J Appl Electrochem (2010) 40:1379–1385
DOI 10.1007/s10800-010-0105-y

ORIGINAL PAPER

Selective determination of dopamine in the presence of ascorbic acid using ferrocenyl-tethered PAMAM dendrimers modified glassy carbon electrode

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Received: 28 September 2009 / Accepted: 14 February 2010 / Published online: 7 March 2010

Abstract Determination of dopamine (DA) in the absence and presence of ascorbic acid (AA) by ferrocenyl-tethered PAMAM G3 dendrimers (Fc-D) modified glassy carbon electrode (GCE) was reported. The modified electrode was characterized with cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Factors influencing the detection processes were optimized and kinetic parameters were calculated. The sensor exhibited excellent catalytic activities for the oxidation–reduction reactions of DA and eliminated the interference of AA. Under optimal condition, the linear range of 1×10^{-5}–1.5×10^{-3} mol L^{-1} and the detection limit of 4.7×10^{-6} mol L^{-1} was obtained. This study provides a new idea for the determination of DA in the presence of AA.

Keywords Dopamine · PAMAM · Ferrocenyl-tethered PAMAM dendrimers · Ascorbic acid · Selective determination

1 Introduction

Dopamine (DA) is an important neurotransmitter with a general catecholamine structure. It is widely distributed in the mammalian central nervous system for message transfer, which plays a particularly important role in the function of central nervous, renal, hormonal, and cardiovascular systems. Several diseases such as epilepsy, senile dementia, Parkinsonism, and HIV infection are attributed to DA abnormal metabolism [1–4]. Therefore, the determination of DA is a very important subject and it is essential to develop rapid and simple methods. The increasing demand led to the development of new techniques, among which electrochemical sensors attracted wide attention due to the advantages of simple, low-cost, and fast analysis in combination with high sensitivity and selectivity. However, a major problem encountered in the determination of DA is the interference from ascorbic acid (AA), which prevented scurvy and was known to take part in several biological reactions. AA was oxidized almost at the same potential as DA, which resulted an overlapping voltammetric response for the oxidation of DA and AA [5, 6]. Nowadays, chemical-modified electrode surface has been proved to be a successful strategy to overcome this problem. Various materials such as Nafion and polypyrrole, have been used to modify various electrodes to selectively determinate dopamine based on the cationic permeability of the polymer [7, 8].

Since they were first synthesized by Tomalia [9] in 1985, polyamidoamine (PAMAM) dendrimers have been studied more and more extensively and deeply. Their terminal groups containing –COOH, $-NH_2$, and –OH are easily functionalized by other substances, and the quite open spherical 3D structures at high generation greatly extend their applications [10–13]. In recent years, based on its high geometric symmetry, easily controlled nano size, controllable surface functionality, and chemical stability [14], PAMAM dendrimers have been widely used to fabricate electrochemical sensors. For example, PAMAM dendrimers were used to construct a novel nitrite biosensor

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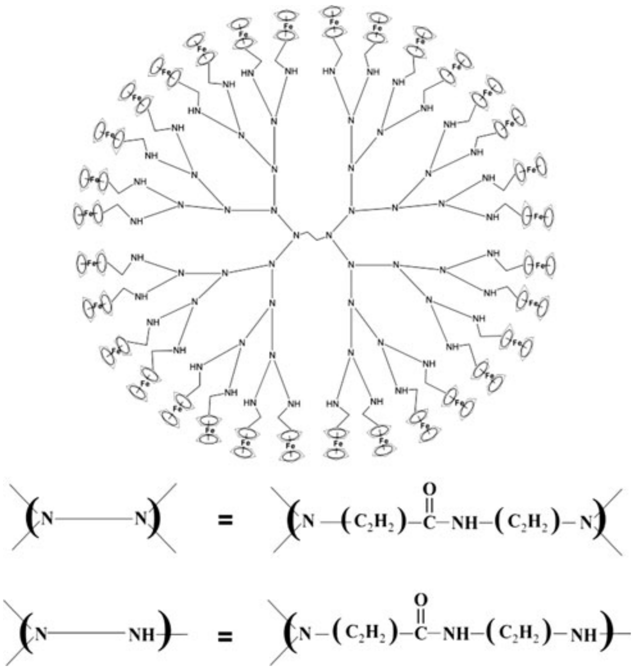


Scheme 1 The structure of the synthesized ferrocenyl-tethered PAMAM dendrimers(Fc-D)

[15] and novel DNA electrochemical biosensor [16], both of which revealed a positive prospect for its application.

In this study, PAMAM G3 dendrimers were modified with ferrocenemonocarboxaldehyde, and the resulting dendrimers (Fc-D) (scheme 1) were used to modify a bare GCE with Nafion acting as the binder. Ferrocene (Fc) and its derivatives possessed a lot of advantages such as stability, readily membrane permeation, the accessibility of a large variety of derivatives, and the favorable electrochemical properties, based on which they were widely used. Herein, the modified electrodes were characterized by cyclic voltammetry and electrochemical impedance spectroscopy. The effects of pH value and scan rate, and the determination of DA in the absence and presence of AA by differential pulse voltammetric (DPV) method were investigated.

2 Experimental

2.1 Reagents and apparatus

DA and AA were purchased from Acros, Nafion (5% w/w solution) from Alfa Aesar, N, N′-Dicyclohexyl carbodiimide (DCC) from Sinopharm Chemical Reagent Co. Ltd. All other materials were at least of analytical grade and used as received. In this study, 0.2 mol L^{-1} phosphate buffer solution (PBS) at different pH values were prepared by mixing the stock solutions of Na_2HPO_4 and NaH_2PO_4. NH_2-terminated G3 PAMAM dendrimers were synthesized according to the reported procedure [9, 17], and ferrocenemonocarboxaldehyde according to the procedure [18]. All aqueous solutions were prepared in doubly distilled water.

Electrochemical impedance spectroscopy (EIS) of the modified electrodes was carried out using CHI 660c (CH Instruments, Chenhua, Shanghai, China), other electrochemical experiments using CHI 832a (CH Instruments, Chenhua, Shanghai, China). EIS was performed in 0.1 mol L^{-1} KCl solution within the frequency range of 10^{-2}–10^{5} Hz with 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) mixture as electroactive probe. A conventional three-electrode system was used in the measurements with a bare or a modified electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a Pt wire as the auxiliary electrode. All experiments were carried out at room temperature.

2.2 Preparation of ferrocenyl-tethered PAMAM G3 dendrimers (Fc-D)

Primary amines of NH_2-terminated G3 PAMAM dendrimers were modified with ferrocenyl groups using the method reported in the literature [19]. Ferrocenemonocarboxaldehyde (0.025 g) and G3 PAMAM (0.025 g) mixed and dissolved in 4 mL methanol. The mixture was slowly stirred for 2 h, and then 5 mg of sodium borohydride was added in one portion and stirred for 1 h to reduce the carbon-to-nitrogen double bonds. The reaction product was purified by lipophilic gel permeation chromatography (Sephadex LH-20, Pharmacia) using methanol as the eluant. The FTIR spectra (not shown) of the composition revealed several major absorption peaks at ~1640, ~1100, ~1000, ~820, and ~480 cm^{-1}, that belong to tertiary amine and ferrocene, indicating the covalent binding between ferrocenemonocarboxaldehyde and G3 PAMAM dendrimers.

2.3 Preparation of the modified electrode

Before modification, the bare GCE (diameter: 0.04 mm) was polished to a mirror-like surface with a 0.05 μm Al_2O_3 slurry. After been cleaned, it was sonicated with 1:1 (V/V) HNO_3, ethanol, and doubly distilled water, respectively. Finally, it was dried under a stream of high purity nitrogen and was then ready for use.

25 μL Fc-D methanol solution (0.05 g/mL) and 100 μL Nafion were added to 1 mL centrifuge tube, diluted to 1 mL with ethanol and mixed homogeneously with the aid of ultrasonic agitation. Then, 5 μL of the mixed solution was dropped on the surface of the cleaned GCE. After dried

in the air at room temperature for 30 min, the modified electrode was rinsed thoroughly with doubly distilled water. The modified electrode was obtained and noted as Fc-D/Nafion/GCE. When not in use, it was stored in 0.2 M pH 7.0 PBS at 4 °C. After every scanning, the modified electrode was regenerated by applying a constant voltage of 0.4 V for 5 min.

3 Results and discussion

3.1 Electrochemical impedance spectroscopy (EIS) of the modified electrode

Electrochemical impedance spectroscopy (EIS) is a highly effective method for probing the features of surface-modified electrodes. In this study, EIS was used to estimate the resistance value and investigate the effect of Fc-D. Figure 1 shows the results of EIS for the bare GCE (a), Nafion/GCE (b), and Fc-D/Nafion/GCE (c) in 0.1 mol L^{-1} KCl solution containing 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1).

The bare GCE showed a typical Nyquist plot for a simple electron transfer reaction with almost straight line at low frequencies corresponding to the diffusion process, and a very small semicircle at high frequencies. The electron transfer resistance (R_{et}) could be estimated about 1,100 Ω since the semicircle is a typical feature signaling an electron transfer-limited process and its diameter corresponds to R_{et}. When the electrode was modified with Nafion, EIS revealed a larger semicircle. R_{et} increased markedly to 6×10^5 Ω, similar to the previous reports [20]. The possible reason is the Nafion film acting as a barrier and blocking interfacial charge transfer makes high resistance on the surface of electrode. The EIS of Fc-D/Nafion/GCE presented as an almost straight line at all frequencies which suggested that the electrode reaction is controlled by diffusion. It could be explained ferrocenyl groups have positive charges in the course of electrode reaction. Therefore, $[Fe(CN)_6]^{3-/4-}$ could be strongly attracted to the modified electrode surface. The significant differences between line b and c suggested that Fc-D was successfully immobilized on the GCE surface.

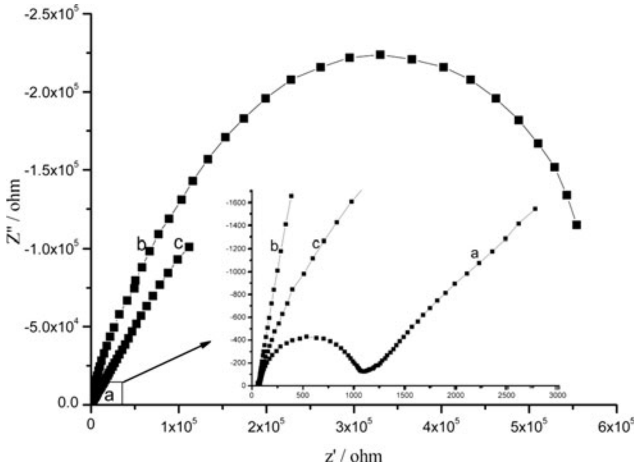


Fig. 1 Nyquist plots of EIS in 0.1 M KCl solution containing 5.0 m mol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) at for bare GCE (**a**), Nafion/GCE (**b**) and Fc-D/Nafion/GCE (**c**)

3.2 Electrocatalytic oxidation of DA at Fc-D/Nafion/GCE

Figure 2 shows CVs for the bare GCE, Nafion/GCE, and Fc-D/Nafion/GCE in 0.2 mol L^{-1} PBS (pH 6.5) containing different concentration of DA. The potential scans range from −0.2 to 0.6 V and the scan rate is 100 mV s^{-1}.

In the bulk PBS solution, both the bare GCE (Fig. 2A (curve a)) and Nafion/GCE (Fig. 2A (curve b)) show no peaks, but a pair of well-defined redox peaks were observed at the Fc-D/Nafion/GCE (Fig. 2A (c)) at 0.27 and 0.17 V. The peaks were then attributed to the electrochemical process of Fc^+/Fc.

As can be seen from Fig. 2B, all the three electrodes showed a pair of redox peaks. However, it is obviously observed that the peak currents of DA enhanced considerably at Fc-D/Nafion/GCE. At the same time, ΔE_p of the Fc-D/Nafion/GCE (0.22 V) was smaller than that of the Nafion/GCE (0.27 V). This result indicates that Fc-D has apparent catalytic effect on the redox reaction of DA, not only makes the response currents increase sharply, but also causes a positive shift of the peak potential. The possible catalytic mechanism of Fc-D could be considered as follows: in weak acidic NaH_2PO_4–Na_2HPO_4 buffer medium (pH 6.5), the $-NH_2$ of DA could be protonated. And the protonated DA would be exchanged into the Nafion film and was then electrostatically attracted. Fc-D was first oxidized to Fc-D^+ in the process of electrochemical oxidation, then the enriched DA was oxidized by Fc-D^+. Therefore, the oxidation peak currents increased sharply.

3.3 The effect of solution pH on the peak currents and formal potential of DA

The effect of solution pH on the peak currents and formal potential of Fc-D/Nafion/GCE was investigated by CVs (Fig. 3). As shown in Fig. 3A, when the pH value changed from 5.8 to 8.0, both the anodic and cathodic peak currents increased with the increasing pH value until it reached to 6.5. Then, the peak currents decreased remarkably. Therefore, pH 6.5 was the optimal pH value and chosen for the electrochemical detection of DA.

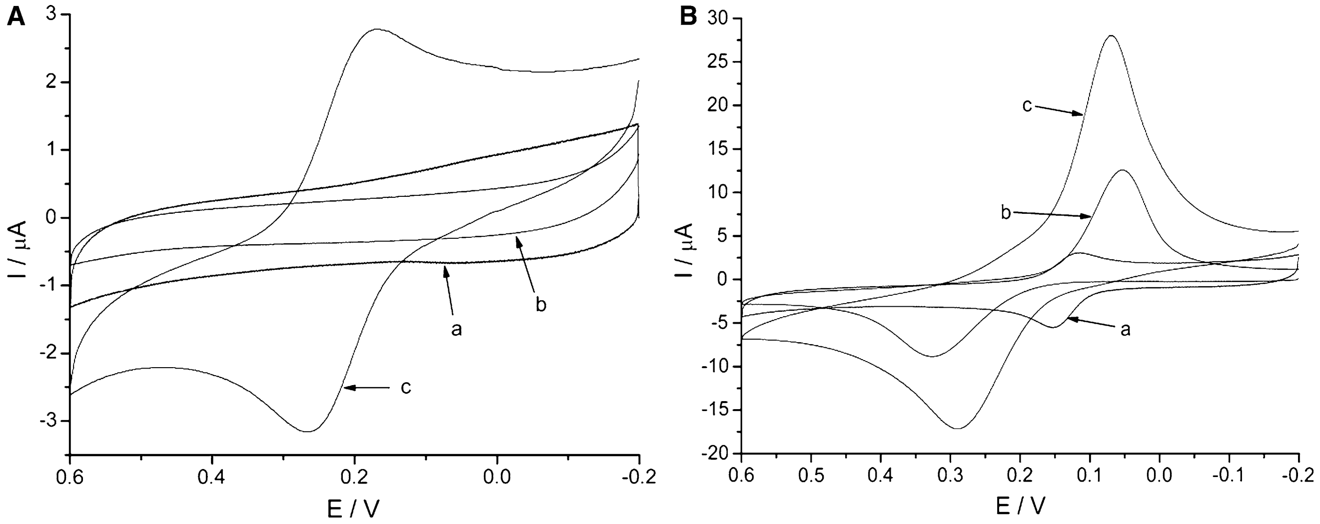


Fig. 2 **A** Cyclic voltammograms of bare GCE (*a*), Nafion/GCE (*b*), and Fc-D/Nafion/GCE (*c*) in 0.2 mol L^{-1} pH 6.5 PBS at 100 mV s^{-1}. **B** Cyclic voltammograms of bare GCE (*a*), Nafion/GCE (*b*), and Fc-D/Nafion/GCE (*c*) in 0.2 mol L^{-1} pH 6.5 PBS containing 1×10^{-4} mol L^{-1} DA at 100 mV s^{-1}

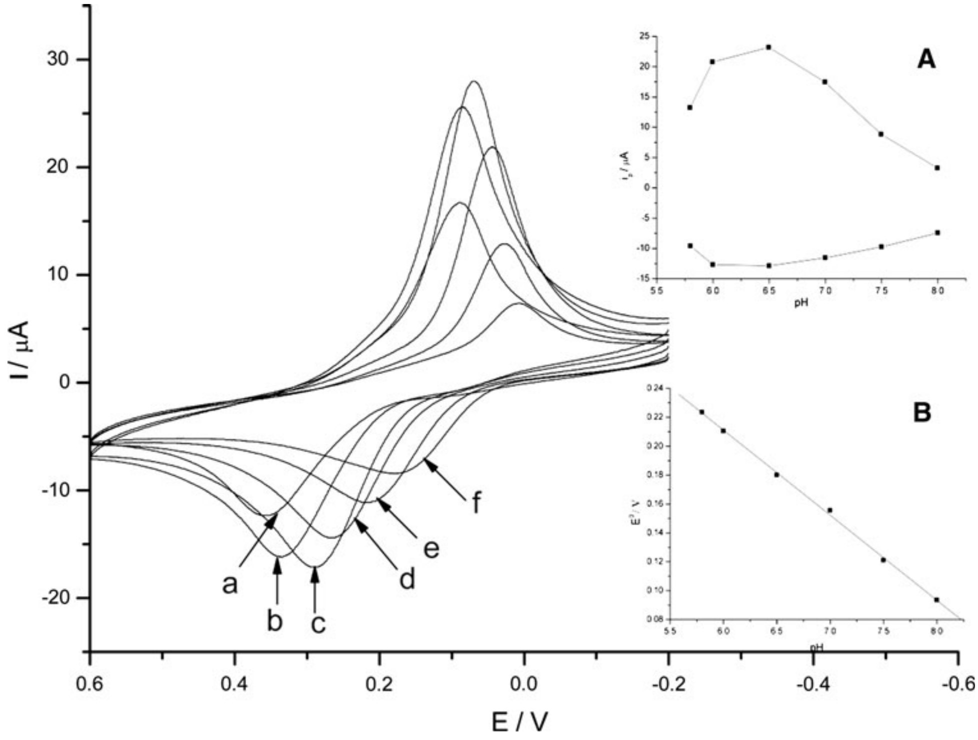
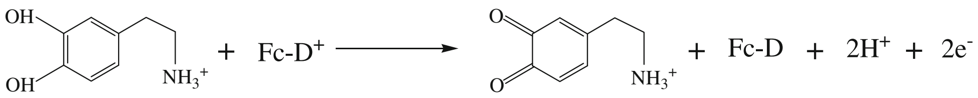


Fig. 3 Cyclic voltammograms of 1×10^{-4} mol L^{-1} DA on modified electrode in various pH value PBS: pH 5.8 (*a*), pH 6.0 (*b*), pH 6.5 (*c*), pH 7.0 (*d*), pH 7.5 (*f*), pH 8.0 (*e*). Scan rate: 100 mV s^{-1}. Inset graph: **A** plots of peak currents (I_p) vs. pH; **B** the plot of the formal potential ($E^{0'}$) vs pH

At the same time, the anodic and cathodic peak potentials shifted negatively with pH value increasing. As shown in Fig. 3B, the formal potential $E^{0\prime}$ was linear as a function of pH in the range of 5.8–8.0, which can be represented by the equation: $E^{0\prime}/\mathrm{V} = 0.5645–0.0589$ pH ($r = 0.9993$). The slope of −58.9 mV/pH, indicated that the electron transfer was accompanied with an equal number of protons in electrode reaction [21]. Since the oxidation of DA involves a two-proton and two-electron process [22], the electrode reaction mechanism could be described as:



3.4 Effect of scan rate on the Fc-D/Nafion/GCE

Figure 4A shows the linear curves of peak currents of 1×10^{-4} mol L^{-1} DA with the square root of scan rate at Fc-D/Nafion/GCE in 0.2 mol L^{-1} PBS (pH 6.5). The potential scan rate was changed from 0.01 to 0.5 V s^{-1}. Both the anodic and cathodic peak currents were linearly proportional to the square root of the scan rate ($\upsilon^{1/2}$) with the equations: i_{pa} (μA) = 2.2953 − 1.2751 $\upsilon^{1/2}$ (V$^{1/2}$) ($r = 0.9920$), i_{pc} (μA) = 1.7135 $\upsilon^{1/2}$ (V$^{1/2}$) − 3.3628 ($r = 0.9982$), indicating the electrode reaction of DA at the modified electrode is mainly controlled by a diffusion control process. Meanwhile, ΔE_p widened slowly when the scan rate increased. This slight deviation from ideal behavior arising even at low scan rate could be attributed to the limitations associated with charge transfer in the Nafion film.

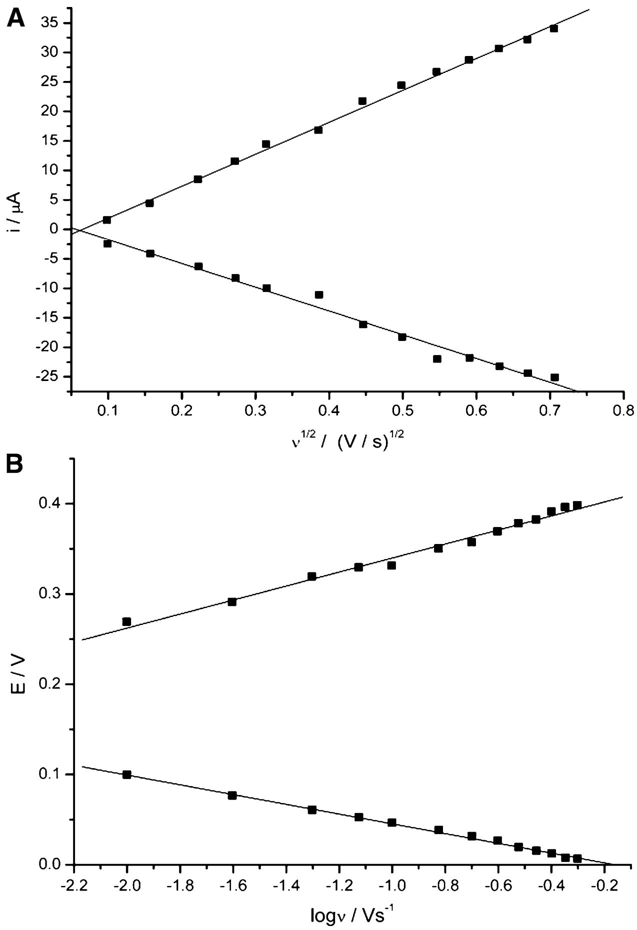


Fig. 4 Effect of scan rate on the voltammograms of the Fc-D/Nafion/GCE in pH 6.5 PBS containing 1.0×10^{-4} mol L^{-1} DA at 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500 mV s^{-1}. Inset graph: **A** plots of peak currents (I_p) vs. the square root of the scan rate; **B** the plot of E_p vs. logυ

By plotting E_p versus logυ (Fig. 4B), two linear relationships were observed with the linear equations: E_{pa} (V) = 0.078 logυ (V s^{-1}) + 0.42 ($r = 0.9936$); E_{pa} (V) = −0.054 logυ (V s^{-1}) − 0.01 ($r = 0.9983$). According to the Laviron's theory [23]: A graph of $E_p = f(\log\upsilon)$ yields two straight lines with a slope equal to $-2.3\ RT/\alpha nF$ for the cathodic peak, and $2.3RT/(1-\alpha)nF$ for the anodic peak. The electron transfer coefficient α could be calculated to be 0.59. Meanwhile, according to Laviron' s another equation [23]:

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/nF\upsilon) - \alpha(1-\alpha)nF\Delta E_p/2.3RT$$

The charge transfer constant, k_s, was calculated to be 0.1 s^{-1}. The smaller charge transfer constant could be attributed to the nonreversibility of the redox reaction of DA at Fc-D/Nafion/GCE.

3.5 Determination of DA

Under optimal conditions, the determination of DA using the Fc-D/Nafion/GCE was performed with DPV (Fig. 5). The peak currents of DA versus its concentration had good linearity in the range of 1×10^{-5}–1.5×10^{-3} mol L^{-1} with the linear equation: i_p (μA) = 0.0221 C_{DA} (μmol L^{-1}) + 1.9671 ($r = 0.9991$). This linear range is much wider than the results obtained at other modified electrodes such as l-cysteine self-assembled monolayer modified gold electrode (9.91×10^{-6}–2.21×10^{-4} mol L^{-1}) [24], or poly (*p*-nitrobenzenazo resorcinol)-modified glassy carbon electrode (8×10^{-6}–2.85×10^{-4} mol L^{-1}) [25], and similar to cetylpyridine bromide (CPB)/chitosan composite film-modified glassy carbon electrode (4.0×10^{-5}–5.0×10^{-3} mol L^{-1}) [4].

The detection limit C_m was determined by the equation [26]: $C_m = 3s_{b1}/m$, where s_{b1} is the standard deviation of the blank response (μA) and m is the slope of the calibration plot (0.0221 μA/μM). In this study, s_{b1} was obtained to be 0.0347 μA after 15 replicate measurements on the blank solution. Hence, C_m was calculated to be 4.7×10^{-6} mol L^{-1}, which is similar to 5×10^{-6} mol L^{-1} obtained at a carbon past electrode in the presence of the surfactant sodium dodecyl sulfate (SDS) [27] and 5.8×10^{-6} mol L^{-1} at an oracet blue-modified glassy carbon electrode [28].

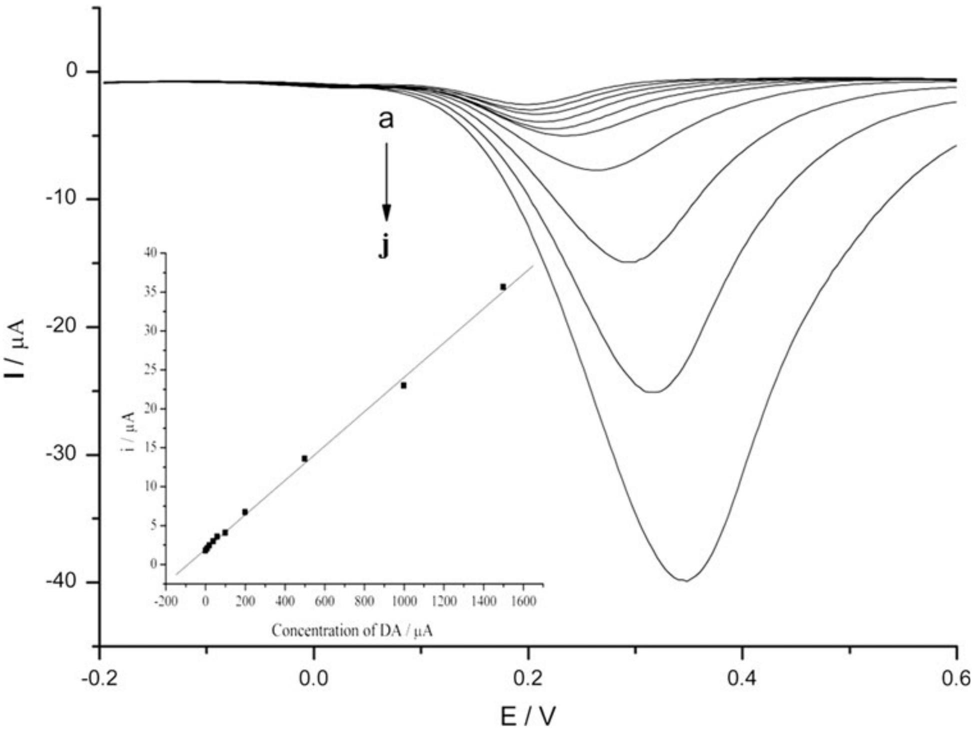


Fig. 5 DPVs of Fc-D/Nafion/GCE in pH 6.5 PBS with different DA concentration (*a*–*j*): 0, 10, 20, 40, 60, 100, 200, 500, 1000, 1500 μM. Amplitude: 0.05 V; Pulse width: 0.05 s; Pulse period: 0.2 s

3.6 Determination of DA in the presence of AA

It is well known that AA widely coexists with DA in real biological matrices, and AA can be oxidized at a potential near to that of DA on most solid electrodes. Therefore, avoiding the interference of AA is an important target for any DA analytical method. Figure 6 shows the DPVs of different DA concentrations in the presence of AA at the Fc-D/Nafion/GCE. The fact that AA has no electrochemical response on Fc-D/Nafion/GCE surfaces was obtained from curves a and a′, indicating AA did not influence the measurement of DA. Moreover, Fig. 6b′ and c′ shows the DPVs of mixed solutions of 4×10^{-5} mol L^{-1} DA + 1×10^{-3} AA and 5×10^{-4} mol L^{-1} DA + 1×10^{-3} mol L^{-1} AA. Compared with curves b and c, the peak potential and peak current of b′ and c′ only changed a little. The determination error of DA concentration was in the permission region (±5.0%). That is to say the electrocatalytic oxidation effect of AA can be eliminated at Fc-D/Nafion/GCE.

3.7 The stability and repeatability of Fc-D/Nafion/GCE

The peak currents of Fc-D/Nafion/GCE were nearly a constant in continuous scanning of 100 cycles in the blank solution. The response current of DA decreased by only 5% of the initial current after the modified electrode was placed for a week. The standard deviation of the peak currents for DA was only 4.2% when the bare electrode was modified in the same treatment method for seven times. All the results showed that the Fc-D/Nafion/GCE has high stability and good reproducibility.

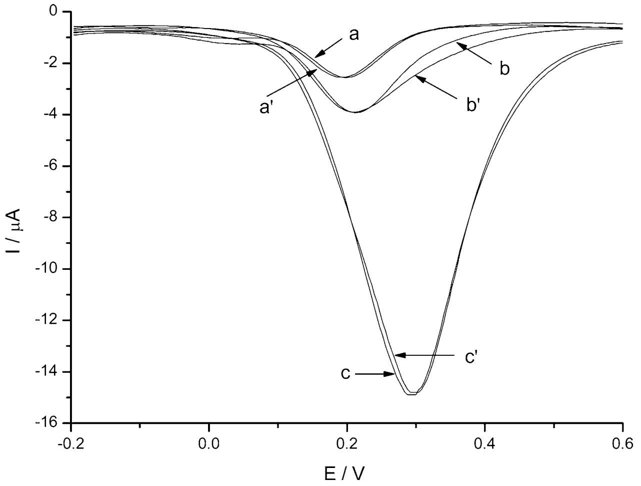


Fig. 6 DPVs obtained for the determination of DA and DA + AA at Fc-D/Nafion/GCE in 0.2 mol L^{-1} PBS (pH 6.5): (*a*) 0 DA; (*b*) 4×10^{-5} mol L^{-1} DA; (*c*) 5×10^{-4} mol L^{-1} DA; (*a*′) 0 DA + 2×10^{-4} mol L^{-1} AA; (*b*′) 4×10^{-5} mol L^{-1} DA + 1×10^{-3} AA; (*c*′) 5×10^{-4} mol L^{-1} DA + 1×10^{-3} mol L^{-1} AA. Amplitude: 0.05 V; Pulse width: 0.05 s; Pulse period: 0.2 s

4 Conclusion

The ferrocenyl-tethered PAMAM G3 dendrimers were synthesized and used to modify the bare GCE with Nafion as the binder. The obtained Fc-D/Nafion/GCE was characterized by EIS which showed that the tunable kinetic barrier was substantially altered. CV results indicated that the modified electrode had a dramatically enhanced electrocatalytic property to DA. Furthermore, the determination of DA in the absence and presence of AA was investigated by DPV, which suggested that the selective and sensitive determination of DA using the modified electrode is feasible. The proposed method provides a new promising and alternative way to detect DA sensitively and selectively.

Acknowledgments This study was supported by the National Natural Science Foundation of China (No.20775044) and the Natural Science Foundation of Shandong province, China (Y2006B20).

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