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Voltammetric Behavior of Dopamine at ct-DNA Modified Carbon Fiber Microelectrode

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Abstract. A sub-micrometer thin-layer DNA modified carbon fiber microcylinder electrode was prepared by electrodeposition of ct-DNA at 1.5 V (vs. Ag/AgCl). The voltammetric behavior of dopamine (3-hydroxytyramine) was investigated at the modified electrode. It was found that the modified electrode exhibits a highly electrocatalytic activity toward dopamine oxidation. Differential pulse voltammetry was used for determination of dopamine in pH 7.4 phosphate buffer solution. A linear response of the peak current versus the concentration was found in the range of 4×10^{-6} to 10^{-4} mol L^{-1} at 10^{-4} mol L^{-1} AA (ascorbic acid) coexistence ($R = 0.9959$) and the range of 6×10^{-5} to 10^{-3} mol L^{-1} at 10^{-3} mol L^{-1} AA ($R = 0.9960$). The presence of a high concentration of ascorbic acid did not interfere with the determination. The proposed method exhibited good recovery and reproducibility. This method can be applied to the detection of DA in real samples.

Key words: Voltammetry; dopamine; DNA; carbon fiber.

The determination of dopamine, a catecholamine, is of great significance for the investigation of its physiological functions and the diagnosis of nervous diseases resulting from abnormal metabolite, such as epilepsy, Parinsonian syndrome and senile dementia, etc [1]. Intensive efforts have been devoted to the in-vitro and in-vivo electrochemical determination for freeing dopamine and other catecholamines from interferences of coexisting substances, especially ascorbic acid [2–4]. One of the most promising strategies for selective determination of dopamine is the use of chemically modified electrodes [5–15].

For the purpose of in-vivo detection, micro-sized electrodes are well suited for advantages including enhanced mass transport, reduced ohmic drop and double-layer charging effect. Furthermore, the small size of microelectrodes not only causes minimal physical damage in living tissues while they are being implanted into the specimen but also permits careful selection of the neuronal or even cellular region to be investigated. Among these, carbon fiber electrodes remain the most commonly used probe for electrochemical detection of neurotransmitters in vivo [16–20].

DNA is a well-known natural macromolecule and a well-defined linear helix, and repetitive structure is found in double strand DNA (ds-DNA). Because of the specific structure, charge migration within the DNA duplex might be possible over a distance of up to 40 Å [21]. Considering the high charge density on the DNA helix and the chelating ability concerning various molecules, DNA may be a useful material for surface modification of electrodes for obtaining selective and sensitive sensing of these catecholamines. With the development of electrochemical DNA biosensors, electrode surface-immobilization techniques for stable and highly dense monolayers with DNA are becoming more important. There are many reported methods for surface-immobilization of

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DNA on electrodes, such as chemical adsorption [22], covalent-binding [23, 24], the antigen-antibody method [25], electrostatic attraction [26–28] and co-polymerization [29].

Here we describe the preparation and characterization of a ct-DNA modified carbon fiber microelectrode. The modification method is efficient for obtaining a uniform and active submicron thin-layer DNA on the micro fiber electrode. This modified DNA layer showed strong electrocatalysis of dopamine oxidation.

Experimental

Reagents

Calf thymus DNA (ct-DNA) was purchased from Sino-American Biotechnology Company. Dopamine (DA) (3,4-dihydroxyphene thylamine hydrochloride) and ascorbic acid (AA) were obtained from Sigma and Chemical Reagent Company of Shanghai, respectively. They were used as received without further purification. All other chemicals were of analytical grade. Solutions of ct-DNA, DA and AA were freshly prepared in 0.1 mol L^{-1} phosphate buffer solution (PBS). To mimic biological environments, pH 7.4 PBS was used for the study. All solutions were prepared using doubly distilled water. Carbon fiber (PAN type, 7 μm in diameter) came from the Jilin Carbon Company LTD (Shanghai).

To demonstrate the applicability of the modified electrode, simulated samples, DA-AA mixtures were prepared by using dopamine hydrochloride injection (DHI) solution and AA for DPV determination. A 2 mL dopamine hydrochloride injection (DHI, 10 mg mL^{-1} DA, 2 mL per injection) was diluted to 100 mL with water. Then, 100 μL of the dilute DHI solution and 1.0 mL 0.0025 mol L^{-1} AA solution were added and diluted to 10.0 mL with pH 7.4 PBS.

Instrumentation

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out with a CHI-660A electrochemistry workstation (CHI, USA). All electrochemical experiments implied the use of a three-electrode cell with a carbon fiber working electrode, a platinum wire auxiliary electrode and an Ag/AgCl wire reference electrode. All reported potentials are against an Ag/AgCl (Sat. KCl) without regard to the liquid junction. Experiments were carried out at 25 ± 1 °C. High purity nitrogen was used for solution de-aeration and for maintaining a nitrogen atmosphere in the cell during the experiment.

SEM was performed with an X-560 electron scanning microscope (Hitachi Japan) operating at 20.0 kV. For SEM analysis of the DNA/CFME (DNA modified electrode), an Au layer of about 25 nm in thickness was applied to the DNA/CFME sample with an Eike IB-3 ion coater. The temperature was controlled at 20 ± 0.1 °C.

Fabrication of Carbon Fiber Microcylinder Electrode (CFME)

The carbon fibers should be pre-treated before use. First, a bundle of carbon fibers of about 4 cm in length were refluxed with acetone for 8 h. Then, the fibers were ultrasonically treated for 10 min in fresh acetone, ethanol and water, successively, and dried in air at room temperature. A piece of pre-treated carbon fiber was connected to a copper wire using silver conductive glue and placed inside a glass capillary and sealed with epoxy. After solidification, a length of 1 ∼ 2 mm of the carbon fiber protruded from the end of the capillary.

Modification of the CFME

The CFME was electrochemically oxidized by applying an electrode potential of +1.5 V for 10 min in 0.1 mg mL^{-1} ct-DNA solution for surface immobilization of the DNA [30, 31]. After rinsing with water, the ct-DNA modified electrode was obtained and labeled as ct-DNA/CFME.

Results and Discussion

Surface Morphology of the ct-DNA/CFME

The ct-DNA/CFME was viewed with SEM as seen in Fig. 1. It shows that the surface of the bare carbon fiber was smooth and uniform, and that after modification

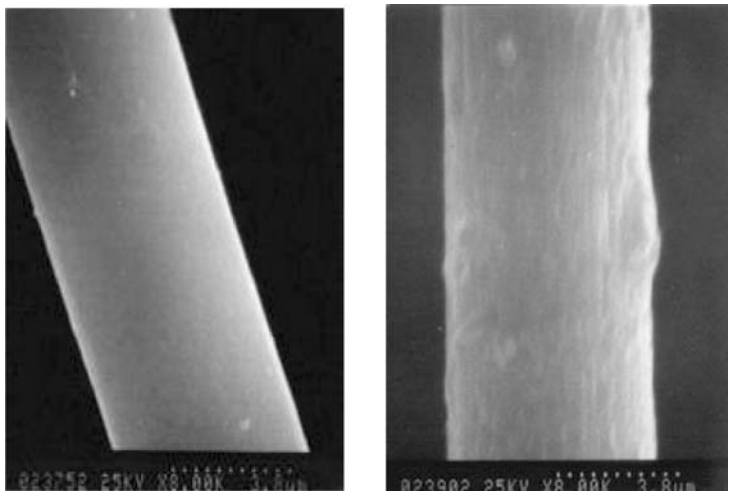
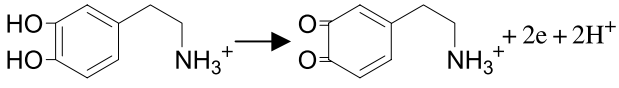


Fig. 1. Scanning electron micrographs of the CFME: before (left) and after electrodeposition of ct-DNA (right)

the surface became a little rough covered in a uniform layer of modifier except for the small protuberances. This suggests that the uniformly covered layer is the functional structure of the modified ct-DNA, but the protuberances may not be. The thickness of the modified layer can be estimated at much less then 1 micron.

Electrocatalytic Redox of DA at CFME

The electrochemical behavior of DA has been reported. The oxidation reaction of DA is described as a two-electron and two-H^+ process at conventional glass carbon electrodes [32, 33]. The reaction can be represented as



Typical cyclic voltammograms of DA at our prepared micro-cylinder electrodes are shown in Fig. 2a. At bare CFME (Fig. 2b), DA did not have any apparent redox reaction in the potential range from -0.2 V to $+0.5$ V. However, at the ct-DNA/CFME, a pair of redox peaks was obtained at $E_m = 0.18$ V with $\Delta E_p = 120$ mV at 50 mV/s (Fig. 2a). The result provides definite information about the catalysis of the CT-DNA modified CFME towards the electrooxidation of DA. A sigmoidal wave was obtained at 5 mV/s, as seen in Fig. 3a, and $E_{1/2} = 0.175$ V and n = 1.9 ($E = E_{1/2} + RT/nF \lg(i/(i_d - i))$ [34] can be calculated from the logarithm analysis (Fig. 3b). It is in good agreement with the 2e steady-state electron

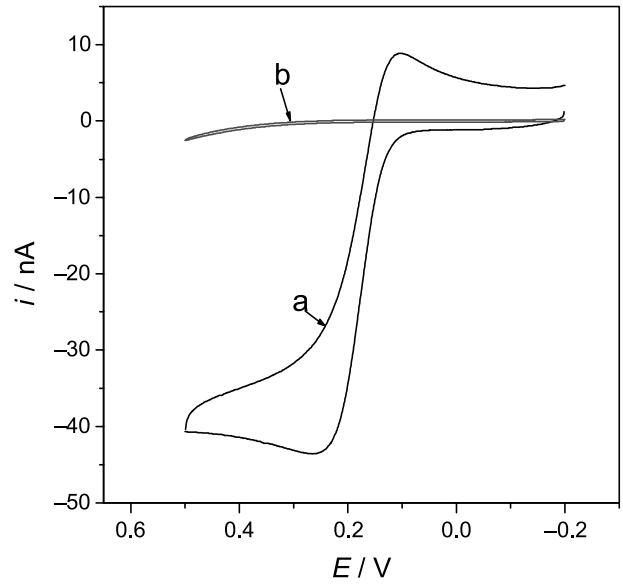


Fig. 2. Cyclic voltammograms of DA in 20 μmol L^{-1} DA solution at ct-DNA/CFME at 50 mV/s (*a*) and bare CFME (*b*)

transfer mechanism. The pH effect was investigated in the range of 3 to 9 (not shown). It revealed that the DPV peak potential of DA oxidation varies linearly with an increase of pH at a slope of -0.057 V per pH unit, which is very close to the theoretical value of -0.059 V per pH unit for the two-electron, two-proton process. For the similarity to the environment in the human body, pH 7.4 was maintained as the condition for determination.

Figure 4 shows CV of DA on DNA/CFME in pH 7.4 phosphate buffer solution. It was obtained after the DNA/CFME was immersed in dopamine solution.

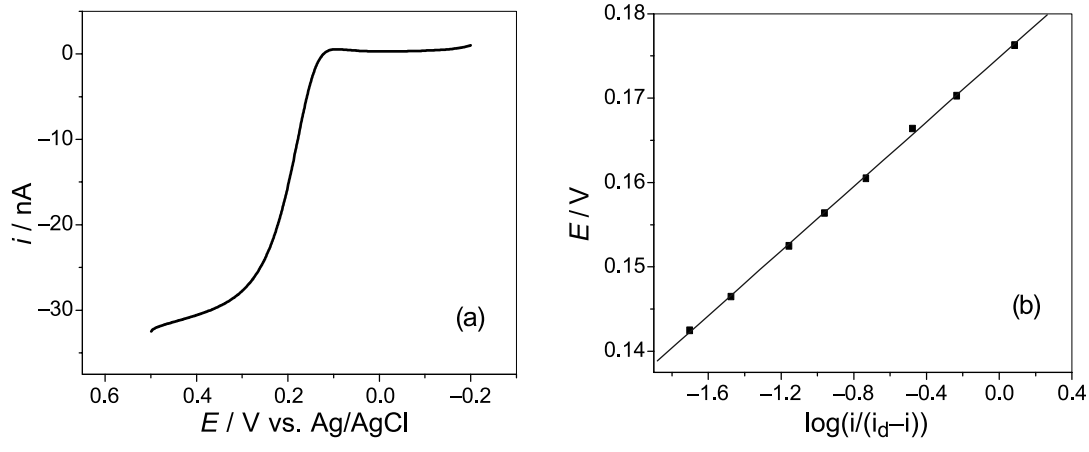


Fig. 3. Cyclic voltammogram of 20 μmol L^{-1} DA in pH 7.4 PBS at 5 mV/s (a) and the relationship of E and $\log(i/(i_d - i))$ (b)

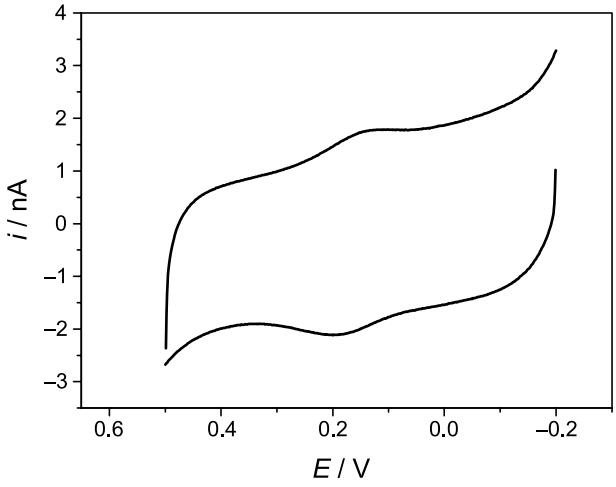


Fig. 4. CV of DA on DNA/CFME in pH 7.4 PBS at 50 mV/s

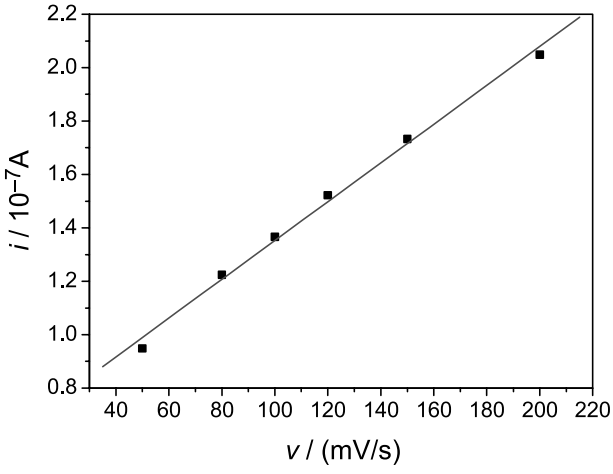


Fig. 5. Relationship between the oxidation peak current of DA and the scan rate ($v = 50$, 80, 100, 120, 150, 180, 200 mV/s). Other conditions: as in Fig. 2

However, the pair of redox peaks disappeared after the DNA/CFME had been immersed in PBS for 30 min. This indicates that there are few DA absorbed on the DNA/CFME. These results indicate that the electrode reaction is controlled by absorption and diffusion at the same time and that DNA can remarkably catalyze the electro-oxidation of DA, maybe due to its interaction with DA. The oxidation peak currents (i_{pa}) are linear with v (Fig. 5) and $v^{1/2}$ (Fig. 6) in the range of $50 \sim 200$ mV/s with a linear correlation coefficient of 0.9974 and 0.9992. This result means that absorption and diffusion occurred at the same time.

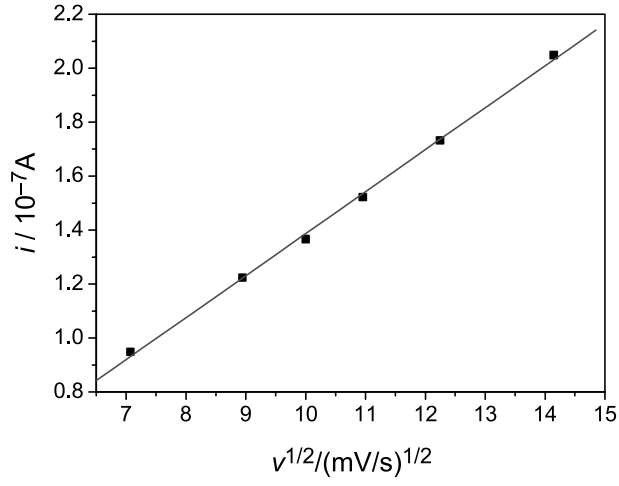


Fig. 6. Relationship between the oxidation peak current of DA and the square root of the scan rate ($v = 50$, 80, 100, 120, 150, 180, 200 mV/s). Other conditions: as in Fig. 2

The effect of ionic strength was also investigated in the range of 0.01 to 0.5 mol L^{-1} (not shown). It was found that the DPV peak potential of DA oxidation shifted in a positive direction with increasing KCl concentration, and the peak current dropped to about 65% when the KCl concentration was larger than 0.5 mol L^{-1}. This suggests that the interaction between DNA and DA is electrostatic [35].

DNA can interact with various molecules, which may be related to the gene expression process of living cells. DA molecules may interact with DNA in some way. The polyanion DNA has a large number of negative charges on its backbone of deoxyribose phosphate, while DA exists mainly in the form of

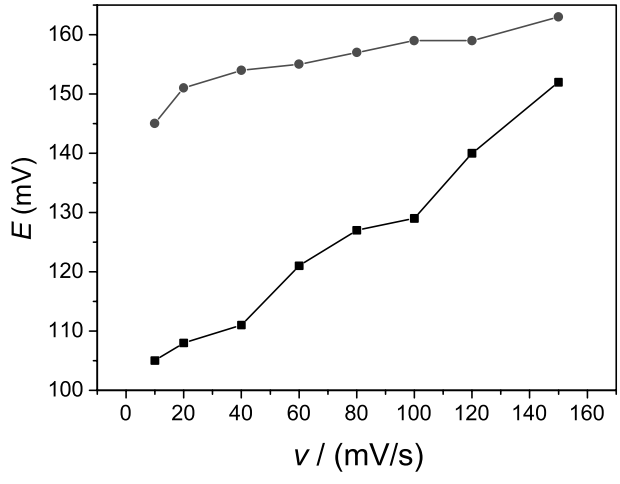


Fig. 7. Scan rate dependent ΔE_p (■), and E_m (●). Conditions: as in Fig. 2

cation in pH 7.4 solution. Thus, the electrostatic interaction always takes place. Obviously, more research is needed to elucidate the mechanism of the interaction of DNA and DA.

Figure 7 summarizes the rate dependent ΔE_p and E_m of this system. The ΔE_p increases with increasing scan rate, yet E_m remains unchanged. The results indicate that the electrode progress is quasi-reversible.

Interferences

Ascorbic acid (AA) is an important bioactive compound in biological fluids. Since the AA concentration in the central nervous system is usually much higher than that of DA, and because AA can also be oxidized at potentials close to that of DA at most solid electrodes, the influences of AA is an important criterion for DA determination [36].

To elucidate the resolution ability of this modified electrode, differential pulse voltammetry (DPV) experiments were carried out with 50 μmol L^{-1} DA and 5 mmol L^{-1} AA in pH 7.4 PBS. A rather broad anodic DPV peak was obtained at about 0.23 V at CFME (Fig. 8), and no individual peaks can be distinguished for DA and AA. However, at ct-DNA/CFME, two well-defined voltammetric peaks appeared at 0.060 V and 0.216 V for the oxidation of AA and DA, respectively, as shown in Fig. 9. The

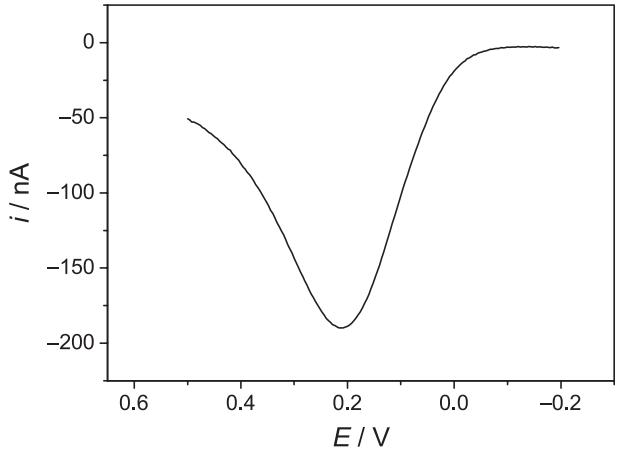


Fig. 8. Differential pulse voltammogram of a mixture of 50 μmol L^{-1} DA and 5 mmol L^{-1} AA in pH 7.4 PBS at bare CFME. Scan rate: 20 mV/s; pulse amplitude: 50 mV; pulse width: 50 ms; pulse time: 200 ms

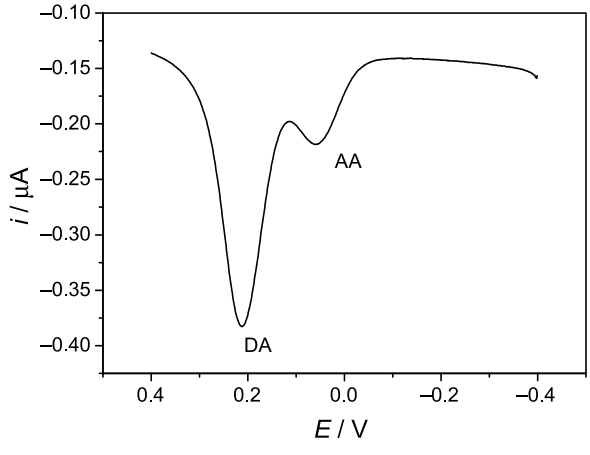


Fig. 9. Differential pulse voltammogram of a mixture of 50 μmol L^{-1} DA and 5 mmol L^{-1} AA in pH 7.4 PBS at ct-DNA/CFME. Conditions: as in Fig. 8

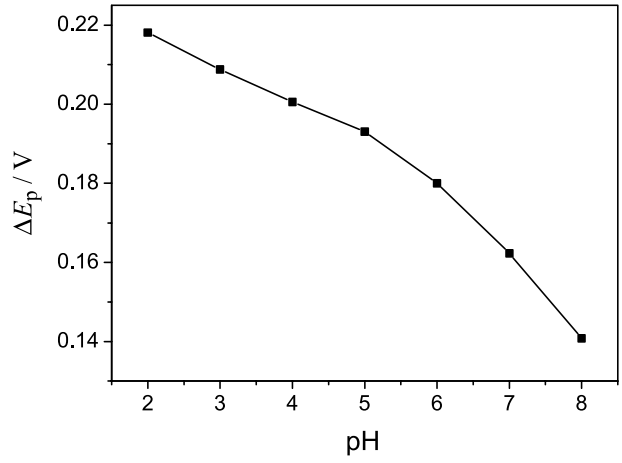


Fig. 10. Effect of pH on the peak potential separation of DA and AA. Other conditions: as in Fig. 8

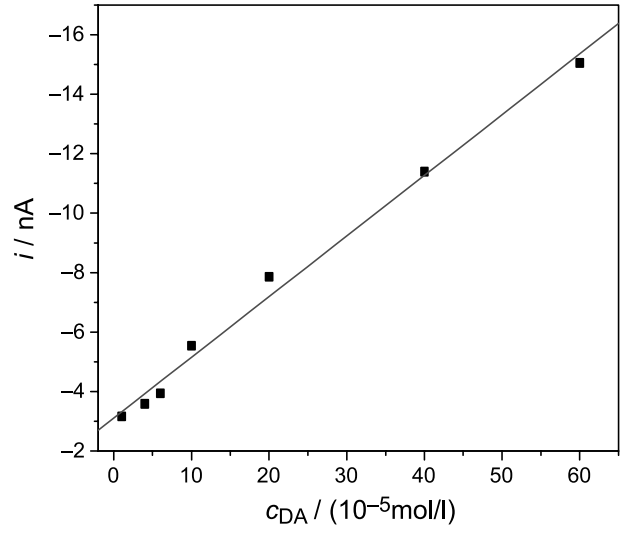


Fig. 11. Response curve of DPV catalytic peak current versus DA concentration. $c_{AA} = 10^{-3}$ mol L^{-1}. Other conditions: as in Fig. 8

Table 1. Determination results of DA in injections

No	DA content ($10\,\mu mol\,L^{-1}$)	AA content ($100\,\mu mol\,L^{-1}$)	DA conc. found ($10\,\mu mol\,L^{-1}$)	AA conc. found ($100\,\mu mol\,L^{-1}$)
1	4.06	2.50	1.02	2.46
2	1.06	2.50	1.03	2.49
3	1.06	2.50	0.96	2.52
4	1.06	2.50	1.01	2.44
5	1.06	2.50	1.03	2.55
Average			1.01	2.47
RSD			2.9%	3.3%

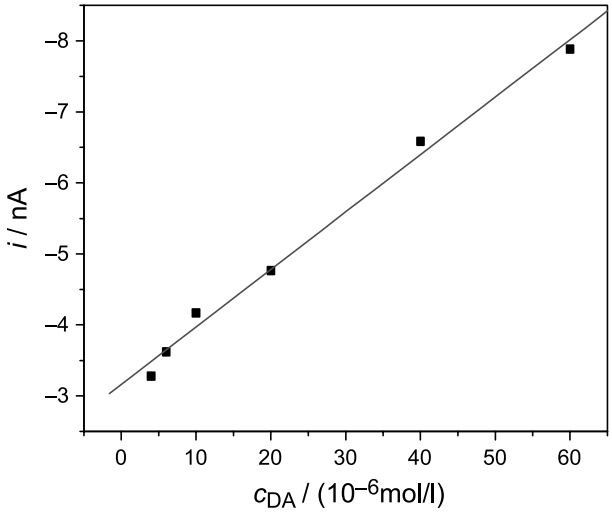


Fig. 12. Response curve of DPV catalytic peak current versus DA concentration. $c_{AA} = 10^{-4}\,mol\,L^{-1}$. Conditions: as in Fig. 8

156 mV peak separation is large enough for simultaneous determination of DA and AA in mixtures. From the current ratio of DPV peaks, the sensitivity of ct-DNA/CFME toward DA can be estimated as 1,000 times higher than for AA. This high selectivity can be attributed to the negatively charged phosphate groups on the helixes of the surface-modified ct-DNA, which may strongly attract DA while repelling AA, since DA exits in its cation forms while AA exits as anions in pH 7.4 solution.

Figure 10 shows the effect of pH on the oxidation peak potential separation of DA and AA. It indicates that the peak potential separation decreases with an increase of pH from 2 to 8 due to the participation of protons in the oxidation process. For the similarity to the environment in the human body, pH 7.4 was maintained as the condition for the determination.

Interferences were also investigated for other compounds. The result showed that for $10\,\mu mol\,L^{-1}$ DA, no interference can be observed for these compounds with the tolerance ratio: glucose (100), citric acid (100), glutamic acid (150), NaCl (400) and NH_4NO_3 (200).

Current Response for DA Concentration

Under optimum conditions, the DPV peak current of DA oxidation was in a linear relationship with DA concentration over the range of 4×10^{-6} to $10^{-4}\,mol\,L^{-1}$ at $10^{-4}\,mol\,L^{-1}$ AA coexistence ($R = 0.9959$) (Fig. 2) and the range 6×10^{-5} to $10^{-3}\,mol\,L^{-1}$ at $10^{-3}\,mol\,L^{-1}$ AA coexistence ($R = 0.9960$) (Fig. 11).

This method was used for analytical applications. The injection of dopamine hydrochloride was analyzed by using this method. The result is shown in Table 1. Good agreement between the measured and the standard content of DA was obtained, which is regarded as a promising feature for the applicability of the modified electrode to determine DA in the presence of large amounts of AA.

Regular sized glassy carbon electrode (GCE) was used for the determination of DA under different surface modifications [13–15]. However, the advantage of using a microelectrode over the regular GCE is the lower detection limit and applicability for fabricating micro-probes for real biological sample detections.

Conclusions

The ct-DNA modified carbon fiber micro-cylinder electrode exhibits highly electro-catalytic activity toward DA. It can separate the DPV peaks of DA and AA for 156 mV, and inhibits AA oxidation significantly, allowing a highly sensitive and selective catalytic determination of DA in pH 7.4 PBS solution by using the DPV technique in the presence of high concentrations of AA. This method can be applied to the detection of DA in real samples.

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