Preparation and Application of Norfloxacin-MIP/ Polysulfone Blending Molecular Imprinted Polymer Membrane

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ABSTRACT: Norfloxacin-molecular imprinted polymer was prepared by bulk polymerization with Norfloxacin (NFXC) as template molecule, Methacrylic acid (MAA) as functional monomer and Trimethylolpropane Trimethacrylate (TRIM) as cross linking agent. And the imprinted polymer membranes of polysulfone-matrix were also prepared by blending method. The structures of Norfloxacin-molecular imprinted polymer were measured and confirmed by spectra of FTIR and TEM, respectively. Although the combination characteristic and mechanism of this molecular recognition membrane were studied by scan electromicroscope (SEM) and combinative equation experiment, the results showed that the molecular recognition membrane represented high selectivity for Norfloxacin. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 1125–1132, 2009

Key words: molecular imprinted technique; norfloxacin; imprinted polymer membrane; specific selectivity

INTRODUCTION

Molecular imprinted technique, combined with polymer science, materials science, biochemistry, chemical engineering, and else multiple knowledge, was a new experimental technology to obtain polymers with space structure and binding sites perfectly matching to a template molecule.[1–9] As the results of its good combinative characteristic, selectivity, and stability, long service life time and various application, the imprinted polymer has been widely used in chromatography, enzymatic catalyze, clinical drug analysis, membrane separation, solid phase extraction, and many other fields. Meanwhile, molecular imprinted technique had important theoretical significance and value for studying enzymatic structure, recognizing receptor–antibody action mechanism. In this work, norfloxacin, as the selected template, is an excellent representative of quinolone antibacterial drugs.[10,11] It has the characteristic of strong antibacterial activity, high bioavailability, good organizational permeability, none cross drug resistance with other bacteriophage and low side effect, as well as it is easily absorbed after be taken orally. Norfloxacin has been widely used in clinical applications. But there are also some shortcomings, such as toxic action and side effect of fetal malformations and renal damage. And improper dosage can result in some drug adverse reaction, for example digestive tract reaction, nervous system reaction, anaphylactic reaction, and so on. So it is important to control the drug dose and test the drug intake in the human body after using drugs with the purpose of achieving the best curative effect and decreasing side effects. Whereas molecularly imprinted polymer has the properties of specific identification and selectivity adsorption to specially appointed molecule, it can be used as the matrix material for drug enrichment, separation, and analysis. Therefore, norfloxacin molecular imprinted polymer was researched on its synthesis, structure, and adsorption in this article, this work was basic for further applied study.

With the advantages of molecular imprinted technology and membrane separation technology, molecular imprinted membrane (MIM) has become a hotspot in the field of molecular imprinted technology in recent years.[12,13] Its two greatest features were as follows. One was predictable to identify the template molecule; another was to aim at separating specific substances. However, present techniques used to prepare molecular imprinted polymers membrane usually resulted in poor flexibility, low mechanic strengthen and fragile in the presence of an amount of cross linking agent aimed at

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maintaining the space structure of recognition site. Therefore, the further research is trying to find a new method to make the molecular imprinted polymer membranes not only with specific recognition but also with high flexibility. Nowadays, it has been reported that plate membrane was served as supporting matrix; molecular template polymer was coated on its surface. So that the molecular template polymer membrane was prepared. Whereas, the interaction between polymer and supporting membrane, as usual, is depended on weak intermolecular forces. After repetitive using, this functional polymer will fall off from the supporting membrane. In this case, the membrane will no longer have selectivity.

In our work, a novel material of Norfloxacin (NFXC) molecular imprinted polymer (MIP) with the specific selectivity of recognition on Norfloxacin (NFXC) both in three-dimensional structure and binding sites, was used as a carrier of recognition agent and blended with PSF to prepare a new type of molecular imprinted polymer membrane. This study laid a foundation for the further applied research and provided a new idea on controlling the drug dose and tests the drug intake in the human body.

MATERIALS AND INSTRUMENTS

Materials

Norfloxacin (NFXC) and Trimethylolpropane Trimethacrylate (TRIM) were of analytical grade available from Beijing Bailingwei (Beijing, China) and used as received. Methacrylic acid (MAA) was obtained from Tianjin Chemical Reagent Research Institute and was purified by vacuum distillation to remove inhibitor before use (Shanghai, China). 2,2-Azo-bis-isobutyronitrile (AIBN) was of chemical grade and was purchased from Shanghai Hewei (Shanghai, China). *N*,*N*-dimethylformamide (DMF) was available from Tianjin Chemical Reagent III (Tianjin, China). Polysulfone (PSF) was utilized as received from motian company of Tianjin Polytechnic University (Tianjin, China).

Instruments

Ultraviolet-visible Spectrophotometer (UV-240) was purchased from Japan Shimadzu (Japan). Speed Adjusting Vibrator (HY-4) was purchased from Tianjin HuaBei experimental instrument (Tianjin, China). Long wave ultraviolent lamp (365 nm) was purchased from Tianjin zijin Special Source (Tianjin, China). Fourier Transform Infrared Spectrophotometer (FTIR) (TENSOR37) was obtained from BRUKER (Germany). Transmission electron microscope (TEM) (H7650) was available from TOSHIBA. Scan electron microscope (SEM)(QUANTA-200) was purchased from FEI (USA).

EXPERIMENTAL

Preparation of norfloxacin (NFXC) molecular imprinted polymer (MIP)

Norfloxacin (NFXC) (0.04785 g, 0.15 mmol) and functional monomer MAA (0.05 mL, 0.625 mmol) were dissolved in *N*,*N*-dimethylformamide (0.5 mL) with shaking for 6 h to form homogeneous solutions, then TRIM (Trimethylolpropane Trimethacrylate) (0.5 mL) and AIBN (7.5 mg) were added into the solution in turns. After prepurged with nitrogen gas for 5 min to displace oxygen and removed nitrogen gas under vacuum for 10 min, the mixture was spread on the glass surface, put it into quartz glass plates, and then irradiated with 365 nm by a UV light under the protection of nitrogen gas for 5–6 h, a homogeneous polymer membrane with the thickness of 100 μm could be obtained. The polymer membrane was washed again and again by using 10% acetic acid-methanol (V) solution to remove the template molecules and cross linking agent, and then skived it into powder. Through 100 mesh sieve, this polymer powder was dried under vacuum for 24 h on standby.

The preparation of non template polymer (NMIP)

The functional monomer MAA was dissolved in *N*,*N*-dimethylformamide with shaking for 6 h to form homogeneous solutions, then TRIM and AIBN were added into the solution in turns. After prepurged with nitrogen gas for 5 min to displace oxygen and removed nitrogen gas under vacuum for 10 min, the mixture was spread on the glass surface, put it into quartz glass plates, and then irradiated with 365 nm by a UV light under the protection of nitrogen gas for 5–6 h, a homogeneous polymer membrane with the thickness of 100 μm could be obtained. The polymer membrane was washed again and again by using 10% acetic acid-methanol (V) solution to remove cross linking agent, and then skived it into powder. Through 100 mesh sieve, this polymer powder was dried under vacuum for 24 h on standby.

Preparation of norfloxacin-MIP/polysulfone blending molecular imprinted polymer membrane

An amount of polysulfone (PSF) (1.3 g) was dissolved in DMF (8.2 g) to form PSF solution. Then the quantitative Norfloxacin-MIP powder (0.5 g) that was ground and sifted was put into the PSF solution and the solution of Norfloxacin-MIP/PSF was rigorously stirred for 24 h. After vacuum deaeration, a certain composition of Norfloxacin-MIP/PSF casting solution could be obtained. The Norfloxacin-MIP/PSF casting solution was cast on glass pane and

coagulated in water at room temperature to form a smooth Norfloxacin-MIP/Polysulfone blending molecular imprinted polymer plate membrane. The membranes were preserved in wet state to prevent the pores of membrane from contracting.

UV spectrometry on the mixture solutions of norfloxacin and functional monomers

Norfloxacin was firstly dissolved in DMF solution to prepare for the concentration of 0.05 mmol/L solution. By adding different doses of α-methacrylic acid (MAA), a series of solution were compounded. The ratios of the molar concentration between Norfloxacin and α-methacrylic acid (MAA) among this set of solutions were: 1 : 0, 1 : 1, 1 : 2, 1 : 3, 1 : 4, 1 : 5, 1 : 6, 1 : 7, and they were shaken for about 4–5 h aiming at sufficient interaction. Then the absorption spectra of these solution were measured on UV-Spectrometer, using the corresponding concentration of MAA-DMF solution without the accession of Norfloxacin as the reference solution.

Analysis of the FTIR

Norfloxacin-molecular powder, non-eluted Norfloxacin-molecular imprinted polymer powder with template molecules, and post-eluted Norfloxacin-molecular imprinted polymer powder without the template molecules were grinded into fine powder. After they were dried completely, made test samples in pressed disc method with KBr. Then scan to obtain their infrared spectra, respectively, using TENSON37-Fourier transform infrared spectrometer. And then the polymers of molecular structure were analyzed, comparatively.

Analysis of the TEM

Put the molecular imprinted polymers with a certain degree of fineness into ethanol to prepare suspension of an appropriate concentration. And scatter for 15 min in the ultrasonic oscillator. Then employ the professional copper net to dredge powder samples, quickly. Or just adopt a point sample method to make samples under test. After removed the volatile ethanol absolutely, they could be observed in the TEM.

Analysis of the SEM

The membranes were immersed into 50% (by volume) glycerol for 24 h. Then taken them out, wiped the glycerol on surface, and dried up. Broken them in liquid nitrogen and gilt in the end. The surface and the transect of these membranes were observed by QUANTA-200 SEM.

Determination of combinative amount of MIP/NMIP to NFXC

Norfloxacin with different mass were added in DMF (10 mL) to form a series of different concentrations of substrate solution, then Norfloxacin-molecular imprinted polymer (20 mg) were added, respectively. The conical flasks which were filled with solutions were set into a shaking table with shaking several hours at room temperature. Then these combined solutions were transferred into the centrifuge tubes. After 15 min, high-speed centrifugation and stewing, supernatant liquor was determined by UV. Under certain wavelength, UV spectrophotometer was used to determine the free concentration of Norfloxacin in these liquid of equilibrium adsorption. According to the mole changes of substrate before and after adding MIP, the combination amount Q of MIP to NFXC could be calculated. The experimental steps of non-imprinted polymer membranes (NIPM) to the substrate (NFXC) were the same as above. The only difference was to add 20 mg NIPM instead of MIP.

RESULT AND DISCUSSION

Choice of monomer

In the preparation process of molecular imprinted polymer, the combination between functional monomer and template was close to the extent possible to fix the template firmly in the polymerization process and array the function groups by the identified position. On the other hand, the template which has been synthesized polymer was removed completely to the extent possible and the functional groups combined with the substrate (such as template compounds) had good interaction. In this article, hydrogen bonding was the main force. On the one hand, the purpose of rapid combination could be achieved. On the other hand, the combination of high specificity could be provided by multiple interactions. In the experiment, α-methyl acrylic acid (MAA) was chose as a functional monomer. It could combine with norfloxacin (NFXC) to form complex substance by using hydrogen bonding.

Analysis of the FTIR

FTIR spectra were one of the important physical methods to determine organic compounds. It was used to conclude the structure of template molecule and molecular imprinted polymer.

Figure 1 indicated IR spectra of eluted (a) and not cleared (b) norfloxacin molecular imprinted polymers. From the (b) curve, the C=N, C–NH, C=C group peaks appeared at 2550.76, 2287.21, and 1486.52 cm^{-1}. The C=C bond stretching of benzene

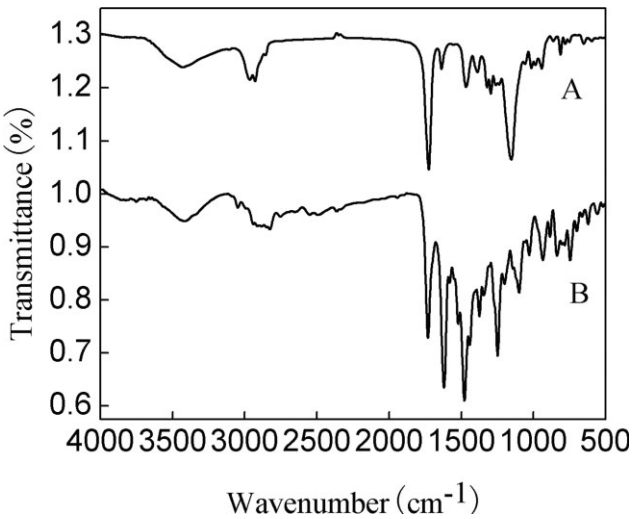


Figure 1 FTIR spectra of NFXC molecular imprinted polymers (NFXC–MIP) (a) before elution and (b) after elution.

showed at 1338.08 cm^{-1}. It can be inferred the structure of not eluted molecular imprinted polymer of norfloxacin combined with the template molecules. Combined with the (a) one, (b) curve showed C=O, C=C group peak at 1725.60 and 1637.56 cm^{-1}. This is shown in the reaction to the post-eluting polymer molecules to be the characteristics of functional groups in line. In theory, Eluted polymers didn't exist template molecules, but they were fully formed by functional monomer α-methyl acrylic acid. Although the C—NH, aromatic amines C—N (1033.79 cm^{-1}) and C—F absorption peak of norfloxacin disappeared (1274.48 cm^{-1}), it showed template molecules of imprinted polymer were eluted cleanly to form a hole.

Analysis of UV spectrum

Nittsson et al. used chromatography method to prove that an ionic interaction between aliphatic amine and carboxylic acid can be generated.[14] And the hydrogen bonds can also form between organic acid. It can be used to speculate the action mode between a molecular template and functional monomer of MAA. In this article, UV-spectrophotometry was utilized for further evidence of the role of hydrogen bonds between a template molecule and functional monomers. To design the appropriate reaction ratio for preparing molecularly imprinted polymers and forecast polymers' selectivity and combination mechanism, a certain concentration of NFXC-DMF solution by adding different concentrations of the MAA were determined by UV absorption spectra, and the changes of these UV spectrums were shown in Figure 2.

Figure 2 shows that, with the concentration of MAA increased in DMF, to a certain concentration

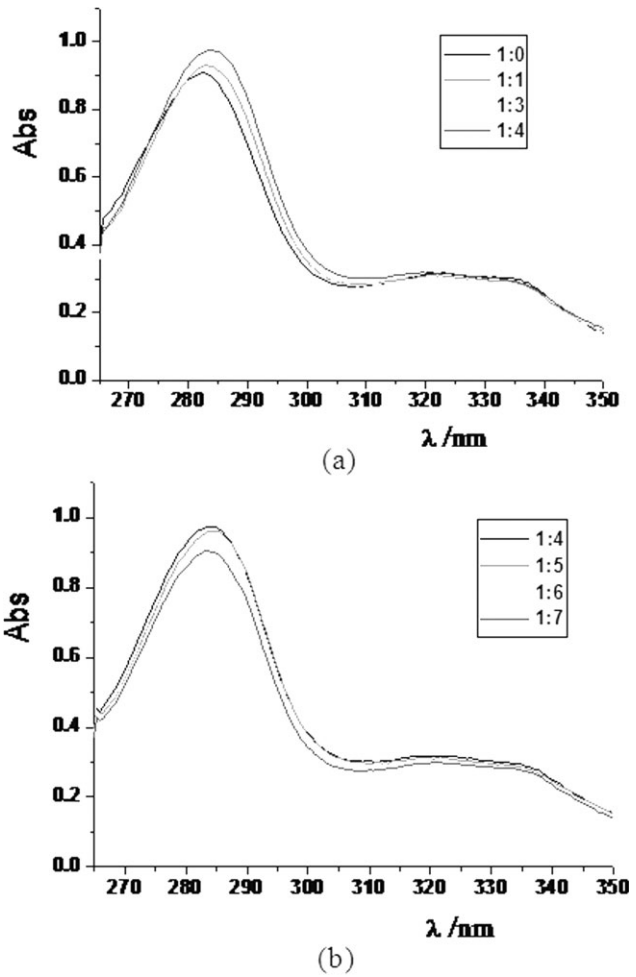
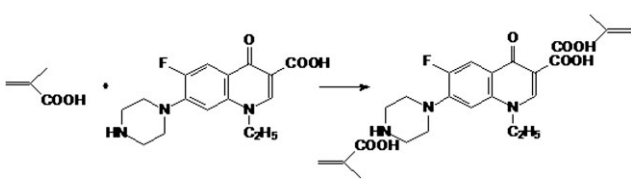


Figure 2 The UV absorption spectrum changes of NFXC after adding MAA in DMF.

of molecular template, maximum absorption wavelength of NFXC red-shift and the absorbency are also increase, gradually. This is caused by the impact of hydrogen bonds to the first $\pi \rightarrow \pi^*$ absorption bands of the elements with chromophore as a proton donor. The red shift phenomenon of UV absorption peak can proof that a complex form under the interaction between template molecule and functional monomers. According to the evidence of the structure both of template molecule and functional monomers, forces between the two should be the hydrogen bonds. The role of NFXC and MAA was as shown in Scheme 1. The above results show that the functional monomer MAA, in solution, can be



Scheme 1 Action between NFXC and MAA.

self-assembly with hydrogen bonds. This subject and object structure after assembly will enter into the polymer matrix through a large number of cross-linking agent, which can form selective binding site to NFXC in the imprinted polymer. Generally speaking, increasing the proportion of functional monomers allows the combination capacity of MIPs become larger. This is because the smaller amount of MAA is only able to form a complex with a small number of NFXC. There are a large number of non-combined elements exist, which will lead to be less identify sites to NFXC in this form of MIPs resulting in lower binding capacity. When increase the amount of MAA, the self-assembly between NFXC and MAA can carry out more fully to form a stable complex. However, the more proportion of functional monomer is not always better. As can be seen from the above chart, when molar ratio of NFXC to MAA is less than 1 : 4, its maximum absorption wavelength and the maximum absorption have reduced. As the excessive MAA would result in non-selective binding site in the identification process, and would lead to self-association of its own, the selective binding sites, however, reduced. Therefore, the ratio of n (MAA) : n (NFXC) = 4 : 1 was chosen as the best volume of functional monomers in this experiment.

Analysis of the TEM

The transmission electron microscope (TEM) revealed the inter-relevance between macro-performance and microscopic character of materials. Figure 3 shown that the molecular imprinted polymer internal structure was observed by TEM. TEM picture appeared many holes in the molecular imprinted polymer. From the TEM picture, we can estimate the diameter of the holes around dozens of nanometers. Contrasted with their microscopic structure, the high adsorption capability of molecular imprinted polymers has been confirmed.

When NFXC had not been washed off from imprinted polymers, there was no hole in it as NFXC combined with functional monomer by non-covalent bonding. After washing off NFXC, it appeared many "holes". The information of template molecules, such as structure, size and spatial position, was imprinted into these "holes". Only template molecules could enter them so as to choose the right template molecules only.[14]

Analysis of the SEM

The different proportions of NFXC molecular imprinted polymer membrane was scanned by SEM. Their structure and morphological constitution changes were shown in Figures 4 and 5.

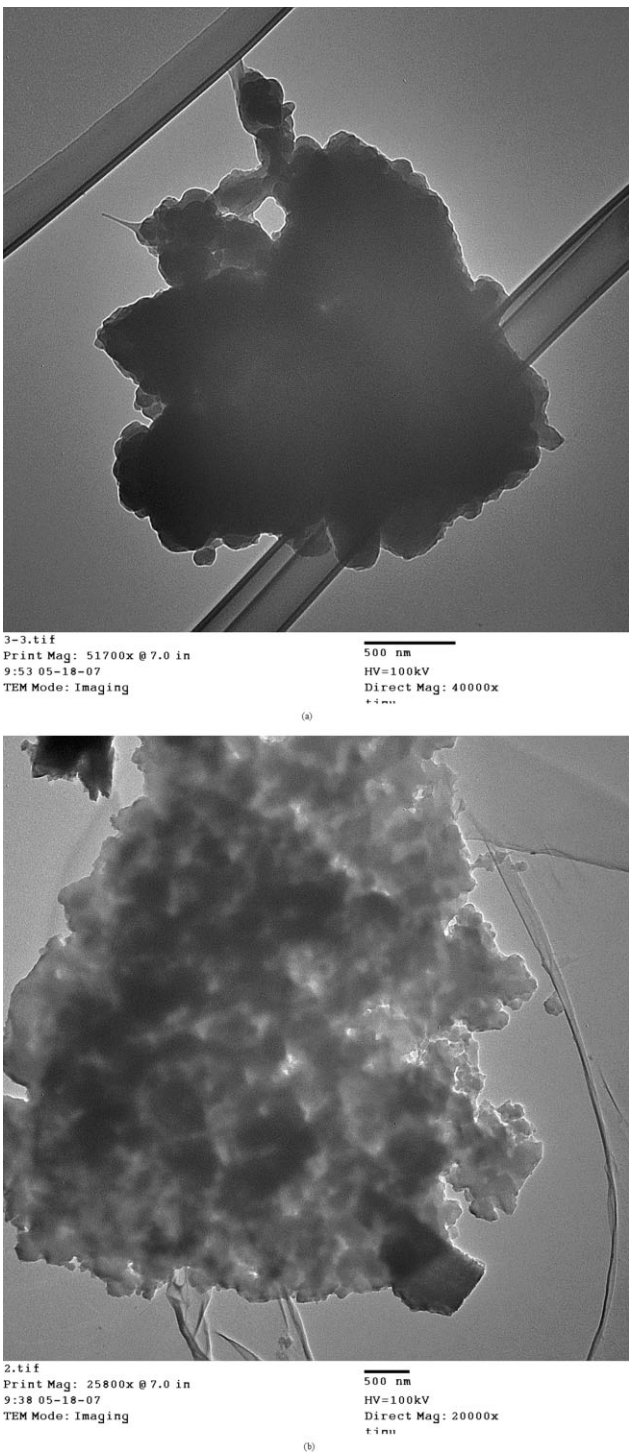


Figure 3 TEM of NFXC molecular imprinted polymers (a) before washing and (b) after washing.

Figure 4 showed molecular imprinted polymers could be more evenly dispersed in the PSF membrane surface.

According to Piletsky's mass transfer mechanism of the "gate" model,[15] with the effect of concentration gradient, imprinted molecule A and other molecules B possibly had the same direction of

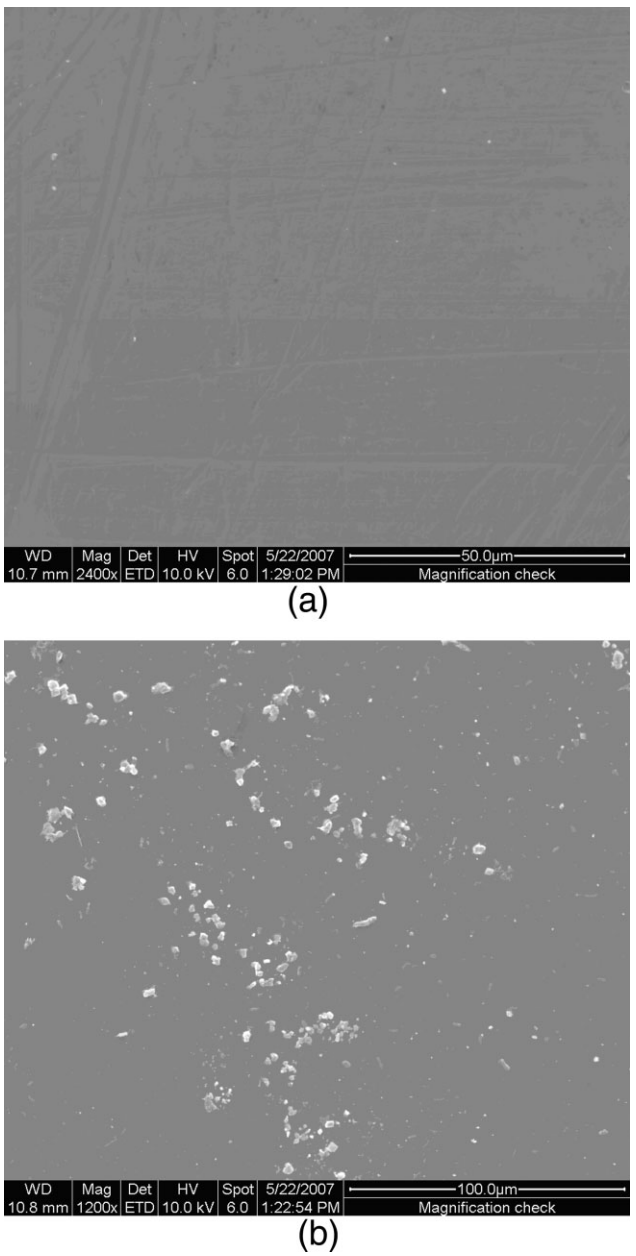


Figure 4 SEM of the surface structure of plate membrane (a) PSF plate membrane (b) NFXC-MIP/polysulfone blending molecular imprinted polymer membrane.

pervasion. But B which was not identified would be blocked off the membrane structure of pores. Only with the interaction between imprinted molecule A and identify sites, imprinted molecule A could move from one binding site to another, and then permeate through molecular imprinted membrane eventually. When the solution of NFXC passed the membrane surface, molecules mainly depended on diffusion to penetrate into the internal membrane. In this process, Molecular imprinted polymers of membrane surface would adsorb NFXC which passing the membrane. When the solution of NFXC entered the interior of membrane, it could be adsorbed by molecular imprinted polymers which dispersed/diffused around membrane pores. Because of the

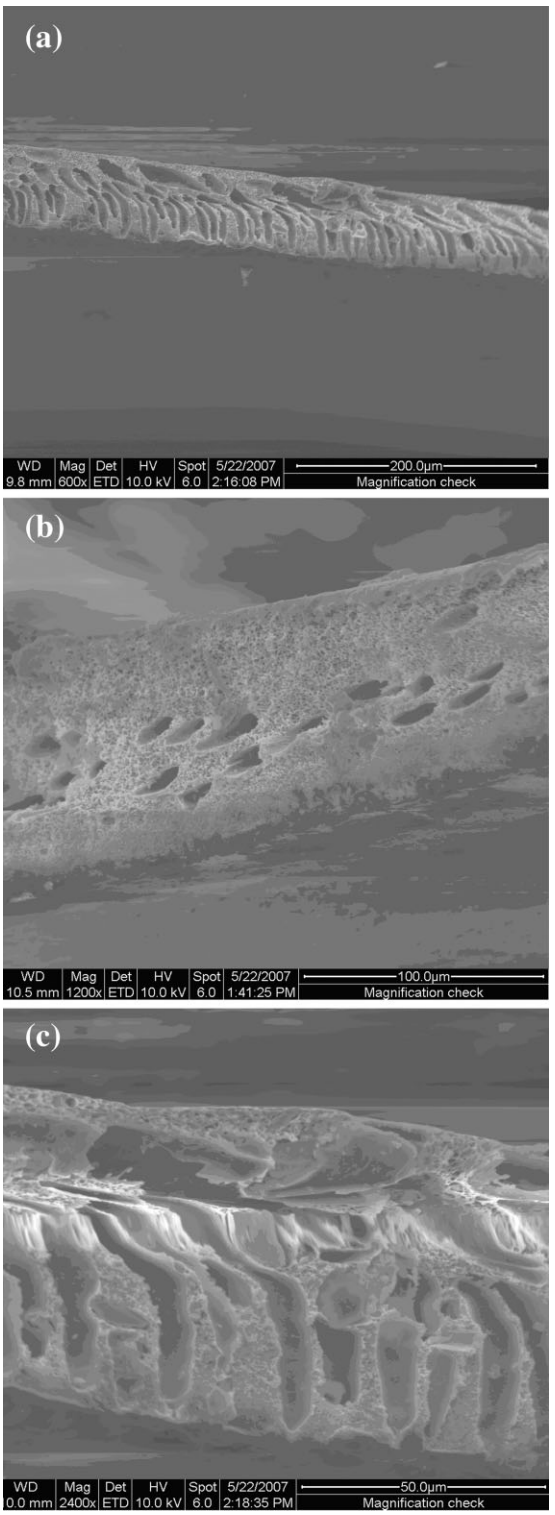


Figure 5 SEM of cross section of NFXC-MIP/polysulfone blending molecular imprinted polymer membrane with deferent content of NFXC-MIP (a) Content of NFXC-MIP : 10% (b) Content of NFXC-MIP : 12% and (c) Content of NFXC-MIP : 15%.

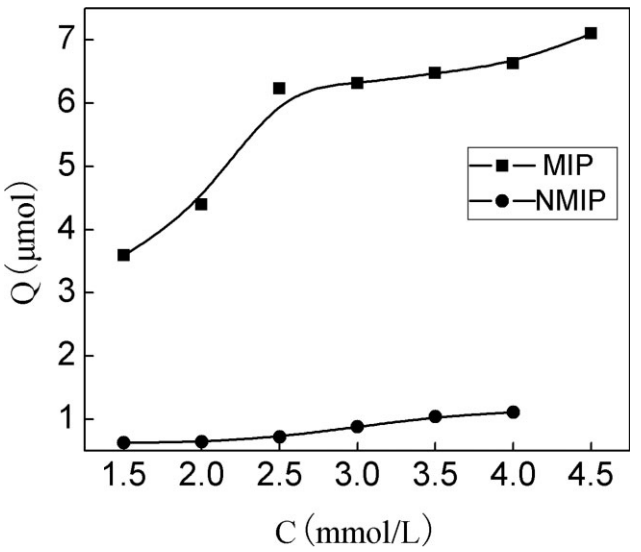


Figure 6 Variation of combinative capacity with initial concentration of NFXC.

interconnection of pores, the solution of NFXC would outflow from the other side. Therefore molecular imprinted polymer membrane had higher adsorption to the template molecule, but the pure/simple polysulfone membrane could adsorb a little NFXC. This process could be inferred by Figure 6. Through the blending process of molecular imprinted polymer and polysulfone, the cavity structure of molecular imprinted polymers had not been disrupted. Meanwhile, it can be seen from Figure 6 that the pore size and pore rate of polysulfone membrane had not been affected greatly.[16]

Recognition abilities of molecular imprinted polymer (MIP)

To easily evaluate the recognition abilities of MIP, a method of balancing combination determination was used. In this experiment, The equilibrium adsorption amount of imprinted polymer, marked as Q, was changed along with the initial concentration of NFXC which varied from 0.5 to 5mmol/L. Table I was to explain the process.

To recognize NFXC, MIP was better than NMIP. Meanwhile, the equilibrium adsorptive amount of MIP was larger than NMIP's in the same circumstances. Scatchard equation, which is commonly used to evaluate the recognition abilities of molecular template polymer, was adopted to analyze the combative data. This equation was as follows:

$$Q/C(NFXC) = (Q_{\max} - Q)/K_d \quad (1)$$

where, Kd was the equilibrium dissociation constants in balance binding site; Qmax was the maximum apparent combination volume in binding sites, C(NFXC) indicated the equilibrium concentration of NFXC in adsorption liquid. According to the Scatchard analysis, the relationship curve of Q/C(NFXC) and Q became almost straight (see Fig. 6). This linear equation was as follows[17]:

$$Y = 216.817 - 33.9x \quad (2)$$

The slope coefficient and intercept were $-1/Kd$ and Qmax/Kd, respectively. It could be learned that MIP existed a group of equivalent binding sites within the extent of concentration we studied, and presented uniform binding capacity to the NFXC. In Figure 7, the slope and intercept of the straight-line could be obtained (Kd = 0.5 mmol/L and Qmax = 129 μmol/g), which were different from the theoretical maximum adsorption capacity. This was mainly because that part of NFXC molecules were embedded in the polymer during the polymerization process, which could not be eluted clean. Another reason was that DMF employed in the solution of NFXC, the polarity of DMF was so strong that it was not easy to form stable hydrogen bonds. Therefore, it had a certain impact on the adsorption of polymers. However, seen from the above data, this impact was not obvious. When compared with non-imprinted polymer, the imprinted polymer had higher adsorption to NFXC.

TABLE I
The Combination Amount of MIP or NMIP with NFXC

Different initial concentration of DMF solutions (substrate: NFXC)	MIP			NMIP		
	C^{M_0} (mmol/L)	C^{M_t} (mmol/L)	Q^M (μmol)	C^{NM_0} (mmol/L)	C^{NM_t} (mmol/L)	Q^{NM} (μmol)
1	5.0	3.76	9.89	5.0	4.71	2.31
2	4.5	3.69	7.10	4.5	4.23	2.16
3	4.0	3.11	6.47	4.0	3.86	1.11
4	3.5	2.71	6.63	3.5	3.37	1.04
5	3.0	2.17	6.31	3.0	2.89	0.88
6	2.5	1.72	6.23	2.5	2.47	0.72
7	2.0	1.45	4.39	2.0	1.92	0.64
8	1.5	1.05	3.59	1.5	1.42	0.64

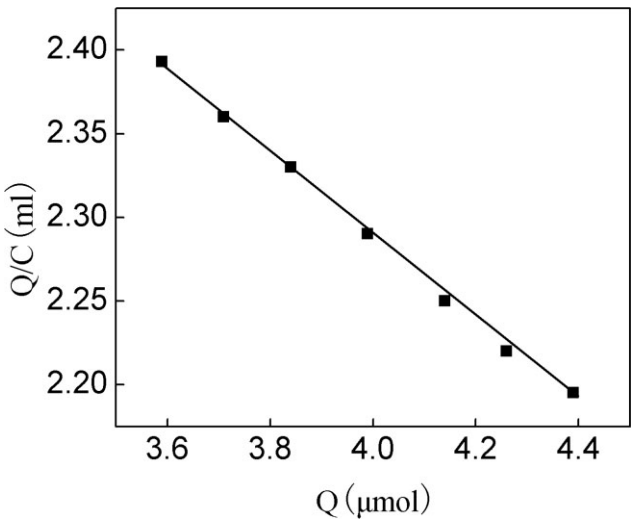


Figure 7 Scatchard curve of NFXC–MIP.

In addition, the adsorptive capacity of non-imprinted polymer (NMIP) raised with the increasing of concentration in the extent of 0.5–5mmol/L. While the imprinted polymer increased first, within this concentration range, and then gradually tended to be saturation. In many binding experiments of the template, as the linear increasing of non-selectivity, the relational curve of combination amount to the initial concentration was often difficult to reach to the saturation point. So it could be learned that the adsorption of NMIP to NFXC was non-selective, but the adsorption of MIP to NFXC was selective. Comparing the synthesis process of these two polymers, it can be inferred that the reason for this selective combination capability of MIP to NFXC was the formation of holes matching with the shape and function position of NFXC in the imprinted polymers inner. Because of the existence of these holes, MIP had good selective combination capacity to the template molecules. It also indicated that the interactional force between template molecules and functional monomers in the molecular imprinted polymers played a very important role in the imprinted polymers' selectivity.

CONCLUSION

From this study, Norfloxacin (NFXC) molecular imprinted polymers were synthesized and characterized by FTIR spectra and TEM. To NFXC molecule, the recognition abilities of polysulfone membranes blended with molecular imprinted polymers were specific. It was confirmed by balanced combination determination and analysis of SEM.

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