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Selective Recognition of Ciprofloxacin Hydrochloride Based on Molecular Imprinted Sensor via Electrochemical Copolymerization of Pyrrole and *o*-phenylenediamine

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A simple and reliable method was proposed for rapid detecting ciprofloxacin, based on molecularly imprinted technology. The molecularly imprinted ciprofloxacin hydrochloride (CPX) sensor was prepared by electropolymerization of pyrrole (Py) and o-phenylenediamine (o-PD) on the pencil graphite electrode (PGE). The performance of the sensor was characterized by square wave voltammetry (SWV) using potassium ferricyanide as molecular probe. Under optimal conditions, the sensor displayed high selectivity and stability towards CPX. A linear relationship between the change in current (Δi) and the logarithm of CPX concentrations from 1×10^{-9} to 1×10^{-3} mol/L was obtained with a detection limit of 7.58×10^{-11} mol/L (S/N=3). Furthermore, the MIP sensor was applied for the analysis of real tablet samples with high accuracies.

Keywords: molecular imprint; copolymerization; cyclic voltammetry (CV); ciprofloxacin; sensor

1. INTRODUCTION

Ciprofloxacin hydrochloride (CPX) is a broad-spectrum synthetic antibiotics which have been widely used for veterinary purposes [1]. It is well known that CPX residues may persist in edible tissues, which is harmful to human health. Various methods have been well-established for the detection of CPX such as high performance liquid chromatography [2-4], spectrophotometry [5-6], capillary zone electrophoresis [7], or micellar liquid chromatography [8]. However, these analysis techniques require expensive instrumentation, complex sample pretreatment and preparation, which cannot meet of large-scale actual testing [9-10]. Thus, a rapid, inexpensive but sensitive method for detecting CPX residues is highly desirable.

Molecularly imprinted polymer (MIP) is a rapidly developing technique for synthesizing molecular recognition materials (molecular imprinted polymers (MIP)) with tailor-made selective recognition sites during the last few decades [11-13]. Because of its unique properties, MIP is widely used in variously analytical methods such as colorimetric sensors [14], drug detection [15], extraction [16], chromatographic separations [17], and catalysis [18] etc. Electrochemical deposition of MIP film on electrode is a simple and useful method. This method has many advantages such as controlling the thickness of film, easily grown and adhered to a transducer of any size and shape, simplicity, and high sensitivity [19]. However, to our knowledge, so far electrochemical sensor based on MIPs for determination CPX has rarely been reported.

In this work, we focused on development of a high selective molecularly imprinted sensor to overcome the drawbacks of the conventional detection methods for CPX. The sensor was fabricated through electropolymerization of Py and o-PD on PGE using CPX as template molecular. The MIP sensor was investigated to confirm its electrochemical properties (selectivity, stability, linearity, repeatability and reproducibility) by SWV. The proposed sensor has been successfully employed to detect CPX in complex samples.

2. EXPERIMENTAL

2.1. Chemicals and apparatus

All electrochemical experiments were performed with a CHI660D electrochemistry workstation (Peking CH Instruments Co., China) at room temperature. A conventional three-electrode system was used, consisting of a bare or modified PGE as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum foil electrode as counter electrode. A pH-meter was also applied for pH adjustment.

Pyrrole (Py, >98%), o-phenylenediamine (o-PD, >98%), ciprofloxacin hydrochloride (CPX, >98%), enrofloxacin (EF), tetracycline (TCH), chloramphenicol (CM, >98%) have been procured from Aladdin Chemistry Co., China. Py has been distilled prior to being used and is kept under dark atmosphere at about 4 ^{o}C to avoid oxidation. All other reagents used were analytical grade. Double deionized water was used in all runs.

2.2. Pretreatment of PGE

Firstly, the surface of PGE with 5 mm long as workspace was polished by metallographic sandpaper. Then the PGE was cleaned by dipping in 2 mol/L H_2SO_4, 6 mol/L HCl and 4 mol/L HNO_3 for 5 min, respectively. Finally, the PGE was washed with double deionized water and dried at room temperature. Before use, the PGE was activated through SWV in 1 mol/L H_2SO_4 for 10 times.

2.3 Preparation of MIP and Non-imprinted polymer (NIP) sensors

The CPX MIP/PGE was prepared by electrochemical polymerization. The pretreated PGE was immersed into the polymerization solution composed of 10 mmol/L CPX, 10 mmol/L Py, 10 mmol/L o-PD in 0.05 mol/L acetic acid buffer (pH=3.5). The copolymerization solution was degassed with nitrogen for 10min. Following this, the electropolymerization was performed by using cyclic voltammetry (CV) in sweeping potential between −0.1 V to +1.2 V at a scan rate 50 mV/s with 20 scan cycles. After copolymerization, the MIP sensor was washed with eluant (alcohol: 1mol/L NaOH=3:1, v/v) for 4 h to remove the template molecules. For comparison, a NIP/PGE was prepared and treated in an identical manner in the absence of CPX.

2.4. Electroanalytical measurements

Square wave voltammetry (SWV) were carried out using a three-electrode system in the probe solution (5 mmol/L $K_3[Fe(CN)_6]$ +0.1 mol/L KCl). Scan ranged from -0.1V to 0.6 V with a step potential of 5 mV, a amplitude of 50 mV, and a frequency of 20 Hz.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of different electrodes

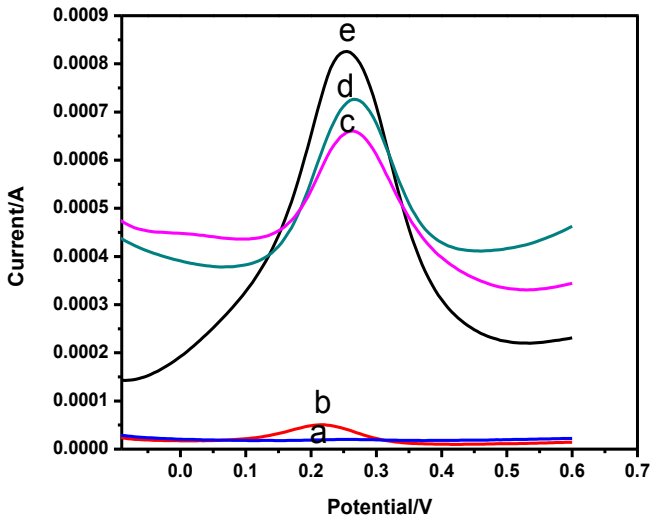

Figure1. SWVs of different electrodes. NIP/PGE (a), MIP/PGE with template(b), MIP/PGE rebinding template(c), MIP/PGE(d) and bare PGE(e).

SWVs of various electrodes were carried out in the probe solution and given in Fig.1. Compared with curves (1e), the peak currents of the MIP/PGE with template (1b) and NIP/PGE (1a) were obviously decreased, indicating that insulating films are formed on the PGE, which agrees with

the result of CV copolymerization (Fig.2). After the MIP/PGE and NIP/PGE washed with the eluant, the peak current of MIP/PGE (1d) was relatively high. It can be attributed to the fact that the imprinted cavies should be favorable for probe $K_3[Fe(CN)_6]$ reaching electrode surface [20]. Moreover, after the electrode of MIP/PGE rebinding CPX, the peak current declined (1c).The above results proved that imprinted cavities were produced in MIP film and the template can be effectively removed with the eluant.

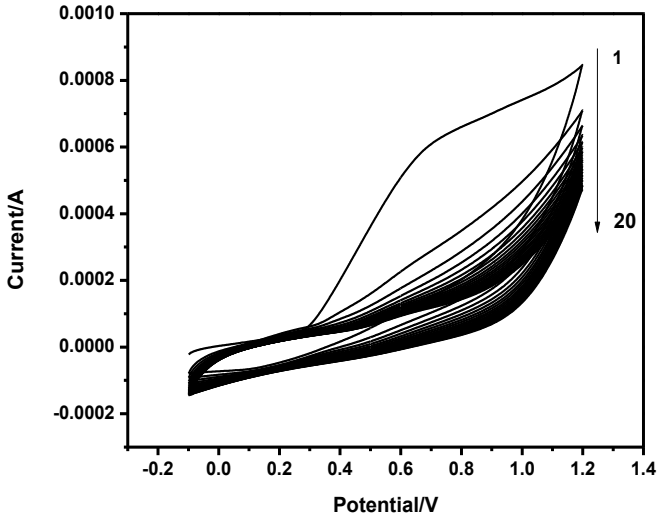

Figure2. Cyclic voltammograms of the copolymerization of Py and o-PD on a PGE.

3.2. Optimization of the parameters for MIP sensor

3.2.1. Effect of pH

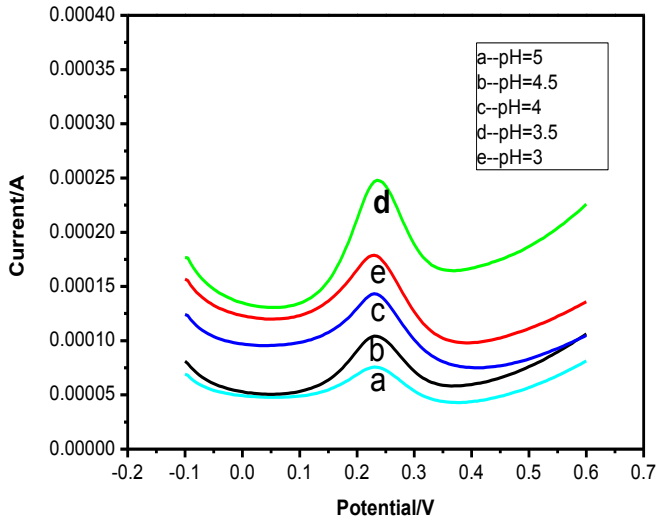

Figure 3. SWV curves of the MIP/PGE prepared in 0.05mol/L acetic acid buffer with different pH

To get the best condition of strong interactions between the template and monomers, the influence of copolymerization solution pH on the electrochemical responses of MIP sensor was investigated in the range of 3.0–5.0 and the results were shown in Fig.3. From the Fig.3, the best response was obtained at pH 3.5, corresponding to the most imprinted cavities in MIP film. This may be attribute to the strong electrostatic and other non-covalent bond interaction between CPX and functional monomer at pH (3.5).

3.2.2. Ratio of functional monomers

In order to obtain best copolymer for the formation of imprinted sites, the Poly(Py-co-o-PD) films were synthesized by electropolymerization of monomers from 0.05 mol/L acetic acid buffer (pH=3.5), containing different feed ratios of monomers (Py: o-PD, 2:1, 1:1, 1:2, 1:3) keeping total monomer concentration constant at 0.02 mol/L, using cyclic voltammetry from −0.1 V to +1.2 V at a scan rate 50 mV/s with 20 scan cycles. From the SWVs (Fig.4), it was found that the copolymer with 1:1 (Py:o-PD) monomer concentration gave the largest current response (fig.4b).

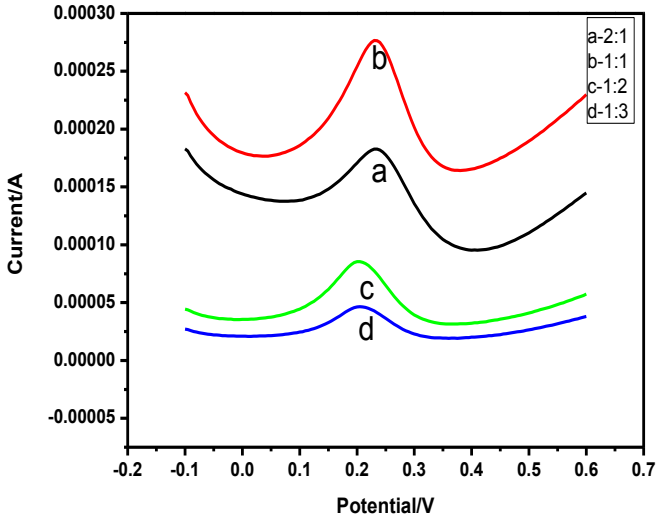

Figure 4. SWV curves of different ratios of Py and o-PD (a-d 2:1, 1:1, 1:2, 1:3).

3.2.3. Effect of electropolymerization time

The numbers of scan during the electropolymerization process affected the film thickness and the active amounts of imprinted sites [21-22]. The peak currents of the sensors synthesized by different cycles were shown in the Fig.5. It was observed that the best response was obtained with 20 cycles. Shorter scans less than 20 cycles resulted in the formation of fewer imprinted cavities in the imprinted films. Moreover, longer scans more than 20 cycles led to increasing of the thickness of the polymer

film, which resulted in the difficulty of removing template hindering the probe ion transfer to the surface of the electrode.

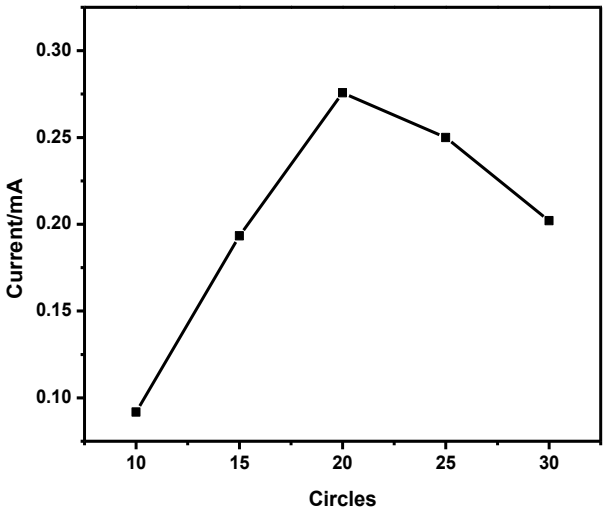

Figure 5. Recorded peak current of the sensor prepared with different CV cycles.

3.2.4. Washing time for template removal

After electropolymerization of Py and o-PD, the MIP sensor were thoroughly rinsed with distilled water to remove any loosely bound all materials.

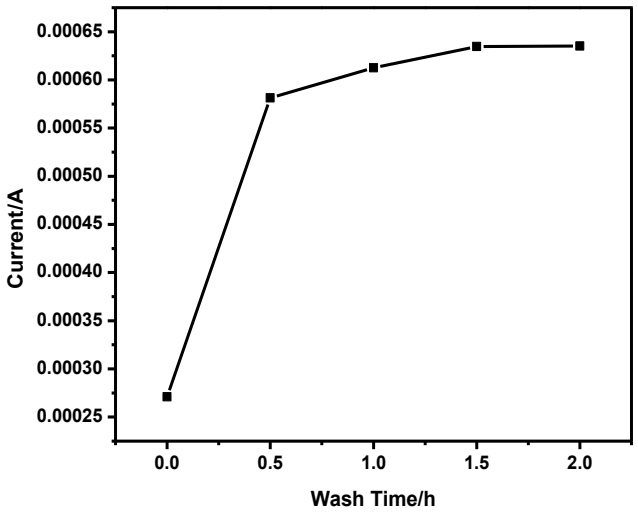

Figure 6. Recorded peak current from the sensor prepared under under optimized conditions immersing into the eluant for different time.

Then, embedded CPX molecules were removed from the polymeric film by the MIP sensor immersing into eluant (alcohol: 1mol/L NaOH=3:1, v/v) for different time, until the electrochemical signals of the sensor reached a plateau, probably indicating a complete removal of all trapped template

molecules [21]. The results (Fig.6) showed that as the immersing time reached 1.5 h, a complete removal of templates was obtained. Thus, 1.5 h was chosen as washing time in subsequent works.

3.2.5. Optimization of incubation time

The incubation time is important for the sensitivity of the sensor [23]. After removal of template molecule, the MIP sensor prepared under optimized conditions was incubated in 0.05 mol/L acetic acid buffer (pH=3.5) containing 5 mmol/L CPX for different time and then the corresponding response was recorded by SWV in the probe solution. As shown in Fig.7, the peak current decreased sharply with the incubation time from 0 to 6 min, which indicated the rapid and effective recognition ability of the MIP sensor for the target molecule. After the incubation time reached 6 min, the peak current leveled off gradually, meaning that the adsorption equilibrium was achieved [24]. In order to respond completely, incubation time was set as 8 min, whenever the measurement using MIP sensor.

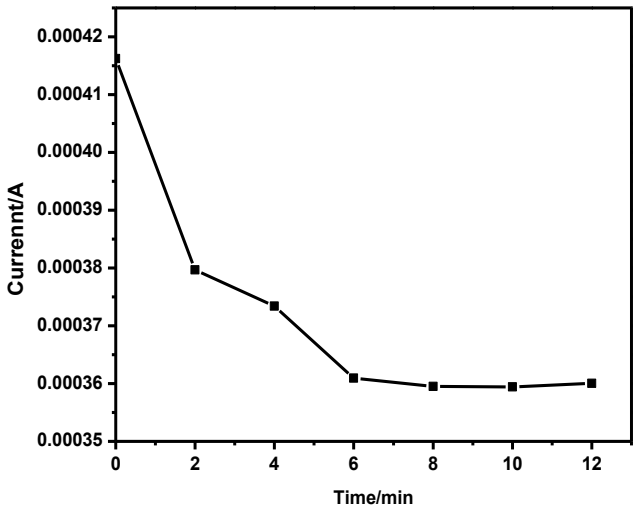

Figure 7. The peak current of the sensor incubated in 5 mmol/L CPX solution for different time.

3.3. Calibration curve and detection limit

To verify the practicality of the MIP sensor for the determination of CPX, the linear range and the detection limit were gotten through drawing the calibration curve. Under the optimum conditions, the SWV peak currents of the MIP sensor were obtained after interaction with various concentrations of CPX in 0.05 mol/L acetic acid buffer (pH=3.5). Fig.8 shows the relationship between the change in peak current (Δi) and the logarithms of CPX concentrations. Herein, $\Delta i=i_0-i_c$, i_0 and i_c are the peak currents when the concentrations of CPX are 0 and c mol/L, respectively. It can be seen that the Δi has a good linear relationship with logarithms of CPX concentrations. The linear regression equation was $\Delta i=1.31166+0.12955\log c$ (Δi: 1×10^{-4}A; c: mol/L) in the concentration range of 1×10^{-9}-1×10^{-3} mol·L^{-1} and the correlation coefficient is 0.99813. The limit of detection is found to be 7.58×10^{-11} mol/L (S/N =3). The performance of the MIP sensor for CPX detection was compared with that of other reported

methods, and the major parameters were summarized in Table 1. Overall, the developed sensor displayed a wider linear range and a lower detection limit, indicating the advantages of the MIP sensor.

Table 1. Comparison of the proposed method with other methods in the determination of CPX.

Method	Functional monomer	Linear range (mol/L)	Detection limit (mol/L)	References
spectrophotometry	-	1.0×10^{-5}-3.8×10^{-5}	-	[25]
capillary electrophoresis	-	1.4×10^{-4}-6.8×10^{-4}	3.3×10^{-5}	[26]
Masss pectrometry	-	1.0×10^{-8}-5.4×10^{-6}	3.0×10^{-9}	[27]
MIP chromatography	methacrylic acid	2.7×10^{-7}-2.7×10^{-4}	8.2×10^{-8}	[28]
MIP sensor	Py and o-PD	1.0×10^{-9}-1.0×10^{-3}	7.58×10^{-11}	present work

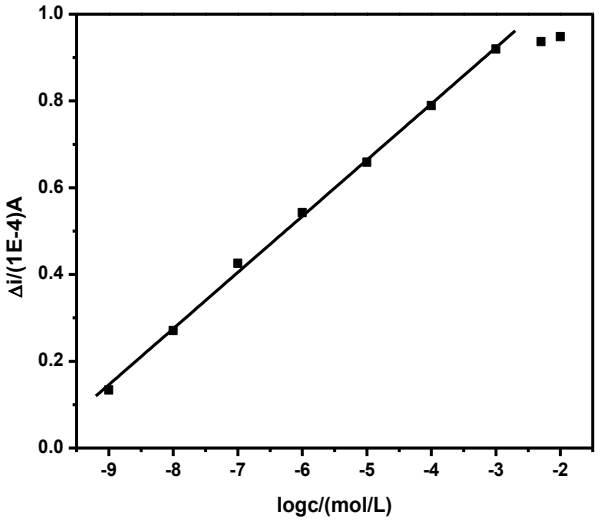

Figure 8. Plot of the peak current response (Δi) versus logarithm of CPX concentration.

3.4. Selectivity, repeatability and stability of the MIP

Selective recognition toward the template molecule was an important capability for a MIP sensor. The capability originated from the specific binding sites and the three-dimensional structure left in MIP [29-31]. To study the selectivity of the MIP sensor, SWV of CPX and structural analogs on MIP and NIP sensor were obtained before and after exposing them to in 0.05 mol/L acetic acid buffer (pH=3.5) containing 10^{-5} mol/L CPX or various analogs and the results were shown in Fig.9. Noticeably, the change of the peak current (Δi) of the MIP sensor was stronger than that of the NIP sensor, and the response of MIP sensor toward CPX was stronger than that toward structural analogs. The results revealed that the proposed sensor exhibited good selectivity toward CPX. This should be ascribed to the selective rebinding of the imprinted sites toward templates.

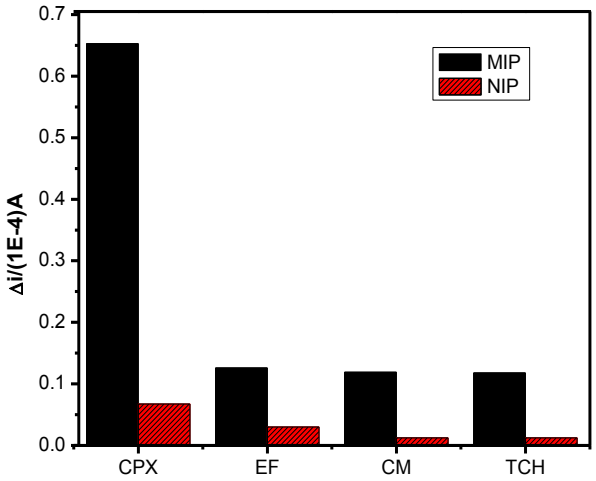

Figure 9. SWV responses of CPX and structural analogs on MIP and NIP sensor.

To investigate the reproducibility of the constructed MIP sensor, five independently prepared MIP sensors were used to detect the same CPX solution (1×10^{-5} mol/L) in 0.05mol/L acetic acid buffer (pH=3.5) by SWV. According to the current responses, the relative standard deviation (RSD) was 0.95%, confirming that the MIP sensor had good reproducibility.

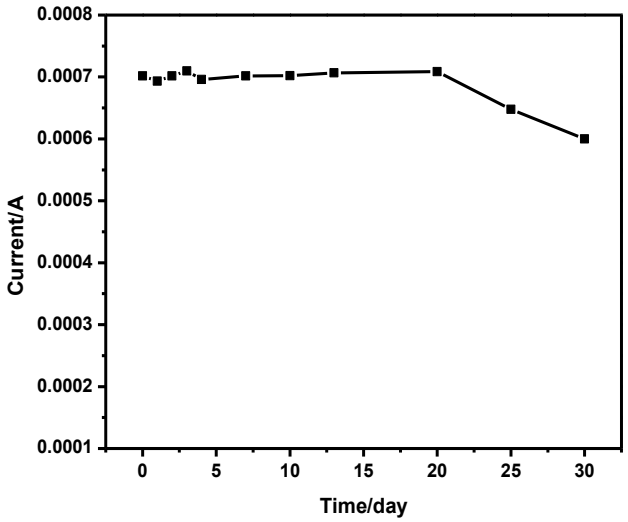

Figure 10. Stability of the MIP sensor stored at ambient conditions over 30 days.

One important factor regarding the molecularly imprinted materials was the stability of the geometry of molecular cavities created inside the polymers which in turn affects both the selectivity and the sensitivity of the imprinted material for template molecules on the long term [32]. For evaluating the long-time stability of the proposed sensor, it was kept in ambient conditions for 30 days. At different storage periods between 30 days, the sensor was used to detect the same CPX solution (1×10^{-5} mol/L) in 0.05mol/L acetic acid buffer (pH=3.5). The obtained results (Fig.10) indicated that

the peak current did not show an obvious decline after storage of 20 days, indicating that the fabricated sensor had a good long-term stability.

3.5. Sample analysis

To evaluate the applicability, the proposed sensor was applied to the determination of CPX in three brands of commercial tablet samples. The tablets were grounded into powder, dissolved in 0.05mol/L acetic acid buffer (pH=3.5), filtered and diluted to certain volume. As can be seen (Table 2), the values obtained by the proposed sensor was in close agreement with the label claim of all pharmaceutical formulations.

Table 2. Determination results of Ciprofloxacin hydrochloride in tablets (n=3)

Sample	Reported content (mg per tablet)	Detected content (mg)	RSD(%)
1	250	252	2.01
2	250	254	3.11
3	250	252	3.27

4. CONCLUSION

In this work, a kind of MIP-based electrochemical CPX sensor was fabricated by electrochemical copolymerization of Py and o-PD in the presence of CPX. Under the optimal experimental conditions, the constructed sensor displayed wide linear range (1.0×10^{-9}-1.0×10^{-3} mol/L), low detection limit (7.58×10^{-11} mol/L), good selective recognition and long-term stability towards CPX detection. Moreover, the MIP sensor has been successfully (RSD<3.27%) applied to determination of CPX in samples of tablets.

ACKNOWLEDGEMENTS
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Rreferences

1. H. Hektoen, J.A. Berge, V. Hormazabal, and M. Yndestad, *Aquaculture*. 133 (1995) 175-184.
2. E. Rodriguez, M.C. Moreno-Bondi, and M.D. Marazuela, *J. Chromatogr: A*. 1209 (2008) 136-144.
3. C. Gonzalez, L. Moreno, J. Small, D.G. Jones, and S.F.S. Bruni, *Analytica Chimica Acta*. 560 (2006) 227-234.
4. K Prakash and K.R. Sireesha, *E-Journal of Chemistry*. 9 (2012) 1077-1084.
5. M.I. Pascual-Reguera, G. Pérez Parra and A.M. Díaz, *J Pharmaceut Biomed*. 35 (2004) 689-695.
6. [6] F. Breton, P. Euzet, S.A. Piletsky, M.T. Giardi and R. Rouillon, *Analytica Chimica Acta*. 569 (2006) 50-57.

7. H.W. Sun, L.Q. Li and X.Y. Chen, *Anal. Bioanal. Chem*. 384 (2006) 1314-1319.
8. F.X. Qiao, H.W. Sun, H.Y. Yan and K.H. Row, *Chromatographia.* 64 (2006) 625-634.
9. R.H.O. Montes, M.C. Marra, M.M. Rodrigues, E.M. Richte and R.A.A. Munoz, *Electroanalysis*. 26 (2014) 432-438.
10. H.L. Tan, L. Zhang, C.J. Ma and Y.H. Song, *ACS Applied Materials & Interfaces* 5(2013) 11791-11796.
11. K. Haupt and K. Mosbach, *Chem. Rev*. 100 (2000) 2495–2504.
12. L.X. Chen, S.F. Xu and J.H. Li, *Chem Soc Rev.* 40 (2011) 2922-2942.
13. M. Bougrini, A. Florea and C. Cristea, *Food Control.* 59 (2016) 424-429.
14. M. Behbahani, S. Salimi, H. Sadeghi Abandansari, F.Omidi, M. Salarian and A. Esrafil, *RSC Adv*. 5 (2015) 59912–59920.
15. M.K. Bojdi, M. Behbahani, A. Sahragard, B. Golrokh Amin, A. Fakhari and A. Bagheri, *Electrochim. Acta*. 149 (2014) 108–116.
16. M. Behbahani, S. Bagheri, M. Amini, H. Sadeghi Abandansari and H.R. Moazami, *J Sep Sci.* 37 (2014) 1610–1616.
17. S. Ambrosini, M. Serra, S. Shinde, B. Sellergren and E. DeLorenzi, *J Chromatogr A*. 1218 (2011) 6961-6969.
18. J. Orozco, A. Cortés, G. Cheng, S. Sattayasamitsathit, W. Gao, X. Feng, Y. Shen and J. Wang, *J.Am.Chem.Soc.* 135 (2013) 5336–5339.
19. L.P. Liu, Z.J. Yin and Z.S. Yang, *Bioelectrochemistry*. 79 (2010) 84–89.
20. M. Torkashvand, M.B. Gholivand and F. Taherkhani, *Materials Science and Engineering C*. 55 (2015) 209–217.
21. H. Hrichi, M.R. Louhaichi, L. Monser and N. Adhoum, *Sensors and Actuators B: Chemical*. 204 (2014) 42–49.
22. M. Zhong, Y. Teng, S.F. Pang, L.Q. Yan and X.W. Kan, *Biosensors and Bioelectronics.* 64 (2015) 212–218.
23. X.C. Tan, Q. Hu, J.W. Wu, X.Y. Li, P.F. Li, H.C. Yu, X.Y. Li and F.H. Lei, *Sensors and Actuators B: Chemical.* 220 (2015) 216–221.
24. I. Bakas, A. Hayat, S. Piletsky, E. Piletska, M. Chehimi, T. Noguer and R. Rouillon, *Talanta*. 130 (2014) 294–298.
25. K.A.M. Attia, M.W.I. Nassar, M.B. El-Zeiny and A. Serag, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 154 (2016) 232–236.
26. M.C.V. Mamani, J. Amaya-Farfan, F.G.R. Reyes, J.A.F. da Silva and S. Rath, *Talanta*. 76 (2008) 1006–1014.
27. H.M.V. Oliveira, F.T.C. Moreira and M.G.F. Sales, *Electrochimica Acta.* 56 (2011) 2017–2023.
28. D. Moreno-González, F.J. Lara, L. Gámiz-Gracia and A.M. García-Campaña, *Journal of Chromatography A*. 1360 (2014) 1–8.
29. Y.K. Yang, G.Z. Fang, X.M. Wang, M.F. Pan, H.L. Qian, H.L. Liu and S. Wang, *Analytica Chimica Acta*. 806 (2014) 136–43.
30. B.L. Li, J.H. Luo, H.Q. Luo and N.B. Li, *Sensors and Actuators B: Chemical.* 186 (2013) 96–102.
31. Z.P. Yang, X. Liu, Y.M. Wu and C.J. Zhang, *Sensors and Actuators B: Chemical*, 212 (2015) 457–463.
32. N.F. Atta and A.M. Abdel-Mageed, *Talanta*. 80 (2009) 511–518.