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Short Communication

Voltammetric Determination of Sulfadiazine Based on Molecular Imprinted Electrochemical Sensor

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Voltammetric determination of sulfadiazine based on molecular imprinted electrochemical sensor is described in this work. The proposed sensor is based on glassy carbon electrode modified with sulfadiazine imprinted polymers. Cyclic voltammetry and differential pulse voltammetry technique were carried out to investigate the electrochemical behavior of sulfadiazine at the modified electrodes. Under the optimal condition, the proposed sensor exhibited a well linear relationship toward the sulfadiazine concentrations in the range of 4 to 50 μM, with a detection limit of 0.68 μM. The proposed sensor was successfully applied for the determination of sulfadiazine in real samples with satisfactory recovery.

Keywords: Molecularly imprinted polymers，Electrochemical sensor，Sulfadiazine

1. INTRODUCTION

Sulfonamides belong to synthetic antibacterial compounds. They were widely used as antibacterial agents in both human and veterinary medicine due to their low cost and high efficiency in the treatment of bacterial diseases [1]. However, sulfonamide residues in treatment of animals may cause allergic or toxic reaction to consumer of animal products. Thus, the European Union Council Regulation sets a safe limit that the combined total residues of all sulfonamide based drugs should not exceed 100 ng/g in animal origin foodstuffs [2]. The maximum sulfadiazine residue limit tolerated by international regulations for milk is 0.07 ppm [3,4].

Therefore, developing the detection method for sulfonamide in animal origin foodstuffs is of great significance. Many methods have been reported for the determination of sulfadiazine, including

spectrophotometry[5], different chromatographic methods[6,7], capillary electrophoresis [8] and biosensing[9]. Electroanalytical techniques have advantages such as simplicity, portability, and sensitivity which make them very attractive for detection of pharmaceutical compounds [10,11].

Molecularly imprinted polymers have high selectivity, strong anti-interference and high stability, which have been widely used for the fabrication of electrochemical sensors[12]. Carbon nanotubes have been used as the carrier in the preparation of imprinted polymers due to their unique properties, such as high stability, large surface area, high conductivity and facilitate the electron transfers [13, 14].

In this work, the multi-walled carbon nanotubes were first acrylamide-functionalized and as a carrier to synthesize molecularly imprinted polymers. The acrylamide on the surface of carbon nanotubes can induce the polymerization to selectively occur on the surface of carbon nanotubes, which could increase the number and density of the imprinting cavities. The proposed molecularly imprinted electrochemical sensor was successfully applied for the determination of sulfadiazine in real samples.

2. EXPERIMENTAL

2.1 Reagents and instrumentation

Multi-walled carbon nanotubes (MWCNT) were obtained from Shenzhen Nanotech Port Co., Ltd (Shenzhen, China) with a diameter about 20 nm. The purity was more than 97%. Sulfadiazine, methacrylic acid (MAA), N, N- dimethformamide (DMF) were obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. Acrylamide (AA), ethyleneglycol dimethacrylate (EGDMA), azodiisobutyronitrile (AIBN) were purchased from Aladdin reagent (Shanghai) Co., Ltd. All other chemical reagents were purchased from Nanjing Chemical Reagent Co. (Nanjing China). All solutions were prepared with double-distilled water.

Electrochemical experiments were performed on a CS350 Electrochemical Workstation (Wuhan Corrtest Instruments CO., LTD, Wuhan, China). Three-electrode system was used for the electrochemical measurements. MIP or NIP modified glassy carbon electrode was used as the working electrode. The saturated calomel electrode and platinum wire electrode were used as the reference and the counter electrode, respectively.

2.2 Modification of MWCNT

The modification of MWCNT was prepared according to our previous report[15]. Namely, 0.8 g MWCNT was added to the mixed acid (V (HNO_3): V (H_2SO_4) = 1: 3) . After ultrasonication for 10 min, the mixture was refluxed 6 h at 75℃, then diluted with distilled water and filtered. The filter cake is repeatedly washed with distilled water to neutral, and at last drying at 70℃ for 24 h to get MWCNT-COOH.

0.5 g MWCNT-COOH was added to the mixed solution of 40 mL $SOCl_2$ and 2 mL DMF , the mixture was refluxed at 70℃ for 24h. After the reaction was completed, the unreacted $SOCl_2$ was distilled out. Then, 40 mL DMF and 2 g acrylamide were added, after ultrasonication for 10 min, the

mixture was refluxed at 60℃ for 12h. After filtration, the filter cake was washed with ethanol and distilled water, and the product was dried at 80℃ to obtain MWCNT-AA.

2.3 Preparation of molecularly imprinted polymers

Fig.1 illustrated the preparation of molecularly imprinted polymers. The procedure is according to our previous report[15] with minor modification. 0.15 g MWCNT-AA, 0.35 g sulfadiazine and 0.3 g MAA were added to 30 mL acetonitrile, after ultrasonication for 10 min and stirring for 2h, 3.5 g EGDMA and 20 mg AIBN were added to the stirring mixture, then bubbled with N_2 to removed O_2.

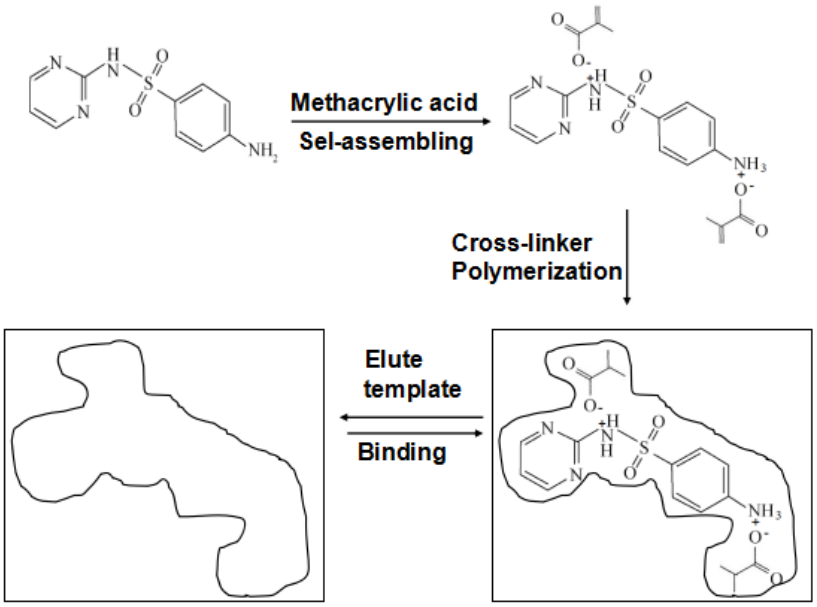

Figure 1. Preparation of molecularly imprinted polymers.

The mixture was stirred for 24 h at 60℃, then the product was cooled to room temperature and collected after centrifugation. Then, acetic acid/ethanol solution (V (acetic): V (ethanol) = 1: 9) was used to elute the template molecules and unreacted functional monomers and crosslinker. Distilled water was used to wash the acetic acid and ethanol. The product was collected and dried at 60℃, thus, the imprinted polymer (MWCNT-MIP) was obtained. The preparation of non-imprinted polymer (MWCNT-NIP) was similar to the preparation of imprinted polymers except sulfadiazine was added.

2.4 Electrode fabrication

Prior to use, the glassy carbon electrode(GCE) was carefully polished with a leather containing 0.05 μm Al_2O_3 slurry, then it was cleaned in ethanol and distilled water. MWCNT-MIP or MWCNT-NIP was dispersed in acetone with the aid of ultrasonication to prepare 3 mg ml^{-1} suspension. Then 10 μl of the suspension was dropped onto the GCE surface and dried in air to prepare MWCNT-MIP /GCE or

MWCNT-NIP /GCE .

2.5 Experimental measurements

The cyclic voltammograms(CV) were performed in a 2.0 mM $K_3[Fe(CN)_6]$ solution containing 0.1 M KCl, the scanning potential range was -0.3 V to +0.6 V and the scan rate was 100 mV s^{-1}. Differential pulse voltammograms (DPVs) were carried out from -0.1 to +0.7 V (step increment of 5 mV, amplitude of 50 mV, pulse period of 0.1 s).

3. RESULTS AND DISCUSSION

3.1 Cyclic voltammograms of the modified electrodes

Fig. 2 shows the cyclic voltammograms of different modified electrodes in 2.0 mM $K_3[Fe(CN)_6]$ solution containing 0.1 M KCl. As can be seen, a pair of redox peaks were observed in curve a, which related to the redox of $K_3[Fe(CN)_6]$. It suggested that the cavities in the MIP after template removal acted as channels for electron transport. Curve b shows the CV of MWCNT-MIP /GCE after incubation with 0.15 mM sulfadiazine.

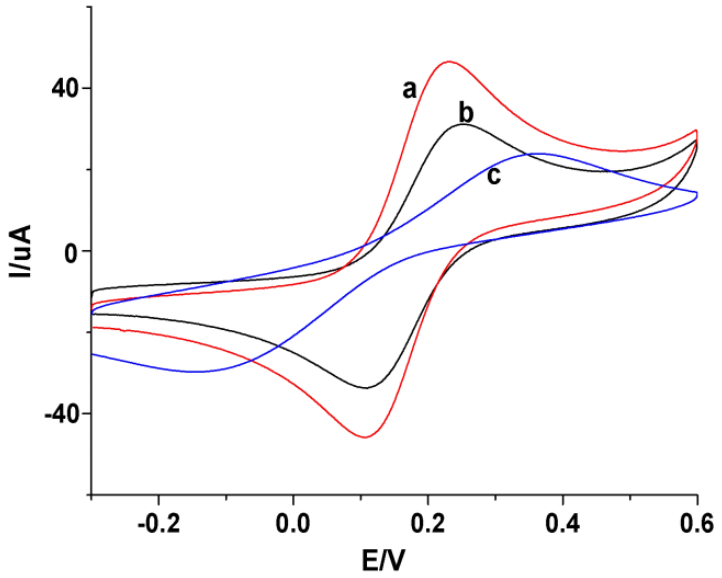

Figure 2. Cyclic voltammograms of 2.0 mM $K_3[Fe(CN)_6]$ at MWCNT-MIP/GCE(a), MWCNT-MIP/GCE after accumulation with sulfadiazine (b), and MWCNT-NIP/GCE (c).

The peak current of the redox peaks were lower than those observed in curve a, which indicated that the binding of sulfadiazine in MIP film resulted in blockage of the cavities, and hence hindered the diffusion of $K_3[Fe(CN)_6]$. However, for the CV of MWCNT-NIP /GCE(curve c), the peaks became very poor and peak current very low, it was because that the polymer film had lower electron transfer rate and

electrical conductivity and limited the diffusion of $K_3[Fe(CN)_6]$ to the electrode surface.

3.2 Optimization of experimental conditions

The MIP amount modified on the surface of GCE could affect the sensitivity of the MWCNT-MIP /GCE. The effect of MIP amount was investigated in the range of 3-15 μL. The results showed that the peak current difference(ΔIp) increases with increasing the MIP amount, then the ΔIp almost keep constant when the MIP amount exceed 10 μL . Therefore, the MIP amount of 10 μL could be suitable to offer the high sensitivity.

Accumulation time is an important factor in the molecularly imprinted electrochemical sensors. The effect of accumulation time was studied. The results showed thatthe ΔIp increases with increasing accumulation time and achieved balance at about 10 min, indicating that the adsorption equilibrium was achieved. Therefore, the accumulation time of 10 min was applied in the following measurements.

3.3 Determination of sulfadiazine

Fig. 3 shows the DPVs of the MWCNT-MIP /GCE at different concentrations of sulfadiazine. As can be seen from the curves in the figure, the peak currents decreased with increasing the concentration of sulfadiazine.

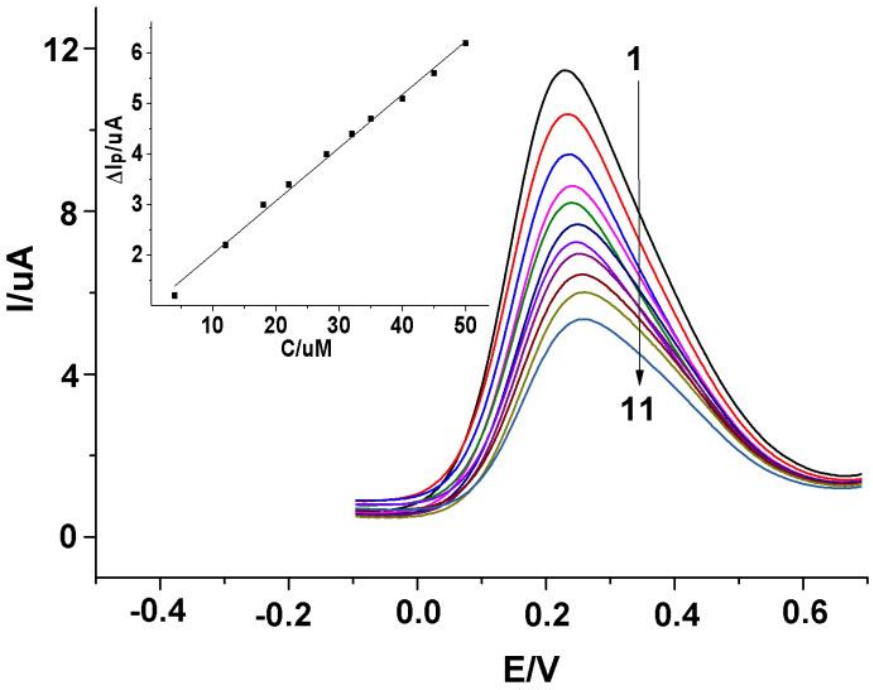

Figure 3. DPVs of the MWCNT-MIP/GCE with different concentrations of sulfadiazine. From 1-11: 0; 4; 12; 18; 22; 28; 32; 35; 40; 45; 50μM. Inset: Plot of the *Δ*Ip *vs* concentration of sulfadiazine.

Fig. 3 inset shows that the *Δ*Ip are linearly to the concentrations of sulfadiazine in the range of 4

to 50 μM, with a detection limit of 0.68 μM. The linear regression equation can be expressed as ΔIp (μA) = 0.967 + 0.105c (μM), with a correlation coefficient r = 0.9948.

Table 1. The determination performance comparison with other sensors for sulfadiazine determination.

Modified electrode	Linear range (μM)	LOD(μM)	References
Hg	2-32	4.9	[1]
GCE	15-60	5.4	[16]
Boron-doped diamond	8-119	2.2	[17]
Bismuth-film electrode	3.2-20	2.1	[18]
Poly-methylthiophene/GCE	20-3200	4.0	[19]
Polypyrrole modified pencil graphite electrode	10-1000	1.0	[20]
GCE	63-340	10.9	[21]
MWCNT-MIP /GCE	4-50	0.68	this work

Table 1 shows a performance comparison of the MWCNT-MIP /GCE with other reported sensors for sulfadiazine detection. It was found that the GCE had relatively high detection limit. The modified electrode could effectively decrease the detection limit. From the comparison, it can be seen that the proposed sensor in our work had high sensitivity, wide linear range, and low detection limit.

3.4 Selectivity of the MWCNT-MIP /GCE

The selectivity of the MWCNT-MIP /GCE was evaluated. For the determination of 8 μM sulfadiazine, the concentration of 20 times of ascorbic acid, uric acid, glucose and 300 times of Ca^{2+}, Mg^{2+}, Na^{+}, SO_4^{2-} and NO_3^{-} did not affect the determination of sulfadiazine. The above results suggested that the MWCNT-MIP /GCE has good selectivity for the determination of sulfadiazine.

3.5 Real sample analysis

To evaluate the applicability of the MWCNT-MIP /GCE to real samples, it was used to the determination of sulfadiazine in the milk and milk powder. The milk and milk powder were pretreated with the procedure according to our previous report[22]. After electrochemical measurements by using the proposed sensor, no sulfadiazine was detected in the real samples. Then, the standard addition method was applied to detect sulfadiazine in real samples.

Table 2. Determination of sulfadiazine in real samples.

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Milk	0	Not detected	-	-
	15	14.3	95.3	3.9
Milk powder	0	Not detected	-	-
	15	14.1	94.0	4.6

As shown in Table 2, the results indicating that the proposed sensor was reliable for the determination of sulfadiazine in real samples.

4. CONCLUSION

In this work, a molecularly imprinted electrochemical sensor for the determination of sulfadiazine was presented, the detection range was 4 to 50 μM with a detection limit of 0.68 μM. The proposed sensor was applied for the detection of sulfadiazine in the milk and milk powder with good recoveries.

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