JOURNAL OF Applied Polymer SCIENCE

Preparation and Characterization of a Pseudo-Template Imprinted Polymer with a Chirality-Matching Monomer for the Separation of Cinchona Alkaloids by High-Performance Liquid Chromatography

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ABSTRACT: A hydroquinidine-imprinted stationary selector with chiral recognition for cinchona alkaloids was prepared by bulk polymerization with (+)-(2R,3R)-*N*,*N*′-diallyl-L-tartardiamide as a functional monomer, and the recognition properties of the polymers for quinidine (QD) were analyzed with high-performance liquid chromatography. By optimizing the preparation conditions and chromatographic conditions, such as the ratio of the mobile phase and flow rate, QD and quinine could be completely separated to the best resolution of 2.25. The chiral molecularly imprinted polymers (CMIPs) combined the virtues of traditional brush-type chiral selectors with existing CMIPs. The results show a substantial synergistic effect between the chiral monomer and the chiral cavity of the resulting CMIPs in chiral recognition. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 3425–3431, 2013

KEYWORDS: synergistic effect; bulk polymerization; chiral separation; cinchona alkaloids; high performance liquid chromatography

Received 17 August 2012; accepted 23 January 2013; published online 27 February 2013
DOI: 10.1002/app.39062

INTRODUCTION

Molecular imprinting is a versatile and facile technique for the preparation of tailor-made materials possessing special recognition for target molecules (templates). As molecularly imprinted polymers (MIPs) have predetermined selectivity, recognition, and feasibility, they are widely used in many applications,[1] including chromatographic separation,[2–5] sensors,[6–9] solid-phase extraction,[10–13] drug delivery,[14–16] and catalysis.[17–19] Before polymerization, a complex is formed by interactions between a functional monomer and the template in a polymerization mixture.[20] After formation of the polymer, the template molecules are washed out, leaving special cavities into which the original template can be re-bound.[21]

In recent years, MIPs have mainly been prepared by noncovalent means, including multistep swelling polymerization, suspension polymerization, precipitation polymerization, surface modification on a silica gel, and bulk polymerization. Compared with these other methods, bulk polymerization is simple, and the optimization of the imprinting conditions is relatively straightforward.[22] MIPs are typically prepared with the bulk polymerization method, where the resulting monoliths are crushed, ground, and sieved to produce microparticles used in various applications.

The separation of chiral compounds is of growing importance and presents a challenge to analytical chemists. Currently, a host of traditional chiral stationary phases in liquid chromatography (cyclodextrins, crown ethers, Pirkle phases, proteins, cellulose derivatives, etc.) are available and are used to investigate the resolution ability of chiral selectors.

Existing molecular-imprinting techniques mainly employ chiral template molecules and nonchiral functional monomers to predetermine chiral three-dimensional cavities and prepare chiral molecularly imprinted polymers (CMIPs) for chiral recognition.[23–25] The most widely used functional monomers are acrylamide, methacrylic acid, and 4-vinyl pyridine. However, these monomers have limited interaction with templates, and this results in a finite ability for molecular recognition, particularly in the case of chiral compounds.[26] However, we propose that good chiral recognition can be achieved on CMIPs with a chiral template and a chiral-matching monomer.

Quinidine (QD) and its stereoisomer quinine (QN) are cinchona alkaloids derived from certain cinchona barks and are typically used in medicines for their antimalarial activity.[27] A review of the analyses of cinchona alkaloids by chromatography was done by McCalley,[28] and reversed-phase high-performance liquid chromatography (HPLC) with octadecyl silane (ODS) columns in combination with acidic mobile phases was found to be the most widely used method. There have been some published works on the imprinting parameters and chiral

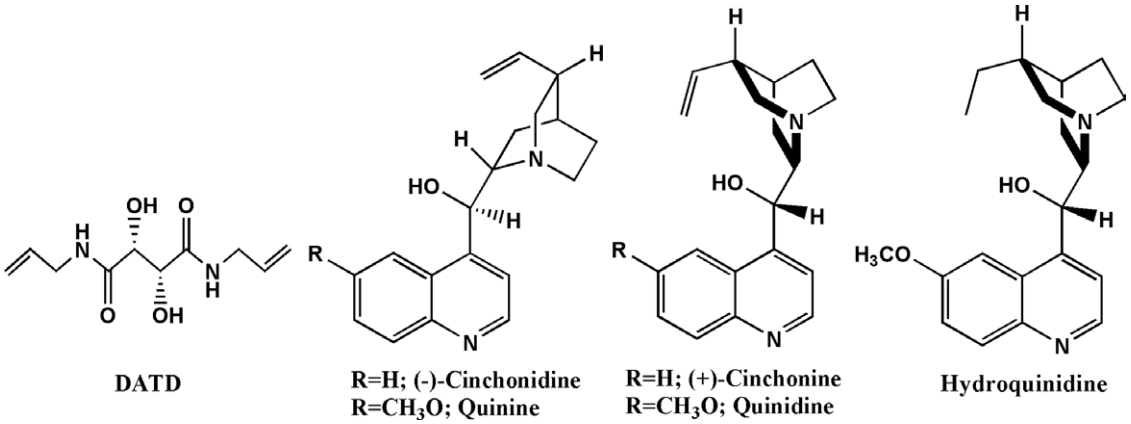

Figure 1. Structures of DATD, QN, CD, QD, CN, and hydroquinidine.

recognition of cinchona alkaloid imprinted polymers.[29,30] However, because the QD molecule contains a terminal double bond, which would be copolymerized with crosslinking monomers[31] and result in a decrease of effective binding sites in MIPs, we speculate that a better imprinting efficiency could be achieved if hydroquinidine were used as the template.

In contrast to a previous study,[32] we used a chiral functional monomer, (+)-(2R,3R)-*N,N′*-diallyl-L-tartardiamide (DATD; Figure 1), which had two chiral centers and would lead to a better resolution for chiral separation, and we used hydroquinidine as the template to synthesize a novel CMIP. The CMIP combined the advantages of chiral stationary selectors with the existing CMIP prepared with chiral templates and nonchiral functional monomers. The objective was to investigate the synergistic effect between the chiral monomer and the chiral cavity of the MIPs for chiral recognition. Under the optimal conditions, baseline separation ($R = 1.83$) was achieved, and this was better than our previous result.

EXPERIMENTAL

Chemicals and Instruments

Hydroquinidine, QD, QN, cinchonine (CN), and cinchonidine (CD) were purchased from Shanghai Ruiyi Medical Technology Co., Ltd. (Shanghai, China). DATD, ethylene glycol dimethacrylate (EGDMA), and 2,2,-azobisisobutyronitrile (AIBN) were acquired from Alfa Aesar (Tianjin, China), and the acetic acid, dimethyl sulfoxide (DMSO), and methanol were purchased from Kermel (Tianjin, China). All of the chemical reagents were analytical or HPLC grade. For HPLC, we used a Shimadzu LC-10AD pump and a Shimadzu SPD-10A UV–visible detector from Shimadzu Co. (Kyoto, Japan).

Preparation of the MIPs

For the preparation of the hydroquinidine-imprinted polymer, 9.6 mmol of the functional monomer DATD was dissolved in DMSO in a 10-mL thick-walled glass tube. The template molecule hydroquinidine, the crosslinking agent EGDMA (38.4 mmol), and the initiator AIBN (0.4 g) were then added to the previous solution. The amount of hydroquinidine used ranged from 0 to 4.8 mmol, as shown in Table I. The solution was purged with oxygen-free nitrogen for 10 min before the glass tube was sealed under nitrogen and placed in a water bath at 70°C. The polymerization was then allowed to proceed for 24 h. The final bulk rigid polymers were ground in a mortar with a pestle and wet-sieved into particles below 35 μm in diameter, which were suitable for HPLC analysis. Fine particles were removed by repeated precipitation in acetone. Finally, the particles were washed with a mixed solution of methanol and acetic acid (90 : 10 v/v) with Soxhlet extraction to remove the template molecules and dried *in vacuo* at 70°C. The chemical scheme for the preparation of the MIPs is shown in Figure 2. The nonimprinted polymers (NIPs) were prepared and treated in the same way as the corresponding imprinted polymers, except for the addition of hydroquinidine in the imprinted polymers.

HPLC Experiments

The sieved and sedimented polymer particles were separated into 2.0-g aliquots and packed into stainless steel HPLC columns (*i.d.* 150 × 4.6 mm) with a conventional slurry method; methanol/chloroform (7 : 3 v/v) was used as the solvent. The residual template and chiral monomer were completely eluted with a mobile phase consisting of a methanol/acetic acid mixture (90 : 10 v/v) at 0.2 mL/min until a stable baseline was obtained. The UV detection wavelength was set at 254 nm. The mobile phase used for the separation studies was methanol/water (97 : 3 v/v) containing triethylamine, and the flow rate of the mobile phase was 0.7 mL/min. For analysis, 10 μL of the 2 mmol/L QD/QN mixture was injected, and all of the HPLC experiments were carried out at ambient temperature. The retention factor (k) was calculated from eq. (1):

$$k = (t_R - t_0)/t_0 \quad (1)$$

where t_R and t_0 are the retention times of the retained and unretained solutes, respectively. t_0 was measured by the injection

Table I. Preparative Composition of the Different Polymers

Polymer	Hydroquinidine (mmol)	DATD (mmol)	EGDMA (mmol)	AIBN (g)
CMIP1	1.2	9.6	38.4	0.4
CMIP2	2.4	9.6	38.4	0.4
CMIP3	4.8	9.6	38.4	0.4
NIPs	—	9.6	38.4	0.4

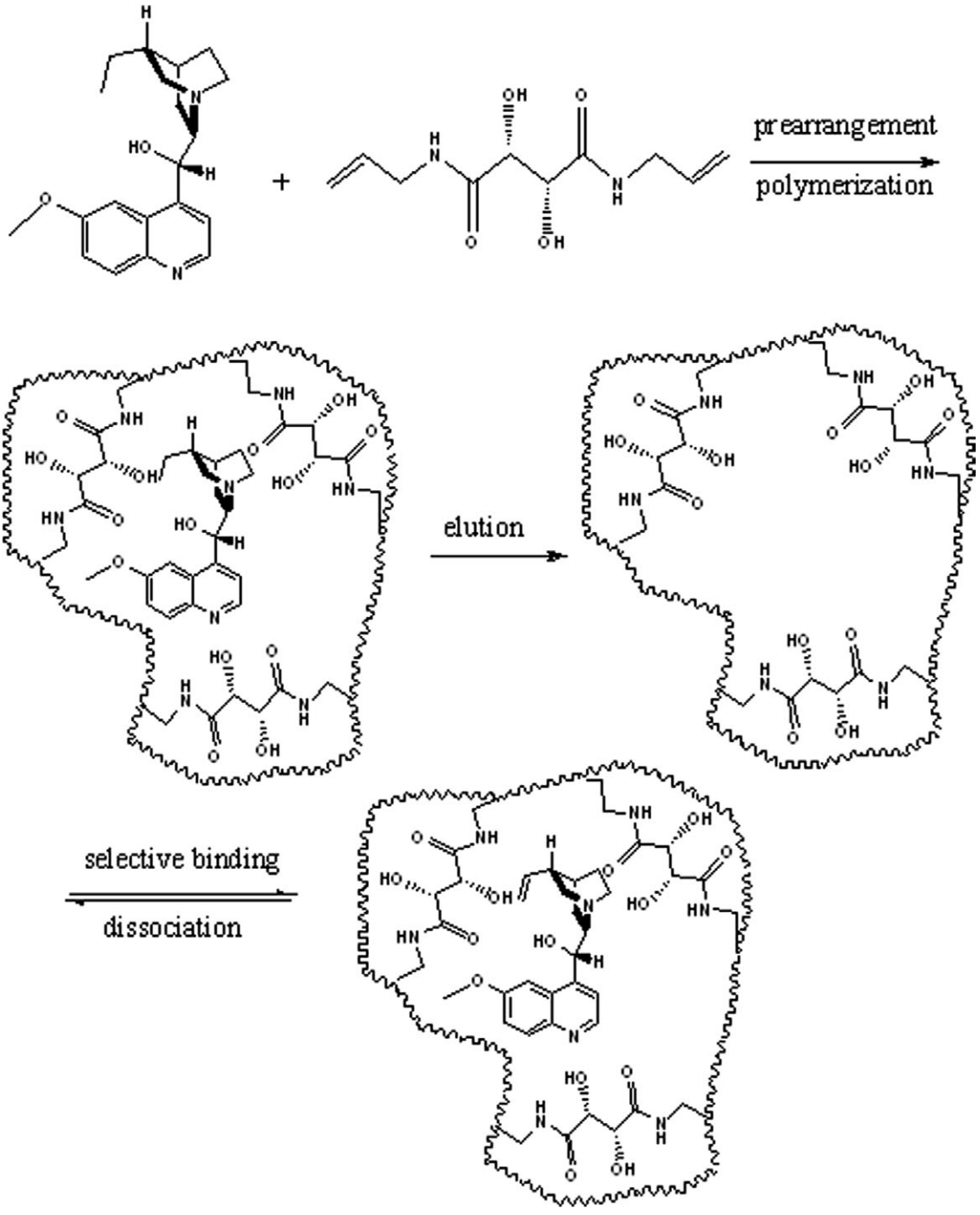

Figure 2. Scheme for the preparation of the MIP.

of acetone. The resolutions and retention times were obtained from Shimadzu LCSolution Lite computer software.

Binding Experiments

The binding properties of CMIP2 to QD were studied with the batch method, where 10.0 mg of washed and dried CMIP2 or NIPs was added to 2 mL of QD at different concentrations. The suspensions were then sealed and oscillated for 24 h at room temperature to ensure equilibration. After centrifugation at 2000 rpm for 10 min, the concentration of free QD in the supernatant was detected by HPLC. The amount of QD bound to the polymer (Q) was calculated by subtraction of the concentration of free QD from the initial concentration according to the following equation:

$$Q = (C_0 - C_t) \times V/W \quad (2)$$

where C_0 and C_t are the initial and the residual concentrations of QD (mg/L), respectively; V is the initial volume of the solution (L); and W is the weight of the CMIP2 or NIPs (g). To do a comparison of the imprinting effect, we defined the

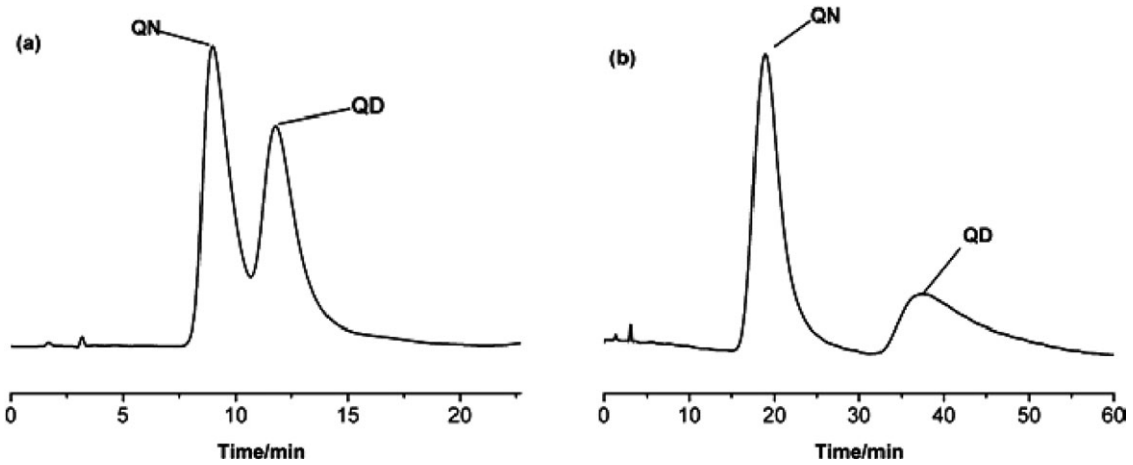

Figure 3. Chromatogram of QD and QN for the (a) NIP column or (b) CMIP2 (injection amount = 10 μL, 2 mmol/L, flow rate = 0.7 mL/min).

imprinting factor (α), as calculated from eq. (3)

$$\alpha = Q_{CMIP2}/Q_{NIPs} \quad (3)$$

where Q_{CMIP2} and Q_{NIPs} are the amounts of bound template on the CMIP and NIPs, respectively.

RESULTS AND DISCUSSION

Optimization of the Preparation Conditions

The strength and positioning of the monomer–template interactions are important for obtaining materials with good molecular recognition properties.[33] Once the chiral functional monomer has been selected, it is important that an appropriate template be selected to construct MIPs with good chiral selectivity. In general, longer retaining times result in stronger interactions between the NIPs and the chiral molecules and result in molecules that are more suitable as the template to improve the chiral recognition. The chiral separation results for QD and QN for the NIP column is shown in Figure 3(a). The NIPs had stronger adsorption for QD than for QN; this indicated that QD matched the chirality of the DATD better than QN. The choice of the molecule–template ratio is another important step during the design of the MIP process; after the functional monomer, crosslinker, and template are chosen, the conditions for MIP formation can be determined by experimental optimization, which involves the synthesis and evaluation of several polymers. In this study, we synthesized three kinds of CMIPs, and the preparation compositions of the different polymers are shown in Table I. The resolutions of QD and QN on the CMIP1, CMIP2, and CMIP3 columns were 1.48, 1.83, and 1.72, respectively. The results suggest that a better chiral separation was achieved with CMIP2, which had a ratio of template to functional monomer of 1 : 4, so this sample was selected for further study and analysis.

Chromatographic Analysis

Effect of the Mobile-Phase Flow Rate. The effect of the mobile-phase flow rate on the chiral separation was investigated at 0.5, 0.6, 0.7, 0.8, and 0.9 mL/min, and we found that the retention times of QD and QN decreased with increasing flow rate. The retention time of QD was 54.4 min at 0.5 mL/min; this value decreased to 28.7 min at 0.9 mL/min. As shown in Table II, the resolution of QD and QN decreased as the mobile-phase flow rate increased. At a flow rate of 0.5 mL/min, the resolution was 1.89; it decreased to 1.41 at 0.9 mL/min. Similarly, at a flow rate of 0.5 mL/min, the resolution value was 2.12; this decreased to 1.98 at 0.9 mL/min. Similar trends with regard to a decrease in the separation time with increasing mobile-phase flow rate have also been reported in the literature for several MIPs. For example, the resolution of phenylalanine enantiomers on the d-phenylalanine (d-Phe) imprinted polymer(methacrylic acid-*co*-ethylene glycol dimethacrylate) [P(MAA-*co*-EGDMA)] microbead stationary phase decreased from 1.39 to 0.87 with an increase in the mobile-phase flow rate from 0.1 to 0.5 mL/min.[34]

Effect of the Concentration of Triethylamine. The effect of the triethylamine concentration in the mobile phase on the chiral separation was investigated next. The experiments were performed in methanol–water (97 : 3 v/v) containing different concentrations of triethylamine in the range of 0.1–1.5% (v/v). As the concentration of triethylamine gradually increased, the retention time of QD dramatically decreased from 109.7 to 30.1 min, whereas the resolution of QD and QN decreased from 2.25 to 1.51. The results suggest that triethylamine bonded to the DATD residues in the polymers and interfered with the hydrogen bonding between QD and the imprinting polymer.

Effect of the Ratio of Methanol to Water. The effect of the mobile phase ratio on the chiral recognition properties of

Table II. Resolution of QN and QD on the CMIP2 Column with Different Flow Rates

Flow rate (mL/min)	Resolution	Retention time of QD (min)
0.5	1.89	54.5
0.6	1.85	43.7
0.7	1.83	37.3
0.8	1.70	31.8
0.9	1.41	28.7

Table III. Resolution of QN and QD with Different Ratios of the Mobile Phase

Methanol/water ratio (1% triethylamine)	k_{QD}	Resolution
90 : 10	13.6	1.42
95 : 5	19.8	1.71
96 : 4	22.8	1.73
97 : 3	26.2	1.83
98 : 2	29.7	1.90
99 : 1	38.4	1.95

hydroquinidine CMIPs was investigated at a flow rate of 0.7 mL/min, and the results are shown in Table III. As the concentration of water in the mobile phase decreased from 10 to 1%, the *k* value of QD on the CMIP2 column gradually increased. On the basis of these results, we suggest that the decrease in the concentration of water in the mobile phase allowed more hydrogen bonding between the CMIP2 and the QD molecule to increase the chiral separation process, whereas the hydrophobic interactions with the polymers' matrix decreased. Nevertheless, hydrogen bonding was predominant and made a major contribution. Thus, the QD retention time increased on the CMIP2 column, and good chiral separation ($R = 1.95$) was obtained at a ratio of 99 : 1 v/v of methanol to water. Taking into account both the chiral resolution and analysis time, we compromised by selecting a mobile ratio of methanol to water of 97 : 3 v/v for further chromatographic study.

Synergistic Effects of the Chiral Separation. The potential synergistic effect of the chiral separation was investigated on the NIP and CMIP2 columns under optimized chromatographic conditions. The resulting chromatograms are presented in Figure 3(a,b). The resolution value of QD and QN for CMIP2 was 1.83 but was only 1.07 on the NIP column. The results demonstrate our original assumption: that there was a synergistic effect in the chiral separation process that came from the chiral functional monomer and the chiral cavities of CMIP2.

Selectivity of CMIP2. The chiral separation of the other cinchona alkaloids, CD and CN, were also investigated for CMIP2. Figure 4(a,b) show the chromatograms obtained on the CMIP2 column by the injection of CD and CN and the mixture of the four cinchona alkaloids, respectively. The resolution of CD and CN was 1.45, which was lower than that of QN and QD. CD and CN are structural analogues of QN and QD, respectively, and lack a methoxyl substituent at the 6 position of the quinoline ring. However, the limited three-dimensional cavity of CMIP2 complemented the size and shape of the CD molecules better than that of QN molecules; a stereohindrance of the methoxyl substituent occurred as QN entered the cavities of the CMIP2 and resulted in CD's longer retention time.

QN and CD could be separated on the CMIP2 column, whereas CN and QD could not be separated at all. One possible explanation was that QD and CN had the same chiral center in accordance with the hydroquinidine molecule, which matched well with the chirality of the cavities. Thus, QD and CN experienced more retention than their stereoisomers. At the same time, CN could enter the cavities of the hydroquinidine-imprinted polymers and form similar multiple-point interactions via hydrogen bonding with CMIP2, as QD did, so CN had almost the same retention time as QD.

Chiral Recognition Mechanism. The chromatographic data from our work suggested that three different factors contributed to the chiral recognition of cinchona alkaloids on CMIP2: (1) matching the chirality of the functional monomer and QD; (2) the size and shape of the imprinted cavity; and (3) the hydrogen bonding between the host and guest.

Binding Characteristics

In general, MIPs exhibit a higher imprinting effect in a porogenic solvent, where the solvent has the lowest swelling effect to disrupt the recognition sites of the polymer.[35,36] In our study, the binding properties of CMIP2 and the NIPs were studied in DMSO, which was used as the porogen in the polymerization. When the initial concentration of QD was increased, the adsorption capacity of QD observed on the CMIP2 and NIP

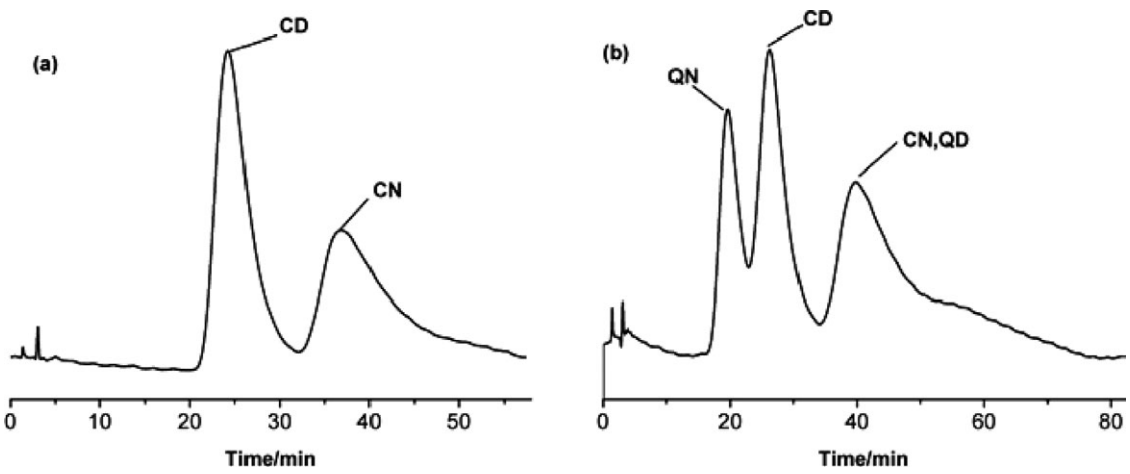

Figure 4. Evaluation of HPLC on the CMIP2 column: (a) CD and CN and (b) CD, CN, QD, and QN. The mobile phase used a methanol-to-water ratio of 97 : 3 v/v (1% triethylamine), a flow rate of 0.7 mL/min, and an injection amount of 10 μL (2 mmol/L).

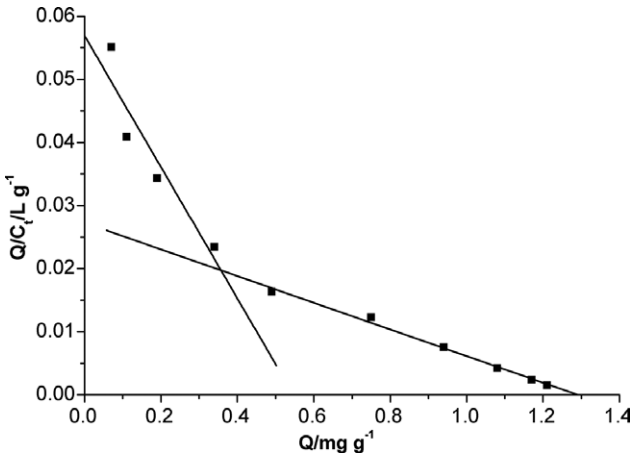

Figure 5. Scatchard plot of the experimental adsorption isotherm for the CMIP2.

columns also increased. The saturated adsorption capacities of the CMIP2 and NIPs were 1.21 and 0.69 mg/g, respectively, and α was 1.75.

The isothermal adsorption data were plotted according to the Scatchard equation[37] to evaluate multiple classes of binding sites. As shown in Figure 5, there were two distinct sections within the plot that could be regarded as straight lines, with two classes of binding sites in the hydroquinidine-imprinted polymers. The equilibrium dissociation constant (K_d) and the apparent maximum number of binding sites (Q_{max}) could be calculated according to the slopes and intercepts in the linear Scatchard analysis. From Figure 5, K_{d1} and Q_{max1} of the higher affinity binding sites were calculated to be 29.62 μmol/L and 0.547 mg/g, respectively. In the same way, K_{d2} and Q_{max2} of the lower affinity bonding sites were calculated to be 0.145 mmol/L and 1.289 mg/g, respectively.

CONCLUSIONS

The chiral functional monomer DATD was successfully used with a bulk polymerization method to prepare a novel synthetic receptor for the chiral recognition of cinchona alkaloids with hydroquinidine as the pseudo-template. The chiral molecular-imprinting technique integrated the merits of the traditional brush-type chiral selector and the existing CMIP and exhibited a significant synergistic effect on the chiral resolution. This technique could be successfully used for the liquid chromatographic separation of cinchona alkaloids. The stereoisomers of CN and CD, QD and QN, were almost completely separated for the CMIP2 sample under optimized mobile phase and polymerization conditions. In addition to molecular shape recognition, the hydrogen bonding of the target with a functional group in the polymers and matching the chirality of the template molecule and the functional monomer could work for the recognition of cinchona alkaloids.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (contract grant number 20875034).

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