Preparation of Poly(9-aminoacridine)-Modified Electrode and Its Application in the Determination of Dopamine and Ascorbic Acid Simultaneously

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ABSTRACT: A novel modified electrode was fabricated with 9-aminoacridine by electropolymerization in the phosphate buffer solution (PBS) (pH 7.4) and was characterized by cyclic voltammetry (CV). The modified electrode showed excellent electrocatalytic effect and high stability toward the electrochemical oxidation of dopamine (DA) and ascorbic acid (AA). Also, it showed a high stability for the determination of DA and AA simultaneously. Well-separated voltammetric peaks were observed for DA and AA on the modified electrode. The separation of two anodic peaks was 170 mV, which was large enough to eliminate the interference of AA and determine DA. The differential pulse voltammograms (DPV) were used for the measurement of DA by means of the poly(9-aminoacridine)-modified electrode in PBS at pH 7.4. A linear response to DA was observed in the concentration range from 1.5×10^{-6} to 3.5×10^{-3} mol L^{-1} with a correlation coefficient of 0.9998 and a detection limit ($S/N = 3$) of 1.0×10^{-7}mol L^{-1}. The proposed method was used to determine DA in DA-hydrochloride injection and showed excellent sensitivity and recovery. The ease of fabrication, good reproducibility, high stability, and low cost of the modified electrode are the promising features of the proposed sensor. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 3864–3870, 2007

Key words: modified electrode; dopamine; ascorbic acid; 9-aminoacridine; determination

INTRODUCTION

DA is one of the most significant catecholamines, which is very important in mammalian central nervous systems. Extreme abnormalities of DA concentration levels may lead to symptoms of several diseases such as Parkinsonism.[1] Therefore, DA is currently the object of intense research for neuroscientists and chemists, and it is essential to develop rapid and simple methods for the determination of the concentration of DA. Generally, the determination of DA is carried out by spectrophotometry,[2] ion-chromatography,[3] liquid-chromatography (HPLC),[4] and ion-exchange column chromatography.[5] And DA can also be determined by electrochemical methods[6,7] because it is an electrochemically active compound. However, a major problem in its determination is the interference from ascorbic acid (AA), which has an overlapping oxidation potential with DA on the solid electrode and largely coexists with DA in biological fluids and brain issue. In biological environments (pH 7.4), DA exists as cations ($pK_b = 8.87$) and AA exists as anions ($pK_a = 4.10$). Therefore, to eliminate the interference of AA with the determination of DA, a convenient way is to fabricate a modified electrode,[8–22] such as self-assembled monolayer modified electrode,[14–16] electropolymers-modified electrode,[17–20] etc. In the recent years, polymer-modified electrode by electropolymerization has attracted much attention because polymer film has good stability and reproducibility.[21,22] On this basis, a number of researchers have employed polymeric-film-modified electrodes to detect DA in the presence AA.

Acridine is structurally similar to anthracene but possesses a nitrogen atom in its central ring. It has been established for many years that the planar structure of tricyclic rings conferred to acridine derivatives,[23] various natural and synthetic compounds of the acridine family have been selected for antibacterial or anticancer chemotherapy. Genetic effects of acridine compounds have been studied in

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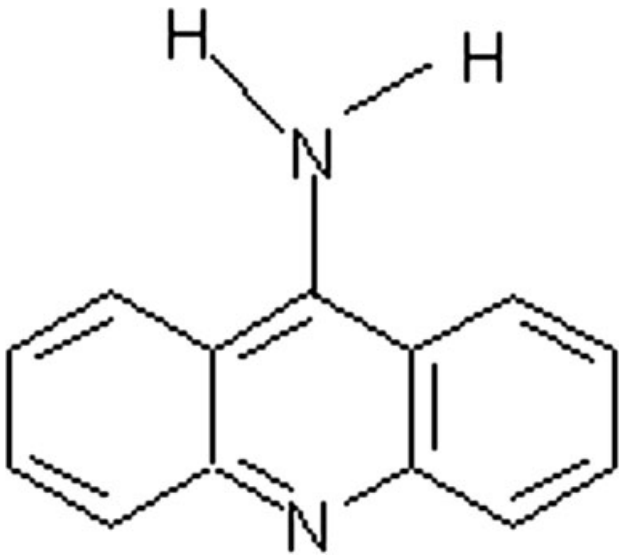

Scheme 1 Chemical structure of 9-aminoacridine.

microorganisms, insects, plants, mammalian cells, and mammals (Ferguson and Denny, 1991). Among their mutagenic effects, the most notable thing is the induction of frame shift mutations, including both base-pair deletions and additions. 9-Aminoacridine is the simplest compound of the acridine family and its structure is described in Scheme 1. Also, it is very important to study its electrochemistry and photochemistry character. However, the usage of 9-aminoacridine as a modifier for the fabrication of biosensor had never been reported until our recent work.

In this article, we apply 9-aminoacridine as a modifier to fabricate a poly(9-aminoacridine)-modified glassy carbon electrode (PAAME) by electropolymerization. And the experiment results show that the modified electrode can separate the anodic peak potential of DA and AA by more than 170 mV. And the differential pulse voltammograms (DPV) were used for the measurement of DA by means of the poly(9-aminoacridine)-modified electrode in phosphate buffer solution (PBS) at pH 7.4. The proposed method was used to determine DA in DA-hydrochloride injection and showed excellent sensitivity and recovery. The ease of fabrication, good reproducibility, high stability, and low cost of the modified electrode are promising features of the proposed sensor.

EXPERIMENTAL

Instruments

All electrochemical experiments were performed with a CHI 660A electrochemical workstation (ChenHua Instruments, Shanghai, China). A conventional three-electrode cell with a saturated calomel electrode (SCE) reference electrode, a platinum wire counter electrode, and a bare glassy carbon or a poly(9-aminoacridine)-modified glassy carbon as working electrode. All potentials reported in this article were reference to the SCE. Oxygen was removed by purging with high-purity nitrogen for 10 min, and a nitrogen atmosphere was kept over the solution during measurement.

Reagents

DA and AA were purchased form Acros and were used as received. 9-Aminoacridine was synthesized and provided by Zhou et al.[24,25] All other reagents used in this investigation were of analytical grade and were used without purification. All aqueous solutions used in this experiment were made using double-distilled water, with a quartz apparatus. The PBS was prepared from KH_2PO_4 and K_2HPO_4 (0.1 mol L^{-1}) and adjusted the pH with 0.1 mol L^{-1} H_3PO_4 and KOH solutions.

Preparation of poly(9-aminoacridine)-modified GCE

The bare glassy carbon electrode (GCE) was polished successively with 0.05 μm Al_2O_3 slurry on emery paper before modification. Then, it was rinsed with doubly distilled water and sonicated in 1 : 1 HNO_3, acetone and doubly distilled water for 5 min, respectively. After rinsed with water, the electrode was activated by 20 cyclic sweepings from −1.0 to 2.0 V at 100 mV s^{-1} in pH 7.4 PBS. Finally, the polymeric film was deposited by cyclic sweeping from −1.2 to 2.5 V at 100 mVs^{-1} for 20 cycles in pH 7.4 PBS containing 5.0×10^{-5} mol L^{-1} 9-aminoacridine solutions.

Electrochemical measurement

A 5.00-mL volume of PBS containing a suitable amount of DA and AA was added to the 10.00-mL voltammetry cell. The solution was purged under nitrogen for 10 min, and the flow of nitrogen over the cell was maintained throughout the experiment. In cyclic voltammetry (CV) and DPV measurement, potential scanning was performed according to the need of experiment. All experiments were conducted at room temperature.

RESULTS AND DISCUSSION

Electrochemical polymerization of 9-aminoacridine films on a GCE

CV was used to form the polymerization film. The potential scan range was the most important factor to prepare the poly(9-aminoacridine) film. The experiment results indicated that if the positive potential value for the polymerization was under 2.0 V or if the negative one was above −0.4 V, no

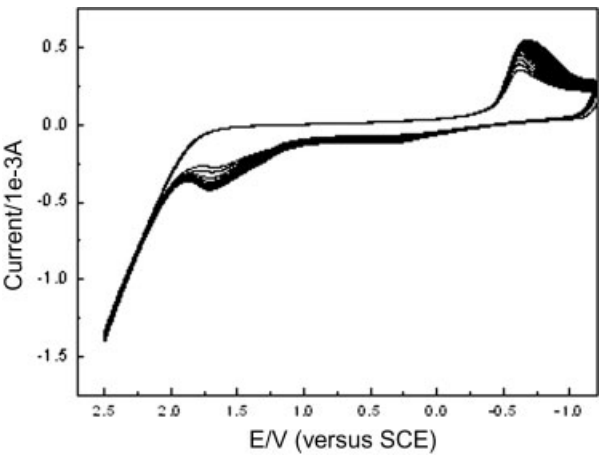

Figure 1 Cyclic voltammograms of 5.0×10^{-5} mol L^{-1} 9-aminoacridine in pH 7.4 PBS. Scan rate: 100 mV/s; sensitivity: 10^{-4} A/V.

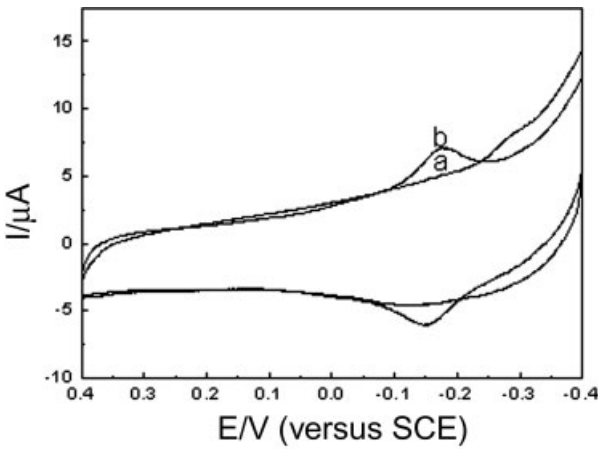

Figure 2 Cyclic voltammograms on a bare glassy carbon electrode (a) and on the modified electrode (b) in 0.1 mol L^{-1} PBS. Scan rate: 100 mV/s; sensitivity: 10^{-5} A/V.

polymer reaction would occur. The peak current of the modified electrode increased with the peak potential shifting positively. When the positive potential value reached 2.5 V and the negative potential value reached −1.2 V, the CV curve was stable. So, we selected the potential scan window from −1.2 to 2.5 V as the electropolymerization condition.

On the other hand, we studied the effect of the bulk solution through the buffer solution of NaAc-HAc, citrate, and NaH_2PO_{4-} Na_2HPO_4 solutions. The experiment results reveal that the CV curve was stable and good when using the PBS as bulk solution. So, we selected the PBS as the experiment bulk solution.

Figure 1 depicts the repetitive cyclic voltammograms of 5.0×10^{-5} mol L^{-1} 9-aminoacridine in PBS (pH 7.4) at the GCE. In the first cycle, a cathodic peak at −0.6 V was observed. From the beginning of the second cycle, we can see two anodic peaks at 1.25 and 1.64 V, respectively. With continuous cycling until 10 cycles, the peak currents increase were observed, which indicated that film formation has occurred. After 20 cycles, no obvious changes in the peak current were observed, which indicating that polymerization had reached saturation. These facts showed that 9-aminoacridine was deposited on the surface of GCE by electropolymerication. After modification, a uniform adherent blue-black polymer film on the GCE surface was observed by naked eye. After polymerization, the poly(9-aminoacridine) film was thoroughly rinsed with doubly distilled water to remove the physical adsorption of 9-aminoacridine and stored in PBS (pH 7.4).

Electrochemical characterization of poly(9-aminoacridine)-modified electrode

Figure 2 is the CVs of the poly(9-aminoacridine) film on the GCE and the bare GCE in the 0.1 mol L^{-1} PBS (pH 7.4). As it shows, on the poly(9-aminoacridine)-modified electrode, there is a chemically reversible redox couple (curve b) of which the potential is −0.148 and −0.178 V, but meanwhile there were no peaks on the bare GCE (curve a). This electrochemical behavior of the modified electrode proved that 9-aminoacridine had been modified onto the GCE.

Electrocatalytic oxidation of DA at the poly(9-aminoacridine)-modified electrode

Figure 3 shows the CVs of DA at the bare electrode and the poly(9-aminoacridine)-modified electrode (PAAME) in 0.1 mol L^{-1} PBS (pH 7.4). The curves (a) and (b) correspond to the oxidation of 2.0×10^{-5} mol L^{-1} DA at the bare GCE and the PAAME, respectively. It can be observed that DA exhibits poor electrochemical response at the bare GCE while

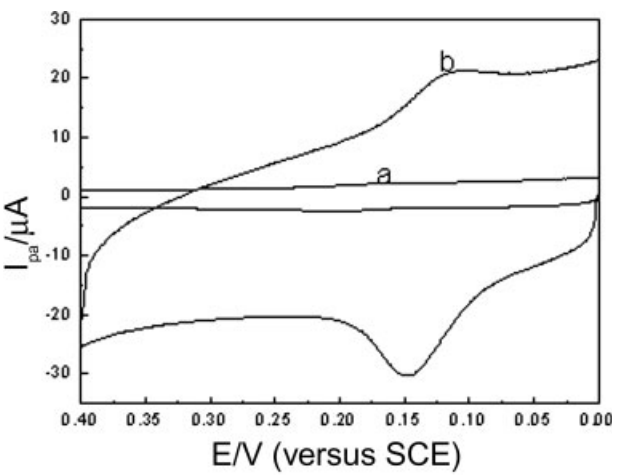

Figure 3 Cyclic voltammograms of 2.0×10^{-5} mol L^{-1} dopamine on a bare glassy carbon electrode (a) and on a poly(9-aminoacridine)-modified electrode (b) in 0.1 mol L^{-1} PBS. Scan rate: 100 mV/s; sensitivity: 10^{-5} A/V.

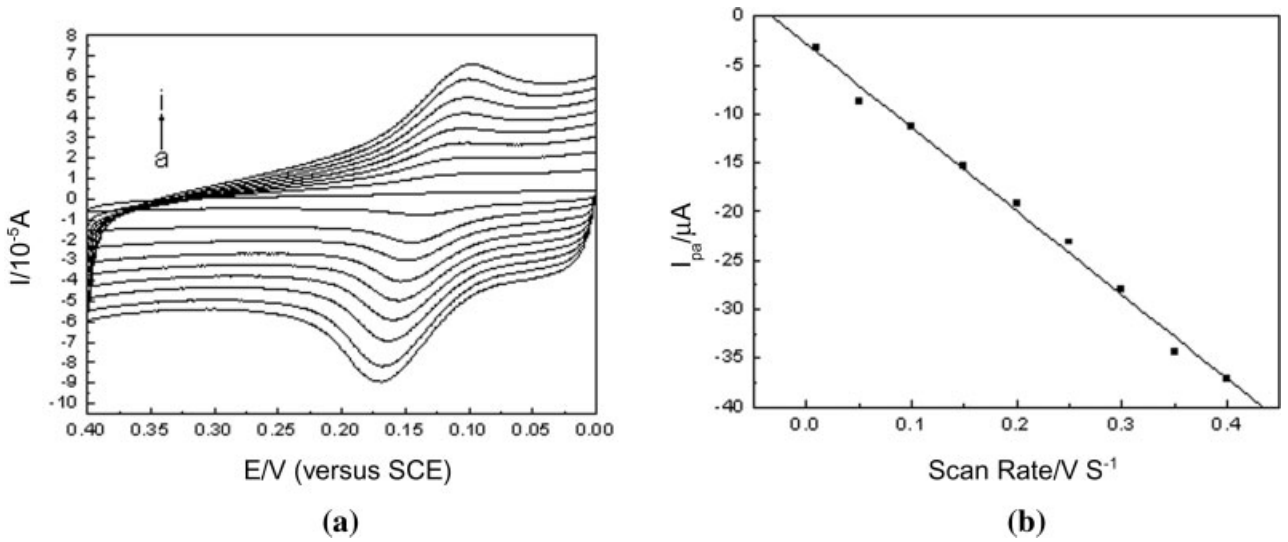

Figure 4 A: Dependence of cyclic voltammograms on the scan rate for the poly(9-aminoacridine)-modified electrode in 0.1 mol L^{-1} PBS (pH 7.4) with 1.0×10^{-5} mol L^{-1} DA. Scan rate: (a) 0.01, (b) 0.05, (c) 0.1, (d) 0.15, (e) 0.2, (f) 0.25, (g) 0.3, (h) 0.35, and (i) 0.4 V s^{-1}. B: Plot of anodic peak current versusscan rate. Scan rate: 100 mV/s; sensitivity: 10^{-5} A/V.

it exhibits good response at the PAAME. At the bare GCE, the peak potential separate value between the anodic peak potential (E_{pa} = 0.201 V) and the cathodic peak potential (E_{pc} = 101 V) was 100mV. But, at the PAAME, the cathodic and anodic peak potential is 147 and 102 mV, respectively. So the peak potential separate value (ΔE_p) of DA on the modified electrode was 45 mV. It can be seen that the anodic overpotential decreased 54 mV. The reason for this may be in biological environment (pH 7.4), DA exists as cations and AA exists as anions. The amidogen of 9-aminoacridine has a lone electron pair, which can attract cations, and there were formed hydrogen bonds between the amidogen of 9-aminoacridine and the hydroxyl of DA. So the DA can reach to the surface of modified electrode easier. Under the same condition, DA has significantly increasing peak currents and a more reversible electron transfer process on the PAAME. The remarkable enhancement in peak currents and the drop in anodic overpotential provide clear evidence of the catalytic effect of poly(9-aminoacridine) film toward DA.

Effect of scan rate

The scan rate effect on the cyclic votammetric response of the poly(9-aminoacridine)-modified electrode in 0.1 mol L^{-1} PBS (pH 7.4) with 2.0×10^{-5} mol L^{-1} DA is shown in Figure 4. As the scan rate increases, the oxidation peak current (I_{pa}) increases. A good linear relationship between I_{pa} and scan rate was obtained over the range of 10–400 mV s^{-1}. The linear regression equation was I_{pa} (μA) = −2.7598–85.8844 V (V s^{-1}) with a correlation coefficient of R = −0.9965. These phenomena suggest that the reaction of DA at the poly(9-aminoacridine)-modified electrode is controlled by adsorption.

Effect of pH

The effect of pH on the response of DA was investigated over the range of 5.1–9.0. Figure 5(a) showed that the anodic peak current increased with pH value increasing until it reached 8.0. When the pH value was more than 8.0, the anodic peak current decreased. Since the physiological pH value is about 7.4, we chose it as the support electrolyte in the electrochemical detection of DA.

Figure 5(b) shows that the variation of peak potential changes in the pH of the electrolyte. It was found that the anodic peak potential shifted with pH value increase, indicating that protons take part in the electrode process. The linear regression equation was E_{pa} (V) = 0.6283–0.0634 pH with a correlation coefficient of R = −0.9954. According to the Nernst equation (T = 293°C), the slope of −63.4 mV/pH suggests that two protons take part in the oxidation of DA.

Determination of DA

To examine the response character of modified electrode to DA, we have undertaken the detection of DA in PBS (pH 7.4). Under the optimum conditions established earlier, the anodic peak current is linearly related to the concentration over the range 1.5×10^{-6} -3.5×10^{-3} mol L^{-1} (Fig. 6). The linear regression equation is I_{pa} (μA) = 0.5284–1.5412 C (10^{-5} mol

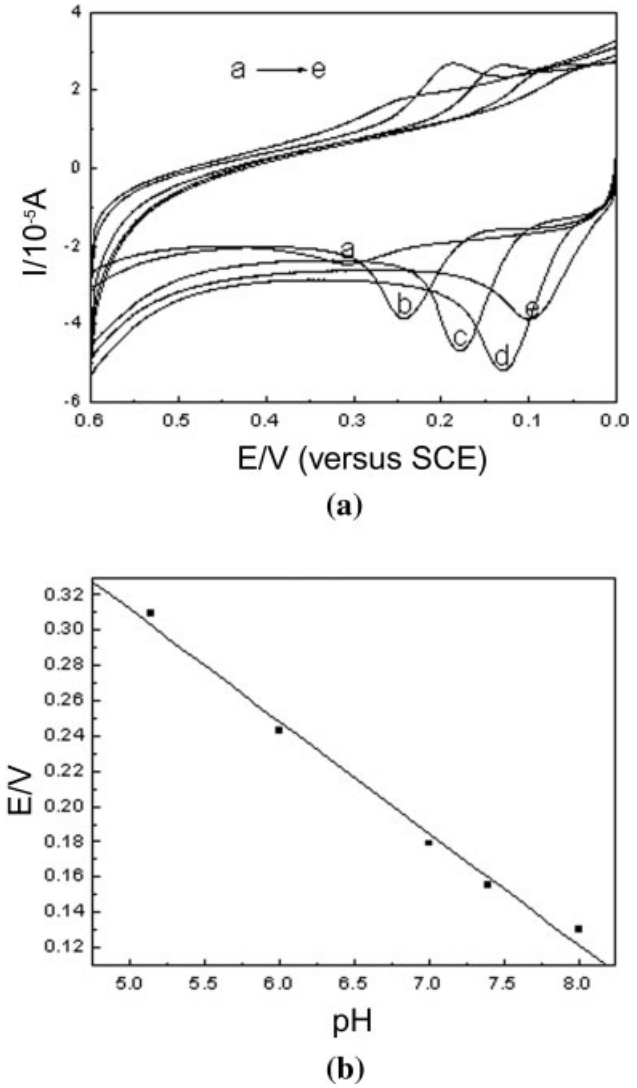

Figure 5 A: Dependence of cyclic voltammograms on the value of pH for the poly(9-aminoacridine)-modified electrode in 0.1 mol L^{-1} PBS with 1.0×10^{-5} mol L^{-1} DA. (a) 5.14, (b) 6.0, (c) 7.0, (d) 8.0, and (e) 9.0. B: Plot of anodic peak potential versus pH. Scan rate: 100 mV/s; sensitivity: 10^{-5} A/V.

L^{-1}) with a correlation coefficient of $R = -0.9998$. And the detection limit ($S/N = 3$) is 1.0×10^{-7} mol L^{-1}.

Simultaneous determination of AA and DA concentrations

To learn more about the electrochemical responses, when DA and AA coexist, we tested the voltammetric behaviors of DA and AA. Figure 7(a) illustrates the DPV of the poly(9-aminoacridine)-modified electrode while simultaneously varying the concentrations of both DA and AA. Under the optimum conditions established earlier, the anodic peak potential for DA and AA at modified electrode was +0.085 and −0.085 V, respectively. The anodic peak current of DA is linearly related to the concentration over the range of 1.5×10^{-6}–2.1×10^{-3} mol L^{-1}, while the anodic peak current of AA is linearly related to the concentration over the range of 1.0×10^{-5}–1.0×10^{-2} mol L^{-1} [Fig. 7(b)]. The linear regression equation of DA is I_{pa}(μA) is −2.613–1.476 C (10^{-5} mol L^{-1}) with a correlation coefficient of $R = -0.9942$, meanwhile the linear regression equation of AA I_{pa} (μA) is −2.983–1.9786 C (10^{-3} mol L^{-1}) with a correlation coefficient of $R = -0.9914$.

Interferences

Interference studies were carried out with other compounds. No interference could be observed for the following compounds: uric acid (200), citric

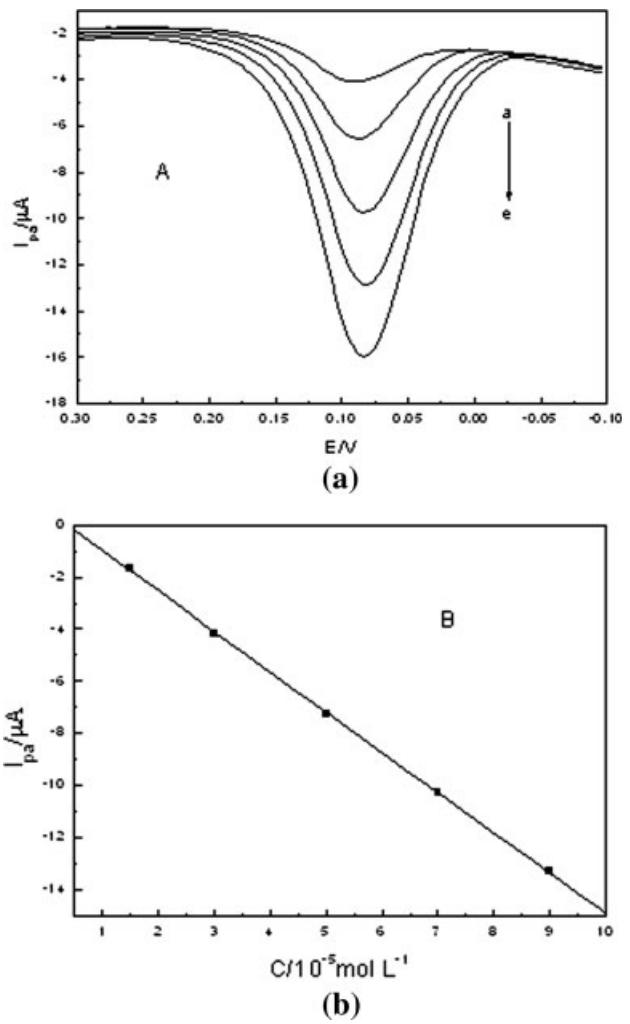

Figure 6 A: Differential pulse voltammograms without correction of background current for different concentrations of DA in PBS (pH 7.4) on PAAME. The concentration of DA: (a) 1.5×10^{-5}, (b) 3×10^{-5}, (c) 5×10^{-5}, (d) 7×10^{-5}, (e) 9×10^{-5} mol L^{-1}. B: Plot of the anodic peak current versus the concentration of DA. Sensitivity: 1.0×10^{-5} A/V; scan rate: 100 mV/s.

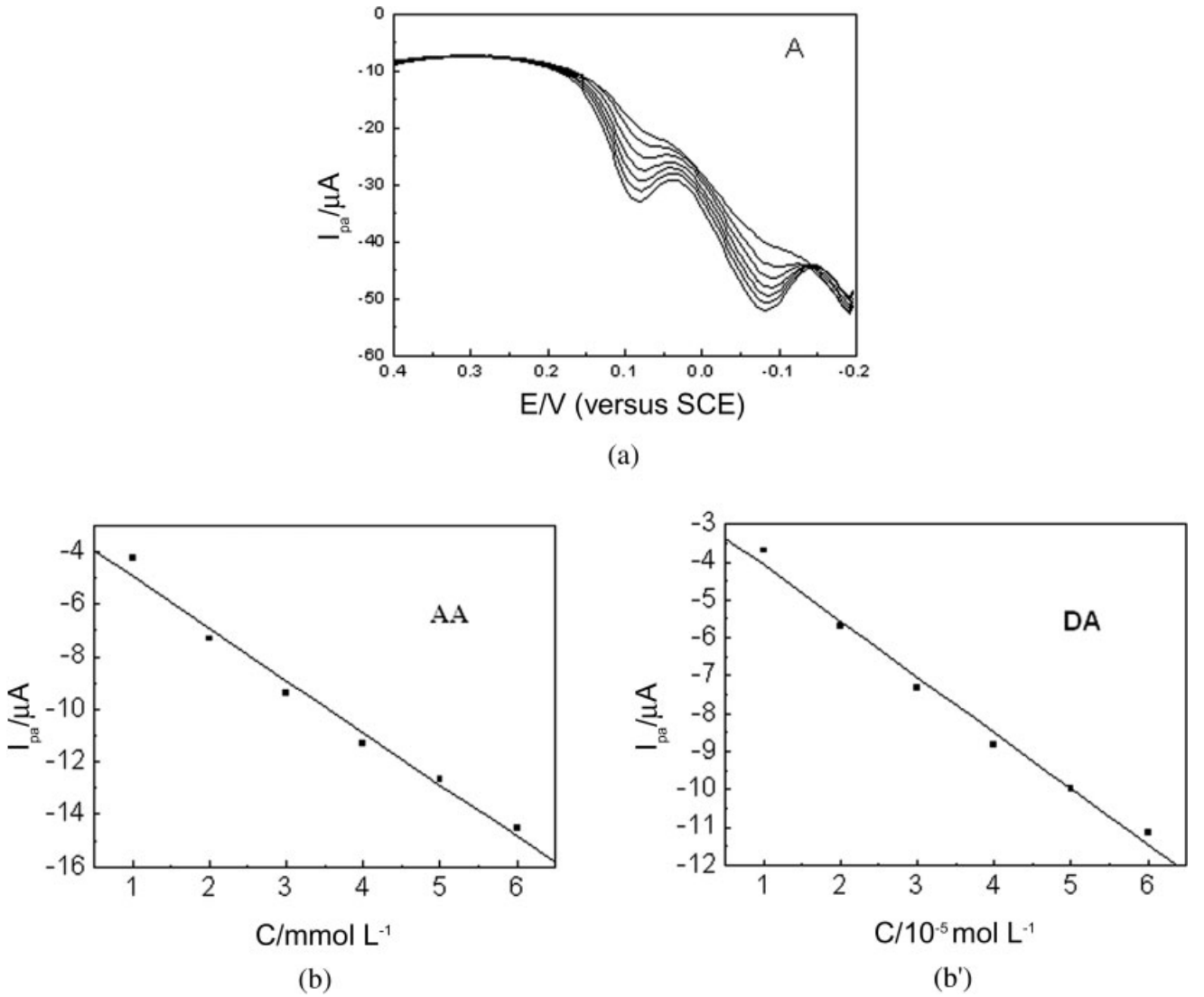

Figure 7 A: The differential pulse voltammograms obtained for AA and DA while simultaneously changing their concentrations in 0.1 mol L^{-1} PBS (pH 7.4) [DA]: each addition increased the concentration by 10 μmol L^{-1}. [AA]: each addition increased the concentration by 1 mmol L^{-1}. B: Corresponding calibration plots for AA and DA. Sensitivity: 1.0×10^{-5} A/V; scan rate: 100 mV/s.

acid (100), cysteine (50), glucose (50), NaCl (400), KCl (350), and $CaCl_2$ (200), where the numbers in parentheses represent the concentration ratios to 1.0×10^{-5} mol L^{-1} DA.

Analytical applications

Using the proposed methods described earlier, the injection of DA hydrochloride was analyzed. A certain value of standard solutions of DA was added into the corresponding injection for testing recovery. The results are shown in Table I. The recovery and R.S.D. were acceptable, showing that the proposed methods could be efficiently used for the determination of DA in injections.

Samples were obtained from the Wannan medical college. Each result was the average of 10 measurements.

Stability and reproducibility of modified electrode

Evaluation of the maintenance, lifetime, and storage of the poly(9-aminoacridine)-modified electrode have been studied by measuring its voltammetric response on storage for a long duration. The modified electrode is very stable in the potential window of 0.4 and 0.4 V in 0.1 mol L^{-1} PBS (pH 7.4) and the capacitance of this electrode is unchanged while cycling the electrode potential in the above-mentioned potential window. It was observed that no apparent decrease in response for nearly 8 days. The modified electrode retained 95, 91, and 90% of its initial response up to 14, 21, and 30 days, respectively. Such a good stability is acceptable for most practical applications. To ascertain the reproducibility of the results, eight different glassy carbon modified

TABLE I
Determination Results of DA in Injections (n = 10)

Sample	Labeled (mg L^{-1})	Found (mg L^{-1}; n = 10)	RSD (%; n = 10)	Recovery (%)
1	10.0	9.9	2.0	99
2	10.0	10.2	2.3	102
3	10.0	10.1	1.9	101
4	10.0	9.9	2.5	99
5	10.0	9.8	2.1	98
6	10.0	10.1	2.0	101

electrodes were modified with 9-aminoacridine and their response toward the oxidation of AA and DA was tested by 10 repeated measurements of the same solution. The peak potential and the peak current obtained in the 10 repeated measurements of four independent electrodes showed a relative standard deviation of 1.0%, confirming that the results are reproducible. As the electrode preparation is very easy and the electrode is very stable, its use in clinical analysis for the determination of DA in the presence of AA is very promising.

CONCLUSIONS

9-Aminoacridine was firstly applied into the electrode modifying to determine DA and AA simultaneously. The modified electrode resolved the merged voltammetric signals of AA and DA into two well-defined peaks with a peak separation of 170 mV. The proposed method has wider linear range, low detection limit, good reproducibility, and stability. The versatility and flexibility of the electrode fabrication and the inherent large number of controllable variables in the fabrication procedure make it promising for a wide variety of sensing materials and applications. These attractive features of the proposed electrode suggest the promising of *in vivo* and *in vitro* applications, which is under progress in our work. This material was firstly applied in electrochemical modified electrode.

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