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Electrochemical Determination of Moxifloxacin Hydrochloride Based on Molecularly Imprinted Polymer Modified Carbon Paste Electrode

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A highly selective molecularly imprinted polymer modified carbon paste electrode was developed. The Moxifloxacin Hydrochloride molecularly imprinted polymer was synthesized by thermal initiation precipitation polymerization, and the modified electrode was prepared by mixing graphite and polymer (90:10). Studied the electrochemical behavior and established the determination method of Moxifloxacin Hydrochloride at the electrode. The results showed that the sensor has high selectivity and sensitivity. and exhibited a linear voltammetric response for Moxifloxacin Hydrochloride in the concentration range of 3.13×10^{-6}~2.0×10^{-4}mol•L^{-1}, with the limit of detection (LOD) of 5.9×10^{-8}mol•L^{-1} (S/N=3). The sensor was applied to determine Moxifloxacin Hydrochloride in Avelox tablets and and human serum samples. The recoveries of Moxifloxacin Hydrochloride ranged from 97.2% to 104.5%.

Keywords: Moxifloxacin Hydrochloride; precipitation polymerization; molecularly imprinted polymer; modified electrode; carbon paste electrode

1. INTRODUCTION

Moxifloxacin Hydrochloride is a kind of broad-spectrum antimicrobial fluoroquinolones [1]. Its chemical structural formula is as shown in Fig.1.

Moxifloxacin Hydrochloride Tablets was firstly registered in Feb. 1999 in Mexico, and was marketing in Germany in Sep. 1999. The infusion solution was marketing in the United States and Germany in Dec. 2001 [2, 3]. 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 [4,5]. As a new generation of antibacterial fluoroquinolone, Moxifloxacin Hydrochloride has strong antimicrobial activity, good clinical effects with little toxicity.

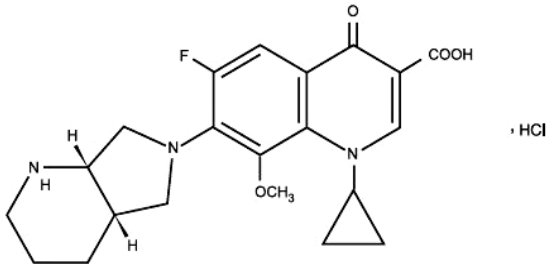

Figure 1. Chemical structure of Moxifloxacin Hydrochloride

The determination method of Moxifloxacin Hydrochloride is mainly as high-performance liquid chromatography (HPLC), which is complicated in measuring process and requires large instrument. It is not convenient for rapid detection [6,7,8]. Chemically modified carbon paste electrode (CMCPE) is composed of graphite and chemical modifier, The chemical modifier can provide preplanned function to the carbon paste electrode, and can improved the sensitivity and selectivity. CMCPE integrated separation, enrichment and determination in one step. Our group has obtained some achievements in CMCPE research as well [9]. Molecularly imprinted polymer (MIP) has been widely applied in chromatographic fractionation, antibody-receptor simulation, biosensor, catalytic synthesis and other fields due to its stereospecific recognition ability [10, 11, 12].

The objective of the present work is to develop a simple and sensitive sensor for Moxifloxacin Hydrochloride determination, study the electrochemical behavior and establish the determination method of Moxifloxacin Hydrochloride on the sensor. Finally, The method has been applied to determine Moxifloxacin Hydrochloride in real samples with satisfactory results.

2. EXPERIMENTAL

2.1 Instrumentations, Chemicals and reagents

All the electrochemical measurements were performed on a Ingsens-1001 electrochemical workstation (Ingsens Instruments (Guangzhou) Co., Ltd.). The three-electrode system consisted of a modified carbon paste electrode or a bare carbon paste electrode as a working electrode, a platinum electrode and a Ag-AgCl electrode were used as the auxiliary and reference electrodes. All experiments were carried out at room temperature ($25 \pm 2C^0$).

Moxifloxacin Hydrochloride (AR) was purchased from Guangzhou Qiyun Biochemical Technology Co., Ltd.; methacrylic acid (MAA, AR), ethylene glycol dimethacrylate (EDMA, AR) , azodiisobutyronitrile (AIBN, AR) , liquid paraffin (AR) were obtained from Aladdin Reagent Co.,

Ltd.; graphite powder(GR) was purchased from Sinopharm Chemical Reagent Co., Ltd.; Avelox tablet(0.4g $tablet^{-1}$) was obtained from Bayer Health Care AG; The human serum samples were obtained from the healthy volunteer and used without further treatment. Other chemicals used in this study were of analytical grade. All solutions were prepared with double distilled water.

2.2 Preparation of MIP

The MIP was prepared with the following steps: Take 1mmol template molecule Moxifloxacin Hydrochloride to dissolve in 120ml methanol. Add functional monomer, crosslinking agent (the molar ratio of template molecule, MAA, EDMA is 1:4:20), 30mg initiator AIBN. After mixing them thoroughly, purge nitrogen to the mixed solution for 10min. Place the mixture into a thermostatic water bath after sealing. Polymerize at 60 C^0 for 24h. Elute the polymer with methanol and acetic acid mixture (7:3, 8:2, 9:1) until no Moxifloxacin Hydrochloride molecule detected. Then wash out the acetic acid with methanol, Dry the polymer at 60 C^0.

The non-MIP (NIP) was prepared with the same method. The only difference is that no template molecule be added.

2.3 Preparation, activation and cleaning up of modified electrode

The MIP modified electrode (MIP-CPE) was obtained by mixing graphite powder, MIP and liquid paraffin according to a certain proportion. Fill the mixture into a glass tube with a radius of 2.0mm and compress it tightly. Fix a copper wire into the electrode. Smooth the electrode surface on a paper.

The NIP modified electrode (NIP-CPE) be made with the same steps.

Activate or clean up the electrode surface by scanning 20 times at a speed of 100mV·s^{-1} from 0.6 to 1.2V in a B.R buffered solution (pH6.5).

2.4 Preparation of Moxifloxacin Hydrochloride standard solution

Take 0.0479g Moxifloxacin Hydrochloride accurately. Dissolve it with methanol in a 100ml volumetric flask. Place it in a refrigerator for cold storage. Dilute it with buffered solution to required concentration for use.

2.5 Pre-treatment of the samples

Take Avelox tablets (labeled amount 0.4g $tablet^{-1}$). Remove the film coating and grind to powder. Take about 0.15g into conical flask. Add 20ml methanol. After supersonic treatment for 30min, filter it. Take 2ml filtered solution into a 100ml volumetric flask. Dilute it with buffered solution for use.

Add certain amount of Moxifloxacin Hydrochloride standard solution into human serum. Take 2ml mixture into a 100ml volumetric flask. Dilute it with buffered solution for use.

3. RESULTS AND DISCUSSION

3.1 Electrochemical behavior of Moxifloxacin Hydrochloride

The electrochemical behavior of Moxifloxacin Hydrochloride at the bare CPE, MIP-CPE and NIP-CPE were investigated in B.R buffered solution (pH 6.5) using cyclic voltammetry, The result was shown in Fig. 2. It can be seen an irreversible oxidation peak of Moxifloxacin Hydrochloride appears at the CPE with a potential of 0.97 V (curve c). No corresponding reduction peak was observed at the reverse scan, indicating that the electrochemical oxidation of Moxifloxacin Hydrochloride was a irreversible reaction at the CPE under the above experimental conditions. The response on the MIP-CPE increased about 2 times than bare CPE with a potential of 1.02 V (curve a), and the NIP-CPE has a smallest response (curve b). Because MIP has specificity recognition capability for template molecule, Moxifloxacin Hydrochloride molecule can spread to the surface of modified electrode through polymer cavity and take redox reaction; at the same time, due to the enrichment of MIP, the sensitivity and anti-jamming ability of electrode have been increased.

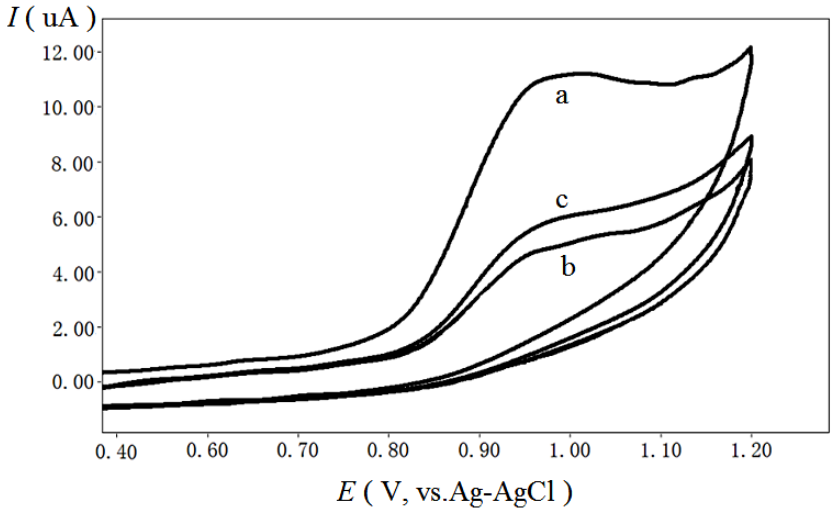

Figure 2. The voltammogram of Moxifloxacin Hydrochloride standard solution at three kinds electrode (a) MIP-CPE (b) NIP-CPE (c) CPE

3.2 Optimization of the test conditions

3.2.1 Influence of polymer ratio

When the proportion of graphite and liquid paraffin is 4:1 (m:m), the Bare CPE will obtain the best response and relatively better peak pattern. Consequently, We make modified electrode with

graphite+polymer and paraffin is 4:1 (m:m), among which the graphite and MIP will be mixed according to proportions 90:10, 80:20, 70:30, 65:35, 60:40, 50:50 (m:m). Scan the electrodes in Moxifloxacin Hydrochloride solution with the same concentration respectively. The results are as shown in Fig. 3. It can be seen that there is almost no current when the proportion of graphite powder and imprinted polymer is 50:50. And when the proportion is 90:10, the peak current will be maximum and the peak pattern will be better. Therefore, the experiment makes modified electrode by graphite+polymer and liquid paraffin as 4:1 (m:m), and the proportion of graphite and MIP as 90:10 (m:m).

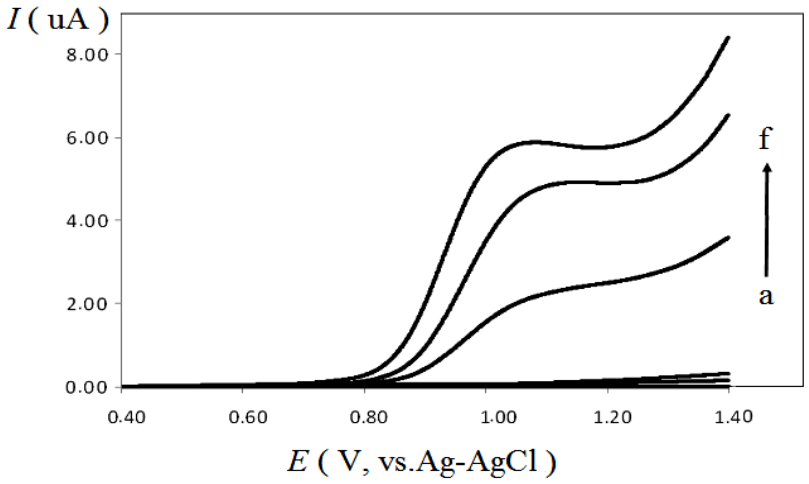

Figure 3. The selection of polymer ratio (curve a-f) : 50:50, 60:40, 65:35, 70:30, 80:20, 90:10

3.2.2 Selection of buffered solution

Perform tests in NaH_2PO_4-NaOH、Na_2SO_4-NaOH、B-R、H_3PO_4、HAc-NaAc HNO_3 H_2SO_4 HCl and other buffered solutions. In B.R buffer solution, it will obtain the best waveform, maximum peak current and better stability. Thus the experiment selects B.R as buffered solution.

3.2.3 Influence of pH

When the pH of B.R buffered solution are different, the peak current will be different. The peak current will increase along with the pH of buffered solution and then decrease after reaching a certain value. When the pH is 6.5, the peak pattern will be better and the peak current will be maximum. Thus pH 6.5 is selected for the B.R buffered solution for the experiment.

3.2.4 Influence of accumulation time

Test the impact of Influence of accumulation time on peak current at two different concentrations of Moxifloxacin Hydrochloride. When the concentration is 1.0×10^{-4} $mol\cdot L^{-1}$, the peak

current will be the maximum at 100s; when the concentration is 1.0×10^{-5} mol·L^{-1}, the peak current will have the maximum at 120s; The smaller as the concentration, the longer accumulation time will be required; the bigger as the concentration, the shorter accumulation time will be required. Thus the experiment chooses 120s as the accumulation time.

3.2.5 Influence of scanning speed

Study the Influence of scanning speed on peak current by cyclic voltammetry method. The results show that the oxidation peak current and scanning rate presents a certain linear relation between 30~500 V·s^{-1}. The results are as shown in Fig. 4. It can be described by the following linear equation: Ip(μA)=0.069v+3.653(r=0.991), which indicates that the oxidation process of Moxifloxacin Hydrochloride is mainly controlled by adsorption. Through the experiment, we can find that with the increasing of scanning speed, the peak current will increase constantly as well, The peak current will move to right continuously. In order to get a better sensitivity and peak pattern, the experiment choose 100mV·s^{-1} as the scanning speed.

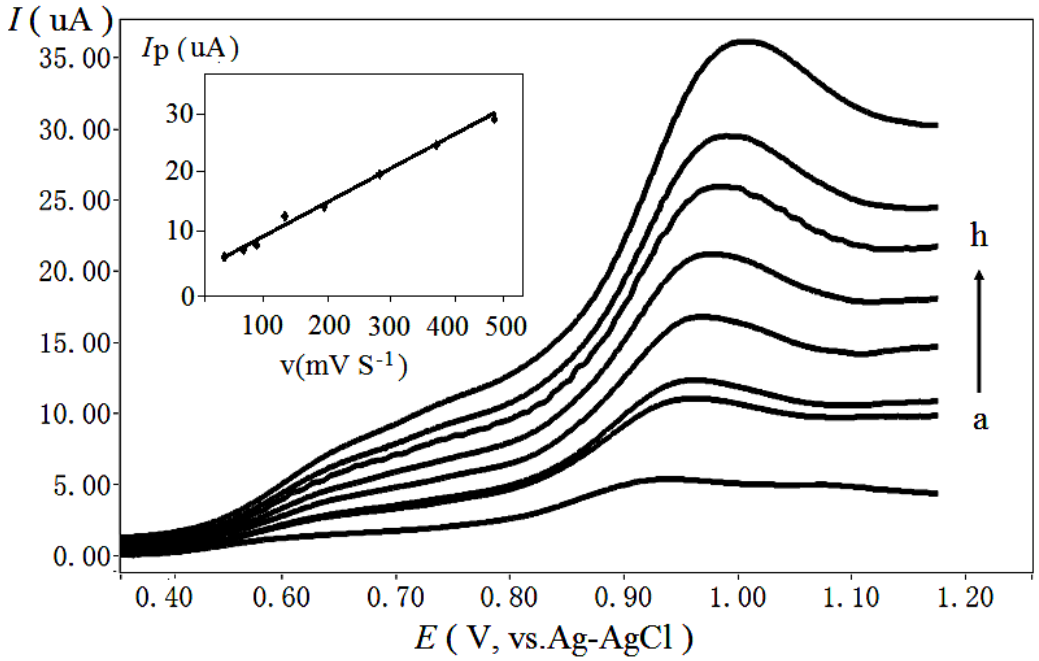

Figure 4. Cyclic voltammograms of Moxifloxacin Hydrochloride at different scan speed, linear relationship between the peak current and scan speed. (curve a-h): 30, 80, 100, 150, 200, 300,400,500 mV s $^{-1}$.

3.3 Interference test

Add 1000 times of Mg^{2+}、Zn^{2+}、K^{+}、Na^{+}、Cl^{-}、CO_3^{2-} into 1.0×10^{-4} mol·L^{-1} into Moxifloxacin Hydrochloride standard solution. The results show that the ions mentioned above have no interference to the determination of Moxifloxacin Hydrochloride on MIP-CPE.

3.4 Calibration curve and detection limit

Under the optimized conditions, When the concentration of Moxifloxacin Hydrochloride ranged from 3.13×10^{-6} to 2.0×10^{-4} mol·L^{-1}. It presents a good linear relationship with peak current. The linear regression equation is : Ip(uA)=62618c +2.819(r=0.999); Based on the signal-to-noise ratio of 3, the detection limit was obtained as 5.9×10^{-8} mol·L^{-1}。

3.5 Sample analysis

In order to evaluate the applicability of the proposed method to the determination of Moxifloxacin Hydrochloride in real samples, the developed method was tested by determining Moxifloxacin Hydrochloride in Avelox tablet and human serum samples. The results are summarized in Table1. The average content of Moxifloxacin Hydrochloride in Avelox tablet is 0.4176g tablet^{-1}. The labeled amount percentage is 104.4%. The results show that the auxiliary materials in tablets, biochemical and inorganic substances in serum have no interference to the determination.

Table 1. Recovery tests of Moxifloxacin Hydrochloride in tablet and human serum

sample	original /×10^{-4} mol·L^{-1}	added /×10^{-4}mol·L^{-1}	found /×10^{-4}mol·L^{-1}	recovery /%	RSD /%
	5.38	5.61	11.24	104.5	2.31
tablet	5.38	4.03	9.57	104.0	3.14
	5.38	6.48	12.02	102.5	2.01
	0.35	0.36	0.70	97.2	3.34
serum	0.35	0.26	0.61	100.0	3.28
	0.35	0.42	0.78	102.4	2.97

The determination method of Moxifloxacin Hydrochloride is mainly as high-performance liquid chromatography, Compared with reference 6, 7 and 8 (table 2), this method has wider linear range and higher recovery with no complicated pre-treatment. It is more convenient for rapid detection.

Table 2. Comparison of this method with references for Moxifloxacin Hydrochloride detection

reference	method	linear range	recovery /%	LOD	RSD /%
[6]	HPLC	0.05-5.0 ug·mL^{-1}	---	0.015 ug·mL^{-1}	---
[7]	HPLC	0.25-10.0 ug·mL^{-1}	97-105	0.03 ug·mL^{-1}	2.9-6.0
[8]	HPLC	15-2700ng·mL^{-1}	94.06-96.63	6ng·mL^{-1}	0.014-4.39

4. CONCLUSION

In summary, an electrochemical sensor based on MIP was developed for the detection of Moxifloxacin Hydrochloride. The sensor showed about 2 times higher current response than the CPE

at the potential of 1.02V. In addition, The sensor exhibited good sensitivity of Moxifloxacin Hydrochloride with the detection limit of 5.9×10^{-8} mol·L^{-1} under accumulation time of 120s. The proposed method was further applied for the detection of Moxifloxacin Hydrochloride in real sample with satisfactory results. It can be applied in determination of pharmaceuticals and biological samples.

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References

1. A.P. de Miranda, C.B. Silva, L.M.J. Mimica, B.K. Moscovici, G.R. Malavazzi, R.Y. Hida, *J Cataract Refr surg* 41(2015) 135-139.
2. S. Ashour, R. Bayram, *Spectrochim Acta A* 140(2015) 216-222
3. L.Q. Zhu, N. Wang, W.J. Yang, Y. Zhang, X.Q. Zhao, S.G. Ji, *J Infect Chemother* 20(2014) 621-626.
4. W. Khan, K.L. Sullivan, J.W. McCann, C.F. Gonsalves, T. Sato, D.J Eschelman, *Am J Roentgenol* 197(2011)343-345.
5. I.C. Gyssens, M. Dryden, P. Kujath, D. Nathwani, N. Schaper, B. Hampel, *J Antimicrob Chemother* 66 (2011) 2632-2642.
6. Y.H. Xu, D. Li, X.Y. Liu, Y.Z. Li, J. Lu, *J Chromatogr B* 878 (2010)3437-3441.
7. A.K. Hemanth Kumar, V. Sudha, R. Srinivasan, G. Ramachandran, *J Chromatogr B* 879 (2011) 3663-3667.
8. S.T. Ulu, *J Pharmaceut Biomed* 43 (2007)320-324.
9. Z.P. Liu, H.Y. Zhai, Z.G. Chen, Q. Zhou, Z.X. Liang, Z.H. Su, *Electrochim Acta* 136 (2014)370-376.
10. C.H. Hu, J. Deng, X.L. Xiao, X.Z. Zhan, K.H. Huang, N. Xiao, S.Q. Ju, *Electrochim Acta* 158 (2015) 298-305.
11. M. Arvand, P. Fallahi, Sensor *Actuat B-Chem* 188(2013)797-805.
12. S. Sadeghi, A. Motaharian, A. Zeraatkar Moghaddam, *Sensor Actuat B-Chem* 168 (2012)336-344.