Journal of New Materials for Electrochemical Systems 21, 077-080 (2018)

Short Communication

Molecularly Imprinted Electrochemical Sensor for the Determination of Sulfamethoxazole

Benzhi Liu[1,3,*], Guangqing Liu[2], Bo Xiao[1] and Jinlong Yan[1,†]

[1]School of Environmental Science and Engineering, Yancheng Institute of Technology, Yancheng, Jiangsu Province, China, Postcode: 224051
[2]School of Environmental Science, Nanjing Xiaozhuang University, Nanjing, Jiangsu Province, China, Postcode: 211171
[3]School of Environment and Safety, Jiangsu University, Zhenjiang, Jiangsu Province, China, Postcode: 212013

Received: December 11, 2017, Accepted: February 28, 2018, Available online: April 23, 2018

Abstract: *A selective and sensitive molecularly imprinted electrochemical sensor was prepared based on the electropolymerization of methylthionine chloride on the multi-walled carbon nanotubes modified glassy carbon electrode. The proposed sensor was applied to the determination of sulfamethoxazole which showed a linear range of 2.4 to 23.6 μM, and a detection limit of 0.81 μM.The determination of sulfamethoxazole in real samples was also studied.*

Keywords: Molecularly imprinted polymers; Sulfamethoxazole; Carbon nanotubes

1. INTRODUCTION

Sulfamethoxazoleis an artificially synthesized ammoniabenzene derivative. It is one of the most frequently useddrugs in the sulfonamide familyfor preventing bacterial infections in veterinary and humantreatments. However, as a result of its abuse, there are a large number residues of drug or its metabolites,which cause severe food safety and environmental problems. Thus, a highly reliable analytical method is necessary to monitor the residues ofsulfamethoxazole in foodstuffs. For the determination of sulfamethoxazole, liquid chromatography [1] and capillary electrophoresis [2] are the most employed methods. However, the above methods are laborious, time consuming and expensive.A good alternative for this purpose is the electrochemical methods , which have the advantages of fast response, low cost, good selectivity in trace analysis [3–5].

Molecularly imprinted polymers (MIP) based electrochemical sensors have received great attention due to their high selectivity and sensitivity [6, 7]. MIP can not only accumulate template molecules on the electrode surface to enhance the sensitivity,but also separate template molecules from the other analytes to improve the selectivity. However, traditional MIP have many limitations of incomplete template removal,low-affinity binding and slow mass transfer. Prepreation of MIP on the surface of nanomaterials could enables the template-imprintingsites and provide the advantages of favorable selectivity andfast association/dissociation kinetics. Carbon nanotubes, owing to theunique structures, high stabilities, and large surface area, are extremely attractive in the field of electrochemical sensors because they increase the surface areas of the electrodes, enhance conductivity and facilitate the electron transfers [8, 9]. They have also been used in the preparation of MIP[10].

In this work, carbon nanotubes were immoblized on the electrode surface, the MIP could be prepared on the surface of carbon nanotubes. For the preparation of MIP, electropolymerization is a simple method which can directly prepare rigid,uniform, and compact MIP film on the electrode surface [11]. Methylthionine chloride is a derivative of thionine. Thionine can undergo electrochemical polymerization in an aqueous solution to produce a stable polymer[12]. Herein, methylthionine chloride was electropolymerized on the surface of carbon nanotubes to prepare MIP. The prepared MIP was used for the electrochemical determination of sulfamethoxazole. It showed wide linear range, low detection limit, high selectivity and sensitivity for the detection of sulfamethoxazole.

To whom correspondence should be addressed:
Email: *benzhiliu@163.com, †yjlyt4788@126.com
Tel:. +86 0515-88298806; Fax:. +86 0515-88298805

2. EXPERIMENTAL

2.1. Reagents and instrumentation

Multi-walled carbon nanotubes (MWCNT) were obtained from Shenzhen Nanotech Port Co., Ltd (Shenzhen, China) with a typical diameter of 10–20 nm. Their purity was more than 98%. Sulfamethoxazole and methylthionine chloride were purchased from Aladdin Industrial Corporation(Shanghai, China). All other chemical reagents were obtained from Nanjing Chemical Reagent Co. (Nanjing China).

All electrochemical experiments were carried out on a CS350 Electrochemical Workstation (Wuhan Corrtest Instruments CO., LTD, Wuhan, China). A conventional three-electrode cell configuration was employed for the electrochemical measurements. A modified glassy carbon electrode (disc diameter of 3 mm) was used as the working electrode. The saturated calomel electrode (Saturated KCl) and platinum wire were employed as the reference and the counter electrode, respectively.

2.2. fabrication of the modified electrode

Prior to use, 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. MWCNT was dispersed in acetone with the aid of ultrasonic agitation to prepare 2 mg ml^{-1} MWCNT suspension. Then 10 μl of the suspension were dropped onto the clean GCE surface and the solvent was evaporated in air to prepare MWCNT/GCE.

For the preparationof MIP/MWCNT/GCE, the MWCNT/GCE was immersed in a 0.2 M Na_2SO_4 solution(pH=2) containing 2.0 mM sulfamethoxazoleand 4.0 mM N-methylthionine for 15 minto pre-assemble between template and monomer. The electropolymerization was carried out using the cyclic voltammetry (CV) method at a scan rate of 50 mVs^{-1} between −0.2 and +1.2 V for 20 cycles. Then, the embedded sulfamethoxazole was removed by scanning between -0.2 and +0.6 V in a 0.2 M Na_2SO_4solution for 6 cycles. Finally,the modified electrode was washed with water and dried for further use.

2.3. Experimental measurements

The cyclic voltammograms(CV)were carried out in a 1.0 mM $K_3[Fe(CN)_6]$ solution containing 0.2 M KNO_3, and the scanning potential range was from +0.7 V to −0.4 V at a scan rate of 20 mV s^{-1}.The square wave voltammetric measurements were performed in the scanning rangefrom +0.7 V to -0.4 V with astep increment of 4 mV, amplitude of 100 mV and frequency of 15 Hz. Prior to electrochemical measurement, the tested electrode was immersed in 0.2 M KNO_3 containing sulfamethoxazole for 9 min under stirring. Aftereach measurement, the template-entrapped electrode wasscanned between -0.2 V and+0.6 V in a 0.2 M Na_2SO_4solution for several cycles to remove the sulfamethoxazole molecules, then the electrode was washed with water for reuse.

3. RESULTS AND DISCUSSION

3.1. Characterization of the modified electrodes

Fig. 1 shows the CVof different modified electrodes in a 1.0 mM $K_3[Fe(CN)_6]$solution containing 0.2 M KNO_3. Curve a shows a pair of well-defined redox peaks related to the redox of $K_3[Fe(CN)_6]$ at MWCNT/GCE. Curve b shows the CV of the MIP film before removing the sulfamethoxazole. Almost no redox peak was observed because the electrode surface was covered by the compact polymer film, which blocked the penetration and access of redox probe to the electrode surface.A pair of redox peaks was observed after the removal of sulfamethoxazole (curve c) . It is due to the fact that the removed template enhanced the diffusion of $K_3[Fe(CN)_6]$and promoted the redox reaction of $K_3[Fe(CN)_6]$ on the electrode surface.However, the peaks current were still lower than those of curve a, which was due to the fact that only the cavities in the MIP after template removal acted as channels for electron transport. Curve d shows the CV of the MIP film after incubation with 0.2 mM sulfamethoxazole. The intensity of this redox peaks were lower than those observed in curve c. It is can be explained that the binding of sulfamethoxazole in MIP film resulted in blockage of the cavities, and hence hindered the diffusion of the redox probe.

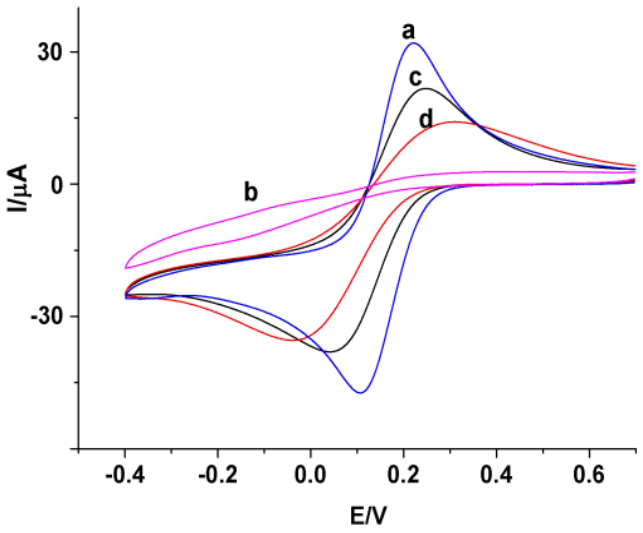

Figure 1. Cyclic voltammograms of 1.0 mM $K_3[Fe(CN)_6]$ at MWCNT/GCE(a),MIP/MWCNT/GCE without removing sulfamethoxazole(b), MIP/MWCNT/GCE after removing sulfamethoxazole(c),and MIP/MWCNT/GCE after incubation with sulfamethoxazole (d).

3.2. Optimization of experimental conditions

The important experimental parameters including scan cycles, mole ratio of template molecules to functional monomers, and incubation time could affect the performance of the MIP sensor.

The thickness of the MIP film would affectthe sensitivity of the MIP sensor. In this work,the thickness of the MIP film can be controlled by the scan cycles during electropolymerization. As can be seen from Fig. 2A, the peak current reduction(ΔIp)increases asthe the scan cycles increases, and the ΔIp reaches the maximum value at 20 cycles. The ΔIp decreased when the scan cycles exceeded 20 cycles, indicating that the film was too thick to completely remove the template molecules. Therefore, 20 cycles could obtain a suitable film thickness to offer the highest sensitivity of the MIP sensor. The molar ratios of template molecules to functional monomers could affect the amount of template molecules embedded in

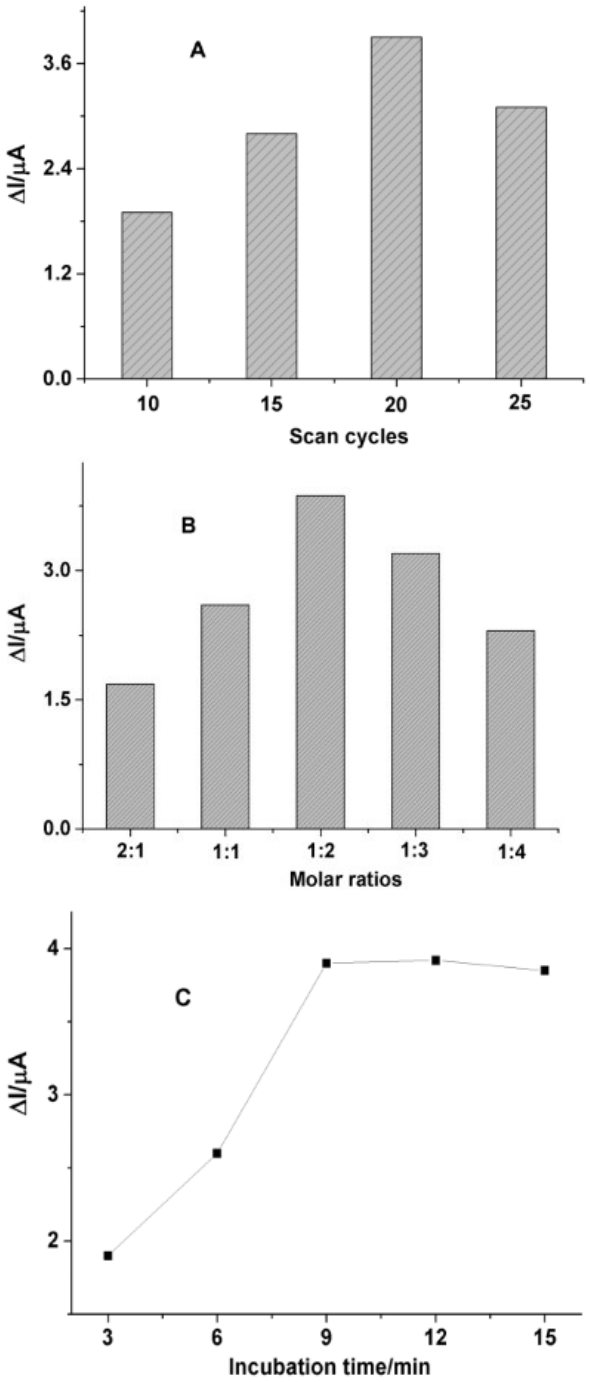

Figure 2. The effect of the scan cycles(A), the mole ratio of template molecules to functional monomers(B), and incubation time(C) on the ΔIp of MIP sensor.

the polymer matrix. The MIP sensors prepared with different molar ratios of sulfamethoxazole to N-methylthionine(2:1, 1:1, 1:2, 1:3 and 1:4) were tested for their current response under the same conditions.As shown in Fig. 2B, a maximum value of ΔIp was achieved at 1:2molar ratio of sulfamethoxazole to N-

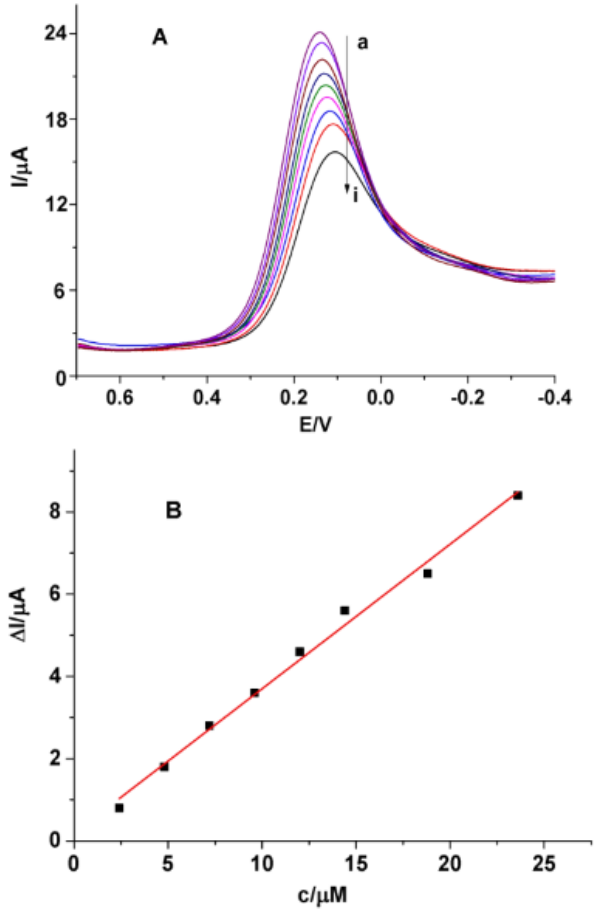

Figure 3. (A) SWVs of the MIP sensor with different concentrations of sulfamethoxazole. From a-g: 0; 2.4; 4.8; 7.2; 9.6; 12; 14.4; 18.8; 23.6 μM. (B) Plot of the ΔIpversus concentration of sulfamethoxazole.

methylthionine. Thus, a sulfamethoxazole to N-methylthionine molar ratio 1:2 was selected and adopted in this work. The effect of incubation time was investigated in the range of 3- 15 min. As shown in Fig. 2C, the ΔIp increases with increasing incubation time and achieved balance at 9 min, indicating that adsorption equilibrium was achieved. Therefore, the incubation time of 9 min was applied in the subsequent measurements.

3.3. Determination of sulfamethoxazole

Square wave voltammetry (SWV) was used for the determination of sulfamethoxazole. Fig. 3A shows SWVs of the MIP sensor with different concentrations of sulfamethoxazole. The peak currents decreased with increasingamount of sulfamethoxazole. The results suggested that imprinted cavities were gradually filled, and mass-transfer diffusion was blocked with increasing template molecules. As shown in Fig. 3B, the ΔIp are proportional to the concentrations of sulfamethoxazole in the range from2.4 to 23.6 μM, with a detection limit of 0.81 μM. The linear regression equation can be expressed as ΔIp (μA) = 0.18 + 0.35c (μM), with a correlation coefficient r = 0.9916.

In addition, the comparative results between the proposed MIP sensor and other previously reported methods for sulfamethoxazole determination are summarized in Table 1. As can be seen, the proposed MIP sensor has a wide linear range and a low detection limit, which makes it suitable for the determination of sulfamethoxazole.

3.4. Reproducibility and stability

To investigate the reproducibility of the MIP sensor, a series of four MIP sensors were prepared in the same manner. They were tested for the determination of 7 μM sulfamethoxazole and the RSD was 3.5%. The stability of the MIP sensor was also studied.The peak current response retained 94.8% of its initial response after it was stored at room temperature for three weeks. The good reproducibility andstability of the proposed MIP sensors make them attractive for fabricating electrochemical sensors.

3.5. Selectivity of the MIP sensor

The selectivity of the MIP sensor was evaluated by investigatingits effecton the detection of 7 μM sulfamethoxazole. The results indicated that the concentration of 10 times of trimethoprim, 50 times of ascorbic acid, dopamine, uric acid, or 200 times of NH_4^+, K^+, Na^+, SO_4^{2-} did not affect the determination of sulfamethoxazole. Thus, the performed MIP sensor showed satisfactory analytical selectivity in the determination of sulfamethoxazole.

3.6. Real sample analysis

To evaluate the applicability of the proposed MIP sensor to real samples,it was applied to the determination of sulfamethoxazole in the milk and milk powder. Themilk and milk powder were pretreated with the following procedure:8 mL milk (or 2 g milk powder) was first mixed with 6 ml of 0.5 mol L^{-1} trichloroaceticacid and 30 ml of methanol. After 10 min shaking and 20 min sonication,the mixture was centrifugedfor 15 min, and the supernatant was filtered through a 0.45 μm filter membrane. The filtrate was then condensed to give a total volume of 10 ml for detection. No sulfamethoxazole was detected in the real samples. Then the standard addition method was adopted to detect sulfamethoxazole in real samples. The data were shown in Table 2. The obtained results indicating that the present MIP sensor was reliable for the determination of sulfamethoxazole in real samples.

4. CONCLUSION

In this work, we presented a molecularly imprinted electrochemical sensor for sulfamethoxazole detection. The detection range was from 2.4 to 23.6 μM and the detection limit was 0.11 μM . The proposed sensor was applied for the detection of sulfamethoxazole in milk products with good recovery.

Table 1. The determination performance comparison with other methods.

Modified electrode	Linear range (μM)	LOD(μM)	References
Boron-doped diamond (BDD)	6.1- 60.1	1.1	[13]
Hydrogen-terminated BDD	3.9-31.6	0.065	[14]
MWCNT/GCE	1.4-118.4	0.4	[15]
GCE	55-395	8.5	[16]
MIP/MWCNT/GCE	2.4-23.6	0.81	this work

5. ACKNOWLEDGMENTS

This work was sponsored by the University Natural Science Research Project of Jiangsu Province of China (16KJB550007), Postdoctoral Foundation of Jiangsu Province (1701105B), and the National Natural Science Foundation of China(21677119) .

REFERENCES

[1] Rossmann J., Schubert S., Gurke R., Oertel R., Kirch W., J. Chromatogr. B, 969, 162 (2014).
[2] Sun H., Qi H., Li H., Food Anal. Methods, 6, 1049 (2013).
[3] Ganjali M.R., Larijani B., Pourbasheer E., Int. J. Electrochem.Sci., 11, 2119 (2016).
[4] Alizadeh T., Ganjali M.R., Akhoundian M., Norouzi P., Microchim. Acta, 183, 1123 (2016).
[5] Sanghavi B.J., Moore J.A., Chávez J.L., Hagen J.A., Kelley-Loughnane N., Chou C.F., Swami N.S., Biotechnol. Bioeng., 78, 44 (2016).
[6] Reddy S.M., Sette G., Phan Q., Electrochim. Acta, 56, 9203 (2011).
[7] Zhong M., Teng Y., Pang S., Yan L., Kan X., Biosens. Bioelectron, 64, 212 (2015).
[8] Benvidi A., Kakoolaki P., Zare H.R., Vafazadeh R., Electrochim. Acta, 56, 2045 (2011).
[9] Kachoosangi R.T., Musameh M.M., Abu-Yousef I., Kanan J.M., Xiao L., Davies S.G., Russell A., Compton R.G., Anal. Chem., 81, 435 (2009).
[10] Zhang H.B., Zhang Z.H., Hu Y.F., Yang X., Yao S.Z., J. Agri. Food Chem., 59, 1063 (2011).
[11]Sharma P.S., Pietrzyk-Le A., Souza F.D., Kutner W., Anal. Bioanal. Chem., 402, 3177 (2012).
[12]Topcu E., Alanyalioglu M., J. Appl. Polym. Sci., 131, 39686 (2014).
[13]Souza C.D., Braga O.C., VieiraI.C., Spinelli A., Sens. Actuators B: Chem., 135, 66 (2008).
[14]Santos Andrade L., Cardozo Rocha-Filho R., Bezerra Cass Q., FatibelloFilho O., Anal. Methods, 2, 402 (2010).
[15]Arvand M., Ansari R., Heydari L., Mater. Sci. Eng.: C, 31, 1819 (2011).
[16]Calaca G.N., Pessoa C.A., Wohnrath K., Nagata N., Int. J. Pharm. Pharm. Sci., 6, 438 (2014).

Table 2. Determination of sulfamethoxazole in real samples

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Milk	0	Not detected	-	-
	12	11.39	94.92	4.1
Milk powder	0	Not detected	-	-
	12	11.53	96.08	3.6