-1}, (d) 80 mV s^{-1}, (e) 100 mV s^{-1}, (f) 120 mV s^{-1}, (g) 140 mV s^{-1}, (h) 160 mV s^{-1}, (i) 180 mV s^{-1}, (j) 200 mV s^{-1}. (B) Plots of peak currents vs. scan rates. (C) Variation of E_p versus the logarithm of the scan rate. (D) Magnification of the same plot for high scan rates.

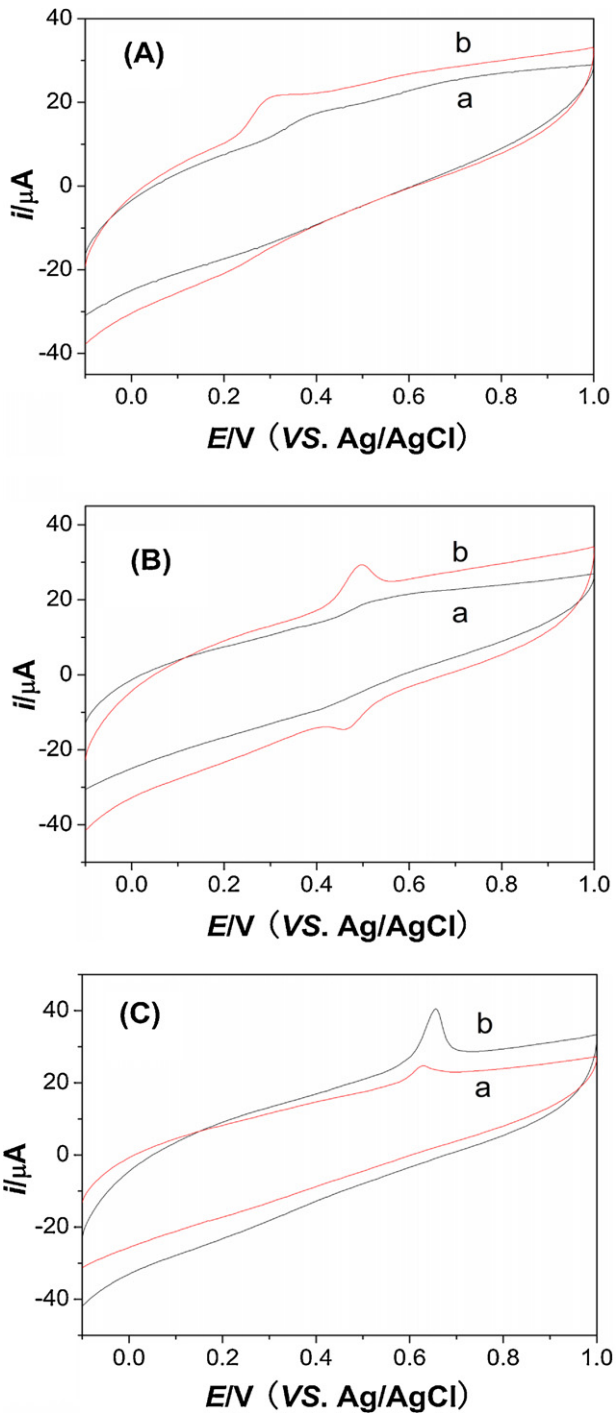


Fig. 5. Cyclic voltammograms of 200 μmol L^{-1} AA (A), 20 μmol L^{-1} DA (B) and 20 μmol L^{-1} UA (C) at a bare GCE (a), and a P-4-ABA/GCE (b) in pH 4.5 PP buffer solution. Scan rate: 100 mV s^{-1}.

in Fig. 5C. These results indicate that the P-4-ABA/GCE have good electrocatalytic properties towards to oxidation of AA, DA, and UA.

3.6. Electrochemical behavior of AA, DA and UA in a mixture at bare and P-4-ABA/GCE

Fig. 6A displays the CV of the mixture of AA, DA and UA at bare GCE and P-4-ABA/GCE, respectively. At the bare GCE, the oxidation peaks of AA, DA completely overlap and the peak potentials for DA, UA are indistinguishable, which revealed that it was impossible to simultaneous determination of these compounds on bare GCE. In contrast to bare GCE, P-4-ABA/GCE oxidized AA, DA and UA at 0.322, 0.494 and 0.659 V, respectively, and the current response is also enhanced. If the DPV method was used to value the system, three sharp and well resolved oxidation peaks for DA, AA and UA appeared at 0.232, 0.440 and 0.576 V, respectively (Fig. 6B). Obviously, this separation was good enough to achieve the simultaneous determination of DA, AA and UA in a mixture solution.

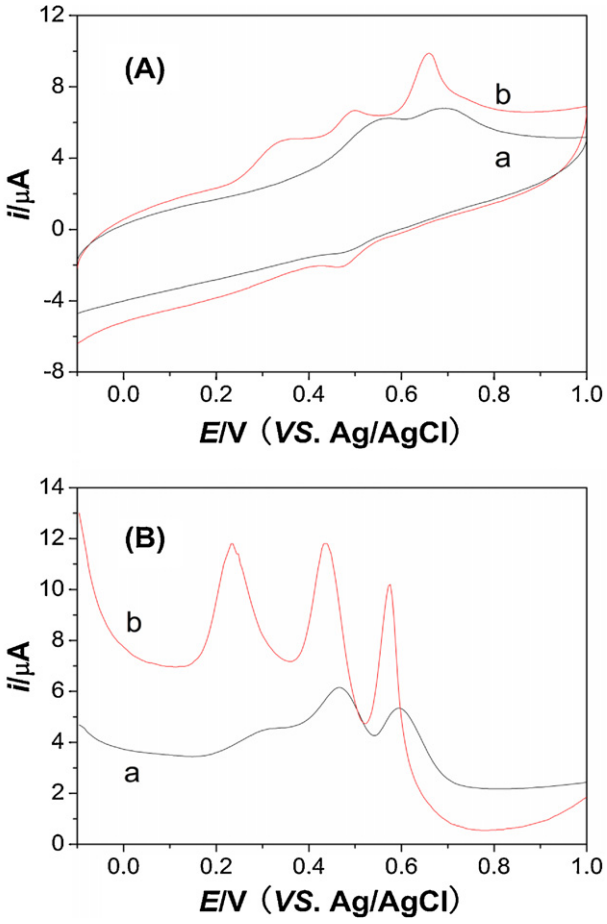


Fig. 6. CV (A) and DPV (B) of 200 μmol L^{-1} AA, 20 μmol L^{-1} DA and 20 μmol L^{-1} UA at a bare GCE (a), and a P-4-ABA/GCE (b) in pH 4.5 PP buffer solution.

3.7. Simultaneous determination of AA, DA and UA using P-4-ABA/GCE

The determination of AA, DA and UA in their mixtures was performed at the P-4-ABA/GCE by using the DPV mode. When the concentration of one species changed, the concentrations of the others were keeping constant. The results are shown in Fig. 7A–C. As shown in Fig. 7A, the peak current of AA was increased linearly with the increasing concentration. However, the changes of AA concentration have no significant influence on the peak currents and peak potentials of the other two compounds. Similarly, as shown in Fig. 7B and C, the oxidation peak currents of DA or UA increased linearly with the increase of its concentration when keeping the concentration of other two compounds constant. The above results confirmed that the oxidation peaks for AA, DA and UA at the P-4-ABA/GCE were well separated with each other when they co-exist in pH 4.5 PP buffer solution. Therefore, it is possible to simultaneously determine AA, DA and UA in mixture samples at the P-4-ABA/GCE. The calibration parameters for the simultaneous determination of AA, DA and UA are listed in

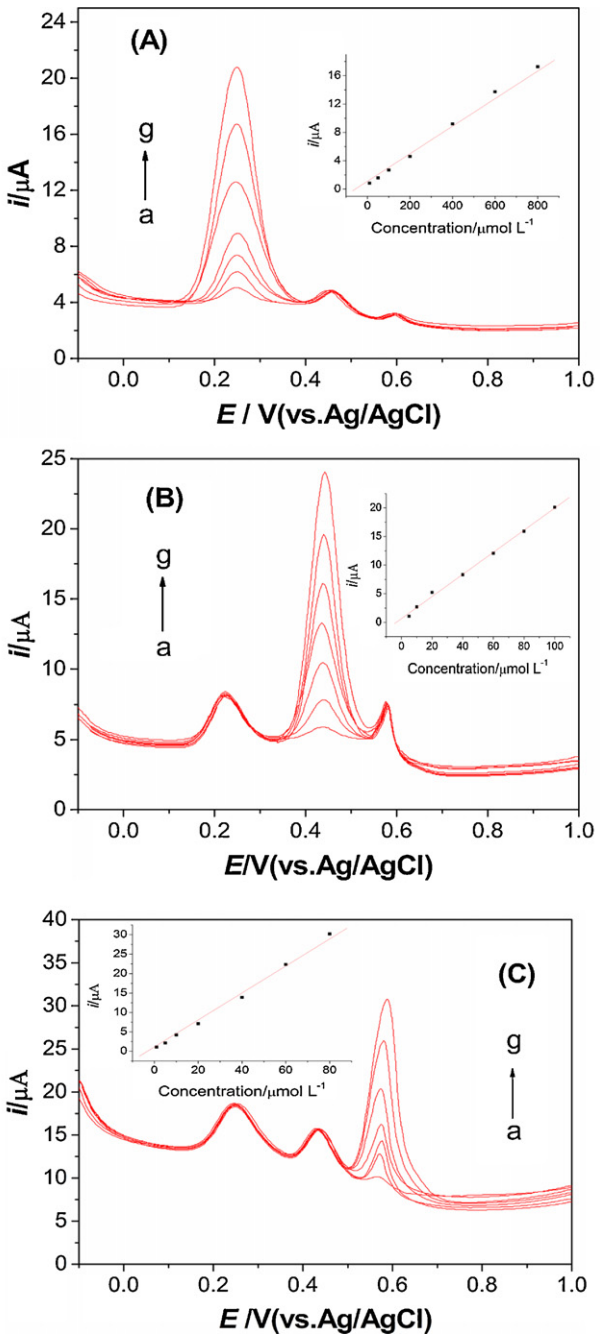


Fig. 7. Differential pulse voltammograms of (A) DPV of (a) 10.0; (b) 50.0; (c) 100.0; (d) 200.0; (e) 400.0; (f) 600.0; (g) 800.0 μmol L^{-1} AA in the presence of 5.0 μmol L^{-1} DA and 2.0 μmol L^{-1} UA (insert A is plot proportionate with these concentrations). (B) (a) 5.0; (b) 10.0; (c) 20.0; (d) 40.0; (e) 60.0; (f) 80.0; (g) 100.0 μmol L^{-1} DA in the presence of 150.0 AA and 10.0 μmol L^{-1} UA (insert B is plot proportionate with these concentrations). (C) (a) 1.0; (b) 5.0; (c) 10.0; (d) 20.0; and (e) 40.0; (f) 60.0; (g) 80.0 μmol L^{-1} UA in the presence of 300.0 AA and 20.0 μmol L^{-1} DA (insert C is plot proportionate with these concentrations) at pH 4.5 using P-4-ABA/GCE.

Table 1
Analytical parameters for simultaneous determination of AA, DA and UA.

Analyte	Linear range (μmol L^{-1})	Regression equation i_p [a], C [b]	R^2	Detection limit (μmol L^{-1})
AA	20.0–800.0	$i = 0.0213C + 0.5686$	0.9986	5.0
DA	5.0–100.0	$i = 0.1979C + 0.6039$	0.9963	1.0
UA	1.0–80.0	$i = 0.3684C + 0.1980$	0.9974	0.5

[a] i_p (μA) is the peak current.
[b] C (μM) is the concentration of the analyte.

Table 2
Comparison of the response characteristics of different modified electrodes.

Electrode materials	Linear range (μmol L^{-1})			Detection limit (μmol L^{-1})			Peak potentia(V)			Reference
	AA	DA	UA	AA	DA	UA	AA	DA	UA	
Poly(Evans Blue)	5–105	1–10	30–110	0.3	0.25	2.0	0.191	0.373	0.553	[2]
Poly(acid chrome blue K)	50.0–1000	1.0–200	1.0–120	10.0	0.5	0.5	0.095	0.288	0.454	[10]
2-Amino-1,3,4- thiadiazole	30–300	5–50	10–100	2.01	0.33	0.19	0.20	0.33	0.49	[27]
Poly-CDDA[a]	5.0–240	5.0–280	0.1–18	1.43	0.29	0.016	0.28	0.44	0.61	[28]
Poly(eriochrome Black T)	150–1000	0.1–200	10–130	10.0	0.02	1.0	-	-	-	[29]
P-4-ABA	20.0–800	5.0–100	1.0–80	5.0	1.0	0.5	0.232	0.440	0.576	This work

[a] Poly(3-(5-chloro-2-hydroxyphenylazo)-4,5-dihy-droxynaphthalene-2,7-disulfonic acid).

Table 3
Interferences of some foreign substances for 200 μmol L^{-1} AA, 20 μmol L^{-1} DA and 20 μmol L^{-1} UA.

Foreign substances	Tolerance level (μmol L^{-1})
K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}	400
Citric acid	200
Cysteine, lysine, and glucose	100

Table 1. As compared with the previous reports (Table 2), improved or comparable performance for the simultaneous determination of UA, AA, and DA can be achieved using the P-4-ABA/GCE. A better separation has been achieved between individual signals, AA and DA.

3.8. Interferences

In order to evaluate the selectivity of the method, several compounds were selected. The tolerance limit was defined as the maximum concentration of foreign substances, with a relative error of less than 5%, for 200 μmol L^{-1} AA, 20 μmol L^{-1} DA and 20 μmol L^{-1} UA. No interference was observed for the following compounds (μmol L^{-1}): K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, citric acid, cysteine, lysine, and glucose. The results are listed in Table 3.

3.9. Stability and reproducibility

The performance of the modified electrode did not change very much when it was stored in 0.2 mol L^{-1} PP (pH 4.5) buffer solution at 4 °C in a refrigerator. There were no obvious decreases (3.4%, 2.7%, and 2.3% for 200 μmolL^{-1} AA, 20 μmol L^{-1} DA and 20 μmol L^{-1} UA, respectively) in the responses after 1 week. It manifested that P-4-ABA/GCE showed good stability.

The reproducibility of the P-4-ABA/GCE was examined by 7 successive DPV measurements for the mixture of 200 μmol L^{-1} AA, 20 μmol L^{-1} DA and 20 μmol L^{-1} UA. The relative standard deviations (RSD) in peak currents were found to be 4.6%, 3.9% and 3.1%, respectively, which indicated that the P-4-ABA/GCE has good reproducibility.

3.10. Sample analysis

Under the optimum conditions, Healthy male urine was selected as biological sample for analysis using the standard addition method. In order to fit onto the linear range of UA, all the urine samples used for detection were diluted 200 times with 0.2 mol L^{-1} PP (pH 4.5) buffer solution. The obtained results are shown in Table 4. No AA or DA has been detected in male urine which was identical with those reported in literature [14,28], and the concentration of UA was in agreement with those reported in literature [2,10,30]. Comparisons of the proposed method with official methods [30] also confirmed the accuracy of the results obtained by our proposed method. The good recoveries of the mixture samples indicate the successful applicability of the proposed method to simultaneous determination of AA, DA, and UA.

4. Conclusions

A novel poly(4-aminobutyric acid) modified glassy carbon electrode was fabricated by electropolymerization method. The modified electrode exhibits highly electrocatalytic activity for the oxidation of AA, DA and UA. Under optimal conditions, the linear response range for AA, DA and UA were 20.0–800.0 μmol L^{-1}, 5.0–100.0 μmol L^{-1} and 1.0–80.0 μmol L^{-1}, respectively, and the detection limits were 5.0, 1.0 and 0.5 μmol L^{-1} (S/N = 3). The modified electrode exhibits good sensitivity, selectivity and reproducibility. The proposed method was successfully applied for the determination of AA, DA and UA in human urine samples.

Table 4
Determination results of AA, DA and UA in real urine samples (n = 5).

Urine		Detected (μmol L^{-1})	Added (μmol L^{-1})	Found (μmol L^{-1})	RSD (%)	Recovery (%) (μmol L^{-1})
1	AA	0	50.0	51.2	2.7	104.0
			500.0	495.8	3.5	99.2
	DA	0	10.0	9.88	2.4	98.8
			80.0	78.4	2.9	98.0
	UA	15.4	5.0	19.7	3.3	96.6
			50.0	66.3	3.7	101.4
2	AA	0	50.0	50.4	2.4	100.8
			500.0	496.4	3.1	99.3
	DA	0	10.0	10.3	2.8	103.0
			80.0	81.2	3.3	101.5
	UA	16.1	5.0	20.2	3.1	95.7
			50.0	65.6	3.5	99.2

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2012.12.115.

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