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# Determination of Dopamine in the Presence of Ascorbic Acid using Poly (Acridine red) Modified Glassy Carbon Electrode

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**Abstract**: Poly (acridine red) modified glassy carbon electrode was used for the detection of dopamine in the presence of ascorbic acid in a pH 7.4 phosphate buffer solutions (PBS) by cyclic voltammetry and differential pulse voltammetry. The major difficulty of the overlapped oxidation potential of ascorbic acid could be overcome through the distinct attractive ability of poly (acridine red) film to cationic dopamine and anionic ascorbic acid. The results showed that the dopamine anodic peak current and the concentration of dopamine had a linear relationship in the range of $1.0\times10^{-7}$ ~ $1.0\times10^{-4}$ mol $dm^{-3}$. The detection limit (S/N=3) obtained by differential pulse voltammetry was $1.0\times10^{-9}$ mol $dm^{-3}$. The relative standard deviation of 10 successive scans was 2.07 % for $1.0\times10^{-6}$ mol $dm^{-3}$ DA. Ascorbic acid had hardly interference with the determination of dopamine. The proposed method exhibits good recovery and reproducibility.

**Keywords:** Glassy carbon electrode; Chemically Modified electrode; Dopamine; Ascorbic Acid; Acridine red

## Introduction

Dopamine (DA) is a kind of neurotransmitter in mammalian central nervous system. Extreme abnormalities of DA levels are symptoms of several diseases such as parkinsonism. So DA is currently the subject of intense research focus to neuroscientists and chemists and it is essential to develop rapid and simple methods for the determination of the concentration of DA. DA can be determined by electrochemical methods because it is an electrochemically active compound. However, a major problem encountered in the detection is the interference from ascorbic acid (AA), because AA largely

coexists with DA in brain issue, therefor it is very difficult to determine DA directly. As we known, DA exists as a cation and AA exists as an anion at physiological pH 7.4. It is a possible way to overcome this problem by coating the electrodes with a thin film of Nafion [1-3] usually suffer from a slow response due to low diffusion coefficients [4-5] of analytes in the films. In order to overcome this problem, double - layer film [6-9] modified electrodes are used, which are coated first with an electroactive material having catalytic effect on the oxidation of DA and then with a Nafion layer.

In recent years, polymer-modified electrodes have attracted a great attention as polymeric film has good stability and reproducibility [10-11]. A number of researchers have employed polymeric film modified electrode to detect DA. So far, different methodologies have been used for depositing polymeric films. Electropolymerization is a good approach to immobilize polymers, because adjusting the electrochemical parameters can control film thickness, permeation and charge transport characteristics.

In the present work, we applied acridine red as a modifier to fabricate a poly (acridine red) modified glassy carbon electrode by electropolymerization method. The modified electrodes showed an electrocatalytic activity for the oxidation of DA. AA had no obvious response at this modified electrode. It is said the existence of AA did not interfere with the determination of DA. Hence the modified electrode can be used to detect DA in the high presence of AA at physiological pH.

## Experimental

### *Reagents*

Acridine red was obtained from Shanghai Reagent Company (China). Dopamine and ascorbic acid were purchased from ICN Biomedicals Inc (USA). They and all the other chemical reagents are of analytical-reagent grade. PBS was prepared by 0.1 mol $dm^{-3}$ $KH_2PO_4$-$K_2HPO_4$ + 0.1 mol $dm^{-3}$ NaCl, and adjusting the pH with 0.1 mol $dm^{-3}$ $H_3PO_4$ and 0.1 mol $dm^{-3}$ NaOH. All aqueous solutions were prepared in double distilled, de-ionized water.

### *Apparatus*

A CHI 660A Electrochemical Workstation (CHI Instruments, Chenhua Corp, Shanghai, China) was used for electrochemical measurements. A conventional three-electrode system was employed with a bare or poly (acridine red) modified electrode (3.0 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum electrode as the counter electrode. All potentials reported in this paper were referenced to the SCE.

### *Procedures*

The bare glassy carbon electrode (GCE) was polished successively with 0.3 and 0.05 μm $Al_2O_3$ slurry on emery paper before modification. Then it was rinsed with double distill water, and sonicated in 1:1 $HNO_3$, acetone and double distilled water for 10 min, respectively. After cleaning, the electrode was disposed by cyclic sweeping from –1.0 to +2.5 V at 100 mV $s^{-1}$ for 10 circles in pH 7.4 PBS containing $1.0\times10^{-4}$ mol $dm^{-3}$ acridine red solution.

All measurements were performed in a 10 $cm^3$ electrolytic cell with 5 $cm^3$ solutions, from which oxygen was removed by purging with high-purity nitrogen for 10 min. All measurements were carried out under a nitrogen atmosphere.

## Results and Discussion

*Electropolymerization of Acridine Red at the GCE Surface*

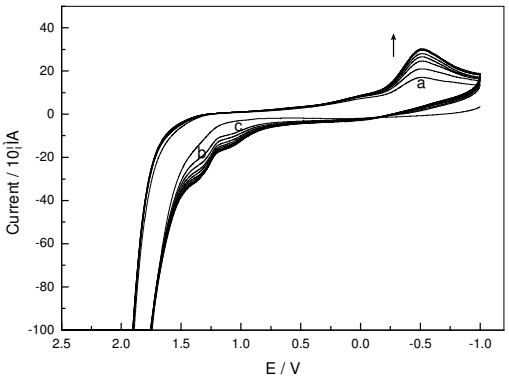



**Figure 1.** Repetitive cyclic voltammograms of $1.0\times10^{-4}$mol $dm^{-3}$ acridine red in pH 7.4 PBS solution; Terminal potential: +2.5 V; Initial potential: –1.0 V; Sensitivity: $1.0\times10^{-4}$ A/V; Scan rate: 100 mV/s.

Cyclic voltammetry was used to form the acridine red film. Fig.1 shows voltammograms of $1.0\times10^{-4}$ mol $dm^{-3}$ acridine red in PBS (pH=7.4) over the potential range from –1.0 V to + 2.5 V at the glassy carbon electrode. In the first potential scan, a cathodic peak (a) was observed near a potential value of –0.52 V. Oxidation peak was hardly observed. From the second cycle on, two obvious anodic peaks (b, c) appeared, larger peaks were observed upon continuous scanning. These facts indicated acridine red was deposited on the surface of GCE by electropolymerization mode. It can be observed that film growth was faster in the six cycles. From the seventh cycle, the film was no longer growth. A uniform adherent carmine polymer film was formed on the GCE surface during electropolymerization. After modification, the poly (acridine red) film electrode was carefully rinsed with doubly distilled water, then storage in air and was prepared to use later.

*Cyclic Voltammograms of DA at Bare and Modified Electrodes*

Figure 2 shows cyclic voltammograms of DA at a bare glassy carbon electrode (GCE) and at a poly (acridine red) modified GCE. At the bare glassy carbon electrode (Fig.2a), DA exhibited a poor electrochemical response. The peak difference ($\Delta E_p$) between anodic peak potential ($E_{pa}$) and the cathodic potential ($E_{pc}$) was 465 mV, but at the poly (acridine red) modified electrode, two couples of redox peaks were raised. Peak 1 and peak 2 was a couple of redox peak, which peak potential difference ($\Delta E_p$) was 58 mV. It could be observed that oxidation peak potential of DA shifted negatively from 365 mV (bare glass carbon electrode) to 136 mV (modified electrode) and the overpotential decreased 229 mV. The oxidation current of DA (peak 1) at the modified electrode was

more 50 times than that of DA at the bare GCE. These results indicated that poly (acridine red) film could accelerate the rate of electron transfer of DA in pH 7.4 PBS. We have employed hippuric acid [12], 2-picolinic acid [13] as modifier to fabricat modified electrode to detect DA in the presence of AA.

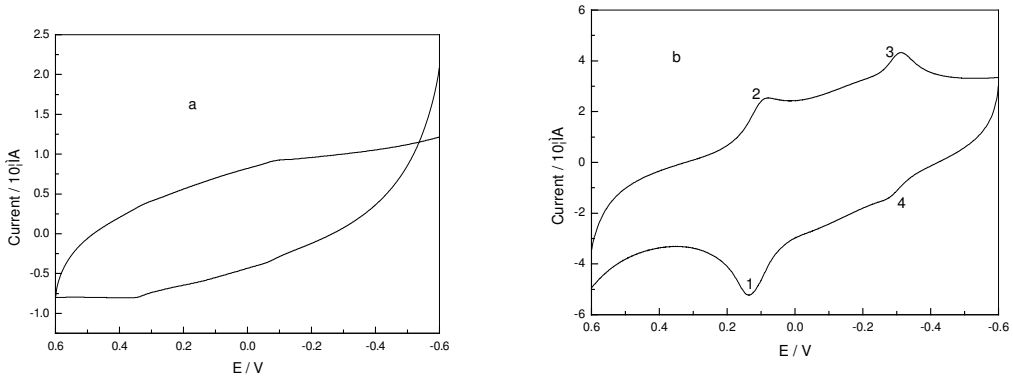



**Figure 2.** Cyclic voltammograms of $1.0\times10^{-4}$mol/L DA in pH 7.4 PBS. (a) At the bare electrode, (b) At the modified electrode. Sensitivity: $1.0\times10^{-4}$ A/V. Scan rate: 100 mV/s.

At hippuric acid or 2-picolinic acid modified electrode, DA exhibited two cathodic and one anodic peak. However at poly (acridine red) modified electrode. DA exhibited two couples of redox peaks. Reference [14] has clearly explained the mechanism of peak 1, peak 2 and peak 3. To establish the existence of peak 4, we successively repeated the cyclic voltammograms of $1.0\times10^{-4}$ mol $dm^{-3}$ DA in PBS of pH 7.4. As is shown in Fig.3.

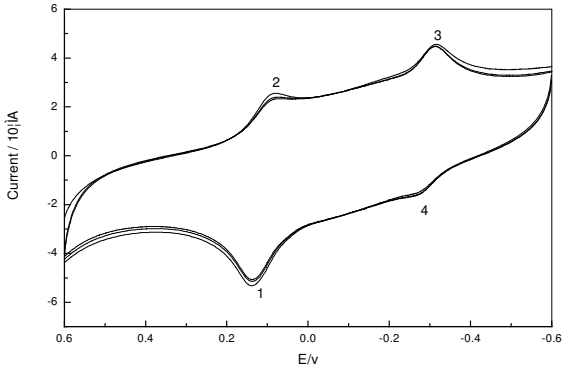
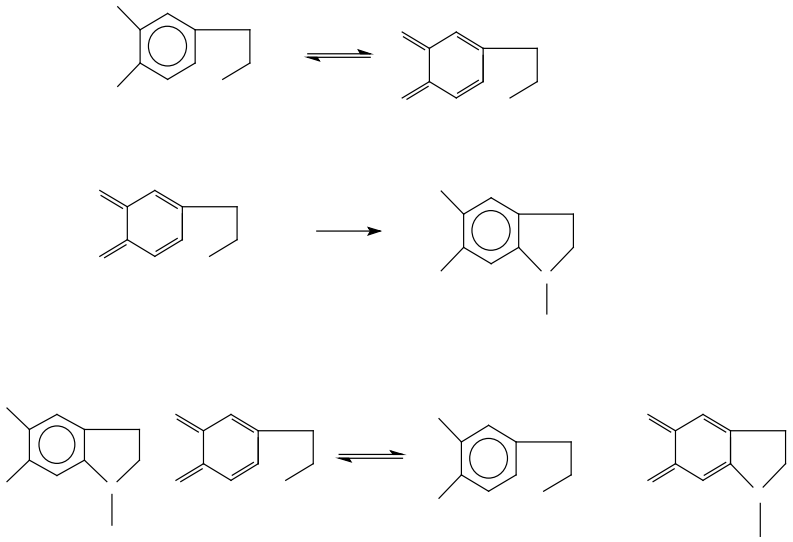



**Figure 3.** Repeated cyclic votammograms (6 scans) of $1.0\times10^{-4}$mol/L DA in PBS of PH 7.4 at the poly (acridine red) film modified electrode. Sensitivity: $1.0\times10^{-4}$ A/V. Scan rate: 100 mV/s.

From Fig.3, it can be found that peak 4 still appears as the scan increasing. According to reference [15-16] we thought peak 4 was corresponding to the oxidation of leucodopachrome (C) to dopachrome (D). The reason of it was as follows:



(A) Dopamine; (B) Dopaminequinone; (C) Leucodopachrome; (D) Dopachrome

In addition, the effect of the scan rate on the anodic peak current of DA was investigated. The $i_p$ was proportional to the square root of scan rate over the range of 40 ~ 220 mV $s^{-1}$. The linear regression equation was $i_p$ (μA) = -1.11253 + 0.71948 $v^{1/2}$ (mV $s^{-1}$)$^{1/2}$, with a correlation coefficient of R = 0.9977. So the electrode process was controlled-diffusion.

*Effect of Solution pH*

The effect of solution pH on the response of DA was investigated over the range of 3 ~ 10.

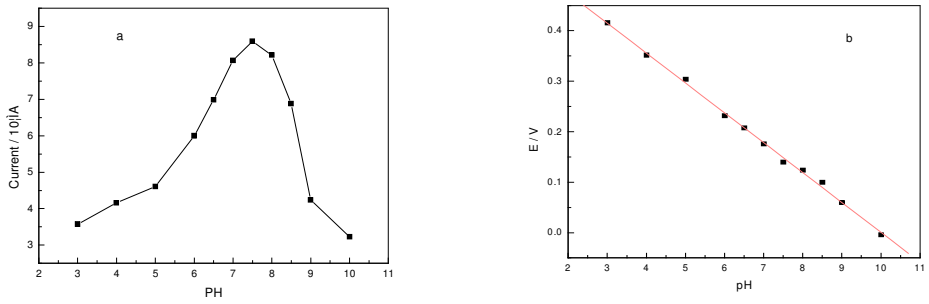



**Figure 4**. Effects of solution pH at the modified electrode on the cyclic voltammetric response of $1.0\times10^{-4}$mol/L DA. Sensitivity: $1.0\times10^{-4}$ A/V. Scan rate: 100 mV/s. (a)Effects of solution pH on the current. (b) Effects of solution pH on the potential.

Figure.4a shows that the anodic peak current increased with the increasing of solution pH until it reached 7.5. Then the anodic peak current decreased with increasing pH. Since it was the physiological conditions, pH 7.4 was chosen in this paper.

Figure.4b shows that the relationship between anodic peak potential ($E_{pa}$) of DA and PH of PBS solution. It can be found that $E_{pa}$ decreased with the increasing of solution pH. The $E_{pa}$ is proportional to the pH over the range of 3 ~ 10. The linear regression equation is $E_{pa}$ (V) = 0.59243 - 0.05918 pH, with a correlation coefficient of R = -0.9990. The slope is 59mV. It indicates two electrons transfer following two protons transfer.

*Determination of DA*

The determination of DA concentration at the poly (acridine red) modified electrode was performed with differential pulse voltammetry. The oxidation peak current of DA was selected as the analytical signal. The results showed that anodic peak current was proportional to the concentration over the range of $1.0\times10^{-7}$ ~$1.0\times10^{-4}$ mol $dm^{-3}$. The linear regression equation is $i_p(\mu A)=1.16006+0.43907C(\mu mol\ dm^{-3})$, with a correlation coefficient of r = 0.9981. The detection limit (S/N = 3) was $1.0\times10^{-9}$ mol $dm^{-3}$. The r elative standard deviation of 10 successive scans was 2.07 % for $1.0\times10^{-6}$ mol $dm^{-3}$ DA, indicating excellent reproducibility of modified electrode.

Furthermore, we also investigated the stability of the poly (acridine red) modified electrode. The peak current could hardly change after storage in air for at least 3 weeks or cyclically scanned in PBS solution.

*Interference Study*

Ascorbic acid (AA) coexists with DA in the extracellular fluid of the central nervous systems and its concentration ($10^{-4}$ mol $dm^{-3}$) is much higher than that of DA ($10^{-8}$ ~ $10^{-6}$ mol $dm^{-3}$). We can use the anodic peak potential for DA detection to eliminate the interference of AA. Since differential pulse voltammetry can improve the selectivity and sensitivity of the determination. So we used it to study the interference of AA and other compounds.

Fig.5 shows differential pulse voltammograms obtained at the polymer modified electrode. Fig.5 A(1) was recorded in $1.0\times10^{-2}$ mol $dm^{-3}$ AA solution. Fig.5 A(2) was recorded in $1.0\times10^{-4}$ mol $dm^{-3}$ DA solution. Fig.5 A(3) was recorded in a solution containing $1.0\times10^{-4}$ mol $dm^{-3}$ DA and $1.0\times10^{-2}$ mol $dm^{-3}$ AA. The anodic peak current of DA was not obvious change in presence of high ascorbic acid, which indicted that $1.0\times10^{-2}$ mol $dm^{-3}$ AA didn't interfere with the determination of $1.0\times10^{-4}$ mol $dm^{-3}$ DA. Fig.5B shows different DA concentration differential pulse voltammograms at modified electrode in the presence of $1.0\times10^{-2}$ mol $dm^{-3}$ AA. Fig.5 B(a) was recorded in $1.0\times10^{-2}$ mol $dm^{-3}$ AA, Fig.5 B(b) was recorded in $1.0\times10^{-4}$ mol $dm^{-3}$ DA and $1.0\times10^{-2}$ mol $dm^{-3}$ AA. The anodic peak current of DA was increased as DA concentration increased in the presence of AA (Fig.5 B (b) to Fig.5 B (e)). We can observe no less than 100 times AA had no interference with the determination of DA. AA only had a very poor response at 0.000V at the modified electrode. The anodic current and potential of DA had no change with the increasing concentration of AA from the Fig.5. It entirely eliminated the interference of AA. The facts indicated DA could be determined in the presence pf high AA

concentration using poly (acridine red) film modified electrode. The reason for these is as follows: DA exists in the cationic form in PBS solution of pH 7.4. The polymeric film exists in a negative charge. Hence, in contrast, AA exists in the anionic form in PBS solution of pH 7.4. The negative charge polymeric film repels it. So it cannot enter the polymer film to the sane extent as DA. And the interference with the determination of DA is diminished.

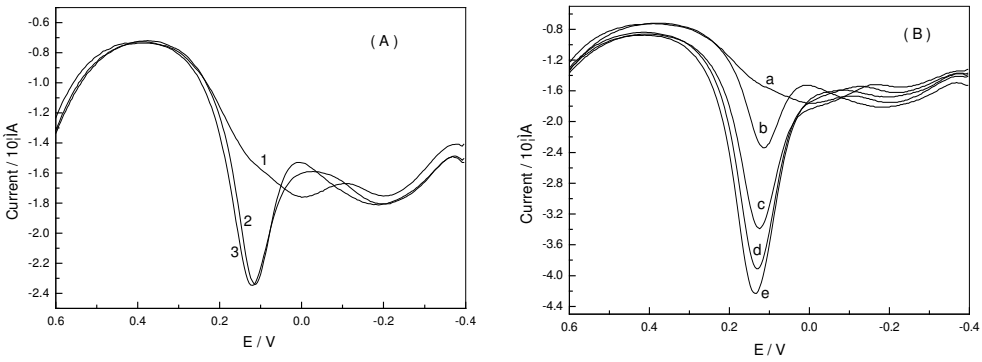



**Figure 5(A).** Differential pulse voltammetric at the modified electrode in pH 7.4 PBS (1): $1.0\times10^{-2}$mol dm$^{-3}$ AA; (2): $1.0\times10^{-4}$ mol dm$^{-3}$ DA; (3): $1.0\times10^{-2}$ mol dm$^{-3}$ AA + $1.0\times10^{-4}$ mol dm$^{-3}$ DA
**Figure 5(B).** DPV recordings of DA at modified electrode and in the presence of $1.0\times10^{-2}$ mol dm$^{-3}$ AA in pH 7.4 PBS. DA concentration ($10^{-4}$ mol dm$^{-3}$): (a) 0 (b) 1.0 (c) 2.0 (d) 3.0 (e) 4.0
Parameter: Amplitude: 50 mV; Pulse Width: 50 ms; Pulse Period: 200 ms;Sensitivity: $2.0\times10^{-5}$ A/V

We also studied interference of other compounds. To $1.0\times10^{-4}$mol dm$^{-3}$ DA, the following compounds had no interference: hippuric acid (100), citric acid (50), cysteine (50), glucose (100), NaCl (300), KCl (300), $CaCl_2$ (100).

*Analytical Applications*

The injection of DA was analyzed by the standard addition method. The results are shown in Table 1. It is accordant with the result by the China Pharmacopoeia method [17], showing that the proposed methods could be efficiently used for the determination of DA in injection.

**Table 1.** Results of DA analysis in injections ( n=10)

| Sample | Content $10^{-5}$ mol dm$^{-3}$ | Found $10^{-5}$ mol dm$^{-3}$ | RSD % | Recovery % | Pharmacopeia Method $10^{-5}$ mol dm$^{-3}$ |
|---|---|---|---|---|---|
| 1 | 1.0 | 0.97 | 2.9 | 97 | 1.01 |
| 2 | 1.0 | 1.01 | 2.0 | 101 | 1.04 |
| 3 | 1.0 | 1.03 | 2.7 | 103 | 1.05 |
| 4 | 1.0 | 0.99 | 3.2 | 99 | 1.02 |
| 5 | 1.0 | 0.98 | 2.2 | 98 | 1.02 |

## 4. Conclusions

The results obtained show poly (acridine red) film modified electrode can promote DA oxidation. Peak current of DA was proportional to the concentration over the range of $1.0\times10^{-7}$ ~$1.0\times10^{-4}$ mol $dm^{-3}$ with a detection limit (S/N = 3) of $1.0\times10^{-9}$ mol $dm^{-3}$.The results of differential pulse voltammetry show that the interference of AA was entirely eliminated in determination of DA in pH 7.4 PBS. And proposed methods can be applied to the detection of DA in samples.

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*Sample Availability:* Available from the authors.