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ORIGINAL PAPER

Simultaneous voltammetric measurement of ascorbic acid and dopamine on poly(caffeic acid)-modified glassy carbon electrode

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Abstract A poly(caffeic acid) thin film was deposited on the surface of a glassy carbon electrode by potentiostatic technique in an aqueous solution containing caffeic acid. The poly(caffeic acid)-modified electrode was used for the determination of ascorbic acid (AA), dopamine (DA), and their mixture by cyclic voltammetry. This modified electrode exhibited a potent and persistent electron-mediating behavior followed by well-separated oxidation peaks toward AA and DA at a scan rate of 10 mV s^{-1} with a potential difference of 135 mV, which was large enough to determine AA and DA individually and simultaneously. The catalytic peak current obtained was linearly dependent on the AA and DA concentrations in the range of 2.0×10^{-5}–1.2×10^{-3} and 1.0×10^{-6}–4.0×10^{-5} mol L^{-1} in 0.15 mol L^{-1} phosphate buffer (pH 6.64). The detection limits for AA and DA were 9.0×10^{-6} and 4.0×10^{-7} mol L^{-1}, respectively. The modified electrode shows good sensitivity, selectivity, and stability and has been applied to the determination of DA and AA in real samples with satisfactory results.

Keywords Poly(caffeic acid)-modified electrode · Ascorbic acid · Dopamine · Simultaneous determination

Dopamine (DA) is an important neurotransmitter molecule of catecholamines, and its deficiency leads to brain disorders such as Parkinson's disease and schizophrenia [1–3]. Similarly, ascorbic acid (AA) has been used for the prevention and treatment of common cold, mental illness, infertility, and cancer [4]. DA and AA are the compounds of great biomedical and neurochemical interest, and they are always present together in biological tissues. Thus, simultaneous determination of DA and AA is a problem of critical importance in field of neurochemistry and biomedical chemistry. Both DA and AA are compounds that can be determined for electrochemical methods based on anodic oxidation. However, a major problem is that the oxidation potentials for AA and DA occur almost in the same potential at unmodified electrodes, which result in overlapped voltammetric responses, making their discrimination highly difficult [3]. Most of the studies on these compounds demonstrated the possibility for the separate determination of either AA [5–7] or DA [8–10] by eliminating the other using different membranes or selecting particular potentials. It was reported that stearic acid [11], Nafion [12], polypyrrole [13], and micellar [14] could selectively detect DA in the presence of AA based on the cationic permeability of the polymer. However, it is most important to develop a sensor, which can determine both AA and DA simultaneously. Some efforts have been taken to fabricate modified electrodes for the simultaneous determination of DA and AA, such as poly (neutral red)-modified electrode [15], poly(phenosafranine) electrode [16], poly (*N*,*N*-dimethylaniline)-modified electrode [17], sol–gel composite electrode [18], chlione- and acetylcholine-modified glassy carbon electrode [19], carbon-polyvinylchloride composite electrode [20], tetrabromo-*p*-benzoquinone-modified carbon paste electrode [21], ferrocene derivative mediators at glassy carbon electrode [22], the positively charged surfactant cetylpyridinium chloride adsorbed onto the electrode surface [23], the coated and intercalated carbon nanotube-modified electrodes [24], poly (benzophenone-4) electrode [25], epinephrine/Nafion-modified electrode [26], multiwalled carbon nanotubes with incorporated β-cyclodextrin combined with polyaniline film-modified electrode [27], poly(3,4-ethylenedioxy)thiophene

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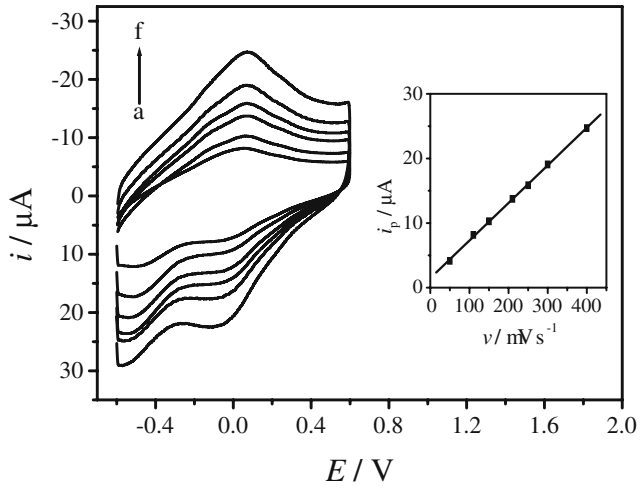

Fig. 1 Cyclic voltammograms at the composite polymer modified electrode in phosphate buffer (pH 7.4) at the scan rate of *a* 110, *b* 150, *c* 210, *d* 250, *e* 300, and *f* 400 mV s^{-1}, respectively. *Inset*: plot of the anodic peak current vs the scan rate

electrode [28], poly (vinyl alcohol)-modified electrode [29], oracet blue modified electrode [30], didodecyldimethylammonium bromide-modified electrode [31], poly(manganese (III)-5-[*o*-(1-imidazolyl) butoxyl] phenyl-10, 15, 20-triphenylporphrine chloride)-modified electrode [32], poly (3,4-ethylenedioxythiophene)-modified electrode [33], and nano-cobalt phthalocyanine-modified electrode [34].

Caffeic acid (3,4-dihydroxycinnamic acid) is a well-known natural phenol. It is very common in plants and can be found in seeds, fruits, tubers, and herbaceous parts of many vegetable species [35]. In the study of simultaneous voltammetric measurement of AA, epinephrine, and uric acid with the poly(caffeic acid) film electrode [36], we found that DA interfered with the determination of epinephrine because of their overlapping oxidant peaks. Because DA often coexists with AA in real samples, studying the simultaneous voltammetric measurement of AA and DA is very significant. The present study relates to the electrochemical deposition of caffeic acid on a glassy carbon electrode to develop a sensor for selective and sensitive detection of DA and AA individually in the presence of the other species. This ability to determine AA and DA in a mixture has a significant attraction in biological and chemical researches.

Experimental

Reagents

Caffeic acid (1.0×10^{-3} mol L^{-1}), DA (1×10^{-3} mol L^{-1}), and AA (0.1 mol L^{-1}) stock solutions were prepared by dissolving 0.0090 g of caffeic acid (Sigma), 0.0095 g of DA (Sigma), and 0.8807 g of AA (Cheng Du Ke Long Chemical Reagents Plant) in water and diluting to the mark in a 50-mL volumetric flask, respectively. AA and caffeic acid solutions were prepared just before use. Phosphate buffers of 0.15 mol L^{-1} with various pH values were prepared by mixing the stock solutions of 0.15 mol L^{-1} NaH_2PO_4 and Na_2HPO_4. All experiments were carried out at room temperature (23±2 °C). All chemicals were of analytical-reagent grade, and doubly distilled water was used throughout.

Apparatus

A CHI 660B Electrochemical Station (CH Instruments) was used for carrying out the electrochemical experiments. A glassy carbon disk electrode with a diameter of 3 mm served as the working electrode, with the Ag/AgCl and platinum wire acting as the reference and counter electrodes, respectively. All potentials were given with respect to the Ag/AgCl electrode. A pHs-3B pH meter (Dazhong, Shanghai, China) was used for measuring pH.

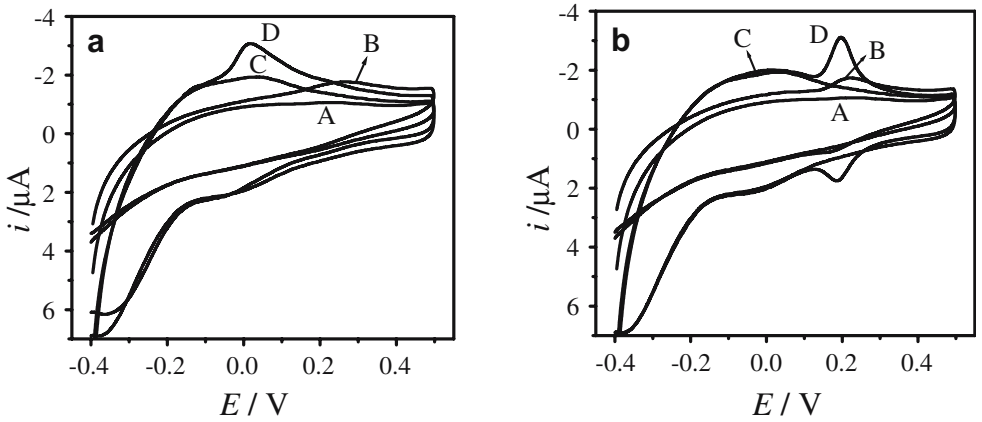

Fig. 2 CVs of the bare glassy carbon electrode (*A*, *B*) and the poly(caffeic acid)-modified electrode (*C*, *D*) at a scan rate of 10 mV s^{-1} in 0.1 mol L^{-1} phosphate buffer (pH 6.64). **a** *A* and *C* represent the absence of AA; *B* and *D* represent the presence of 0.2 mmol L^{-1} AA; **b** *A* and *C* represent the absence of DA; *B* and *D* represent the presence of 0.02 mmol L^{-1} DA

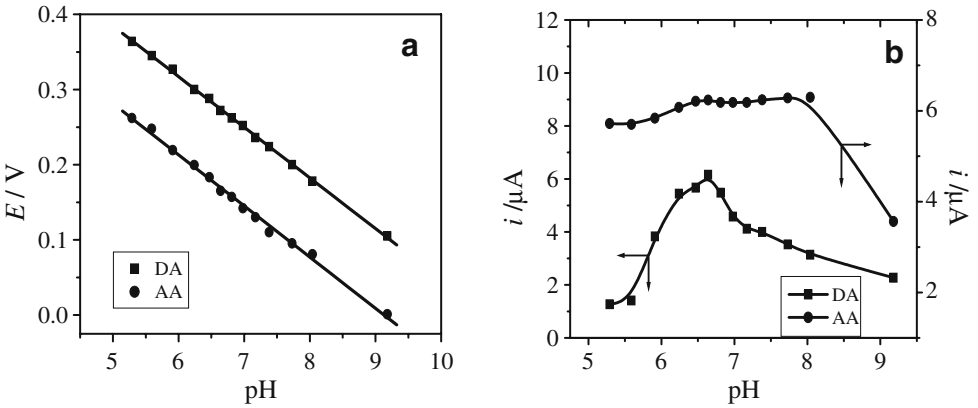

Fig. 3 Effects of pH on peak potential (**a**) and peak current (**b**) of the electrochemical oxidation of DA and AA at the poly (caffeic acid)-modified glassy carbon electrode

Electrode preparation

Before each experiment, the glassy carbon electrode was first polished with 0.05-μm alumina in a water slurry using a polishing cloth and rinsed with 1:1 HNO_3, acetone and water, respectively. Subsequently, the electrode was placed in a pH 7.0 phosphate buffer solution containing 0.2 mmol L^{-1} caffeic acid and modified by deposition at the potential of +2.0 V for 30 s. Then, the poly(caffeic acid)-modified glassy carbon electrode was prepared.

Electrochemical measurement procedure

The poly(caffeic acid)-modified glassy carbon electrode, the reference, and counter electrodes were immersed into 0.15 mol L^{-1} phosphate buffer solution (pH 6.64) containing different concentrations of DA and AA. Freshly prepared solution of DA and AA were used for the experiments. The cyclic voltammograms (CVs) were recorded in a suitable potential range under various conditions.

Sample analysis

The modified electrode was applied for the measuring of AA in AA tablets (Southwest China Pharmaceutical). Each tablet was powdered in a mortar and dissolved with water, and the solution was displaced to a 100-mL volumetric flask and diluted to the mark with water. For the simultaneous determination of DA and AA in mixed real samples, the mixed real samples contained a known amount of AA (from AA tablet, 100.0 mg/tablet) and DA (from DA injection, 10.00 mg mL^{-1}). Aliquots of the mixture samples were added to 10-mL calibrated flasks and diluted to the mark with 2.0 mL of phosphate buffer (6.64) and water. The following procedure was the same as the electrochemical measurement procedure described above.

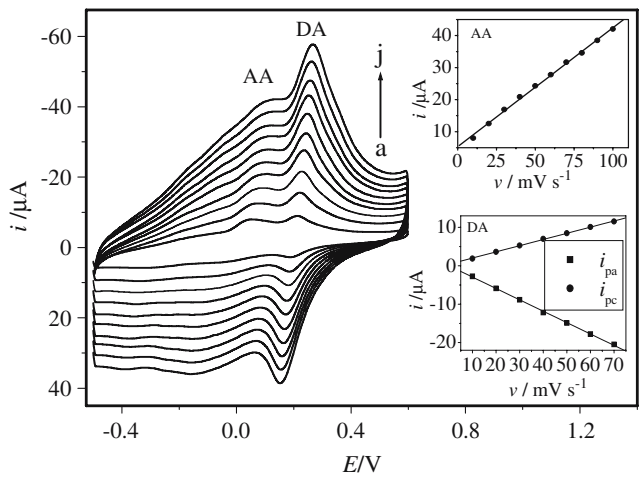

Fig. 4 Cyclic voltammograms at the composite polymer modified electrode in phosphate buffer (pH 6.64) at different scan rates: *a* 10, *b* 20, *c* 30, *d* 40, *e* 50, *f* 60, *g* 70, *h* 80, *i* 90, and *j* 100 mV s^{-1}. *Inset*: plot of anodic peak current vs scan rates

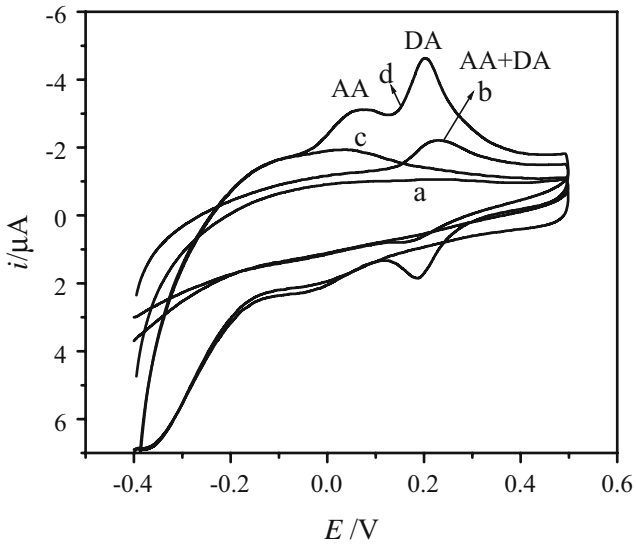

Fig. 5 CVs of phosphate buffer (pH 6.64) in the absence (*a*, *c*) and presence (*b*, *d*) of the mixture containing AA (0.2 mmol L^{-1}) and DA (0.02 mmol L^{-1}) at the bare glassy carbon electrode (*a*, *b*) and the poly (caffeic acid)-modified electrode (*c*, *d*). Scan rate, 10 mV s^{-1}

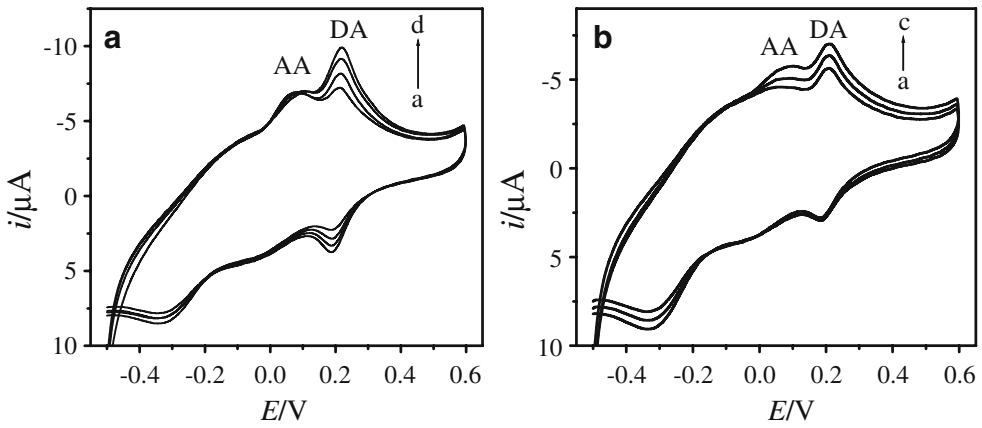

Fig. 6 CVs for the mixture containing AA and DA with different concentrations in phosphate buffer (pH 6.64) at the poly(caffeic acid)-modified electrode. **a** AA, 0.5 mmol L^{-1}; DA (from *a* to *d*), 10.0, 20.0, 30.0, 40.0 μmol L^{-1}); **b** DA, 0.01 mmol L^{-1}; AA (from *a* to *c*), 100.0, 200.0, 300.0 μmol L^{-1})

Results and discussion

Characteristics of the deposited caffeic acid film-modified electrode

The CVs of the poly(caffeic acid)-modified glassy carbon electrode at various scan rates in phosphate buffer (pH 6.64) in the potential range of −0.6 to 0.6 V are shown in Fig. 1. It can be seen from Fig. 1 that a couple of redox peaks appeared at 0.065 and −0.052 V, respectively, at a scan rate of 150 mV s^{-1}, and the peak currents were directly proportional to the scan rates in the range below 500 mV s^{-1}, indicating that this was a surface-confined redox couple.

The modified electrode exhibited a high stability whenever it was placed in a dry state or in the phosphate buffer. No loss of electroactivity of the electrode was found for the continuous cyclical sweep for 8 h. The modified electrode was also not deteriorated even for as long as 2 months.

Individual electrocatalytic oxidation of AA and DA

The poly(caffeic acid)-modified electrode can singly determine AA and DA. Figure 2 illustrates the CVs of AA (Fig. 2a) and DA (Fig. 2b) at the bare and poly(caffeic acid)-modified glassy carbon electrodes in phosphate buffer (pH 6.64). As can be seen from Fig. 2a, at the bare electrode, the CV exhibited a broad peak at a higher potential about 250 mV with poor current response for AA oxidation. In contrast, at the poly(caffeic acid)-modified electrode, a negative shift of the oxidation potential to 18 mV and an increase in peak current were observed, which indicated that the poly(caffeic acid)-modified electrode possessed a strong electrocatalytic activity for the oxidation of AA.

From Fig. 2b, it can be seen that a broad oxidation peak at about 225 mV and a small reduction peak response at 160 mV appeared at the bare electrode. However, a negative shift of the oxidation potential for DA to about 190 mV was observed, and the peak current was enhanced at the modified electrode, indicating a good catalytic activity of the modified electrode for the oxidation of DA. The CV response at the bare electrode was rather broad, whereas it was very sharp and well defined at the poly(caffeic acid)-modified electrode, indicating that the electron transfer reaction was accelerated.

Effect of pH on the oxidation of AA and DA

The effects of pH on the catalytic responses of AA and DA in 0.1 mol L^{-1} phosphate buffer in the pH range from 5.29 to 9.18 at the modified electrode were studied (Fig. 3). The results showed that the anodic peak potentials for the

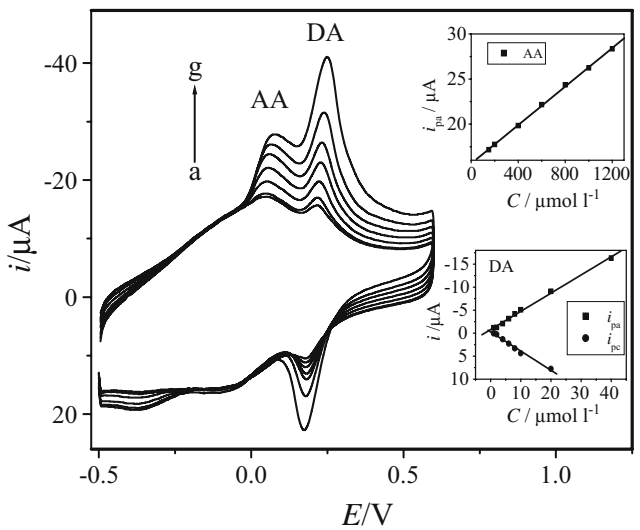

Fig. 7 AA and DA concentrations dependence of the cyclic voltammetric response of the poly(caffeic acid)-modified glassy carbon electrode in 0.15 mol L^{-1} phosphate buffer (pH 6.64) at 50 mV s^{-1}. Concentrations of the two compounds (from *a* to *g*): 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mmol L^{-1} for AA; 2.0, 4.0, 6.0, 8.0, 10.0, 20.0 and 40.0 μmol L^{-1} for DA. *Inset*: plot of anodic peak current vs. AA concentration (*upper*); plot of the anodic and cathodic peak current vs. DA concentration (*lower*)

Table 1 Analytical characteristics for simultaneous determination of AA and DA by the proposed method

Analyte	Linear range (mol L^{-1})	Linear regression equation (i: 10^{-6}A, C: μmol L^{-1})	Correlation coefficient	Detection limit (mol L^{-1})
AA	2.0×10^{-5}–1.2×10^{-3}	$i_{AA}=15.6251+0.0107\ C$	0.9995	9.0×10^{-6}
DA	1.0×10^{-6}–4.0×10^{-5}	$i_{paDA}=0.7941+0.3951$ C	0.9984	4.0×10^{-7}
	1.0×10^{-6}–2.0×10^{-5}	$i_{pcDA}=0.0655+0.0314$ C	0.9943	5.0×10^{-7}

oxidation of AA and DA shifted toward a negative direction with increasing pH, showing that protons have taken part in the electrode processes. In addition, the anodic peak current of AA reached the maximum and kept constant in the pH range of 6.2–8.0. When pH was above 8.0, the anodic peak current decreased. The pK_{a1} and pK_{a2} values of AA are 4.04 and 11.34, respectively [37], and thus it existed as a negatively charged species under our experimental condition. The reported values for pK_{a1}, pK_{a2}, and pK_{a3} of caffeic acid are 4.4, 8.5, and 11.2, respectively [38]. In the pH range from 5.29 to 8.0, the hydrophobic interaction between the poly(caffeic acid) film and AA molecules was the main factor. The modified surface had more negative charge when pH was above 8.0. Therefore, the peak current of AA decreased. As far as DA is concerned, the anodic peak current reached the maximum at pH 6.64. When the pH was higher or lower than the value, the anodic peak current decreased. The pK_{a1}, pK_{a2}, and pK_{a3} values of DA are 6.8, 7.2, and 8.8, respectively [39]. The negative group on the poly(caffeic acid)-modified electrode surface was increased with increasing pH, so more DA cations were attracted to the electrode surface. The DA anodic peak current increased with the increase of pH over the range 5.29 to 6.64. Then, the protonated degree of DA decreased with the increase of pH. When pH of the solution was higher than 6.64, the negative group of the modified electrode surface increased, but the DA cations deceased more; therefore, the static attraction interaction between DA and the modified electrode decreased. Considering the influence of pH on both AA and DA response mentioned above, pH 6.64 was chosen for the simultaneous determination of AA and DA in this paper.

Effect of scan rate on the oxidation of AA and DA

Figure 4 shows the CVs of the caffeic acid modified glassy carbon electrode at various scan rates obtained in 0.1 mol l^{-1} phosphate buffer (pH 6.64) containing 0.2 mmol l^{-1} AA and 0.02 mmol l^{-1} DA. The peak current was proportional to the scan rate in the range of 10−100 mV s^{-1} with a correlation coefficient of 0.9997 and in the range of 10−70 mV s^{-1} with a correlation coefficient of 0.9995 for the anodic oxidations of AA and DA, respectively, indicating that the catalytic reactions were controlled by absorption. In addition, the anodic potentials shifted positively with the increase of scan rate, indicating the quasi-reversible nature of the electrode reaction.

Electrocatalytic oxidation of mixture of AA and DA

To evaluate the sensitivity and selectivity of the present system for the quantification of AA and DA, the electrochemical behavior of mixture of AA and DA at the poly (caffeic acid)-modified electrode was studied. The results showed that at the bare glassy carbon electrode, the CV of phosphate buffer (pH 6.64) had no oxidation peak (curve a in Fig. 5) and that of the mixture of AA and DA in phosphate buffer had only an oxidation peak at 240 mV (curve b in Fig. 5), illustrating that the oxidation peaks of AA and DA could not be separated at the bare electrode. In contrast, two well-defined anodic peaks at the potentials of 70 and 205 mV were observed for the oxidation of AA (0.2 mmol L^{-1}) and DA (0.02 mmol L^{-1}), respectively, at the modified electrode (curve d in Fig. 5). The observed CV changes indicated that the modified electrode had a good

Table 2 Determination of DA in DA injection and AA in AA tablet

Number	DA injection[a] (mg/2 mL)	DA (n=4)			AA tablets[b] (mg/tab)	AA (n=3)		
		Found (mg/2 mL)	Recovery (%)	RSD (%)		Found (mg/tab)	Recovery (%)	RSD (%)
1	20.00	19.66±1.14	98.3	4.93	100.00	99.78±7.73	99.8	4.6
2	20.00	20.03±0.79	100.2	3.37	100.00	99.13±5.84	99.1	3.5
3	20.00	20.99±1.04	105.0	4.24	100.00	101.26±6.82	101.3	4.0

[a] From Jiangsu Ya Bang Pharmaceutical, Chongqing, China.
[b] From Southwest China Pharmaceutical

Table 3 Simultaneous determination of DA and AA in a mixture

Number	DA added[a] (10^{-5} mol L^{-1})	AA added[b] (10^{-4} mol L^{-1})	DA (n=5)			AA (n=5)		
			Found (10^{-5} mol L^{-1})	Recovery (%)	RSD (%)	Found (10^{-4} mol L^{-1})	Recovery (%)	RSD (%)
1	1.05	5.68	1.08±0.038	102.8	3.7	5.78±0.22	101.8	4.1
2	2.11	8.52	2.05±0.096	97.2	4.9	8.48±0.34	99.5	4.2
3	3.16	11.36	3.28±0.13	103.8	4.2	11.16±0.49	98.2	4.7

[a] From Jiangsu Ya Bang Pharmaceutical, Chongqing, China.
[b] From Southwest China Pharmaceutical

catalytic activity for the oxidation of AA and DA and could clearly distinguish AA and DA.

The electro-oxidation processes of AA and DA in the mixture have also been investigated when the concentration of one species changed, and the results are shown in Fig. 6. Examination of Fig. 6a shows that the peak current of DA increased with an increase in DA concentration when the concentration of AA was kept constant; the anodic peak current of AA did not change. Similarly and obviously, as shown in Fig. 6b, keeping the concentration of DA constant, the oxidation peak current of AA was positively proportional to its concentration, while that of DA did not change. Although the anodic charge current was enhanced after AA was oxidized, the anodic and cathodic peak currents of DA did not change. From the experimental results described above, it was known that in the mixture containing AA and DA, the oxidation peaks of the two compounds were clearly separated from each other.

If the concentrations of AA and DA increased synchronously, the peak currents at the modified electrode increased synchronously as shown in Fig. 7. It can be seen that the peak currents for the two analytes increased linearly with their concentrations. The DA concentration is linear with not only the anodic peak current but also the cathodic peak current. The voltammetric responses of the modified electrode toward the simultaneous determination of AA and DA are listed in Table 1. The relative standard deviations of the determination of 1.0×10^{-4} mol L^{-1} AA and 1.0×10^{-5} mol L^{-1} DA repeated for eight times were 1.2 and 2.0%, respectively.

Effect of foreign substances

The influence of various foreign species on the determination of 1.0×10^{-4} mol L^{-1} AA and 1.0×10^{-5} mol L^{-1} DA were investigated. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5% relative error in the determination. The tolerated ratio of the foreign substances was 1,000 for NaCl and KCl, 200 for L-lysine and chitosan, 100 for glycin and glutamic acid, 50 for uric acid and glucose, and 20 for L-asparagine, $Ca(NO_3)_2$, L-cystine, and $MgCl_2$.

Analytical application

Using the proposed methods described above, the injection of DA hydrochloride (Jiangsu Ya Bang Pharmaceutical) was analyzed. The results are shown in Table 2. The average determination result of DA in the injection was 10.11 mg mL^{-1}, which was quite corresponding to the value specified on the injection (10.00 mg mL^{-1}). Different standard concentrations of DA were added to the diluted DA injection (1.05×10^{-5} mol L^{-1}), and the recovery was between 103.8 and 104.4% for eight measurements. This method was also used to determine the AA in AA tablets (Southwest China Pharmaceutical), and the determination results are listed in Table 2. The poly(caffeic acid)-modified glassy carbon electrode was applied to measurement of DA and AA in mixed real samples. The CVs were recorded, and the anodic peak currents were measured at the oxidation peak potentials of AA and DA. The determination results are listed in Table 3. A good recovery obtained with the present system indicates the reliability of the method for application to monitoring of AA and DA.

Conclusions

The poly(caffeic acid) film was prepared and used as a modified electrode in a near-neutral solution for the determination of AA, DA, and their mixture by CV. A clear separation of oxidation peaks of AA and DA could be achieved, indicating that the poly(caffeic acid) electrode facilitated the simultaneous determination of AA and DA with good stability, sensitivity, and selectivity. The proposed method was applied to the simultaneous determination of DA and AA concentrations in real samples with satisfactory results.

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