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Molecularly Imprinted Sensor based on o-phenylenediamine for Electrochemical Detection of Sulfamethoxazole

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Molecularly imprinted electrochemical sensor for the detection of sulfamethoxazole was prepared by electropolymerization with sulfamethoxazole as template molecule and o-phenylenediamine as functional monomer. Electrochemical performance of molecularly imprinted sensor was studied by cyclic voltammetry in 0.1 mol/L KCl solution containing 5 mM $K_3Fe(CN)_6$. On the optimal experimental conditions, square wave voltammetry was used to detect sulfamethoxazole. The square wave voltammetric peak current difference of the sensor has a good linear relationship with the concentration of sulfamethoxazole in the range of 0.2 to 1.4 uM, and the detection limit is 0.05 uM. The molecularly imprinted sensor has good selectivity, repeatability and stability.

Keywords: Sulfamethoxazole; o-phenylenediamine; Molecularly imprinted sensor

1. INTRODUCTION

Sulfamethoxazole has strong antimicrobial activity, it is often added to animal feed as a veterinary antibiotic. However, residues of sulfamethoxazole or its metabolites will cause severe food safety and environmental problems. Therefore, a sensitive, stable and reliable detection method is needed to monitor the residues and metabolites in food and environment. At present, the main analytical methods for sulfamethoxazole including chromatography[1-5], immunoassay [6], capillary electrophoresis [7] and electrochemical methods [8-10]. For the above methods, electrochemical methods have the advantages of fast response, low cost, and good selectivity[11-15] .

In recent years, molecularly imprinting technique has developed rapidly. For the molecularly imprinting technique, the affinity matrix is prepared by the polymerization of functional monomers and crosslinkers in the presence of template molecules. The template molecules are removed from the polymer by eluting. Thus, the molecularly imprinted polymer (MIP) is obtained with the specific

cavities, which could selectively recognize template molecule. The molecularly imprinted electrochemical sensors have received great attention due to their high selectivity, sensitivity, chemical stability, reproducibility and low detection limit [16-21]. For the preparation of imprinted polymers, electropolymerization is widely used because of its simple preparation process, thin and uniform film, fast response and high sensitivity [22, 23]. Therefore, in this study, sulfamethoxazole molecularly imprinted electrochemical sensor was prepared by electropolymerization on a glassy carbon electrode with o-phenylenediamine as functional monomer. A simple, rapid and sensitive electrochemical sensor was established for the detection of sulfamethoxazole.

2. EXPERIMENTAL

2.1 Reagents and instrumentation

Sulfamethoxazole and o-phenylenediamine were purchased from Aladdin Industrial Corporation(Shanghai, China). All other chemical reagents were obtained from China Pharmaceutical Group Chemical Reagents Co., Ltd. The sulfamethoxazole stock solution of 1.0×10^{-3} mol/L was prepared by dissolving 0.0256g sulfamethoxazole in ethanol with water. All other standard solutions of sulfamethoxazole were diluted from the stock solution.

All electrochemical experiments were carried out on a LK2005 Electrochemical Workstation (Tianjin Lanlike Chemical Electronics High Technology Co., Ltd., Tianjin, China). A conventional three-electrode system was used for the electrochemical measurements including glassy carbon electrode as the working electrode, saturated calomel electrode as reference electrode and platinum wire as counter electrode.

2.2 Preparation of sulfamethoxazole molecularly imprinted electrochemical sensor

Firstly, the glassy carbon electrode (GCE) was carefully polished with a leather containing 0.05 μm Al_2O_3 slurry and then ordinal ultrasonically cleaned in ethanol and distilled water. Then, the GCE was transferred to 30 ml 0.1 mol/L Na_2SO_4 solution containing 0.03g o-phenylenediamine and 0.03 g sulfamethoxazole. The electropolymerization was performed by using cyclic voltammetry. The potential range was 0.3 - 1.0V, the scan rate was 100 mV/s and the number of scan cycles was 10. After electropolymerization, the electrode was cleaned with distilled water to remove the unreacted o-phenylenediamine and sulfamethoxazole. Finally, the electrode was eluted in 0.1 mol/L NaOH solution by magnetic stirring for 15 minutes to remove the sulfamethoxazole template embedded in the polymer film. The electrode was cleaned with distilled water and stored for further use. This electrode was named as sulfamethoxazole molecularly imprinted electrode(MIP/GCE).

The preparation of non-imprinted electrode(NIP/GCE) is exactly the same as the previous process, except that the template molecule sulfamethoxazole was not added to the solution.

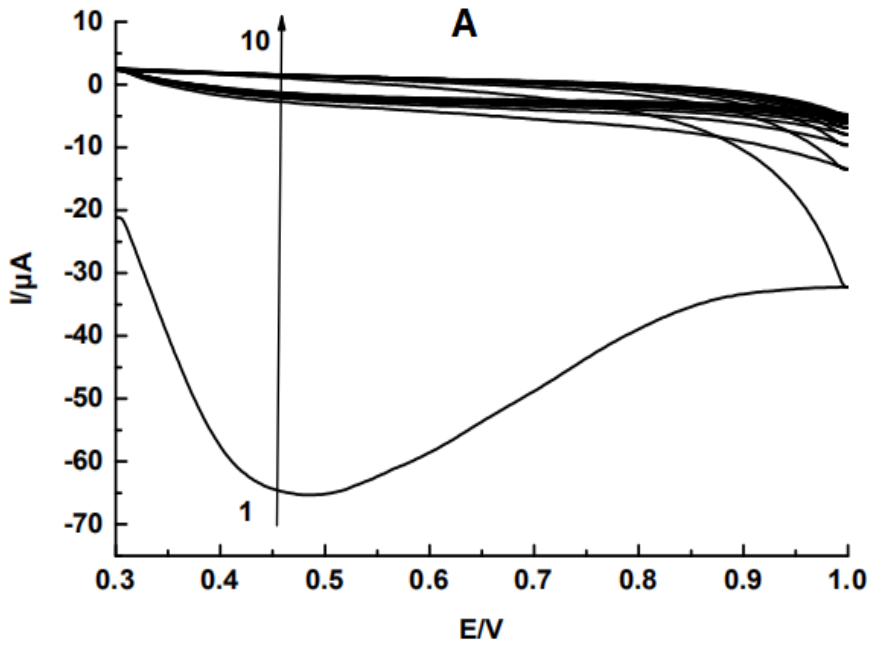
2.2 Electrochemical experiments

The electrochemical experiments was performed by using cyclic voltammetry and square wave voltammetry. For the cyclic voltammetry, the potential range was -0.4 - 0.9V, the scan rate was 100 mV/s. For the square wave voltammetry, the potential range was -0.4 - 0.9V, the potential increment was 5 mV, square wave frequency was15 Hz and square wave amplitude was 0.1 V. After each use, the MIP/GCE was immersed in 0.1 mol/L NaOH solution and eluted for 15 minutes to remove the sulfamethoxazole from the polymer film for reuse.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammogram of electropolymerization

Cyclic voltammetry was used for the electropolymerization of molecularly imprinted polymers, where sulfamethoxazole as template molecule and o-phenylenediamine as functional monomer. As can be seen from figure 1A, the peak current of the first cycle was very large (about 65.6 uA) and the peak potential was 0.49 V. However, the current intensity decreased sharply in the second cycle, and then the current intensity decreased gradually with increase the number of cycle. The phenomenon is similar to the previous reports[24,25]. The results indicated that the electropolymerization of o-phenylenediamine and sulfamethoxazole on glassy carbon electrode is a completely irreversible process, and when scanned for 10th cycle, the peak current was very stable. It is proved that the surface of glassy carbon electrode is covered with a dense and weak conductive polymers.



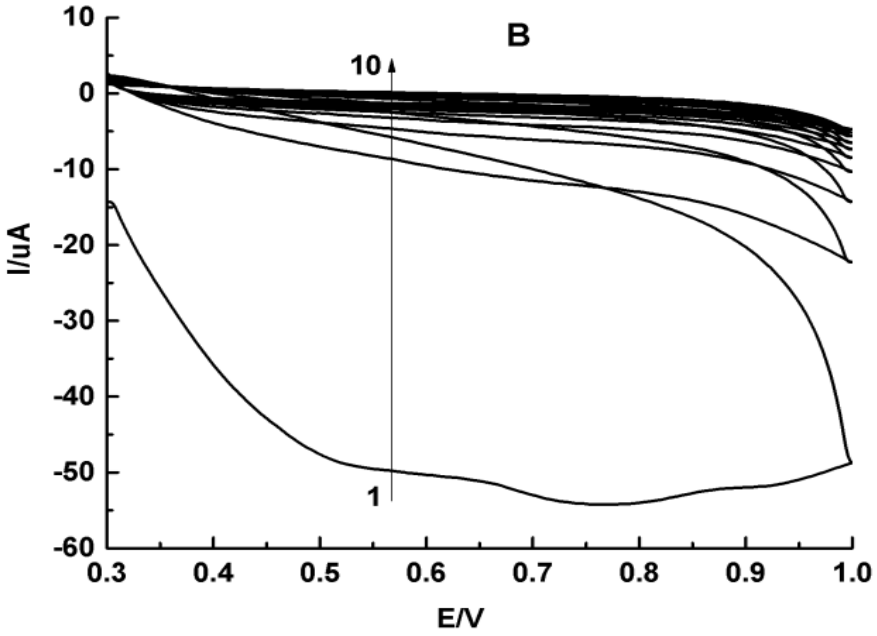


Figure 1. Sulfamethoxazole molecularly imprinted electropolymerization(A) and non-imprinted electropolymerization (B).

The cyclic voltammogram of non-imprinted electropolymerization (Fig. 1B) is similar to the imprinted electropolymerization. This indicates that the template molecule sulfamethoxazole does not appear electrochemical activity in the electropolymerization process. The electropolymerization of o-phenylenediamine is not affected by sulfamethoxazole and the polymers molecular structure is not changed in the electropolymerization process.

3.2. Cyclic voltammogram of different electrodes

Cyclic voltammetry is also used to study the electron transfer performance for a modified electrode[26]. Figure 2 showed the cyclic voltammogram of bare electrode(a), electropolymerized electrode(b), template-eluted electrode(c) and incubated electrode(d) in 5 mmol/L $K_3Fe(CN)_6$ solution. As can be seen, the bare electrode (curve a) has a pair of well-defined redox peaks related to the redox of $K_3Fe(CN)_6$. For the electropolymerized electrode (curve b), no redox peak was observed because the electrode surface was covered by the compact polymer film, which blocked the redox probe $K_3Fe(CN)_6$ access to the electrode surface. After 10 minutes elution with 0.1mol/L NaOH, the redox peak of the electrode (curve c) was appear. It can be explained that the elution of sulfamethoxazole from the polymers left many cavities, which make the access of $K_3Fe(CN)_6$ to the electrode surface. However, only the cavities in the polymers after elution of sulfamethoxazole acted as channels for electron transport. The peak currents were lower than that of bare electrode. When the template-eluted electrode was immersed in sulfamethoxazole solution for 10 minutes, the sulfamethoxazole molecule will reoccupy the cavities. Thus, the peak current (curve d) was lower than that of template-eluted electrode. The results are consistent with our previous work[18, 23].

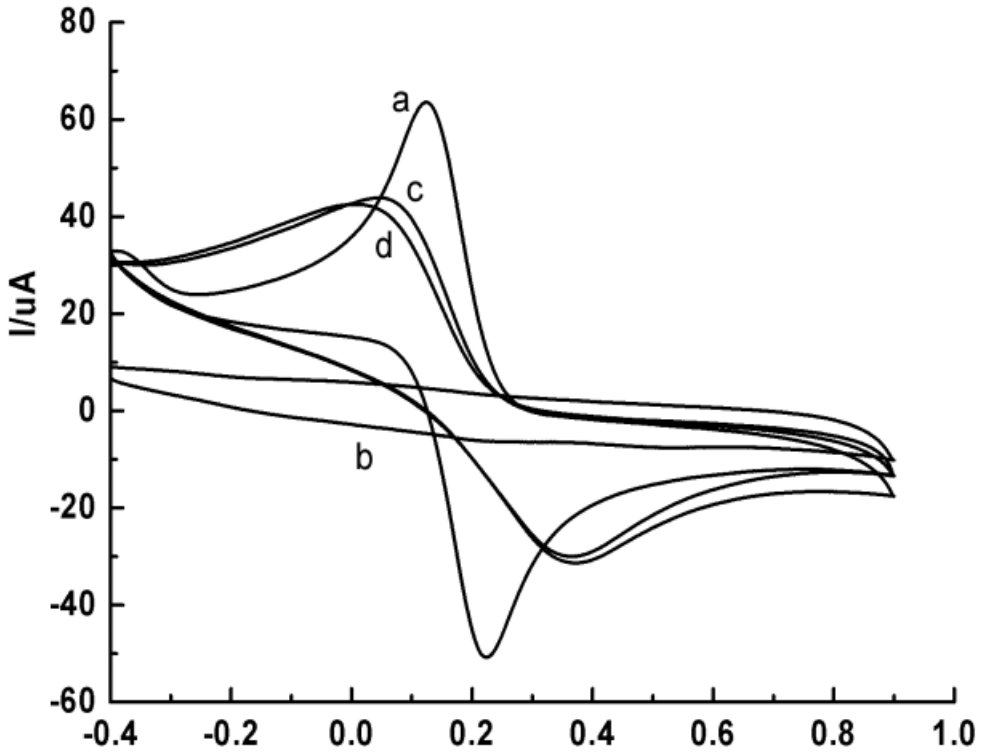


Figure 2. The cyclic voltammogram of bare electrode(a), electropolymerized electrode(b), template-eluted electrode(c) and incubated electrode(d) in 5 mmol/L $K_3Fe(CN)_6$ solution.

3.3. Study of scan rates

The reaction process of molecularly imprinted electrode(MIP/GCE) in $K_3Fe(CN)_6$ solution was studied. Fig. 3A showed the cyclic voltammogram of MIP/GCE in $K_3Fe(CN)_6$ solution with different scan rates. As can be seen, the peak current increases with the increase of scan rates, and the oxidation peak potential shifts positively and the reduction peak potential shifts negatively. The peak potential difference increased obviously between the oxidation peak and the reduction peak.

Fig. 3B showed the plot of scan rates and the corresponding peak current. It can be seen from the graph that the peak current Ip (uA) is linearly with scan rates *v* (mV/s) in the range of 20 to 200 mV/s. The linear equation is Ip = 20.62 + 0.16*v*, *r* = 0.9851, and Ip = -13.21 - 0.17*v*, *r* = -0.9928, respectively. The results showed that the reaction process of MIP/GCE in $K_3Fe(CN)_6$ solution is surface controlled in the range of scan rates[27, 28].

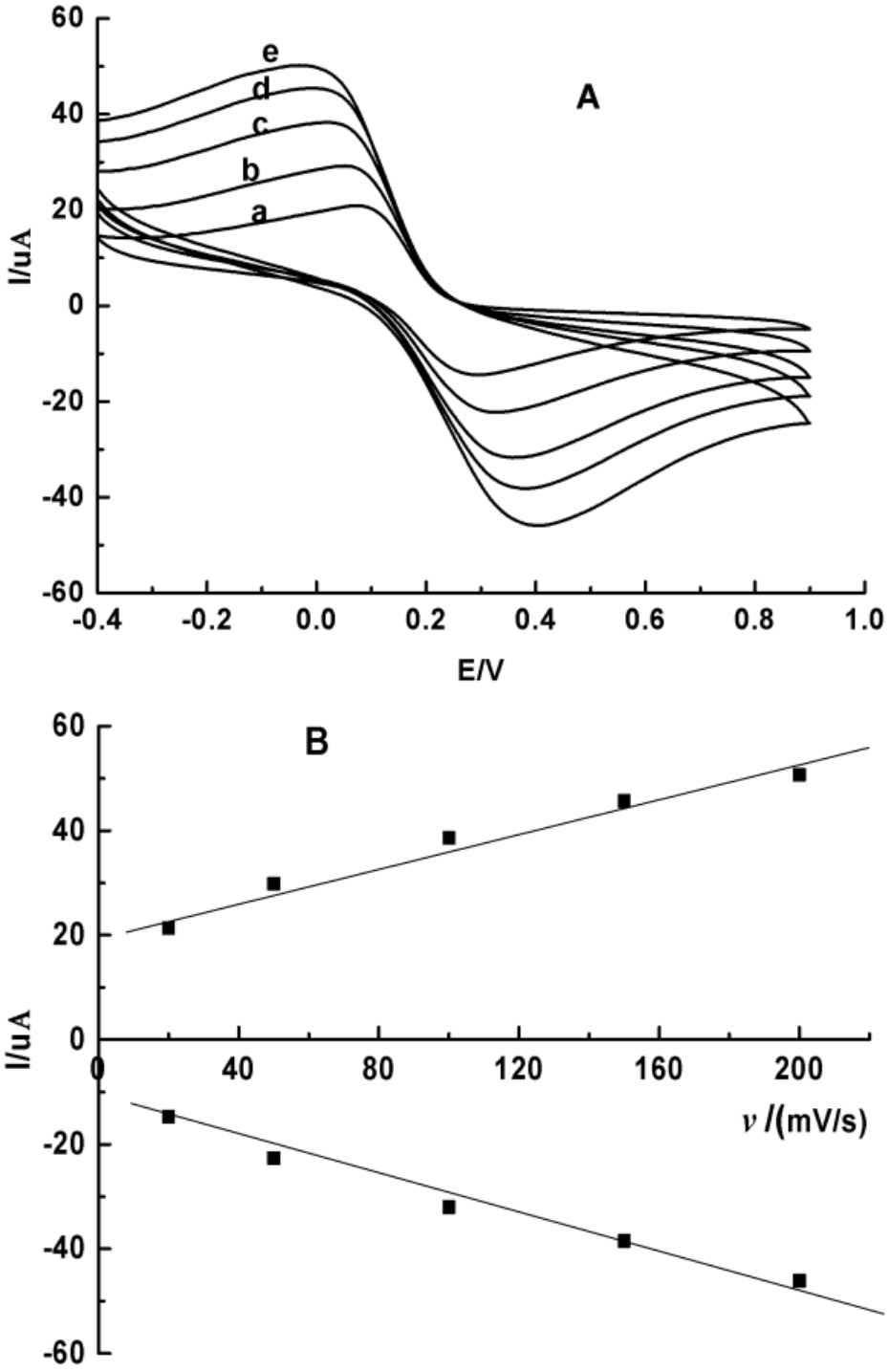


Figure 3. The cyclic voltammogram of MIP/GCE in $K_3Fe(CN)_6$ solution with different scan rates(A), from a-e: 20, 50, 100, 150 and 200 mV/s and the plot of scan rates with corresponding peak current(B).

3.4. Determination of sulfamethoxazole

Square wave voltammetry(SWV) was employed for the determination of sulfamethoxazole in this work. Fig. 4A showed the SWVs of the MIP/GCE with different concentrations of sulfamethoxazole.

It can be seen from the figure, with increasing the sulfamethoxazole concentration, the peak current decreases gradually. This is due to the fact that sulfamethoxazole molecules entered the cavities of the polymers, resulting in less $K_3Fe(CN)_6$ access to the surface of the electrode and lower the peak current. When the concentration of sulfamethoxazole exceeds 1.4 uM, the peak current decreases slowly, which suggesting that the cavities of the polymers tends to be saturated.

Fig. 4B showed the plot of peak current difference(Δ Ip) with corresponding sulfamethoxazole concentration. As can be seen, the Δ Ip is linearly to the sulfamethoxazole concentration in the range of 0.2 to 1.4 μM, with a detection limit of 0.05 μM. The linear regression equation is Δ Ip (μA) = 14.05 + 6.66c (μM), and the correlation coefficient is r = 0.9968.

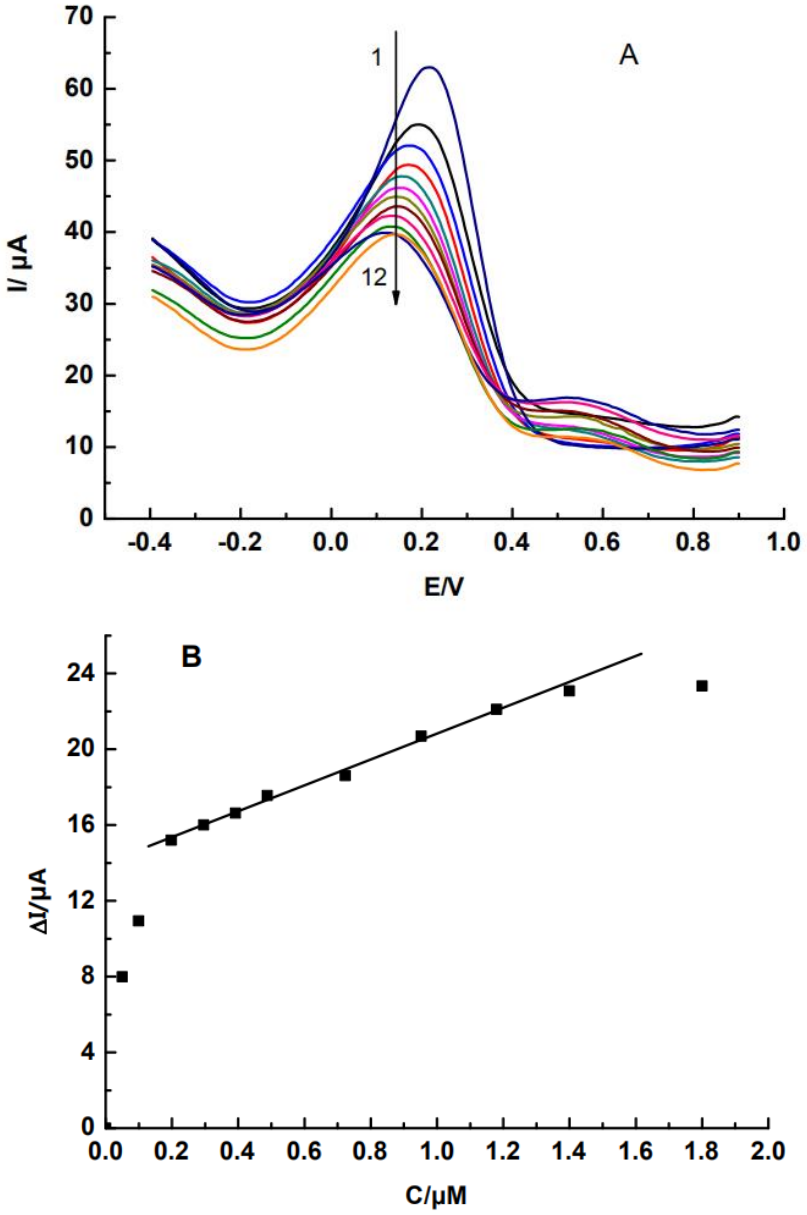


Figure 4. The SWVs of the MIP/GCE with different concentrations of sulfamethoxazole(A), from 1-12: 0; 0.05; 0.15; 0.2; 0.3; 0.4; 0.5; 0.72; 0.95; 1.2; 1.4; 1.8 uM, and the plot of peak current difference(ΔIp) with corresponding sulfamethoxazole concentration(B).

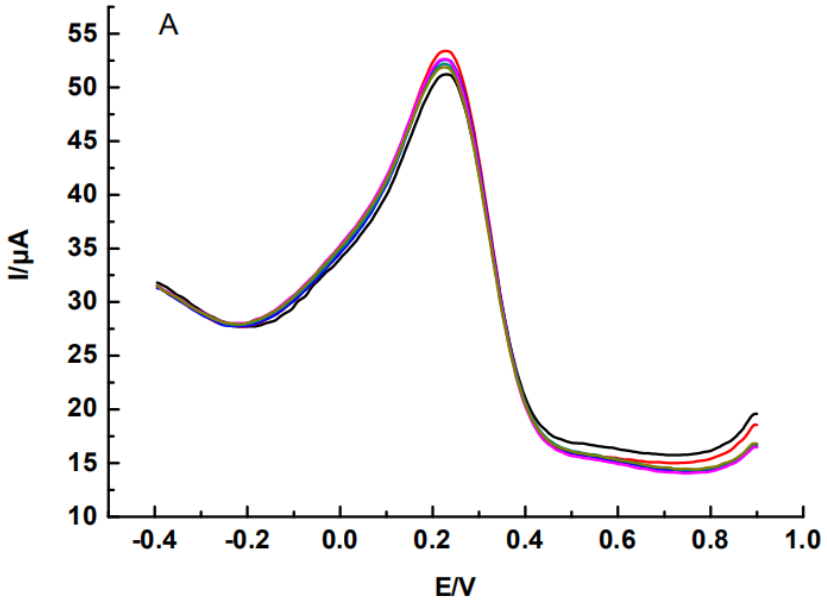
The sulfamethoxazole determination performance was compared with other similar methods/sensors. The comparative results are summarized in Table 1. As can be seen, the proposed MIP sensor has a low detection limit, which makes it suitable for the determination of sulfamethoxazole with low concentration level.

Table 1. The sulfamethoxazole determination performance comparison with other methods.

Modified electrode	Linear range (μM)	LOD(μM)	References
Boron-doped diamond (BDD)	6.1- 60.1	1.1	[8]
MWCNT/GCE	1.4-118.4	0.4	[9]
GCE	55-395	8.5	[10]
MWCNT-MIP /GCE	4-50	0.68	[23]
Hydrogen-terminated BDD	3.9-31.6	0.065	[29]
o-phenylenediamine MIP	0.2-1.4	0.05	this work

3.5. The repeatability and stability of MIP/GCE

The repeatability and stability of MIP/GCE were studied. Fig. 5 showed the SWVs and peak current of sulfamethoxazole detection in parallel for six times. It can be seen from the figure that the peak currents of six detection are 50 to 55 uA, and the relative standard deviation of the results is 1.43%, which indicating the good repeatability of MIP/GCE.



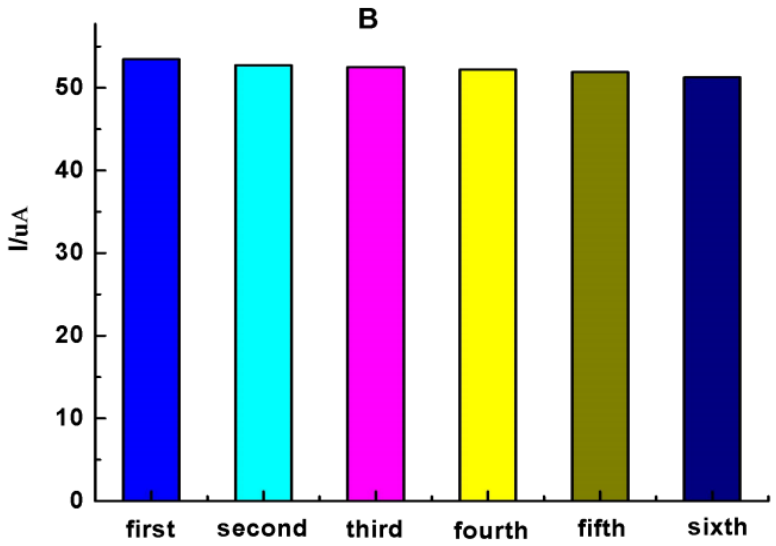


Figure 5. The SWVs (A) and peak currents plot(B) of sulfamethoxazole detection in parallel for six times.

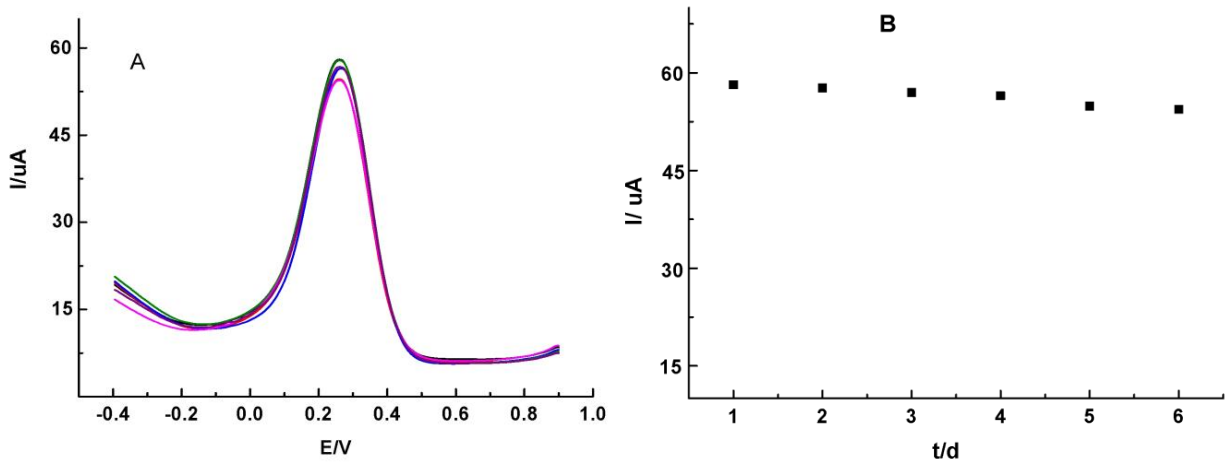


Figure 6. The SWVs (A) and peak currents plot(B) of continuous tests for six days.

The MIP/GCE electrode was stored in distilled water at room temperature and the SWV was measured every day under the same conditions. Six curves were obtained after continuous tests for six days, the SWVs and the peak currents were shown in Fig. 6. As can be seen, there was little decrease within six days. It is about 93.8% of current response on the 6th day, which suggesting a good stability of MIP/GCE.

3.6. The selectivity of MIP/GCE

The selectivity of MIP/GCE was investigated. For the 30 fold concentration of ascorbic acid, aminophenol, urea, or 100 fold concentration of K^+, Na^+, NH_4^+, Cl^-, NO_3^-, SO_4^{2-} did not affect the

detection of sulfamethoxazole. Therefore, the MIP/GCE sensor has good selectivity for the detection of sulfamethoxazole.

3.7 Real sample analysis

To evaluate the applicability of the proposed sensor to real samples, it was used to the determination of sulfamethoxazole in the lake water. After electrochemical measurements by using the proposed sensor, no sulfamethoxazole was detected in the water samples. Then the standard addition method was applied to evaluate the recovery. The data were shown in Table 2. The good recovery indicating that the proposed sensor was reliable for the determination of sulfamethoxazole in real samples.

Table 2. Determination of sulfamethoxazole in real samples.

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Lake water 1	0	Not detected	-	-
	0.60	0.63	105	3.7
Lake water 2	0	Not detected	-	-
	1.20	1.15	95.8	4.1

4. CONCLUSION

In this work, molecularly imprinted sensor based on o-phenylenediamine for electrochemical detection of sulfamethoxazole was prepared by electropolymerization. Sulfamethoxazole template molecules were eluted with 0.1 mol/L NaOH solution. The proposed molecularly imprinted sensor has low detection limit, good selectivity, repeatability and stability.

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