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RESEARCH ARTICLE

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Synthesis and characterization of molecularly imprinted polymers for recognition of ciprofloxacin

Abstract A molecularly imprinted polymer (MIP), with special molecule recognition properties of ciprofloxacin (CIP), was prepared by thermal polymerization in which ciprofloxacin acted as template molecule, α-methacrylic acid (MAA) acted as functional monomer and trimethylolpropane trimethylacrylate (TRIM) acted as crosslinker. The optimized ratio was determined to be *n*(CIP): *n* (MMA):*n*(TRIM) = 1:6:16 by investigation of the effects of different concentrations of functional monomer and the crosslinker on the MIP's recognition properties. Equilibrium binding experiment was used to investigate the adsorption dynamics, the binding ability to template molecule and the substrate selectivity. Scatchard analysis was used to study the MIP's binding characteristic to template molecule. The results indicated that MIP has higher adsorption ability and selectivity. The equilibrium distribution coefficient K_D was 41.64 and the separation factor α was 1.62. Scatchard analysis showed that two different kinds of binding sites were produced in the polymer matrix and their dissociation constants were calculated to be $K_{d1} = 5.249 \times 10^{-5}$ mol·L^{-1}, $K_{d2} = 2.237 \times 10^{-3}$ mol·L^{-1}.

Keywords molecularly imprinted polymer, ciprofloxacin, adsorption characteristic

Molecular imprinting technology (MIT) refers to the preparation of a polymer with special recognition properties of a specific target molecule (template molecule, imprinted molecule) [1]. Wulff [2] gave the first report of a synthetic molecularly imprinted polymer in 1972, and Mosbach [3] issued a molecularly imprinted polymer of theophylline which was prepared under the intermolecular interaction principle in Nature in 1993. Since then the molecular imprinting technique started to develop flourishingly. Molecularly imprinted polymers prepared through MIT have many characteristics, such as high affinity and selectivity, strong ability in an anti-adverse environment, good stability, long service life, wide application range and so on; they have been used in many fields, including chromatographic analysis [4,5], solid phase extraction [6,7], biosensors [8,9], membrane technology [10,11] and so on.

Quinolones (QNs), which have undergone rapid development in the past 20 years, are a kind of important broad-spectrum antibiotics. They are used extensively for treatment, prevention and growth promotion [12]. As one of the most representative third-generation quinolones, ciprofloxacin has excellent antibacterial activity. Thus far, cyclopropyl has been considered as the best 1-substituent. This change in structure is significant in the history of the development of quinolone antibiotics [13]. Close attention has been widely paid to the residue problem, such as drug resistance of pathogenic bacteria and potential carcinogenicity of some quinolones [12–14], but it is difficult to analyze the residue of QNs accurately due to severe matrix interferences and low levels of drug residue. The authors wish, through this paper, to achieve the elimination of matrix interference and establish effective purification by using MIP with special recognition properties. Caro and Marcé [15,16] synthesized an MIP using ciprofloxacin and enrofloxacin as template molecules, respectively, by non-covalent ways. By using the MIP, they separated ciprofloxacin and enrofloxacin from human urine and pig liver through a two-step solid phase extraction method and got good purification results. Based on references [17–20], we synthesized an MIP with recognition properties by using ciprofloxacin (CIP) as template molecule and trimethylolpropane trimethylacrylate (TRIM) as crosslinker. In order to greatly reduce the amount of crosslinker while keeping the crosslinking degree unchanged, the larger number of binding sites per unit mass of MIP was thus adopted. The effects of different concentrations of functional monomers and crosslinker on the MIP's recognition properties were

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investigated. The optimized ratio of the polymerization was experimentally determined. Equilibrium binding experiment was used to investigate the adsorption dynamics, the binding ability to the template molecule, and the substrate selectivity. Scatchard analysis was used to study the MIP's binding characteristic of the template molecule. This paper provides a basis for the residue analysis of ciprofloxacin in biological samples.

1 Experimental

1.1 Instruments and chemicals

TU-1810 UV-Vis Spectrophotometer (Beijing Purkinje General Instrument Co., Ltd); LG10-2.4A Centrifuge (Beijing Lab Centrifuge Co.,Ltd); THZ-82 Vibrator with Homothermal Water Bath (Changzhou Guohua Electric Appliance Co., Ltd); KQ-250B Ultrasonic Cleaner (Kunshan Ultrasonic Equipment Co., Ltd); BF-2000M Nitrogen Evaporator (Beijing Bafang Century Technology Co., Ltd).

Ciprofloxacin (CIP), Levofloxacin (LVFX)(Beijing Institute for the Control of Pharmaceutical and Biological Products); α-Methylacrylic Acid (MAA), Trimethylolpropane Trimethylacrylate (TRIM), Methylbenzene; Methanol, Acetic Acid, all analytically pure, were obtained from Tianjin; Azobisisobutyronitrile (AIBN, chemically pure, Shanghai).

1.2 Preparation of MIP of ciprofloxacin

0.331 g CIP (template molecule) and a certain amount MAA (functional monomer) were weighed into a flask, dissolved in 6 mL methylbenzene, and finally ultrasonicated to ensure complete association. TRIM (a certain amount) and AIBN (0.056 g) were added to the flask and the mixture was subjected to further ultrasonication. The flask was flushed with nitrogen and sealed immediately, and then left to polymerise in a water bath at 60°C for 24 h. The resultant white rod polymer was crushed, ground and then sifted through sieves with mesh sizes of 200–300 to obtain polymer particles with diameters between 54 and 74 μm. The material was washed with methanol-acetic acid (8:2 *V*/*V*) solution under ultrasonication in order to eliminate template molecules. After ultrasonication, the mixture was centrifuged and the supernatant was removed. The washing procedures were repeated until no template molecule in the supernatant could be detected via UV-Vis spectrophotometer. After that, methanol was used to wash the particles until pH 7. The MIP was obtained by drying at room temperature.

A non-imprinted, control polymer (NIP) was prepared in the same way as the MIP, except for the absence of ciprofloxacin.

1.3 Determination of adsorption properties

Aliquots of MIPs of ciprofloxacin (50 mg) were weighted respectively and put into several 25 mL colorimetric tubes, and then a series of aqueous solutions (5 mL) with different substrates were added. The tube was put in a 25°C water bath and homothermally vibrated for a few minutes. After vibration, the supernatant was used to analyze the concentrations of substrates by UV spectrophotometry. The calculation of the adsorption capacity of every polymer was based on the concentration change before and after the adsorption.

2 Results and discussion

2.1 Optimization of polymerization conditions

2.1.1 Effect of concentration of crosslinker on properties of MIP

We had tried to synthesize the MIP with the condition that the molar ratio of TRIM to CIP was 4:1. However, the polymer obtained appeared very soft due to the small amount of crosslinker. The diameter of the polymer was below 54 μm ever after, and the polymer was only slightly ground. As shown in Fig. 1, the binding capacity of MIP increased first with the increasing of the amount of TRIM, reached the maximum when *n*(TRIM):*n*(CIP) = 16:1, and then decreased gradually after that. The explanation is as follows: As the amount of crosslinker was small, although the long free polymer chains with few crosslinking sites were of large pore diameters and low mass transfer resistance, the aggregation morphology of the polymer would have an adjustment after elution, and the polymer could not maintain the original shape and size of its cavities. This changed the binding sites and resulted in bad binding effects for ciprofloxacin. An increased amount of crosslinker would

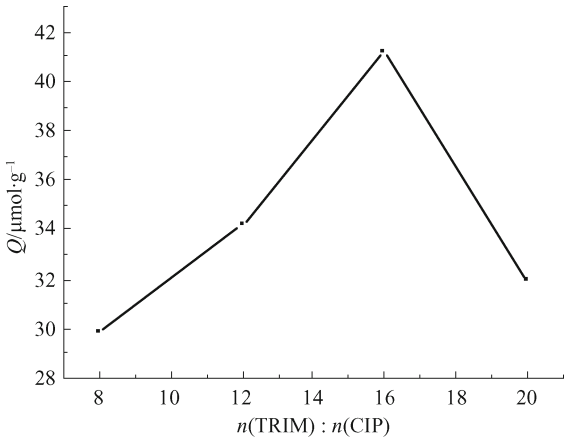

Fig. 1 Effect of different concentrations of cross-linker on the binding capacity of MIP

enhance the rigidity of the polymer and reduce the swelling in different solvents. However, the pore diameter would become smaller although the formed pore structure was more stable when excessive crosslinker was used. The mass transfer of the imprinted molecule in the polymer system would be hindered by a highly crosslinked net and the imprinted molecule could not get into the interior of the polymer so that they could not be bound with recognition sites, resulting in a decrease of the binding properties. After optimization, when n(TRIM) : n(CIP) = 16 : 1, the prepared polymer has a moderate crosslinking degree and rigidity and shows the best binding characteristics for ciprofloxacin.

2.1.2 Effect of concentration of functional monomer on MIP properties

Ciprofloxacin belongs to the pyridonecarboxylic acids; it has two ionizable functional groups, i.e. an acidic carboxyl group and an alkaline piperazine group. It could be concluded that there were hydrogen bonding and ionic interactions between CIP and MAA. The effect of the amount of MAA on MIP properties can be seen in Fig. 2. When a low concentration of functional monomer was used, only a small portion of CIP could form stable complexes with MAA, and most of the CIP molecules were still in the free state. Under such a condition, the density of the formed stereo cavities which could match with the structure of CIP was low and the recognition sites were small; therefore, the binding capacity was low. With the amount of functional monomer being increased, the self-assembly between CIP and MAA could be performed more sufficiently. More stable complexes could be formed, and more stereo cavities matching the structure of CIP could be obtained and the adsorbance increased. The Q-value reached maximum when n(MAA):n(CIP) = 6:1. If the ratio of functional monomer to CIP increased continuously, excess MAA could be left in the MIP. This could make the freely distributed –COOH group increase. In the process of recognition, MAA would interact with CIP in a non-spatial specific form and compete with the imprinting recognition interaction in the MIP. Moreover, the excess functional monomer would also initiate self-association, and this would result in the decrease of selective recognition sites and decreased Q-value.

In conclusion, after the experiment, the ratio of n(CIP): n(MAA):n(TRIM) was optimized as 1:6:16.

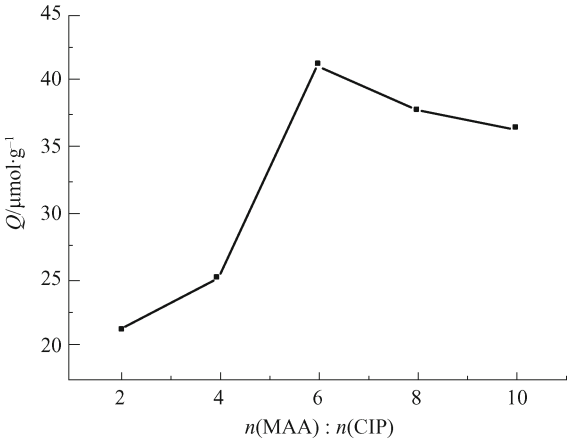

Fig. 2 Effect of different concentrations of functional monomer on binding capacity of MIP

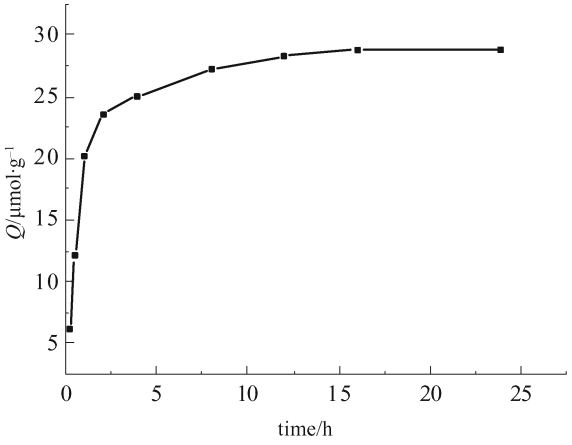

Fig. 3 Effect of adsorption time on amount of adsorption

2.2 Binding kinetics of MIP

To determine the binding rates of MIP for CIP, an equilibrium binding experiment was adopted to detect the binding capacity of MIP with CIP in the solution of CIP under different times at 25°C. The obtained adsorbance-time curve is shown in Fig. 3.

As shown in Fig. 3, the adsorbance of all samples increased rapidly in the first 2 h with the adsorbance covering 82% of the total adsorption, and then increased at a much slower rate with prolonged time. After 8 h, the adsorption process reached equilibrium and the ratio of adsorbance to total adsorption was 94%. The reason for this is that the stereo cavities in MIP formed by the crosslinker and the functional monomer distributed at different depths. In the early stage, the adsorption for template occurred mainly on the outer surface of particles and therefore the binding rate of adsorption was fast. However, when the binding on the outer surface reached saturation, the template molecule diffused inward through pore canals and then was adsorbed. Because the adsorption rate was affected by the mass transfer resistance, the binding rate was decreased. It is shown *via* the decreased slope of the adsorption curve. After 8 h, the process reached adsorption equilibrium.

2.3 Equilibrium adsorption experiment

The static equilibrium adsorption experiments for the imprinted and non-imprinted polymers were carried out

by varying the initial concentration of ciprofloxacin in the range of 0.1–4 mmol·L^{-1}. The adsorption isotherms are shown in Fig. 4.

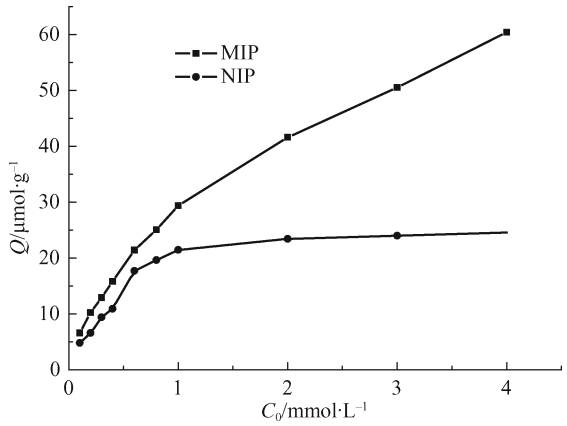

Fig. 4 The adsorption equilibrium isotherms of MIP and NIP for CIP

It can be seen from the curve in Fig. 4 that, the adsorbance of MIP increased with increasing of the initial concentration, and the adsorbance of NIP reached saturation when the initial concentration of CIP was beyond 1 mmol·L^{-1}. Obviously, the adsorbance of MIP was bigger than that of NIP, which indicated that the cavities formed on MIP by selective bonding and the active binding sites in cavities determined that high affinity and specific recognition of MIP on the template were much larger than the non-selective bonding interaction.

In studies on molecule imprinting, the Scatchard Model was often used to evaluate the binding characteristics of MIP, and the Scatchard equation can be described as [21]

$$Q/C_{CIP} = (Q_{max} - Q)/K_d \quad (1)$$

where K_d is the dissociation constant of the binding site, Q_{max} is the maximum binding capacity of the binding site, and C_{CIP} is the equilibrium concentration of the substrate in the supernatant.

Q/C_{CIP} was plotted versus Q in Fig. 5. As shown in Fig. 5, Q/C_{CIP} versus Q shows an apparent nonlinear relationship. It illustrates that the binding sites of MIP for CIP were heterogeneous, but there were good linear relationships at both ends of the graph. According to this, it can be concluded that there existed two classes of binding sites with different affinities in the range of the different concentrations. It is probably because there were various interactions between the functional monomers and imprinted molecules, and the interactions formed many kinds of complexes with different components. Various complexes have binding sites with different properties after polymerization. The data can be fitted according to the two sections of the linear relationship to get the fitting linear equation: the higher affinity binding site $Q/C = 314.054 - 19.051Q$ (correlation coefficient $R = 0.978$) and the lower affinity binding site $Q/C = 44.158 - 0.447Q$ (correlation coefficient $R = 0.964$). $K_{d1} = 5.249 \times 10^{-5}$ mol·L^{-1}, $Q_{max,1} = 16.5$ μmol·g^{-1}; $K_{d2} = 2.237 \times 10^{-3}$ mol·L^{-1}, $Q_{max,2} = 98.8$ μmol·g^{-1} can be calculated from the slope and intercept of the linear equation.

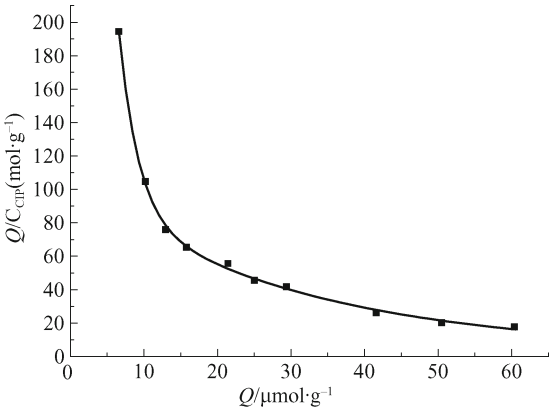

Fig. 5 Scatchard plot of MIP

For the MIP with different binding sites, there were errors in the analysis with the Scatchard Model. Therefore, a Multi-Point Model was used to get the data fitted according to the Multi-Point Model Formula [22]:

$$Q = Q_{max,1}C_{CIP}/(K_{d1} + C_{CIP}) + Q_{max,2}C_{CIP}/(K_{d2} + C_{CIP}) \quad (2)$$

By substitution of the dissociation constant of the Scatchard Model into the Multi-Point Model Formula, curve fitting was done and the result is shown in Fig. 6. The obtained fitting curve fitted well with the experimental results.

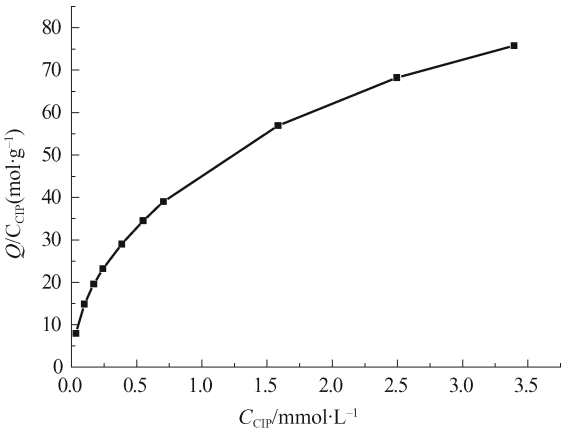

Fig. 6 Non-linear fitting for the multi-binding model

2.4 Studies on the selectivity of MIP

An equilibrium binding experiment was used to determine the binding capacity of MIP and NIP for substrates by choosing CIP and LVFX, with similar structure to CIP, as

substrates. The binding capacity was characterized by distribution coefficient K_D and separation factor α [23] of equilibrium adsorption.

The definition of distribution coefficient K_D was described as:

$$K_D = C_p / C_s \quad (3)$$

where *C*p is the concentration of substrates on polymer (μmol/g polymer), *C*s is the concentration of substrates in solution (μmol/mL).

The definition of separation factor α was described as:

$$\alpha = K_{Di} / K_{Dj} \quad (4)$$

where i and j represent the template molecule and substrate, respectively, $\alpha = 1$ when I = j. The experimental results are shown in Table 1.

Table 1 Adsorption amount *Q*, distribution coefficient K_D and separation factor α of CIP and LVFX on MIP and NIP

	MIP			NIP		
	$Q/(\mu mol \cdot g^{-1})$	$K_D/(mL \cdot g^{-1})$	α	$Q/(\mu mol \cdot g^{-1})$	$K_D/(mL \cdot g^{-1})$	α
CIP	29.4	41.64	1	21.4	27.24	1
LVFX	21.4	25.66	1.62	19.6	22.86	1.19

As shown in Table 1, the adsorption amount and distribution coefficient of MIP on substrates were larger than that of NIP. It revealed that the MIP has an apparent imprinting effect on the template molecule. There were functional groups bound with CIP and cavities in special shapes complemented with the structure of CIP. Both of them determined the selective binding properties of MIP on the template molecule. Nevertheless, the functional groups in NIP distributed at random and there were no molecular recognition sites complemented with the template molecules. Therefore, NIP showed no specific recognition properties on template molecules. It further suggested that the imprinting effect of the template molecule plays a very important role in the preparation of MIP.

3 Conclusion

The MIP was prepared by MIT with ciprofloxacin as template. The polymers showed high adsorption ability and selective recognition property for ciprofloxacin. The adsorption amount *Q* was 29.4 $\mu mol \cdot g^{-1}$ and separation factor α was 1.62. The polymers have advantages of strong thermal stability, high mechanical stability and long-term usage. These polymers can be used as potential solid phase extractants to recognize, separate and enrich the ciprofloxacin in animal-origin foods. This was convenient for the detection of CIP. It provided a new approach for selective enrichment and analysis of CIP in biological samples with a complex matrix.

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