Journal of Chromatographic Science 2012;50:499–508
doi:10.1093/chromsci/bms028 Advance Access publication April 23, 2012

Article

Molecularly Imprinted Stationary Phase Prepared by Reverse Micro-Emulsion Polymerization for Selective Recognition of Gatifloxacin in Aqueous Media

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Received 19 September 2010; revised 7 June 2011

A new stationary phase for selectively recognizing gatifloxacin in aqueous media based on molecularly imprinted microspheres (MIMs) has been prepared by water/oil reverse micro-emulsion polymerization. The MIMs were prepared using gatifloxacin as the template, *N*, *N*′-methylenebisacrylamide as cross-linker and acrylamide and acryloyl-β-CD (β-CD-A, synthesized by ester reaction of acrylic acid with β-CD) as combinatorial functional monomers. The surface morphology of MIMs was characterized by scanning electron microscopy. The properties of MIMs recognition for gatifloxacin in water were studied by adsorption kinetics, adsorption isotherms combined with Scatchard analysis and selective recognition experiments. The results showed that the synthesized MIMs had an excellent ability to selectively recognize gatifloxacin in aqueous media. MIMs were employed as the chromatographic stationary phase to successfully separate the template gatifloxacin from its analogues. Discovering the mechanism of the MIMs recognition revealed that the memory cavities in the surface of the MIMs and hydrophobic effects between the template and the cavities of the β-CD residues were the primary contributions to the special recognition process.

Introduction

Molecular imprinting is a widely studied and rapidly developing technique for preparing highly cross-linked polymeric materials by the incorporation of the template molecule during the polymerization process (1). Molecularly imprinted polymers (MIPs) possess a three-dimensional memory cavity for the used template molecule. The memory cavity is complementary in shape, size and functional group orientation with respect to the template molecule, so MIPs can specially recognize the template molecule from mixtures (2, 3). Based on these advantages, MIPs have been widely used in separation and analytical applications such as chromatographic stationary phase (4), extraction of active ingredients from natural products (5), sample preparation (6), solid-phase extraction (SPE) (7) and various sensor strategies, among others (8–9).

MIPs have primarily been prepared by conventional bulk polymerization. The preparation process is wasteful and time-consuming. The shape and some binding sites of the obtained MIPs may be destroyed in the grinding process, creating lower loading capacity for the MIPs. Additionally, the irregularly shaped particles obtained are suboptimal for the stationary phase of chromatography (10). These problems have been improved by using different preparative processes such as bead polymerization techniques (11), suspension (12), multistep swelling process (13) and precipitation polymerization (14), in addition to imprinting on the surface of spherical polymer or silica (15). However, these methods are often complex. Water/oil (W/O) reverse micro-emulsion polymerization can be developed to directly prepare uniformly sized molecularly imprinted microspheres (MIMs) in the form of spherical particles of a controlled diameter. The synthesis method is better at protecting the shape and binding sites of cavities. Additionally, the obtained spherical particles can be directly used as the stationary phase for high-performance liquid chromatography (HPLC) and SPE.

Until now, most of MIPs were synthesized and used aprotic and low polar organic solvents. However, many important recognitions and separation primarily occur in aqueous systems and high polarity organic solvents, especially for HPLC systems (16, 17). Previous studies have discovered that MIP, which was prepared based on hydrogen bonding interactions, lost its selective recognition ability to the template when it was used in aqueous media or high polarity organic solvents. To make MIPs selectively recognize the template in aqueous media, it is necessary to exploit other intermolecular interactions such as hydrophobic effects or electrostatic interaction (18, 19). Recently, β-cyclodextrin (β-CD) and its derivatives have been chosen as functional monomers to achieve molecular imprinting in aqueous solution because of its doughnut-shaped cyclic oligosaccharide, which has a hydrophilic external and a hydrophobic internal cavity (20–22). When β-CD-MIPs are prepared for some comparatively big templates, β-CD molecule residues in the polymers are complementarily placed to the template, and the assembled β-CD molecules can work as a whole molecule to precisely recognize the template based on hydrophobic interaction (23). Some reports have used β-CD as functional monomer to prepare MIPs possessing high affinity and selectivity for the template in water (24–27).

Gatifloxacin, a fluoroquinolone antibiotic, is effective in treating infections that are resistant to other antibiotic families. The analysis of fluoroquinolones is a difficult task in some matrices, because they are micro-content and may bind to the lipoproteins in biological samples. To achieve the selective extraction of fluoroquinolones in aqueous samples, some water-compatible MIPs have been synthesized by conventional bulk or *in situ* polymerization in methanol–water systems (28–32).

However, these quinolone-MIPs have all used olefinic acid compounds as functional monomers, which are easily destroyed by water after interaction with the template by electrostatic interaction and hydrogen bond.

In this paper, for preventing the destruction of hydrone and protecting the shape and binding sites of imprinted polymers, we used acryloyl-β-CD as special functional monomer to prepare MIMs for gatifloxacin by W/O reverse micro-emulsion polymerization. The obtained imprinted microspheres can be directly used as chromatography stationary phase to specifically recognize gatifloxacin in the aqueous system. Furthermore, we discuss the special binding mechanism of the β-CD-imprinted microspheres for gatifloxacin in aqueous media.

Experimental

Materials

β-CD (Sinopharm Chemical Reagent Co., Shanghai, China) was recrystallized three times by distilled water and dried under vacuum at 110°C for 24 h. *N*, *N*/-Dicyclohexyl carbodiimide (DCC), *N*, *N*-dimethylformamide (DMF), acrylamide (AAM), *N*, *N*/-methylenebiacrylamide (MBAA), cyclohexane and Span 60 were purchased from Sinopharm Chemical Reagent Co. Acrylic acid (AA) and potassium persulfate were obtained from Shanghai Lingfeng Chemical Reagent Co. (Shanghai, China). Gatifloxacin, ciprofloxacin, lomefloxacin, cefuroxime and cefaclor were purchased from Sinopharm Chemical Reagent Co. All solvents were of analytical grade and used as received.

Apparatus

An ultraviolet-visible (UV-VIS) spectrometer-1800 (UV-1800) and Fourier transform infrared spectrometer-8400S (FT-IR-8400S) was from Shimadzu (Kyoto, Japan); a scanning electron microscope-3000 (SEM-3000) was purchased from Hitachi Corporation in Japan.

Synthesis of acryloyl-β-CD (β-CD-A)

According to the literature (33), β-CD-A was synthesized by ester-exchange reaction. The synthesis procedure is described as following: 3.4102 g β-CD and 1.0314 g DCC were dissolved in 25 mL of DMF and stirred for 1 h at 35°C. Then AA, with the same molar ratio as DCC, was slowly dropped into the mixture solution in 30 min. The mixture was stirred at 30°C for 12 h. When the reaction was ended, the precipitated solids were removed by vacuum filtration. Acetone was added to the filtrate to precipitate the crude product. The obtained precipitation was successively washed with diluted acetic acid and potassium carbonate solution, then dissolved in methanol solution and purified by acetone to obtain the white solid product (β-CD-A). Finally, IR and UV measurements were carried out to confirm the structure of the obtained products.

Molecularly imprinted microspheres preparation

The gatifloxacin-MIPs were prepared using a method described by Li *et al.* (34) with few modifications:

(i) Oil phase preparation: 0.2593 g Span 60 emulsifier was dispersed in 30 mL cyclohexane and stirred at 60°C;
(ii) Water phase preparation: 0.0376 g gatifloxacin was dissolved in 10 mL of deionized water, and then combinational functional monomers consisting of 0.0734 g β-CD-A and 0.0284 g AAM were added to the mixed solution. The mixture was stirred for 30 min at 60°C; and then sonicated for 30 min to ensure the complete interaction of the template with the monomers. Then 0.2775 g MBAA and 0.0432 g $K_2S_2O_3$ were dissolved in the mixed solvent to form the water phase;
(iii) Preparation of imprinted microspheres by W/O reverse micro-emulsion polymerization: the water phase prepared in Step 2 was added into the oil phase prepared in Step 1 drop by drop in 20 min. The mixture in the sealed round bottom flask was reacted for 8 h at 60°C by stirring under a nitrogen atmosphere. After polymerization reaction, the micro-emulsions were demulsified by acetone to obtain MIMs. The resultant micro-balls were washed with methanol in an ultrasonic cleaning machine to remove the template and emulsifier, and then filtered off. This procedure was repeated several times until the template could not be detected by UV in the filtrate. The remaining micro-balls were dried under vacuum at 60°C and could be used for the subsequent studies.

Non-imprinted polymer microspheres (NIMs) used as reference, which did not contain the template, was also prepared in parallel with the MIMs by using the same synthetic process. Finally, SEM was applied to observe the morphology of MIMs and NIMs.

Adsorption dynamic experiments

MIMs or NIMs (20.00 mg) were suspended in 10.00 mL of an aqueous gatifloxacin solution at concentration of 200 mg/mL in 15-mL test tubes. The tubes were then shaken at 30°C. Ten samples were taken at defined time intervals (at 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h, respectively) and centrifuged for 10 min. The obtained supernatant (0.5 mL) was diluted to 10.0 mL with distilled water. The amount of gatifloxacin adsorbed by MIMs or NIMs was determined by UV-VIS spectrophotometer.

According to the variance of gatifloxacin concentration before and after adsorption, the equilibrium adsorption capacity (Q, μg/mg) of gatifloxacin bound to the imprinted microspheres is calculated by

$$Q = (C0 - C1) \cdot V/m \quad (1)$$

where C0, C1, V and m represent gatifloxacin initial solution concentration and the final solution concentration (the concentration after adsorption) (μg/mL), the volume of the solution (mL) and the weight of the microspheres (mg), respectively. The average data of triplicate independent results were used for the following discussion.

After obtaining the equilibrium adsorption capacity (Q) of MIMs and NIMs, we can draw the adsorption kinetic curve of MIMs and NIMs for gatifloxacin. The specific recognition property of MIP can be evaluated by the imprinting factor (α), which is defined as:

$$A = QMIMs/QNIMs \quad (2)$$

where QMIMs and QNIMs are the equilibrium adsorption capacity of the template on MIMs and the corresponding NIMs, respectively. Imprinting factor (α) represents the special recognition performance of MIMs for gatifloxacin, and its larger value means that MIMs have better recognition characteristic and stronger imprinting effect.

Adsorption isotherm experiments

In the adsorption isotherm experiments, 20.00 mg of MIMs was equilibrated with 10 mL initial gatifloxacin at varied concentrations (5 ~ 50 μg/mL) in 15-mL test tubes. The tubes were shaken and incubated at 30°C. After 20 h, 10 samples were taken and centrifuged for 10 min. The obtained supernatant (0.5mL) was diluted to 5.00 mL with distilled water. The residual concentration of the adsorbate was measured by UV-VIS spectrophotometer, and the equilibrium adsorption capacity of MIMs for gatifloxacin adsorption was calculated by Equation (1).

Selective recognition experiments

The selective performance of imprinted microspheres for gatifloxacin was estimated by using ciprofloxacin, lomefloxacin, cefaclor and cefuroxime as analogues. The process of these experiments is the same as described in the "Adsorption Isotherm Experiments" section.

Chromatographic experiments

The MIMs and NIMs were packed into glass columns (150 × 10 mm i.d.), respectively. The columns were washed with methanol (20%) aqueous solution (mobile phase) at a flow rate of 0.06 mL/min at room temperature and normal pressure until the eluant had the same UV absorption spectra as the mobile phase. The sample containing gatifloxacin, lomefloxacin and cefaclor (mass ratio of 1:1:1) was loaded on the top of columns packed with the MIMs and NIMs. The loaded columns were washed with the mobile phase, and the eluant was collected section-by-section with each hour intervals. Silica gel-G-thin-layer chromatography (TLC) and UV were used to detect the eluant. The developing conditions of TLC were set at room temperature and normal pressure. The developer was chloroform, methanol and ammonia water with the volume ratio of 20:8:1. Iodine was used as the chromogenic agent.

Results and Discussion

Characteristics of β-CD-A

Figure 1 shows FT-IR spectrograms of β-CD-A and β-CD. By comparing them, we observed that strong and broad hydroxyl stretching vibration absorption peaks have emerged at approximately 3,410 cm^{-1} that indicate the hydroxyl group of β-CD before and after modification. However, the width of the β-CD-A hydroxyl band is slightly narrower than that of β-CD, and this can be because the introduction of the acryloyl group reduces the number of β-CD hydroxyl groups. Acryloyl group of β-CD-A was confirmed by the absorption peak at 1,700 cm^{-1} (carbonyl characteristic peak) and 1,656 cm^{-1} (alkenyl characteristic peak), as shown in Figure 1A. Therefore, these

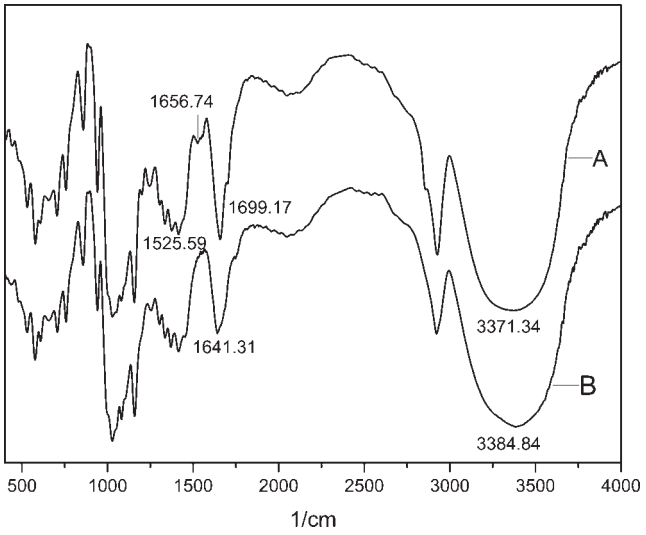

Figure 1. FT-IR spectrogram of (A) β-CD-A and (B) β-CD.

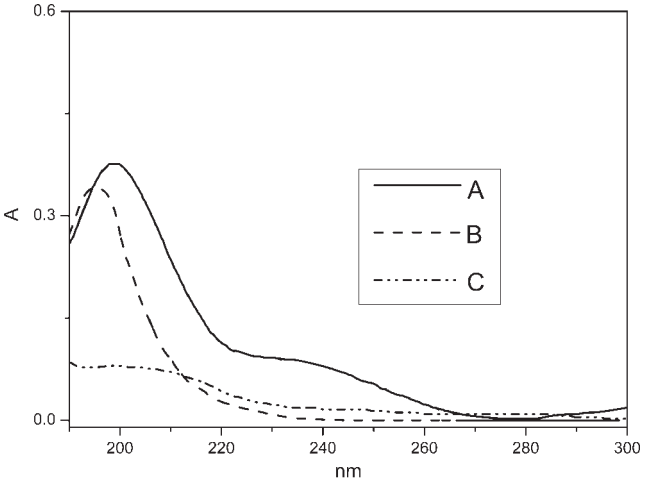

Figure 2. UV spectrogram of (A) β-CD-A; (B) AA and (C) β-CD.

characteristic peaks reveal that the acryloyl group was introduced into the structure of β-CD.

The UV-VIS spectrophotometer scans of β-CD-A, AA and β-CD are shown in Figure 2. β-CD does not have the UV absorption. Compared with AA, the maximum absorption wavelength of β-CD-A has the red shift, because the auxochrome (introduction of −OR) induces the chromophore (acryloyl group) to the red shift and hyperchromic effect. Therefore, the information of IR and UV analyses indicate that β-CD-A was successfully synthesized.

Characteristics of MIMs

SEM is used to observe the surface and bulk pore structures of imprinted microspheres (Figure 3).

As shown in Figure 3, there are some significant differences in the morphologies between MIMs and NIMs. Compared with NIMs, the MIMs have a rough morphology and mass with irregular pore structures in their surface. The irregular rough morphology and pore structures of the imprinted microspheres

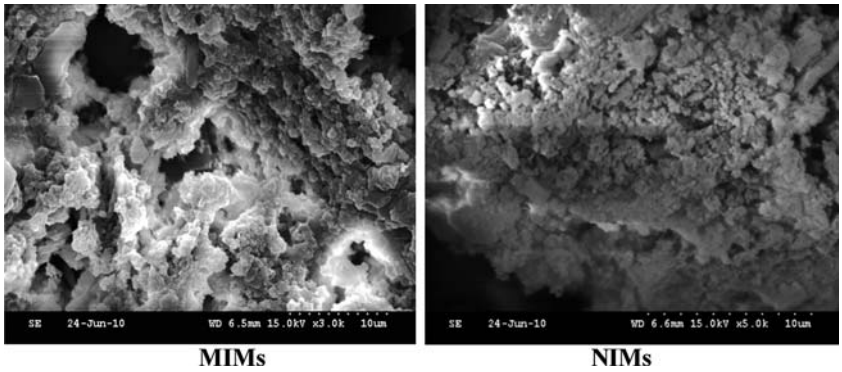

Figure 3. SEM micrographs (5,000 ×) of polymers: MIMs and NIMs.

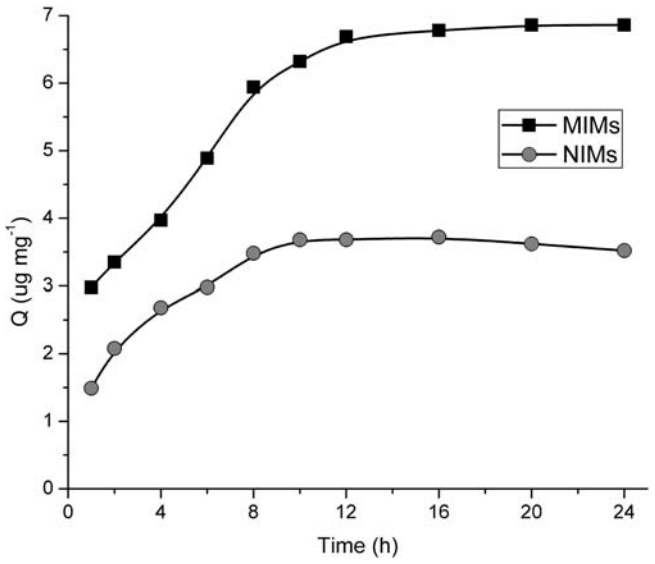

Figure 4. Adsorption kinetic curves of MIMs and NIMs. Experimental conditions: 10 mL of 200 μg /mL gatifloxacin with 20.0 mg of MIMs or NIMs at different times and at 30°C, respectively. The measurements were repeated three times.

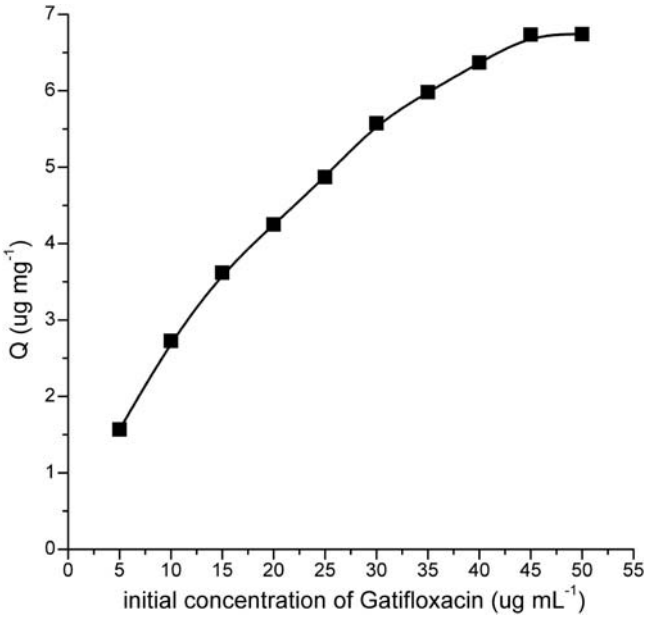

Figure 5. The adsorption isotherms of gatifloxacin on MIMs. Experimental conditions: 10 mL of 5–50 μg/mL gatifloxacin with 20.0 mg of the MIMs for 20h at 30°C. The measurements were repeated three times.

may be the appearance of memory sites left by the templates. However, the shape and surface of the NIMs are more uniform, compact and smooth than those of the MIMs. The regular structure of the non-imprinted microspheres is probably due to non-specific binding sites created by the templates.

Binding kinetics

Adsorption dynamic experiments are conducted to investigate the kinetic adsorption processes of MIMs and NIMs for gatifloxacin. Figure 4 shows the adsorption kinetic curves of MIMs and NIMs. As shown in Figure 4, MIMs have much higher binding affinities for the templates than NIMs. This indicates that MIMs have much better imprinting ability than NIMs. Figure 4 also shows that the adsorption process of MIMs for gatifloxacin could be divided into two steps at the eighth hour: a quick absorbing step before 8 h and a slow absorbing step 8 h later. In the first step, the adsorption rate is fast, and the binding capacity increased quickly; in the subsequent step, the capacity of adsorption increased slowly with time extension. After approximately 16 h, adsorption process reaches equilibrium. At this time, the equilibrium adsorption capacity of MIMs (QMIMs) was 6.86 μg/mg, and imprinting factor (α) was 1.95. Compared with MIMs, NIMs have less adsorption capacity for gatifloxacin and reach adsorption saturation earlier. During the adsorption time, some of the substrate is released, so the adsorption of NIMs for the template is not strong. This phenomenon may be caused by the adsorption mechanism of NIMs. NIMs only follow the common adsorption mechanism, so the rate of adsorption curve increases significantly during the initial adsorption process, and the adsorption saturation is achieved after a much shorter period of time. However, MIMs primarily abide by the imprinting mechanism to bind gatifloxacin because it matches well with the template in chemical groups and the spatial structure. As a result, the initial rate of adsorption is lower than the common binding process, and the adsorption-reached equilibrium needs a longer time in this rebinding process. Therefore, the difference in binding mechanism results in the different adsorption performances observed between MIMs and NIMs.

Langmuir adsorption isotherm

Adsorption isotherm, which can be used to measure the concentration-dependent recognition behavior of a system, was employed to illustrate the adsorption properties of MIMs for template gatifloxacin. The adsorption capacity (Q) of the adsorbate bound to MIMs according to the initial concentration of adsorbate is shown in Figure 5. The adsorption isotherm of MIMs for the template is a convex curve. The adsorption capacity of MIMs rises with the increase of gatifloxacin concentrations. When all recognition sites of imprinted microspheres are occupied by gatifloxacin, the adsorption capacity tends toward saturation. The convex adsorption isotherm indicates that there are different kinds of active recognition sites on the surface of MIMs. Gatifloxacin prefers to occupy the strong active recognition sites when its concentration is very low. However, with the high gatifloxacin concentrations, almost all the specific imprinted sites are saturated, and the residual template molecules have to interact with the weak active sites. As a result, the distribution ratio of template molecules between the MIMs and the solvent decreases. This binding process results in the formation of the convex adsorption isotherm.

To further study the binding properties of the MIMs for the template, the Scatchard analysis is adopted to estimate the recognition characteristics of the MIMs according to the Scatchard equation:

$$Q/C = (Q_{max} - Q)/Kd \quad (3)$$

where Kd (μg/mL) is the equilibrium dissociation constant; Q_{max} (μg/mg) is the apparent maximum number of binding sites; and C (μg/mL) is the equilibrium concentration of gatifloxacin. According to the result of the adsorption experiments, the Scatchard curve is drawn by Q/C versus Q (Figure 6). As shown in Figure 6, there are two distinct linear sections for the MIMs, which indicate that the MIMs have two different kinds of recognition sites. The results of Scatchard analysis, including two linear equations, correlation coefficient (r), the apparent maximum binding capacity (Q_{max}) and the equilibrium dissociation constant (Kd), are listed in Table I, respectively.

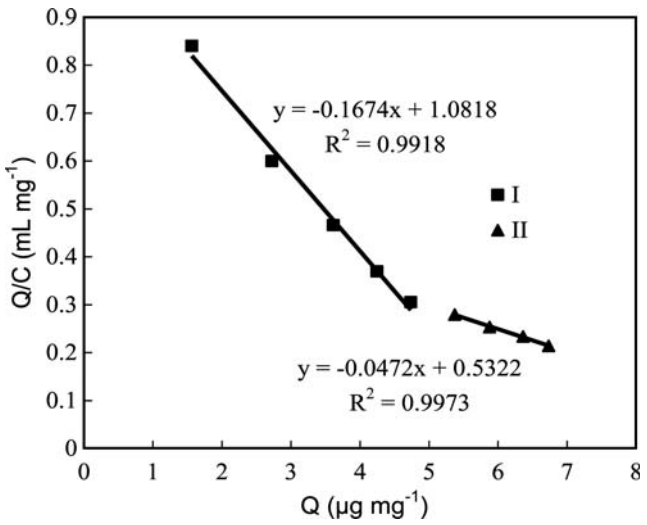

Figure 6. Scatchard plot of MIMs selective for gatifloxacin.

Table I
Results of Scatchard Analysis

Binding site	I*	II†
Linear equation	$y = -0.1674x + 1.0818$	$y = -0.0472x + 0.5322$
Correlation coefficient(r)	0.9959	0.9986
Apparent maximum binding amount [Q_{max} (μg/mg)]	6.46	11.28
Equilibrium dissociation constant [K_d (μg/mL)]	5.97	21.19

*For a higher affinity binding site.
†For a lower affinity binding site.

Table II
Adsorption capacity (Q) and Imprinting Factor (α) of the Tested Substrates on MIMs and NIMs under Equilibrium Binding Conditions*

Substrates	QMIMs (μg/mL)	QNIMs (μg/mL)	Imprinting factor (α)
Gatifloxacin	6.855	3.502	1.96
Ciprofloxacin	4.049	2.545	1.59
Lomefloxacin	3.269	2.452	1.33
Cefaclor	1.329	1.305	1.02
Cefuroxime	1.123	1.101	1.01

*Experimental conditions: 10 mL of 200 μg/mL gatifloxacin, ciprofloxacin, lomefloxacin, cefuroxime and cefaclor with 20.0 mg of MIMs or NIMs at 30°C and different times, respectively. The measurements were repeated three times.

As shown in Figure 6 and Table I, the Scatchard analysis allows the following conclusions: (i) there are two types of strong and weak affinities during the interactive process between the template and the MIMs: strong affinity plays a major role at low concentrations of the substrates, while the weak just plays an ancillary role at high concentrations; (ii) the strong binding sites may be caused by imprinting effect, whereas the weak binding sites are caused by the common adsorption mechanism.

Because the MIMs contain two types of binding sites, the Scatchard analysis does not consider the contribution of the lower affinity binding sites to the adsorption capacity of the MIMs at low concentrations of substrates in the determination of higher affinity binding parameters. Similarly, adsorption capacity of higher affinity binding sites is ignored in the determination of lower affinity binding parameters at a high concentration of substrates. Therefore, the binding parameter values obtained by the Scatchard analysis are less accurate in these systems because the last ones have two classes of binding sites.

Selective recognition

To investigate the selective recognition of the MIMs for the template and analogues, selective recognition experiments were carried out to determine the adsorption capacity (Q) of MIMs and NIMs for the analogues containing ciprofloxacin, lomefloxacin, cefuroxime and cefaclor, respectively. The imprinting factor (α) is used to evaluate the specific recognition effect of the imprinted microspheres for the substrates. The value of Q and α are listed in Table II. Molecular structures of the template and analogues are shown

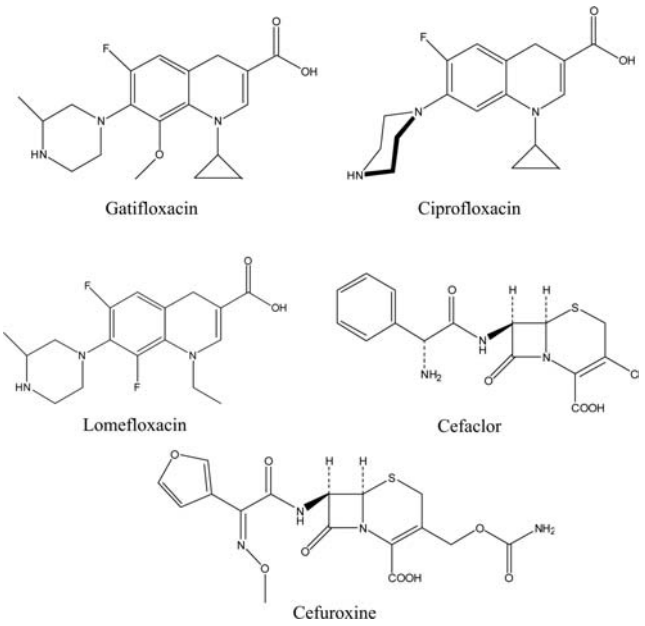

Figure 7. Structures of the template and analogues.

Table III
Results for Selectivity Factor (β) of MIMs on Gatifloxacin and its Analogues

Analogues	Gatifloxacin	Ciprofloxacin	Lomefloxacin	Cefaclor	Cefuroxime
Selectivity factor (β)	1	1.23	1.47	1.66	1.77

in Figure 7. Figure 7 and Table II show that the value of Q and α of MIMs decrease progressively, with the structure difference between the template and the analogues becoming more and more obvious.

The selective recognition performance of MIMs is evaluated by the selectivity factor (β), which is defined as:

$$\beta = \alpha_{tem}/\alpha_{ana} \quad (4)$$

where α_{tem} is the imprinting factor towards the template molecule; α_{ana} is the imprinting factor towards the analogue. If the valve of β is much higher, the MIMs have better selectivity for the template and can more easily separate the template from the analogues. The selectivity factor (β) of MIMs on the analogues is listed in Table III.

Table III shows that the selectivity recognition of MIMs on the five substances is the following: gatifloxacin > ciprofloxacin > lomefloxacin > cefaclor > cefuroxime.
Ciprofloxacin has the same active chemical groups and a similar structure to gatifloxacin, so MIMs exhibit relatively strong adsorption ability. However, because the methoxy group is missing in the C4 site of ciprofloxacin, the structural shape of the ciprofloxacin cannot be completely matched with the three-dimensional cavity of the MIMs. This resulted in a weaker adsorption capacity than gatifloxacin. Compared with ciprofloxacin, lomefloxacin is far more different than gatifloxacin in

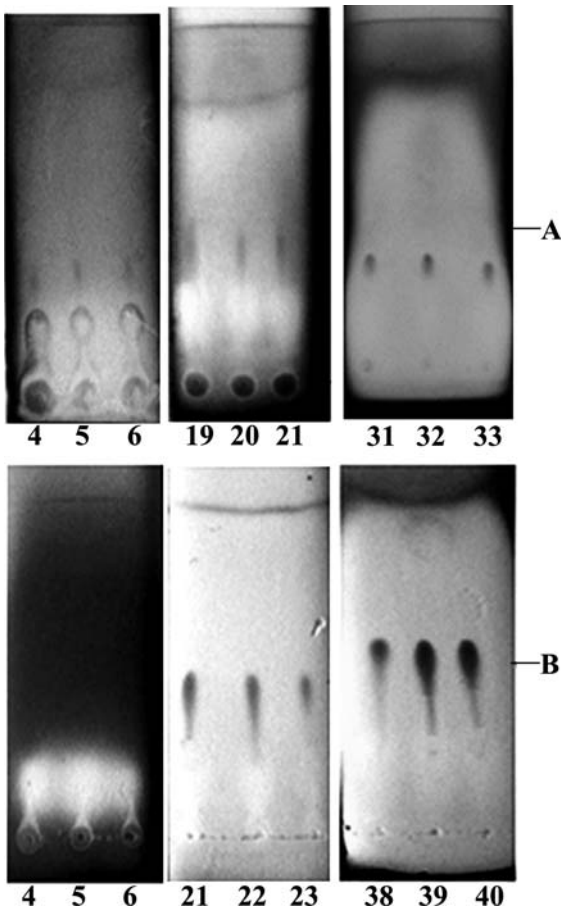

Figure 8. The Silica gel-G-TLC images of the eluent from both of chromatographic columns packed with the (A) NIMs and with (B) the MIMs, respectively.

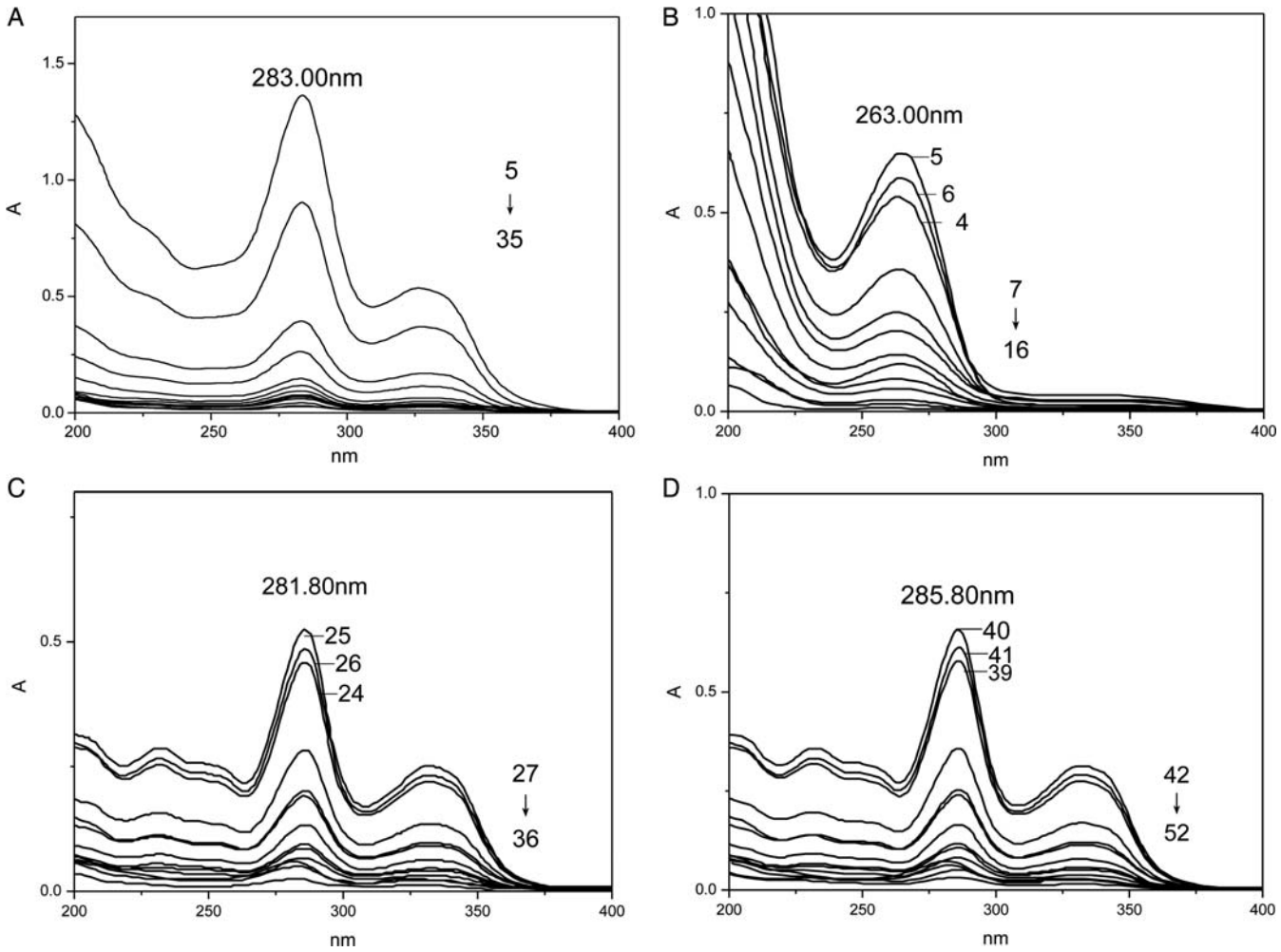

Figure 9. UV spectrograms of the eluent collected in the way of section-by-hour from chromatographic column packed with (A) the NIMs and (B, C and D) the MIMs.

the molecular structure. Because lomefloxacin still possesses the same bicyclo-quinolone as gatifloxacin, the MIMs maintain a certain extent of adsorption capacity for lomefloxacin. The difference of the branched groups between lomefloxacin and gatifloxacin not only decreases the active recognition sites interacting with the MIMs, but also keeps the shape and size of lomefloxacin from matching the spatial cavity of the MIMs. As a result, the selectivity factor of lomefloxacin was greater than that of ciprofloxacin. The chemical structures of cefaclor and cefuroxime are completely different from gatifloxacin (Figure 7), and their shape and size do not match the spatial cavity of the MIMs. They enter the cavities with difficulty, and their amide and carboxy groups, prone to hydrogen bonding, are easily destroyed. Therefore, the MIMs have almost no special recognition performance for them.

Chromatographic analysis

In the following discussion, the MIMs are employed as the chromatographic stationary phase to investigate the feasibility of the imprinted microspheres that can separate the template from the analogues. The results of chromatographic separation are shown in Figures 8 and 9. The UV spectrograms of the standard substances are shown in Figure 10. The eluted orders of each section of eluent represent the retention times of substrates on the chromatographic columns.

The eluent from chromatographic column packed with the NIMs was detected by TLC and UV. Figure 8A shows that there are always three substances in each of eluent section. In comparison with Figure 10, it can be concluded that the UV spectrogram of the eluent in Figure 9A is similar to that of the standard substances consisting of gatifloxacin, lomefloxacin and cefaclor with mass ratio of 1:1:1. Therefore, the chromatographic column packed with the NIMs cannot separate the template from the analogues. The results may be explained by no special recognition sites for the template in the surface of the NIMs. Figures 8B and 9B, C and D show that the three substances have been washed out of the chromatographic column packed with the MIMs different time periods. The UV spectrograms in Figures 9B, C and D are the same as those of cefaclor, lomefloxacin and gatifloxacin standards in Figure 10, respectively, so the effluent priority of the three substances is cefaclor > lomefloxacin > gatifloxacin. These results may be due to the imprinting effect and the molecular volume of the analytes. The imprinting cavities on the surface of the MIMs are more suitable for gatifloxacin, so the retention time of

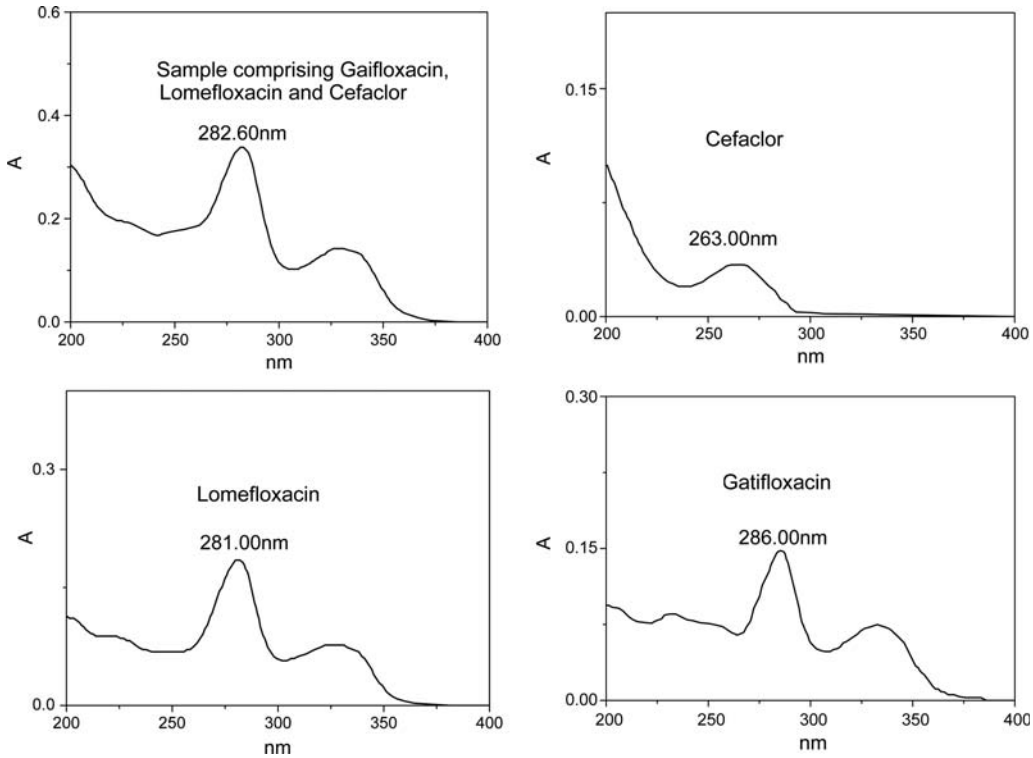

Figure 10. UV spectrograms of the standard substances.

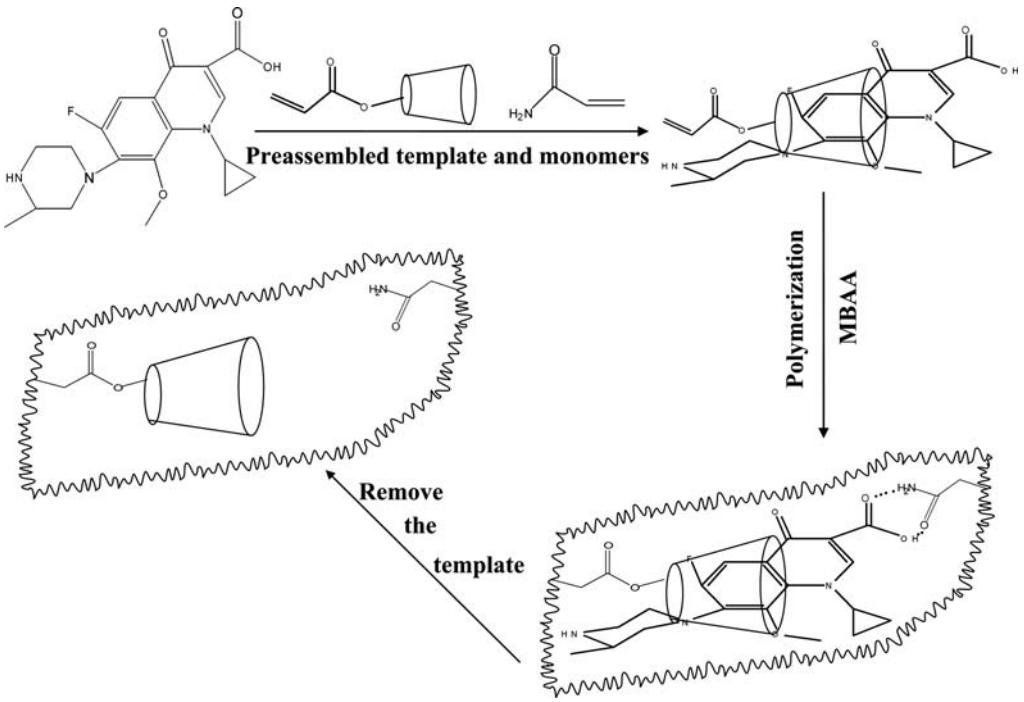

Figure 11. Schematic representation of the preparation processes of MIMs and the mechanism of binding specificity.

gatifloxacin is the longest one. Lomefloxacin and gatifloxacin have similar structures, while cefaclor has different structure from gatifloxacin, so the retention time of lomefloxacin is longer than cefaclor.

Recognition mechanism of the MIMs in aqueous media

MIPs can exhibit the remarkable recognition properties for the template because they matching the template and the spatial structure in chemical groups. It is very important for the

template molecule and functional monomer to form the stable complex structure because the complex structure not only increases the number of binding sites of the obtained MIPs, but also reduces the number of non-specific interactive sites (35). In aqueous media, hydrogen bonding interaction between template and imprinted microspheres is easily destroyed. Therefore, the MIMs achieve special recognition for the template by requiring other intermolecular interactions, such as hydrophobic effect, conjugated effect and electrostatic interaction (36). The β-CD molecule is a conic tube-shaped cyclic oligosaccharide with an external hydrophilic and an internal hydrophobic cavity. This structure allows β-CD to form stable inclusion compounds with guests in water via hydrophobic interactions. Therefore, in this study, β-CD is modified via the introduction of acrolyl group and successfully utilized as functional monomer in MIMs for gatifloxacin. Combining with the previously discussed analytical results of experiments, the mechanism of β-CD-MIMs achieve binding specificity for the template gatifloxacin in water is discussed further.

As shown in Figure 11, the preparation process of MIMs and the mechanism of binding specificity may be the following: in the pre-polymerization solution, each of the β-CD molecules is bound to a portion of gatifloxacin, while acrylamide interacts with the carboxyl group. Then, the cross-linker is used to fix the positions and mutual conformations of β-CD and acrylamide. After removing the template from the MIP, the formed micro-cavities can selectively bind the template. In the process of recognition, some parts of the template are inserted in the complementary micro-cavities on the surface of the MIMs, while other parts are inserted in the cavities of the β-CD residues due to the hydrophobic effect.

Conclusions

In this work, MIMs for selectively recognizing gatifloxacin, acting as a novel stationary phase for column liquid chromatography, are successfully synthesized by W/O reverse microemulsion polymerization using β-CD-A and AAM as the functional monomers. The obtained molecularly imprinted stationary phase can separate the template from the analogues under aqueous mobile phase condition. A series of adsorption experiments and chromatographic separation tests of the imprinted microspheres showed that the prepared MIMs have high selective binding for gatifloxacin. The results of experimental analysis show that the selective recognition of MIMs for gatifloxacin is primarily due to the complementary micro-cavities and the hydrophobic cavities of the β-CD residues on the surface of the MIMs.

Acknowledgment

The authors acknowledge the research grant (No. JKY2009059) provided by the Fundamental Research Funds for the Central Universities and Supported by Zhejiang Provincial Natural Science Foundation of China (Grant No. Y4110235).

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