

# Electrochemical preparation of poly(bromothymol blue) film and its analytical application

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**Abstract** The poly(bromothymol blue) film was prepared using a simple cyclic voltammetry method. Field emission scanning electron microscopy and electrochemical methods were employed to characterize this film. When the film was electropolymerized on an electrode, it showed excellent catalytic activity toward the oxidation of dopamine (DA) in pH 4.0 phosphate buffer solution. In addition, the overlapped voltammetric responses of dopamine and ascorbic acid were resolved into two well-defined peaks by the film-modified electrode with a large peak separation of 0.17 V. A calibration curve for DA was obtained by the amperometric method in the range of 3.0–195.0  $\mu\text{M}$  with a correlation coefficient of 0.9997. The film-modified electrode has been successfully used to measure the concentration of DA in injections and human serum samples.

**Keywords** Poly(bromothymol blue) · Electrochemical · Preparation · Dopamine

## 1 Introduction

Bromothymol blue (BTB) (Scheme 1) is an acid–base indicator that has been used to probe alterations in the structure and physical properties of proteins [1], phospholipids vesicles [2], and biomembranes [3]. However, to the

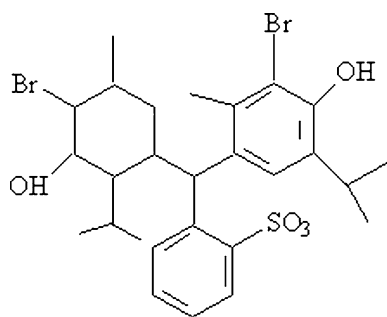
best of our knowledge, there is little literature on the electropolymerization of BTB and its application.

As a member of the catecholamine family, dopamine (DA) plays an important role in mammalian central nervous systems [4]. Low levels of DA can lead to neurological disorders such as Parkinsonism and schizophrenia [5, 6]. Thus, it is especially important to perform sensitive measurements of DA, and tremendous effort has been put into this area over the past 30 years [7–10]. Because DA has an intrinsic redox nature, it can be analyzed by electrochemical techniques [11, 12]. However, since other coexisting species, such as ascorbic acid (AA), may have close redox potentials, their voltammetric responses would overlap with those of DA and interfere with the direct detection of DA [13, 14]. In order to overcome this problem, a number of approaches have been developed. Most of them are based on the modification of electrodes with different materials, such as self-assembled monolayers [15–18], carbon nanotubes [19–21], nanoparticles, organic polymers [21–23], and polymer membranes [24–26].

Electropolymerization is an attractive method for the development of membranes, because it allows reproducible, precise, uniform, and thickness-controlled polymer coatings without limiting the size, area, and geometry of the surfaces [27–32]. Conducting polymers, such as polyaniline [33], polypyrrole [34], poly(3-methylthiophene) [35], polycarbazole [36, 37], and poly(carbazole-*co-p*-tolylsulfonyl pyrrole) films [38] have been used for the analysis of DA. Besides conducting polymers, other films have also been prepared by the electropolymerization method and been used successfully. Lin and Chen studied the electro-catalytic activity of poly(cresol red) [39], poly-BTB [40], and poly(Evans Blue) [41] modified electrodes toward the oxidation of DA and AA. Similarly, we have

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**Scheme 1** The structure of Bromothymol blue

prepared a poly(acid chrome blue K) (ACBK) modified electrode and applied it in the measurement of DA [42].

In this work, we prepared poly-BTB by electropolymerization and evaluated its application for the analysis of DA. The surface morphology of poly-BTB coated electrode was characterized by field emission scanning electron microscopy (FESEM). The electrochemical behavior of DA and AA on the poly-BTB film modified electrode was studied. The oxidation peaks of DA and AA could be well resolved in the modified electrode. Based on the improved catalytic activity of the modified electrode toward DA oxidation, a sensitive, selective, and simple method was set up for routine analysis.

## 2 Experimental

### 2.1 Chemicals

Bromothymol blue was purchased from Shanghai Chemical Reagents Company (China). DA and AA were purchased from Sigma (USA). All reagents were of analytical grade and used without any further purification. Phosphate buffer solutions (PBS) were prepared by mixing stock solutions of 0.1 M  $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ , and then the pH was adjusted with 0.1 M  $\text{H}_3\text{PO}_4$  or 0.1 M NaOH. All solutions were prepared with double-distilled water. DA and AA solutions were freshly prepared prior to measurements.

### 2.2 Apparatus

CHI 660B electrochemical workstation (Shanghai Chenhua Instruments, China) was used for all the electrochemical measurements. A conventional three-electrode system was used throughout the experiments. The working electrode was either a bare or a poly-BTB modified glassy carbon electrode (GCE) with a surface area of  $0.071\text{ cm}^2$ . The auxiliary electrode was a platinum wire and the reference electrode was a saturated calomel electrode (SCE). Before each experiment, the GCE was polished with 0.3 and

$0.05\text{ }\mu\text{m}$  alumina slurries, rinsed with double-distilled water, and then purified sequentially by  $\text{HNO}_3$  (1:1, v/v), ethanol (1:1, v/v) and distilled water. FESEM images were obtained using an S-4800 Field Emission Scanning Electron Microscope (Hitachi, Japan).

### 2.3 Preparation of poly-BTB modified GCE and determination of DA

The poly-BTB modified GCE was prepared from a pH 7.6 solution containing  $0.75\text{ mmol L}^{-1}$  BTB by cycling the potential between  $-0.8$  and  $1.8\text{ V}$  at a scan rate of  $0.1\text{ V s}^{-1}$  for 20 cycles. After electropolymerization, the modified electrode was thoroughly rinsed with distilled water and then dried in air for further experimentation.

The concentration of DA in  $10.0\text{ mL}$  of stirred PBS (pH 4.0,  $0.1\text{ M}$ ) was determined by the amperometric method at  $0.3\text{ V}$ . After the base solution reached a steady-state current, an aliquot of DA stock solution was injected using a microsyringe at regular intervals. The concentration of DA was calculated based on the difference between the baseline and the steady-state current after injection.

## 3 Results and discussion

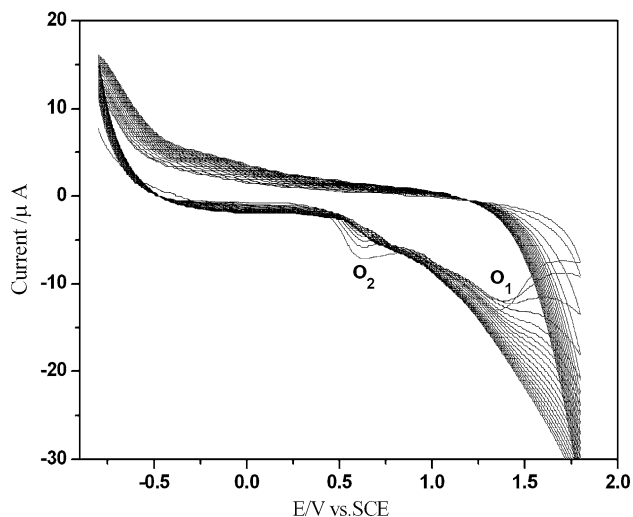
### 3.1 Electropolymerization of poly-BTB modified GCE

Figure 1 illustrates the continuous cyclic voltammograms for the electrochemical polymerization of BTB on a GCE. The BTB monomer shows an oxidation peak at about  $1.30\text{ V}$  ( $\text{O}_1$ ) during the first anodic scan. With potential cycling, the peak current of  $\text{O}_1$  decreased greatly and a new oxidation peak appeared at about  $0.60\text{ V}$  ( $\text{O}_2$ ) due to formation of the polymer film. It shows that the electrochemical behaviors of BTB at the GCE are completely irreversible. Thus, poly-BTB film can be prepared by the cyclic voltammetric method.

Figure 2a shows the FESEM images of a bare (a) and a poly-BTB modified GCE (b). The surface morphology of the poly-BTB film (b) is dense and composed of many nanoparticles, compared with that of the bare GCE (a).

### 3.2 Electrochemistry of DA and AA at the poly-BTB modified GCE

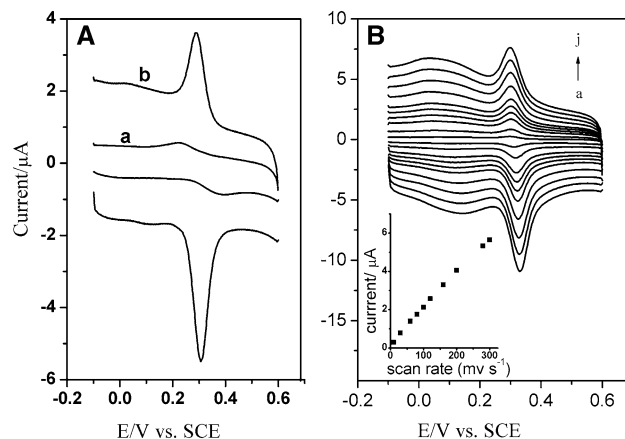
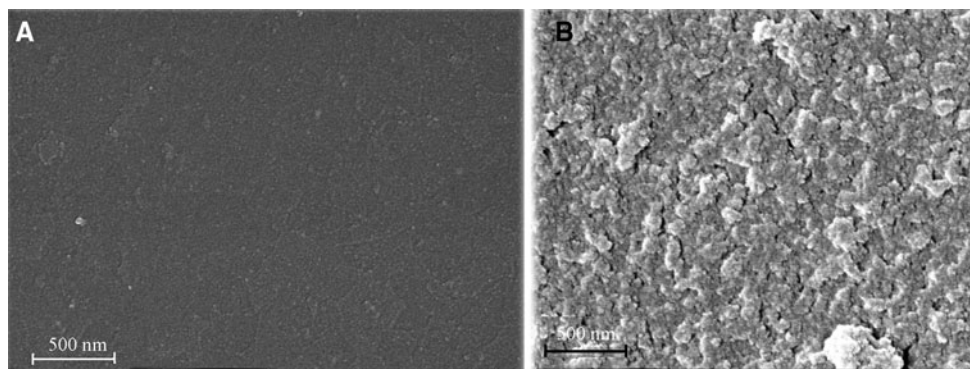
The poly-BTB modified GCE shows excellent catalytic activity toward the oxidation of DA and AA. Figure 3A shows the cyclic voltammograms of  $12.0\text{ }\mu\text{M}$  DA in pH 4.0 PBS at a bare GCE (curve a) and a poly-BTB modified GCE (curve b). At the bare GCE, DA shows a sluggish and much smaller CV peak response. The oxidation and reduction peak potentials are at  $0.395$  and  $0.220\text{ V}$  (curve a),



**Fig. 1** Cyclic voltammograms of BTB ( $0.75 \text{ mmol L}^{-1}$ ) in pH 7.6 solution on the glassy carbon electrode in the potential range of  $-0.8$  to  $+1.8 \text{ V}$  at a scan rate of  $100 \text{ mV s}^{-1}$ . The cycling number is 20

respectively. The separation of the redox peak potentials ( $\Delta E_p$ ) is  $0.175 \text{ V}$ . On the poly-BTB modified electrode, the oxidation peak potential shifts negatively to  $0.310 \text{ V}$  and the reduction peak potential shifts positively to  $0.295 \text{ V}$ , which results in a well-defined redox wave of DA with a  $\Delta E_p$  of  $0.015 \text{ V}$ . In addition, substantial increases in the peak currents of DA were also observed. The increase in current and shift in potential indicated that poly-BTB could improve the electrochemical response of DA on the electrode. Figure 3B shows the effect of scan rate on the oxidation peak current of  $12.0 \mu\text{M}$  DA at the poly-BTB modified electrode in  $0.1 \text{ M}$  pH 4.0 PBS. The oxidation peak currents ( $I_{pa}$ ) increase with the scan rates. A good linear relationship between  $I_{pa}$  and scan rates was obtained over the range of  $0.01\text{--}0.3 \text{ V s}^{-1}$ . The linear regression equation was  $I_{pa} (\mu\text{A}) = 0.0182v (\text{mV s}^{-1}) + 0.262$ , with a correlation coefficient of  $0.9990$  (Fig. 3B inset). The results suggest that the redox reaction of DA on the modified electrode was a surface-controlled process. It has been well-documented that the peak potential and current of the

**Fig. 2** FESEM image of **a** the bare GCE and **b** the poly-BTB film modified GCE

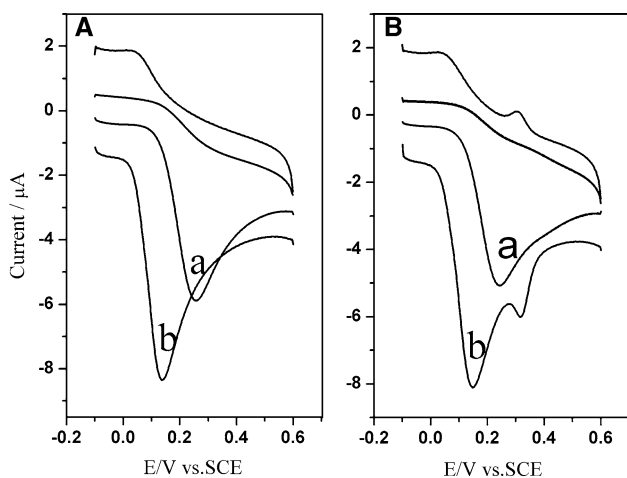


**Fig. 3** **A** Cyclic voltammograms of  $12.0 \mu\text{M}$  DA at (a) a bare GCE and (b) a poly-BTB modified GCE in pH 4.0 PBS. **B** Cyclic voltammograms of  $12.0 \mu\text{M}$  DA on the poly-BTB modified glassy carbon electrode at various scan rates from  $10$  to  $300 \text{ mV s}^{-1}$ . The inset shows the plot of anodic peak current ( $I_{pa}$ ) versus the scan rate

adsorbed species are dependent upon the number of the adsorption and electroactive sites [43, 44]. The nano-structured poly-BTB film (as seen in Fig. 2b) may provide more active sites for the adsorption of DA and consequently leads to increased peak currents and decreased potential separation.

Figure 4A shows the cyclic voltammograms of  $250.0 \mu\text{M}$  AA in pH 4.0 PBS at a bare (curve a) and a poly-BTB modified GCE (curve b). At the bare electrode, the oxidation peak potential of AA is about  $0.258 \text{ V}$ . The oxidation peak potential is  $0.137 \text{ V}$  at the poly-BTB modified GCE. This peak is much sharper than that on the bare GCE. The  $0.121 \text{ V}$  negative potential shift and enhanced current of the oxidation peak show the strong catalytic effect of the poly-BTB modified layer toward the oxidation of AA.

Since it is normally difficult to obtain separate voltammetric waves for AA and DA in the presence of each other, the ability of the modified electrode to promote the voltammetric resolution of AA and DA was investigated. Figure 4B shows the cyclic voltammograms of the mixed



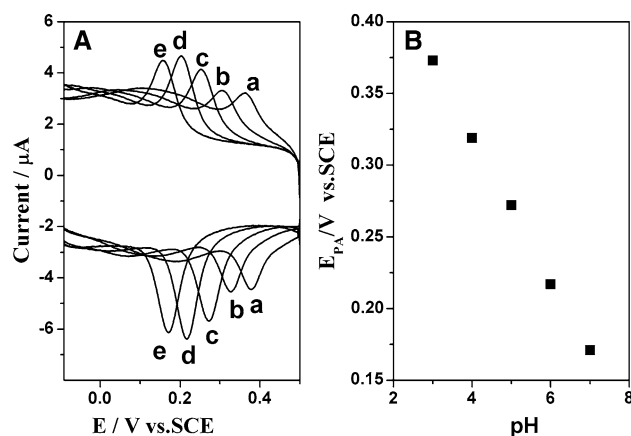
**Fig. 4** **A** Cyclic voltammograms of 250.0  $\mu\text{M}$  AA at (a) a bare GCE and (b) a poly-BTB modified GCE in pH 4.0 PBS. **B** Cyclic voltammograms of 12.0  $\mu\text{M}$  DA, 250.0  $\mu\text{M}$  AA at (a) a bare GCE and (b) a poly-BTB modified GCE in pH 4.0 PBS. In all the experiments, the scan rate was  $100 \text{ mV s}^{-1}$

solution containing 12.0  $\mu\text{M}$  DA and 250.0  $\mu\text{M}$  AA in pH 4.0 PBS at a bare (curve a) and a poly-BTB modified GCE (curve b). In Fig. 4B-a, the CV of the mixture only gives a broad anodic peak at the bare GCE, and the peak potentials of DA and AA are indistinguishable. In Fig. 4B-b, the electrochemical responses of DA and AA on the poly-BTB modified electrode occur at different potential windows. The merged voltammetric anodic peaks were resolved into two well-defined peaks at potentials around 0.310 and 0.140 V for DA and AA, respectively. The large peak separation of the anodic peaks of DA and AA makes it feasible to detect DA in the presence of AA.

### 3.3 Effect of pH on the oxidation of DA

The effect of pH (3.0–7.0) on the formal potential and anodic peak current of DA was investigated by cyclic voltammetry in the solution containing 4.5  $\mu\text{M}$  DA. The anodic and cathodic potential of DA decreased with increasing pH from 3.0 to 7.0 (Fig. 5A a–e). This implies that the protons took part in the electrode reaction. As shown in Fig. 5B, the plot of the anodic peak potential ( $E_{\text{pa}}$ ) versus pH is linear in the pH range of 3.0–7.0 with a slope of  $52.9 \text{ mV pH}^{-1}$  ( $r = 0.9996$ ), which reveals that the proportion of electrons and protons involved in the reaction is equal [24].

The effect of pH on the electrochemical responses of 4.5  $\mu\text{M}$  DA on the poly-BTB modified GCE is shown in Fig. 6a. It can be seen that the anodic peak current of DA reached a maximum at pH 6.0 and then decreased. The reduction of currents at higher pH values may correspond to the instability of DA in less acidic conditions [45]. To lower the interference, it is also necessary to seek a large

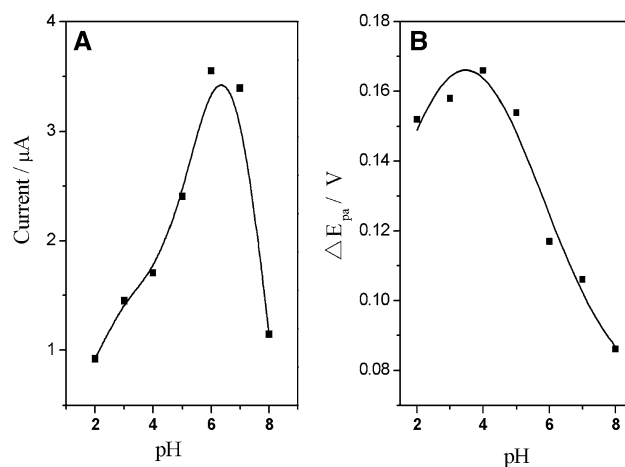


**Fig. 5** **A** Cyclic voltammograms of 4.5  $\mu\text{M}$  DA at poly-BTB modified GCE in different pH solution: (a) 3.0, (b) 4.0, (c) 5.0, (d) 6.0, and (e) 7.0. **B** Plot of the peak potential as a function of pH. Scan rate was  $100 \text{ mV s}^{-1}$

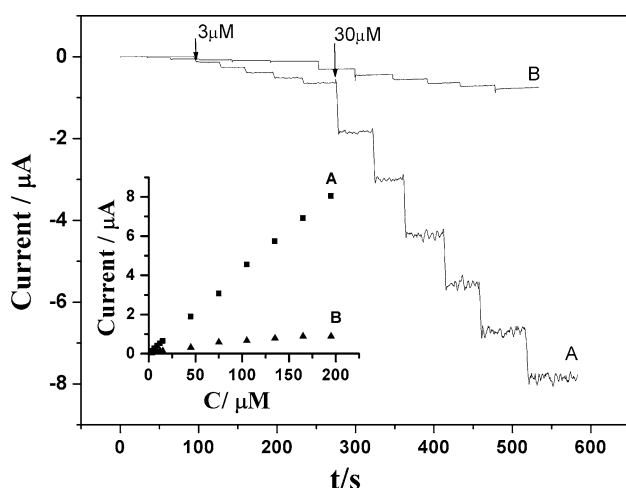
separation in peak potential. The separations of the oxidation peak potentials for DA and AA ( $\Delta E_{\text{pa}}$ ) were 0.168 and 0.117 V at pH 4.0 and 6.0, respectively (Fig. 6b). Based on the selectivity and sensitivity, a pH of 4.0 PBS was chosen for the following analysis.

### 3.4 Analytical performance of the poly-BTB modified electrode for DA detection

The detection of DA was performed at the poly-BTB modified GCE (Fig. 7A) and bare GCE (Fig. 7B) by amperometric methods under constant stirring. At an applied potential of 0.3 V, when DA was added, the anodic



**Fig. 6** **a** Effect of pH on the oxidation peak current of 4.5  $\mu\text{M}$  DA at poly-BTB modified GCE by cyclic voltammograms. **b** Effect of  $\Delta E_{\text{pa}}$  (the separation of the anodic peak potentials for DA and AA) at various pH



**Fig. 7** Amperometric detection of DA using (A) the poly-BTB modified electrode and (B) the bare GCE under successive additions with different concentrations of DA. Inset was the calibration curves of (A) poly-BTB and (B) bare GCE modified electrode for the analysis of DA

current on the poly-BTB modified electrode increased sharper than that on the bare GCE and reached a steady state within 10 s (Fig. 7A). The results demonstrated clearly that the poly-BTB modified electrode had a rapid response and high sensitivity for DA detection. The higher efficiency of the modified electrode may be due to the larger surface areas caused by the poly-BTB nanoparticles. The dispersed nanoparticles on the electrode surface presumably provided a good physical dispersion of the catalytic centers, which led to a highly active surface suitable for efficient catalysis. To verify the linear relationship between peak current and DA concentration, six calibration graphs were constructed under optimum conditions. In all cases the current and concentration relationship was linear over a concentration range of 3.0–95.0  $\mu\text{M}$ . The equation for the curve in the linear range was  $I_{pa} (\mu\text{A}) = 0.025 + 0.042C_{DA} (\mu\text{M})$  with a correlation coefficient of 0.9997 for  $n = 6$  (Fig. 7A inset).

According to the IUPAC definition [46], the detection limit (DL) is related to the smallest response that can be detected with reasonable certainty. For a given analytical method, the DL is

$$DL = \frac{kS_B}{b}$$

where  $S_B$  is the standard deviation of the blank measurement,  $b$  is the sensitivity of the method (determined as the slope of the calibration curve), and  $k$  is a statistical constant (a value of  $k = 3$  is strongly recommended by IUPAC, based on the confidence interval). The slope obtained from a least-squares regression was 0.042, yielding a DL of 0.5  $\mu\text{M}$  ( $3\sigma$ ,  $n = 7$ ) for DA.

### 3.5 Interferences

In order to evaluate the interference, the amperometric response of the poly-BTB modified electrode toward DA and other interfering species was studied. Figure 8 displays the responses of 40.0  $\mu\text{M}$  DA and the interferences. No interference is observed for AA (100), cysteine (650), glucose (650), lysine (650), citrate (650),  $\text{K}^+$  (4000), and  $\text{Na}^+$  (4000) (Fig. 8), where the data in the parenthesis are the concentration ratios of those interferences to DA. The results indicated that the poly-BTB film has good selectivity for the determination of DA.

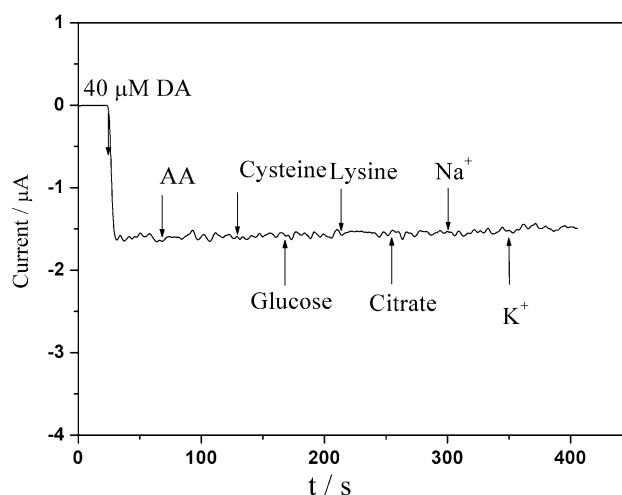
### 3.6 Reproducibility and stability

The reproducibility of the poly-BTB modified GCE was investigated by repeatedly measuring its response to 40.0  $\mu\text{M}$  DA by ten consecutive analyses, yielding an average current of 1.71  $\mu\text{A}$  with an RSD of 2.5%. Furthermore, the GCE was re-modified five times with poly-BTB. The average current of 40.0  $\mu\text{M}$  DA obtained from these modified electrodes was 1.73  $\mu\text{A}$  with the RSD of 3.7%.

The stability of the poly-BTB modified GCE was investigated by storing it at room temperature and then measuring its response to 40.0  $\mu\text{M}$  DA. The modified GCE was stable for 2 weeks, after which a gradual decrease of current (5%) was found. These results suggest that poly-BTB modified GCE possesses remarkable reproducibility and good stability.

### 3.7 Determination of DA in dopamine hydrochloride injections and human serum samples

The poly-BTB modified GCE for real sample analysis was investigated by direct analysis of dopamine hydrochloride



**Fig. 8** Amperometric responses of DA and different interferences

**Table 1** Determination result of DA in injections and human serum sample

Analyte	Content ( $\mu\text{M}$ )	Added ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	RSD (%)	Recovery (%)
Injection	10.00	0	10.25	3.7	102.5
		2.0	12.47	3.2	103.9
		4.0	14.45	2.8	103.2
Serum sample	20.00	0	20.52	3.9	102.6
		5.0	24.39	4.1	97.6
		10.0	30.92	2.6	103.1

injection ( $10 \text{ mg mL}^{-1}$ , 2 mL per injection, batch no: 081209). Using the method described above, the injection of dopamine hydrochloride was analyzed using a calibration plot. In addition, different volumes of standard solutions were added into the corresponding injection for a recovery test. The results are listed in Table 1.

Since DA concentration in blood serum is very low, a recovery test was also used to evaluate the performance of the proposed electrode in practical analytical applications. Fresh human serum sample was obtained from Yangzhou No. 1 People's Hospital. The serum sample was filtered and diluted using pH 4.0 PBS. The diluted serum sample was spiked with various concentrations of DA and the anodic currents were obtained by the amperometric method at the modified electrode. The recovery results for DA spiked to the diluted serum samples were in the range of 97.6–103.1% (Table 1). This suggests a promising feature for the applicability of the modified electrode for the direct determination of DA in real samples.

#### 4 Conclusions

In conclusion, a novel poly-BTB film-modified electrode was fabricated by the cyclic voltammetry method. The as-formed poly-BTB film exhibited remarkable catalytic activity toward the oxidation of DA. Large peak separations between DA and AA allow the proposed electrode to determine DA with good selectivity, sensitivity, and stability. Moreover, the proposed method could be applied for the determination of DA in real samples with satisfactory results. The poly-BTB can be used as a promising material for other analytical applications as well.

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