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A novel sensor based on bifunctional monomers molecularly imprinted film at graphene modified glassy carbon electrode for detecting trace of moxifloxacin

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Abstract

A novel selective and sensitive electrochemical sensor for moxifloxacin (MFLX) detection based on bifunctional monomers molecularly imprinted polymer (MIP) membranes on a glassy carbon electrode (GCE) modified with graphene was constructed. A suspension of graphene was deposited on the GCE surface. Subsequently, a molecularly imprinted film was prepared by electropolymerization, via cyclic voltammetry of o-phenylenediamine and L-lysine as the functional monomers in the presence of MFLX as the template molecule. A control electrode (NIP) was also prepared. The electrochemical properties of the MIP and non-molecularly imprinted polymer (NIP) sensors were investigated via cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), in which $[Fe(CN)_6]^{3-/4-}$ was used as an electrochemical active probe. The surface morphology of the imprinted film was characterized by scanning electron microscopy (SEM). The fabrication conditions that affect the performance of the imprinted sensor have been discussed. Under the optimal experimental conditions, the imprinted sensor had good linear current responses to moxifloxacin concentrations in the ranges from 1.0×10^{-9} to 1.0×10^{-8} M and 1.0×10^{-8} to 5.0×10^{-5} M, with a detection limit of 5.12×10^{-10} M ($S/N = 3$). The developed sensor was successfully applied to detect moxifloxacin in tablets and human urine samples. Moreover, the fabricated sensor possessed a good selectivity and stability, providing a promising tool for immunoassays and clinical applications.

Keywords: molecularly imprinted polymer; o-phenylenediamine; L-lysine; graphene; moxifloxacin

1. Introduction

Moxifloxacin (MFLX) is a fourth-generation fluoroquinolone antibacterial agent active against a broad spectrum of Gram-positive and Gram-negative ocular pathogens, a typical microorganisms and anaerobes.[1] It is mainly applied in the treatment of acute bacterial sinusitis caused by sensitive microbes, acute bacterial chronic bronchitis, mild to moderate community intravenous pneumonia, and skin and soft tissue infection without complications.[2,3] Moxifloxacin has been detected by various methods, such as spectrophotometry,[4,5] spectrofluorimetry,[6] atomic absorption spectrometry,[7] high performance liquid chromatography (HPLC),[8-11] capillary electrophoresis (CE)[12] and electrochemical methods.[13-15] Electrochemical sensors, as one of the electrochemical methods, are reported as ecofriendly and considered as highly sensitive, selective and convenient tool with fast response and low cost as compared to the other routine analytical techniques. Various electrochemical sensors have been used for the moxifloxacin determination.[16-18] However, the presence of higher concentration of some structurally related analogues such as gatifloxacin, ciprofloxacin, ofloxacin and norfloxacin strongly interfere in the selective determination of moxifloxacin in biological samples. Thus, the aim of this study was to prepare a sensor for the selective and sensitive determination of moxifloxacin in human biological fluids.

As a typical approach for high affinity and specific recognition, molecularly imprinted polymers (MIPs) have gained a considerable attention in the recent years and have been found most promising in the field of electrochemical sensors.[19,20] The integration of electrochemical devices and MIPs, which demonstrates good sensitivity and selectivity, is an attractive approach for the development of biochemical sensors.[21-23] As most MIPs commonly were prepared with strategies such as bulk polymerization, precipitation polymerization and sol–gels often have some limitations including slow mass transfer, incomplete template removal and heterogeneous distribution of binding sites.[24-26] So, the approach of electropolymerization for the proper design of the MIP-modified electrode is one of the efficient ways to solve these limitations by generating a rigid, uniform and compact molecularly imprinted film with controlled thickness.[27,28] For the construction of a molecularly imprinted polymer (MIP)-based sensor by an electropolymerization technique, the choice of functional monomer is important. The electropolymerization of o-phenylenediamine (OPD) has been widely used for the preparation of molecularly imprinted electrochemical sensors,[29,30] due to its excellent biocompatibility and the feasibility of immobilising different compounds. L-lysine is an essential α-amino acid with basic properties. L-lysine modified electrodes have the advantages of stability and positive surfaces,[31,32] which could provide fast electron transfer. At the same time, those charged molecules are more easily adsorbed on the surface of the sensor. To further increase the amount of effective binding sites in the sensor, an attempt to use both of these monomers to form MIP was made.

Although MIPs are excellent in improving selectivity, sensitivity is also a fundamentally important feature of an electrochemical sensor. In some cases, MIPs resulted in a reduced sensitivity. So, materials such as multi-walled carbon nanotubes (MWCNTs),[33,34] metallic nanomaterials[35,36] and, more recently, graphene,[37,38] have been used as a substrate layer in MIPs preparation. Among these materials, graphene are considered an ideal supporting material because they promote electron transfer reactions due to their significant mechanical

strength, high electrical conductivity, high surface area and good chemical stability.

Herein, for the first time, we designed a rapid, selective and sensitive sensor based on MIP for the determination of moxifloxacin. The GR as a supporting material, moxifloxacin as template molecule, OPD and L-lysine as the functional monomers have been used to construct the MIP film on the surface of glassy carbon electrode by electropolymerization. After the removal of the embedded template moxifloxacin by extraction with an 50% ethanol (*V/V* = 1:1) solution, the MIP/GR/GCE sensor was finally obtained. The adsorbed moxifloxacin is detected by electrochemical signal of $[Fe(CN)_6]^{3-/4-}$ due to the binding of moxifloxacin blocking electron transfer of $[Fe(CN)_6]^{3-/4-}$ at the electrode surface. The electrochemical signal intensity is related to the concentration of moxifloxacin. The whole preparation procedure is shown in Scheme 1. The sensor could recognize template molecule from its analogs with a good selectivity and sensitively detect moxifloxacin with a wide linear range and a low detection limit. Meanwhile, the sensor was used to detect moxifloxacin in real samples with satisfactory results.

2. Experimental

2.1. Reagents and apparatus

Moxifloxacin, gatifloxacin, ciprofloxacin, ofloxacin and norfloxacin were purchased from Wuhan Yuancheng Gongchuang Technology Co., Ltd. (China). L-lysine was purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Graphene (1.0 mg/mL) was purchased from XFNANO, INC (Nanjing, China). o-phenylenediamine (OPD), potassium ferricyanide, potassium ferrocyanide and ethanol were purchased from Sinopharm Chemical Reagent Co. Ltd. (China). The phosphate buffer solution (PBS) was prepared by mixing stock solutions of NaH_2PO_4 and Na_2HPO_4 and adjusting the pH values with either 0.10 M HCl or NaOH solutions. Tablets containing moxifloxacin manufactured by Bayer Pharma AG (Germany) were purchased from the local market of Chong Qing. Fresh urine samples obtained from healthy person were supplied by Southwest University Hospital. All other chemicals and solvents used in the experiment were of analytical grade and double distilled water was used throughout the experiments.

Electrochemical experiments including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a LK 2006AZ electrochemical workstation (Tianjin Lanlike Co., Ltd., China), with a conventional three-electrode system including a MIP/GR/GCE as working electrode, a Pt wire counter electrode and a saturated calomel electrode (SCE) reference electrode. All potential values given below were referred to the SCE. The scanning electron micrograph (SEM) measurement was carried out on scanning electron microscope (JSM-6510, Japan). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660D electrochemical workstation (Chenhua Corp. Shanghai, China). A digital pH/mV/Ionmeter (CyberScan model 2500, USA) was used for the preparation of the buffer solution.

2.2. Preparation of the graphene-modified electrode

The bare GCE (3 mm in diameter) was polished with 0.05 μm Al_2O_3 slurry before it was used, and rinsed ultrasonically with 1:1 HNO_3, ethanol and ultrapure water respectively until a mirror-like surface was obtained. The electrode was then washed with ultrapure water and allowed to dry at room temperature before use. Two microliters of the graphene suspension

(1.0 mg/mL) was dropped onto the surface of the GCE and dried in the vacuum oven at 60 °C for 1 h.

2.3. Construction of the MIP/GR/GCE, MIP/GCE and NIP/GR/GCE

The GR/GCE was immersed in 10.0 mL phosphate buffer solution (pH = 5.7) containing 1.0 mM OPD and 1.0 mM L-lysine as the functional monomers, and 0.10 mM of template moxifloxacin and was electrochemically polymerized via cyclic voltammetry (CV) in the potential range of −0.2 to 0.8 V at a scan rate of 50 mV/s for 20 cycles. After the electropolymerization, the polymers modified electrode was incubated into an 50% ethanol (*V/V* = 1:1) solution for 3 min to extract the template moxifloxacin to obtain the MIP/GR/GCE. The procedure for the preparation of the MIP/GR/GCE is depicted in Scheme 1. As a control, none-imprinted polymer sensor (NIP/GR/GCE) and MIP/GCE were prepared by the same procedure but without the addition of moxifloxacin and GR, respectively.

2.4. Electrochemical characterization and measurements

Different modified electrodes were characterized by EIS in a solution of 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) containing 0.10 M KCl using an alternating current voltage of 10 mV and recorded at a bias potential of 200 mV within a frequency range of 10^{-1} to 10^5 Hz.

$[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ was also chosen as an electrochemical active probe to study the performance of the prepared sensor due to the poor electroactivity of moxifloxacin. Imprinted cavities formed in the MIP/GR could provide pathway for the diffusion of probe into and out of the MIP matrix, which then is oxidized or reduced at the electrode and produce an electrochemical signal. The MIP/GR/GCE was immersed into moxifloxacin solution with different concentrations, and incubated for 10 min to ensure moxifloxacin molecule rebound by MIP/GR/GCE. Then, CV and DPV methods were conducted for electrochemical determination of moxifloxacin in 2.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.10 M KCl (pH = 7.0). CV was performed over a potential range from −0.2 to 0.6 V with a scan rate of 100 mV/s. DPV measurement was carried out between −0.2 and 0.6 V, pulse width 50 ms, and an amplitude of 50 mV. All the electrochemical experiments were conducted at room temperature (RT, 25 ± 1 °C).

2.5. Preparation and determination of real samples

A proposed sensor for evaluating the accuracy of the content of moxifloxacin in commercial tablets (400 mg of moxifloxacin in each tablet, from Bayer Pharma AG) was determined using the DPV method. Ten tablets of moxifloxacin drug were accurately weighed in order to find the average weight of each tablet. Then, the tablets were powdered in a mortar and carefully mixed. A quantity equivalent to one tablet was weighed, dissolved into double distilled water and transferred to a 100 mL volumetric flask, and diluted to the mark with double distilled water. The resulting solution was centrifuged at 5000 rpm; then, the supernatant was collected and diluted to 100 mL and used as a stock solution of the sample.

Urine samples were collected in sterile bottles. The samples were spiked with known concentration of moxifloxacin, centrifuged (3000 rpm, 10 min) to removal of proteins and diluted to 50% with 0.10 M phosphate buffer solution (pH = 7.0). The samples were then analysed without further treatment, using the conditions described in Section 2.4.

3. Results and discussion

3.1. Characterization of the different modified electrodes

SEM was performed to obtain an insight into the surface morphology of the different modified electrodes, as shown in Fig. 1. Fig. 1A–C shows the SEM of GR/GCE, MIP/GR/GCE, and NIP/GR/GCE, respectively. Fig. 1A can be realized from the images that GR has large surface area which makes it easy for the electron transfer. It can be found rough and multihole structure in Fig. 1B, which provided a large recognition sites in removing the MIP/GR/GCE. However, there are no imprinted cavities in removing the NIP/GR/GCE.

The extraction of moxifloxacin from the MIP layer on the surface of electrode has resulted in the formation of imprinted cavities in the MIP/GR/GCE. The formed imprinted cavities could act as channels and allow access for the diffusion of $[Fe(CN)_6]^{3-/4-}$ into and out of the polymeric network, which could be oxidized or reduced at the electrode and produce an electrochemical signal. As an effective method for probing the features of a surface modified electrode, electrochemical impedance spectroscopy (EIS) was employed to characterize the stepwise construction process of the sensor. Fig. 2A shows the EIS curves of different electrodes. Bare GCE (a) shows a very low charge transfer resistance. With the modification of the MIP before elution (b), the resistance to charge transfer (Ret) is large becuse the film modified on the electrode is nonconductive. When the template moxifloxacin was removed out of the imprinted film (c), the Ret reduced significantly, which suggested that the removal of moxifloxacin from the MIP film decrease the electron transfer resistance. This phenomenon could be attributed to the formation of imprinted cavities after the removal of template moxifloxacin, leaving channels for the penetration of $[Fe(CN)_6]^{3-/4-}$ through the MIP film to reach GCE for the further oxidation. The Ret of MIP/GR/GCE after elution (d) is less than that of MIP/GCE after elution (c), which verifies that the grapheme facilitates electron transfer. Compared with MIP/GR/GCE after elution (d), the NIP/GR/GCE after elution (e) shows larger diameter semicircle which relates to the presence of template moxifloxacin.

The current change of $[Fe(CN)_6]^{3-/4-}$ on the different electrodes recorded by CV method confirmed the same result. As shown in Fig. 2B, the MIP/GCE (b) before elution hardly has current response. After removal of the temperate, the current response of MIP/GCE (c) increases, which suggests that the cavities are formed in the MIP membranes. The current response of the CV of the $[Fe(CN)_6]^{3-/4-}$ at the MIP/GR/GCE (d) is larger than that of MIP/GCE (c) after the addition of graphene. Graphene shows high conductivity which allows $[Fe(CN)_6]^{3-/4-}$ to reach the electrode surface easily. Compared with the MIP/GR/GCE (d), the NIP/GR/GCE (e) without the template has very small current response. The results might be attributed to the NIP membranes that block the electrontransfer.

3.2. Choice of electropolymerized monomer for MIP/GR/GCE

In order to choose an efficient monomer, OPD, L-lysine, and OPD–lysine were employed as different monomer to prepare three sensors and the specific rebinding properties were investigated. From Fig. S2, sensor prepared using OPD or L-lysine as monomer showed the specific adsorption since the current of $[Fe(CN)_6]^{3-/4-}$ on NIP/GR/GCE was really low, implying that the OPD or L-lysine could been applied individually in the MIP preparation for determination of moxifloxacin. The sensor prepared using OPD and L-lysine as monomer showed the highest current of $[Fe(CN)_6]^{3-/4-}$ compared with other sensors, which could be explained that the synergistic effects of OPD and L-lysine in MIP film could rebind lots of moxifloxacin molecules. The imprinted factors (IF, $IF = \Delta I_{MIPs}/\Delta I_{NIPs}$) have been calculated and compared on the three kinds of sensors, which were 2.6, 5.5, and 10.2, corresponding to

the sensors prepared using L-lysine, OPD, and OPD–lysine as monomer, respectively. These results fully illustrated the advantage of OPD–lysine as a polymerized monomer.

3.3. Optimization of conditions for MIP/GR/GCE preparation

3.3.1 Effect of the volume of graphene suspension

The effect of volume of graphene suspension on the peak current in the MIP/GR/GCE was initially studied. As shown in Fig. S3(A), the $[Fe(CN)_6]^{3-/4-}$ current responses increased from 0 to 2.0 μL and then decreased sharply with further increase of the graphene volume. A large volume of graphene on the GCE can increase the sensor response. However, an increase of the graphene volume to above the threshold value leads to a decrease, probably because of the thick graphene membrane which decreases the electrode surface conductivity.

3.3.2 Effect of function monomer to template ratio

The monomer concentration in the electropolymerization process could affect not only the thickness of the polymer matrix but also the amount of imprinted molecule, which in turn influences the electrochemical behavior of the sensor. To investigate the effect of monomer concentration on the MIP/GR/GCE, the electrodes were electropolymerized in different monomer concentrations in the range of 0.80–2.0 mM with a constant moxifloxacin concentration of 0.10 mM. As shown in Fig. S3(B), the highest peak current of $[Fe(CN)_6]^{3-/4-}$ on the MIP/GR/GCE was observed when the concentration of monomer was at 1.0 mM. A lower peak current was found when monomer concentration was lower than 1.0 mM, which may be due to the less capture of moxifloxacin during electropolymerization. Additionally, a considerable decrease in the current response on MIP/GR/GCE was observed when the concentration of monomer was above 1.0 mM because the electropolymerized film was too compact to form imprinted caves after elution. Thus, the optimum concentration of monomer for preparing MIP/GR/GCE was 1.0 mM.

3.3.3 Scan cycles and scan rate of electropolymerization

Scan cycles and scan rate of electropolymerization are both important factors for the fabrication of MIP/GR/GCE, which would influence the thickness and compactness of the imprinted polymers, respectively. To investigate the effect of scan cycles on the polymer thickness, 5–30 scan cycles were carried out. From the results of Fig. S3(C), higher cycles lead to thicker films with less accessible imprinted sites. The optimum polymerization cycles was selected as 20 according to the peak current of $[Fe(CN)_6]^{3-/4-}$. Fig. S3(D) showed the influence of scan rate on electropolymerization. On the one hand, at a slower scan rate the imprinted polymer formed a tight polymer that decreased the accessibility of removing template moxifloxacin to form imprinted sites. On the other hand, a loose and rough film with a low recognition capacity was formed at a faster scan rate. Thus, the optimum scan rate of electropolymerization was set to be 50 mV/s.

3.3.4 Template removal treatment

To remove the template molecules completely is a very important step in the preparation of molecularly imprinted electrochemical sensors. An 50% ethanol (V/V = 1:1) solution was used to elute the template molecules. $[Fe(CN)_6]^{3-/4-}$ was used as a probe molecule and scanned the differential pulses corresponding to different elution times. Fig. S3(E) is the elution curve of the MIP/GR/GCE. As shown in Fig. S3(E), as the elution time increased, the current gradually increased until it approached a stable value after more than 3 min of the elution time. It indicated that the template molecules were removed completely from the MIP.

So we choose an 50% ethanol (*V*/*V* = 1:1) solution and 3 min as the best solvent and time for template removal. Fig. S3(F) is the elution curve of the MIP/GCE. Compared with the MIP/GCE, the current response of MIP/GR/GCE after elution is larger. The results further indicated that graphene could improve the conductivity of molecularly imprinted polymers.

3.4. Optimization of determination conditions

3.4.1 The pH effect of rebinding solution for MIP/GR/GCE

The pH effect of rebinding solution was investigated by DPV method at constant concentration of moxifloxacin (1.0×10^{-8} M) in PBS with the pH value ranging from 5.7 to 7.4. As shown in Fig. S4(A), the response current increased from pH 5.7 to 6.5 and decreased above pH 6.5. The highest current changes (ΔI) of $[Fe(CN)_6]^{3-/4-}$ was observed when the pH value of rebinding solution was adjusted to 6.5. Thus, we chose the pH value as 6.5.

3.4.2 The effect of incubation time

The incubation time is important for the sensitivity of the sensor. After removal of template molecule, the MIP/GR/GCE was incubated in 4.0×10^{-9} M moxifloxacin solution at different times. The test results are shown in Fig. S4(B). The peak current decreased sharply with the incubation time from 0 to 15 min, which indicates the rapid and effective recognition ability of the MIP film for the target molecule. When the incubation time reached 10 min, the oxidation peak current levelled off gradually. So 10 min was chosen as the incubation time in this experiment.

3.5. Electrochemical behavior of the electrochemical active probe

The electrochemical mechanism can usually be obtained from the relationship between the peak current and the scan rate. The CV curves of the imprinted sensors in the $[Fe(CN)_6]^{3-/4-}$ solution at different scan rates were investigated in the range of 10–100 mV/s. As seen in Fig. S5, the peak currents of the CV in the imprinted sensor increased with the increment of the scan rate. The anodic (I_{pa}) and cathodic (I_{pc}) peak currents were nearly independent of the scan rate and can be expressed as: I_{pa} (μA) = 8.51 + 5.17$v^{1/2}$ (R^2 = 0.998) and I_{pc} (μA) = –9.40 – 4.03$v^{1/2}$ (R^2 = 0.998) (where v is the scan rate with units mV/s), suggesting typical surface controlled electrochemical behavior.

3.6. Calibration curve

Under the optimum conditions, the detection of various concentrations of moxifloxacin was investigated with DPV using the MIP/GR/GCE sensor. As shown in Fig. 3, the peak current decreased as the moxifloxacin concentration increased, and the reduction in ΔI for $[Fe(CN)_6]^{3-/4-}$ was proportional to the moxifloxacin concentrations for the ranges 1.0×10^{-9}–1.0×10^{-8} M and 1.0×10^{-8}–5.0×10^{-5} M, respectively. The linear regression equations are: ΔI (μA) = 11.3logC_{MFLX} (M) + 106.3 (R^2 = 0.998) and ΔI (μA) = 1.59logC_{MFLX} (M) + 28.81 (R^2 = 0.997). The imprinted sensor had a detection limit (S/N = 3) of 5.12×10^{-10} M for moxifloxacin. Table S1 shows the comparison of the performance of this sensor with other sensors for moxifloxacin detection. The results indicated that the prepared MIP/GR/GCE possessed an excellent sensitivity and a high selectivity for moxifloxacin determination.

3.7. Repeatability and stability

To evaluate the reproducibility of the MIP/GR/GCE sensor, the net response of the sensor before and after incubation in 1.0×10^{-8} M moxifloxacin solution was measured with five replicates. The relative standard deviation (RSD) was 1.2% for the five successive assays. On the other hand, five sensors were prepared and tested under the same conditions, and the

RSD of five tests was 3.3%. Furthermore, the storage stability of the sensor was investigated. The results showed that the sensor lost only 6.0% of its initial response after it was stored in refrigerator for 25 days. Therefore, the MIP/GR/GCE sensor has good reproducibility and stability.

3.8. Selectivity

The selectivity of sensor towards moxifloxacin (MFLX) was evaluated by DPV using compounds with structures similar to moxifloxacin such as gatifloxacin (GFLX), ciprofloxacin (CPLX), ofloxacin (OFLX) and norfloxacin (NFLX). As shown in Fig. S1, the current variation (ΔI) ($\Delta I = I_0 - I_c$, where I_0 is the original current and I_c denotes the current response of MIP/GR/GCE incubated in a solution of concentration) of MIP/GR/GCE was higher than that at NIP/GR/GCE. The current response of the sensor to different analytes was measured at a concentration of 4.0×10^{-9} M. It is found that the sensor had stronger response towards moxifloxacin template than those structurally related analogues, suggesting that the sensor had special recognition and selectivity to moxifloxacin due to the imprinted effect. The imprinting and selecting factors are defined as

$$\alpha = (\Delta I/I_0)_{MIP}/(\Delta I/I_0)_{NIP} \quad (1)$$

$$\beta = \alpha_{MFLX}/\alpha_{analog} \quad (2)$$

where the α value of the sensor to template molecule is much higher than that to the other substances. The calculated results are given in Table S2, which suggest that the size and the conformation of cavities match with moxifloxacin in the MIP network.

3.9. Applications

The sensor was evaluated by carrying out the determination of moxifloxacin in the real samples solution obtained from tablets and human urine samples using the standard addition method under optimized conditions. The moxifloxacin content of real samples was determined using the MIP/GR/GCE, and the results were shown in Table 1. The recoveries of 96–103% and the relative standard deviation less than 2.0% for the proposed sensor in real sample analysis indicate the acceptable precision for the voltammetric determination of moxifloxacin using the MIP sensor. Therefore, the MIP/GR/GCE is successfully applied to the monitoring of moxifloxacin in biological and pharmaceutical samples.

4. Conclusions

In this study, we have developed a new electrochemical sensor for moxifloxacin determination using a novel graphene-molecular imprinted polymers composite as recognition element. There are several advantages of the developed MIP/GR/GCE sensor. First, preparation of MIP/GR/GCE sensor simply involved electrochemical polymerization of o-phenylenediamine and L-lysine, in the presence of moxifloxacin on the surface of GR/GCE, which was really convenient and inexpensive. Second, the resultant MIP/GR/GCE sensor can selectively recognize the template moxifloxacin and revealed a remarkably wide linear range with a low detection of limit down to 5.12×10^{-10} M. Third, short response periods, satisfactory reproducibility and stability were also demonstrated. Moreover, MIP/GR/GCE sensor has been successfully used to determine moxifloxacin in real samples with satisfactory

results.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the supporting information.

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414 Table 1 Determination of moxifloxacin in real samples (n = 3).

Sample	Added (μM)	Found[a] (μM)	Recovery[b] (%)	RSD[c] (%)
Tablet	0	5.36	–	1.9
	2.00	7.40	102	1.1
	4.00	9.33	99	1.3
	6.00	11.4	101	1.7
Human urine	2.00	1.91	96	0.8
	4.00	4.13	103	1.0
	6.00	5.80	97	0.6
	8.00	7.85	98	0.9

415 [a] Average value of three determinations.

416 [b] Recovery (%) = (found concentration / added concentration) × 100.

417 [c] RSD: relative standard deviation.

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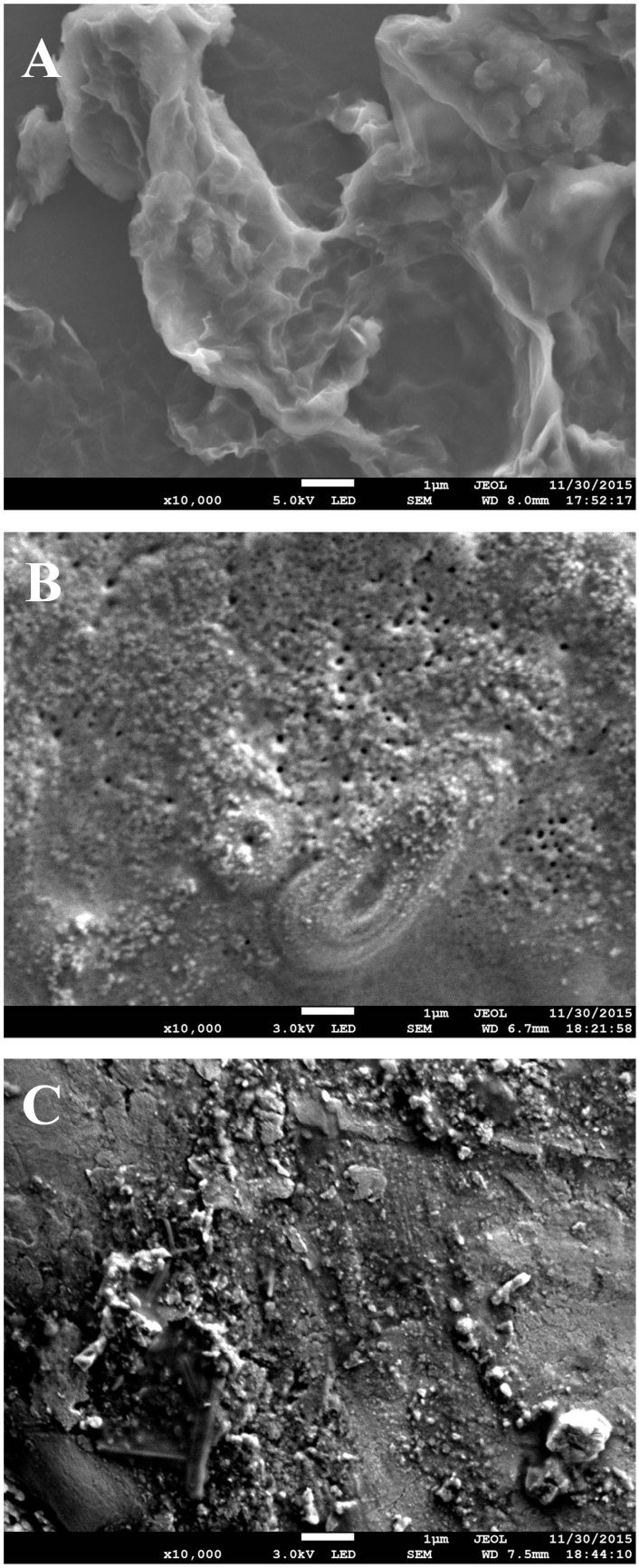

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Fig. 1. SEM images of GR/GCE (A), MIP/GR/GCE (B) and NIP/GR/GCE (C).

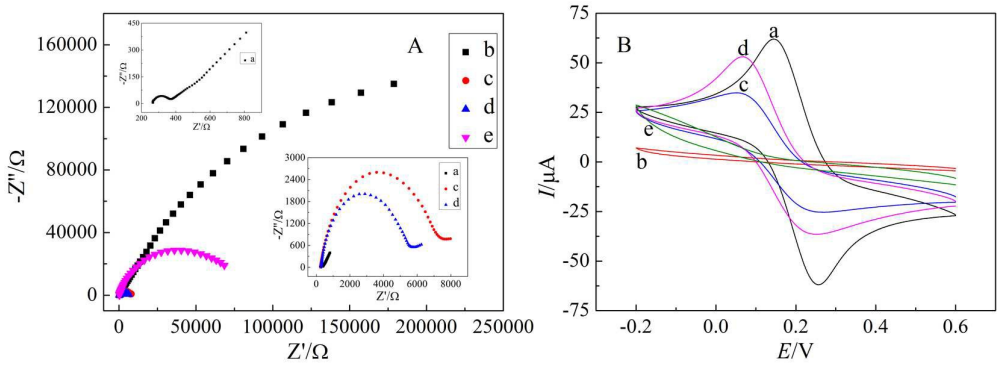


Fig. 2. (A) EIS of different modified electrodes in 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.10 M KCl and (B) CV of different modified electrodes in 2.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.10 M KCl: the bare GCE (a), MIP/GCE before elution (b), MIP/GCE after elution (c), MIP/GR/GCE after elution (d) and NIP/GR/GCE after elution (e). Insert: the bare GCE.

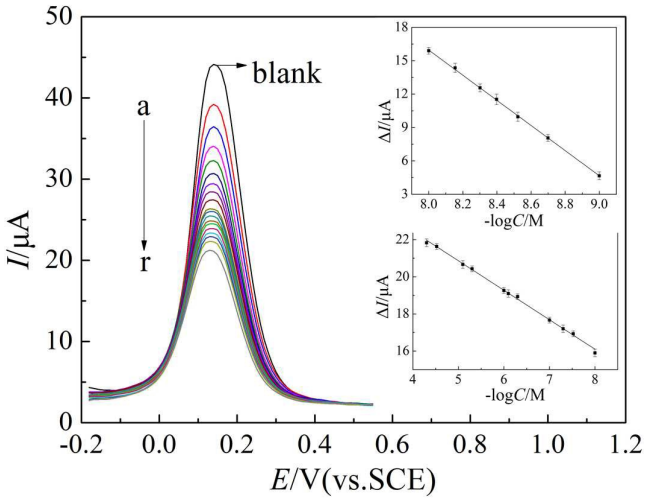
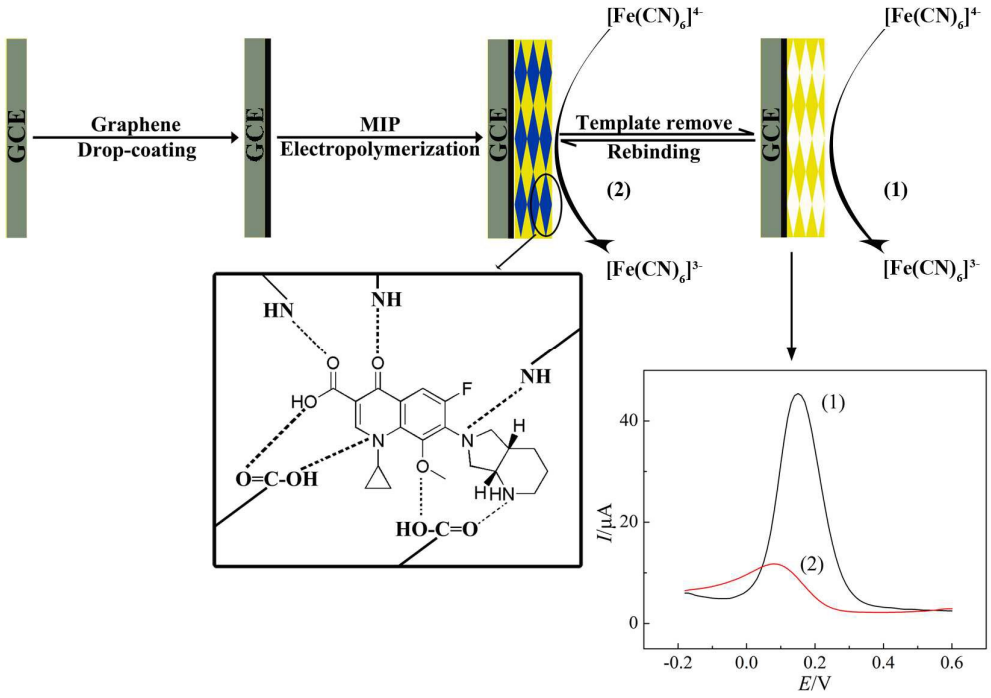


Fig. 3. Different pulse voltammograms of different moxifloxacin concentration on the sensor in 2.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.10 M KCl. Insert: plot of ΔI vs. (a–r) concentration of moxifloxacin from 1.0×10^{-9} M to 5.0×10^{-5} M.



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435 Scheme 1. Schematic illustration of stepwise electrode modification.