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Cyclodextrin-Based Molecularly Imprinted Polymers for the Efficient Recognition of Pyrethroids in Aqueous Media

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ABSTRACT: Recent years have witnessed growing applications of the molecular imprinting technique for the detection of pesticide residues in environmental and food samples. In this study, molecularly imprinted polymers (MIPs) for pyrethroids, a class of popular insecticides, were synthesized by the crosslinking of β-cyclodextrin (β-CD) with 1,6-hexamethylene diisocyanate (HMDI) or toluene-2,4-diisocyanate (TDI) in dimethyl sulfoxide, with lambda-cyhalothrin (LCT) as a model template. Equilibrium batch-rebinding tests were operated in different aqueous solutions. The results indicate that MIP prepared with TDI (MIP–TDI) possessed a much greater binding activity to LCT than MIP based on HMDI (MIP–HMDI), and MIP–TDI displayed a remarkably specific binding to LCT (with an imprinting factor of around 3) in an acetonitrile/water (4:7 v/v) mixture. The adsorption of LCT by MIP–TDI reached equilibrium after 3 h; this demonstrated comparatively rapid adsorption kinetics. Also, MIP–TDI could be regenerated eight times at least; this implied that the robust β-CD polymer has the potential for practical applications. Furthermore, a cross-selectivity study indicated that the high adsorption of LCT and its analogues by MIP–TDI in aqueous media must have been ascribed to the cooperative effects of CD inclusion interaction and stereoshape memory. This study paved the way for the use of β-CD as a functional monomer for preparing smart artificial receptors for the efficient recognition of pyrethroids under aqueous conditions. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 128: 4014–4022, 2013

KEYWORDS: molecularly imprinted polymer; ***β*-cyclodextrin**; lambda-cyhalothrin; pyrethroids; aqueous media

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INTRODUCTION

Analytical methods with high sensitivity and selectivity for the detection of pesticide residues in environmental and food samples have been long-cherished for their practical monitoring purposes. To date, the molecular imprinting technique, a fruitful approach to the construction of tailor-made recognition sites for ligands,[1] has been increasingly applied to pesticide detection. Related studies have predominantly focused on three types of agrochemicals: triazines,[2–5] organophosphates,[6–8] and urea-based herbicides.[9–11] Additionally, most molecularly imprinted polymers (MIPs) explored as artificial receptors for pesticides have often taken effect in organic solvent systems, but selective and efficient recognition in aqueous media is still limited. The primary obstacle to successful binding in aqueous media is the nature of the hydrogen bond. As the most universal interaction for noncovalent molecular imprinting, hydrogen bonding between templates and functional monomers, however, can be partly destroyed in aqueous solutions. To circumvent this problem, nonpolar forces (e.g., hydrophobic effects) are introduced in the molecular imprinting strategy, particularly in the case that the template is hardly capable of polar interaction.[12]

As a kind of important supramolecular host compound, cyclodextrins (CDs) offer strong inclusion interactions toward numerous guest molecules with suitable polarities and dimensions in aqueous media or highly polar solvents because of CDs' physical characteristics, that is, their lipophilic internal cavity and hydrophilic external surface. Various kinds of intermolecular interactions are involved (e.g., hydrophobic effects, van der Waals forces, dipole–dipole interactions, and hydrogen bonding), which are beneficial for obtaining high-affinity binding sites during the process of molecular imprinting.[13] Compared with conventional functional monomers in the preparation of MIPs, CDs and their derivatives have proven to be attractive candidates for achieving artificial receptors with specific recognition abilities toward organic compounds in aqueous media, even toward nanometer-scaled large biomolecules in water.[14] Furthermore, the combination of CDs and general functional monomers in the imprinting process could improve the adsorption capacity of MIPs[15] and even promote the

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selectivity of MIPs in aqueous solutions.[16–19] However, CD-based MIPs for nonpolar pesticides have rarely been reported or highlighted.

Pyrethroids are known as a class of effective and popular insecticides for pest control in agriculture and public health, but they have been confirmed to cause developmental neurotoxicity and potential endocrine disruption effects in human beings.[20,21] Accordingly, the maximum residue limits for pyrethroid residues are strictly set by governments worldwide, and the exploitation of novel functional materials for the simple, rapid, and high-sensitive detection of trace pyrethroids still remains a challenge, especially in complicated sample matrices. As judged from their chemical structures, most pyrethroid molecules have very limited polar interaction sites available for precise molecular recognition (which is typically based on hydrogen bonding); this makes specific and high-affinity binding to them rather difficult by means of traditional molecular imprinting based on common functional monomers. Probably for this reason, until now, there have been only a few publications on the molecular imprinting of pyrethroids.[22–26]

Studies on β-CD inclusion complexes with pyrethroids can be traced back to reports by Yamamoto and coworkers,[27,28] who put forward an approach for stabilizing pyrethroids by making β-CD inclusion compounds. In this study, we aimed to use β-CD inclusion interactions to synthesize MIPs for pyrethroids. Lambda-Cyhalothrin (LCT) was chosen as the model template on account of its widespread use. Binding assay results show that the developed MIPs displayed a high adsorption to LCT and its analogues in the optimal solutions. The binding mechanism and further comparisons with other related reports are discussed.

EXPERIMENTAL

Materials

Figure 1 presents the chemical structures of the relevant pesticides used in this study. LCT (95.7%) was provided by Jiangsu Yangnong Chemical (Yangzhou, China), and the other pesticide standards were supplied by National Standards (Tianjin, China). β-CD (>98%) and dimethyl sulfoxide (DMSO; > 99.9%) were purchased from Sangon Biotech (Shanghai, China). Toluene-2,4-diisocyanate (TDI) was obtained from Sigma-Aldrich (St. Louis, MO), and 1,6-hexamethylene diisocyanate (HMDI) was from Sinopharm Chemical Reagent (Shanghai, China). All of the chemicals and solvents were analytical grade or better, unless otherwise stated. Deionized water was used throughout.

Before use, β-CD and LCT were dried *in vacuo* at 50°C for 24 h, and DMSO was distilled under a reduced pressure after it was dried with a 4A molecular sieve.

Polymer Synthesis

The β-CD-based MIPs were prepared through bulk imprinting according to a previously reported procedure[29] with some modifications. LCT (0.5 mmol, as the template) and β-CD (1.5 mmol) were dissolved in dry DMSO (20 mL), and the mixture was magnetically stirred at room temperature (RT) for 2 h. Subsequently, the crosslinking agent, HMDI or TDI (9 mmol), was carefully and slowly added. After the reactants were stirred at 65°C for 24 h, the resultant polymer was collected, ground, and

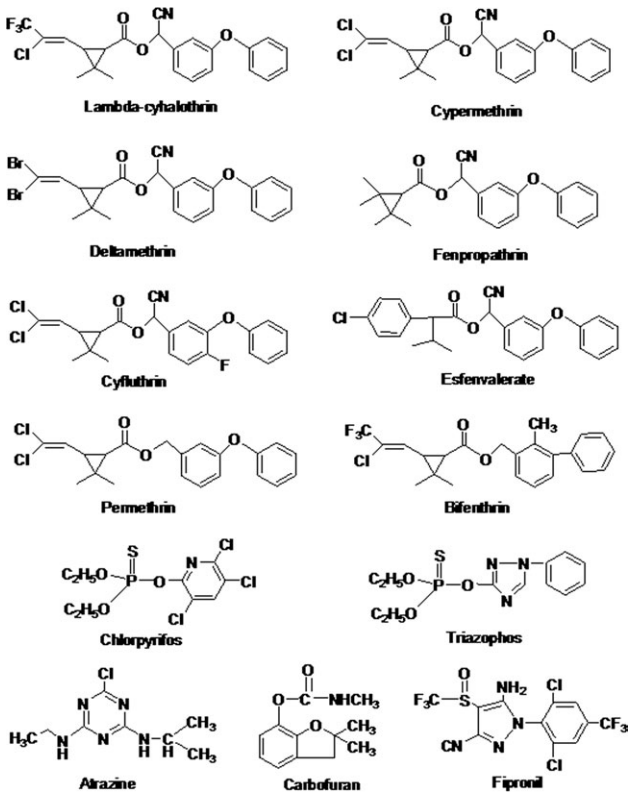

Figure 1. Chemical structures of pesticides used in this study.

sieved (particle size ≈ 32–65 μm). Fine particles were further removed through sedimentation in acetone. Then, the polymer particles were washed by batchwise solvent extraction in an ultrasonic cleaner with acetone, hot water, and hot ethanol, respectively, to sufficiently remove the template LCT, free β-CD, and unreacted crosslinker. The complete removal of LCT from the polymer matrix was ensured until no more LCT could be detected in the elution solvent by gas chromatography coupled with electron-capture detection (Agilent GC-6890 series, Palo Alto, CA). Finally, the polymer was dried *in vacuo* at 50°C for 24 h and kept in a desiccator before use. As a control, the corresponding nonimprinted polymers (NIPs) were prepared in a similar way, except without LCT. Consequently, two pairs of β-CD polymers were obtained, namely, MIP–HMDI/NIP–HMDI and MIP–TDI/NIP–TDI (listed in Table I).

Characterization of Polymers

Surface area and porosity analyses for polymers were carried out with a Micromeritics TriStar II 3020 instrument (Norcross, GA) on the basis of nitrogen sorption porosimetry. In brief, around 0.3–0.4 g of the polymer sample was degassed at 100°C overnight *in vacuo*. The surface area was measured by the Brunauer–Emmett–Teller (BET) method, and the pore size distribution was determined on the basis of a *t*-plot with the Harkins–Jura average thickness equation and the Barret–Joyne–Halenda (BJH) model.

Scanning electron microscopy (SEM) imaging of the polymer particles was done in an environmental scanning electron microscope (ESEM-XL-30, Philips, Eindhoven, The Netherlands). Before the SEM measurement, the polymer particles were

Table I. Surface Area and Pore Analysis of the Crosslinked β-CD Polymers Prepared in DMSO

β-CD polymer	Template	Crosslinker	BET surface area (m^2/g)	Pore volume (cm^3/g)[a]	t-Plot micropore volume (cm^3/g)	Average pore diameter (nm)[b]
MIP–HMDI	LCT	HMDI	8.95	0.061	1.09×10^4	27.97
NIP–HMDI	—	HMDI	2.5	0.024	1.56×10^4	32.65
MIP–TDI	LCT	TDI	1.73	0.010	1.47×10^4	27.82
NIP–TDI	—	TDI	12.19	0.081	5.60×10^5	28.05

[a]BJH adsorption cumulative pore volume of pores between 1.7 and 300 nm.
[b]BJH adsorption average pore diameter (4 × pore volume/surface area) of pores between 1.7 and 300 nm.

sputter-coated with gold by an ion coater (EIKO IB-3, Hitachi, Tokyo, Japan).

Fourier transform infrared (FTIR) spectra of the polymers were obtained by an FTIR spectrometer (AVATAR 370, Thermo Nicolet, Madison, WI) with KBr tablets in the 4000–500-cm^{-1} region. The FTIR spectroscopic analysis of β-CD was also followed for comparison.

Guest-Adsorption Tests

Each 10 mg of polymer particles was incubated with 1.5 mL of a guest solution at a known concentration in a 2-mL Eppendorf tube on a rocking table for a certain period at RT. After centrifugation (8000 rpm) at 4°C for 5 min, 1 mL of the supernatant was filtered through a 0.22 -μm filter, and the free concentration of the guest in the liquid was measured by reversed-phase, high-performance liquid chromatography with a diode array detector (Shimadzu LC-20AT system, Kyoto, Japan). Each experiment was performed in triplicate.

The percentage of the guest adsorbed or bound by the polymer was defined as the guest-binding activity of the polymer, that is, Bound% = $(C_{total} - C_{free})/C_{total} \times 100\%$, where C_{total} and C_{free} represent the initial total concentration of the solution and its final free concentration after adsorption, respectively. The template-induced promotion of binding (i.e., specific binding) is expressed as ΔBound% by subtraction of the NIP–TDI binding activity ($Bound\%_{NIP-TDI}$) from the MIP–TDI binding activity ($Bound\%_{MIP-TDI}$), which mirrors the imprinting efficiency. Beyond that, the partition coefficient (K) and the imprinting factor ($IF = K_{MIP}/K_{NIP}$, where $K = n/C_{free}$ and n is the amount of guest bound per gram of dry polymer) were figured out, as they are also important parameters frequently used to evaluate the performance of MIPs.[30]

Regeneration Experiments

The guest-adsorbed polymers were collected and sufficiently washed with an acetone/ethanol (1:1 v/v) mixture in a shaker at RT; this was followed by vacuum filtration to remove the solvent. The regenerated polymers were dried *in vacuo* at 50°C for 24 h and reused in the next cycle of adsorption tests. The guest-binding activity was checked after each cycle to assess the reusability of the β-CD polymers.

RESULTS AND DISCUSSION

Polymer Preparation

Inspired by reports[27,31,32] on inclusion interactions between β-CDs and pyrethroids, we expected β-CD would be a good functional monomer for making pyrethroid MIPs. Some studies have revealed that two different types of inclusion complexes, that is, with 1:1 and 1:2 stoichiometries between the pyrethroid molecule and the β-CD unit, appeared or coexisted in aqueous solutions, depending on the concentration of β-CD.[33,34] Because the pyrethroid molecule is too large to be accommodated in the cavity of a single β-CD residue, two or more β-CDs may be required to bind it completely. Herein, a molar ratio of LCT to β-CD of 1:3 was adopted for the preparation of the MIPs.

For polymerization, the crosslinker and the solvent should also be rationally chosen. Diisocyanates and epichlorohydrin are commonly used to crosslink β-CDs.[29,35] However, the crosslinking of β-CDs with epichlorohydrin must be achieved under highly alkaline conditions (e.g., NaOH solution), where pyrethroids are prone to decompose and the corresponding complexes with β-CDs are likely to dissociate. Accordingly, only diisocyanates (HMDI and TDI) were used in this research. With regard to the reaction medium, it should neither disturb the polyaddition nor obstruct the interaction between β-CD and the template. Thereby, a polar solvent, DMSO,[36] was used for the imprinting in this study. In addition, a crosslinker/β-CD ratio of 6 was selected for the polymerization in light of a previous investigation.[37]

Actually, in this study, the reaction phenomenon of β-CDs crosslinked by HMDI was different from that by TDI as the polymerization proceeded. With the use of HMDI, a gel was formed and was chopped into pieces for the subsequent steps. Interestingly, no gel appeared during the whole reaction for either MIP–TDI or NIP–TDI, but only a bright yellow and homogeneous solution was observed. The different reaction phenomena could be roughly understood by polymerization with unequal reactivities between HMDI and TDI. Apparently, polymerization-induced phase separation took place in the case of HMDI, whereas the synthesized TDI-based polymers were miscible with the final solution. After it was cooled to RT, the MIP–TDI or NIP–TDI solution was poured into a large amount of acetone under stirring, and then, the resulting white precipitate was collected and washed extensively. To our surprise, the white powder coagulated to be rigid solid when it was dried

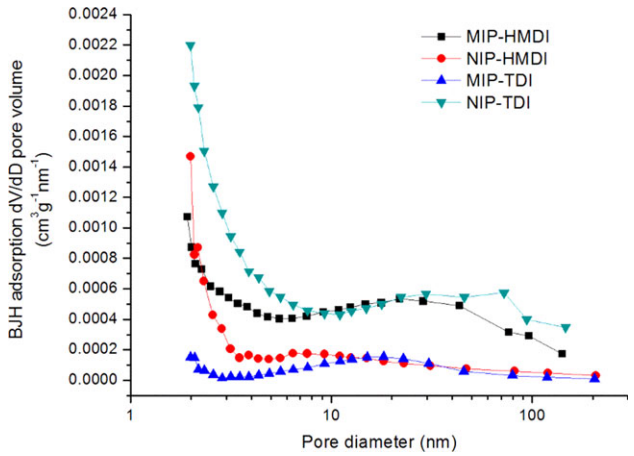

Figure 2. Pore size distribution of the β-CD polymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

out, and hence, it underwent further treatment of grinding and sieving. These distinct phenomena could have been responsible for the morphological differences between the two types of polymers (see later discussion).

Morphological Characterization of Polymers

Although the binding affinity and selectivity of MIPs in batch rebinding or chromatographic mode are not directly dependent on the morphology of MIPs, applications in drug delivery may rely on mass-transfer kinetics, which is closely related to the porosity.[38] Generally, nitrogen sorption porosimetry is useful for analyzing detailed medium-sized (meso-) and small-sized (micro-) pores, whereas SEM is conducted to image macropores.[39] The results of porosity measurement (Table I and Figure 2) show that these crosslinked β-CD polymers were full of mesopores, with an average pore diameter of 27–33 nm (the IUPAC definitions of pore size are as follows: Micropores $<$ 2 nm; 2 nm $<$ Mesopores $<$ 50 nm, and Macropores $>$ 50 nm), but the proportions of micropores were rather limited. Thus, their BET surface areas were much lower than those of traditionally formulated MIPs (in the range 100–400 m^2/g).[38] As observed by SEM, the HMDI-based and TDI-based polymers were mostly amorphous clusters, and the latter had a more compact matrix than the former did; this probably resulted from the powder agglomeration mentioned previously. Figure 3 presents only the SEM images of MIP–HMDI and MIP–TDI, as their reference polymers (NIP–HMDI and NIP–TDI) were similar in appearance. Anyhow, the mesoporous polymer network formed by crosslinking the hollow β-CD units in DMSO must have contributed to the comparatively high mass-transfer rates and gave rise to the relatively easy removal of the template and fast rebinding of the guest (see the Adsorption Kinetics section).

FTIR Spectra of the Polymers

The FTIR spectra of β-CD and the two types of polymers are provided as Figure S1. We observed the following characteristic peaks: for β-CD, 3500–3300 cm^{-1} [ν (O—H)] and 2928 cm^{-1} [ν (C—H)]; for MIP–HMDI/NIP–HMDI, 3500–3300 cm^{-1} [ν (O—H)], 2934 and 2856 cm^{-1} [ν (C—H)], 1621 cm^{-1} [ν (C=O)], and 1575 cm^{-1} [δ (N—H)]; and for MIP–TDI/NIP–HMDI, 3500–3300 cm^{-1} [ν (O—H)], 2923 cm^{-1} [ν (C—H)], 1646 cm^{-1} [ν (C=O)], 1540 cm^{-1} (δ (N—H)], and 1601, 1449 and 1415 cm^{-1} (aromatic ring). The existence of C=O and N—H groups could have been evidence of β-CDs being crosslinked by diisocyanates.

Binding Performance

Batch-binding analysis is a prevailing method for characterizing the ligand recognition properties of MIPs. Because LCT is sparingly soluble in water, the addition of an organic solvent was needed to provide homogeneous solutions. Hence, water-miscible solvents (e.g., THF, acetonitrile, and methanol) were chosen to make the carrier solutions for the batch-binding experiments.

In a series of 2-mL polypropylene microcentrifuge tubes, the two types of polymer particles (each 10 mg) were individually suspended in 1.5 mL of mixtures of different solvents and water (1:1 v/v) containing 20 μM LCT. After incubation overnight, the binding activities of the polymers were determined and compared (Figure 4). With respect to the carrier solutions, all polymers had highest binding activity to LCT in the mixture of 1:1 v/v acetonitrile/water. This fact demonstrated that a proper

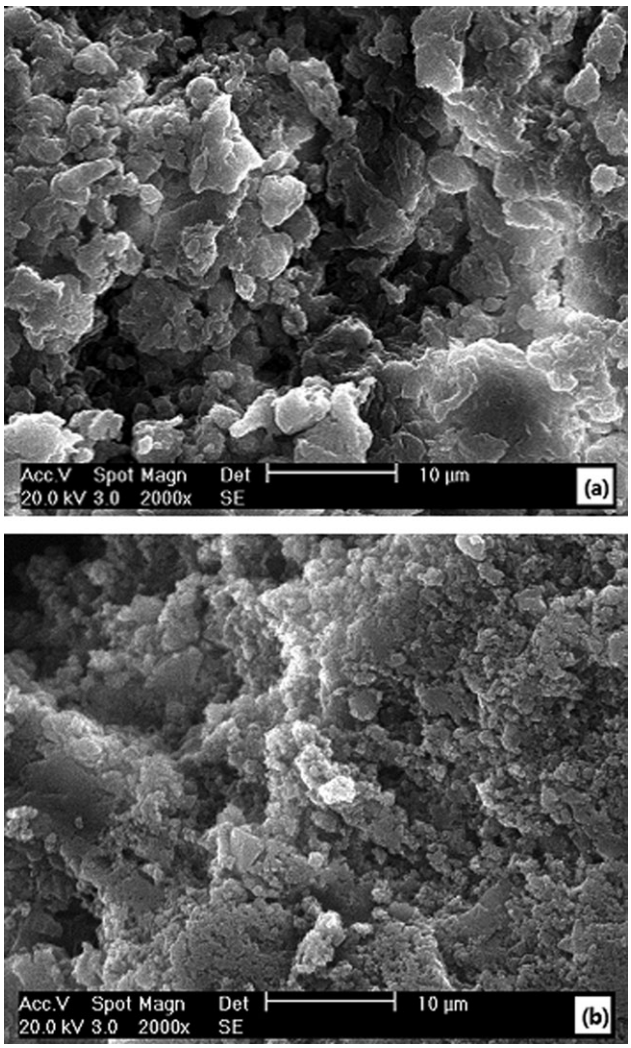

Figure 3. SEM images of (a) MIP–HMDI and (b) MIP–TDI.

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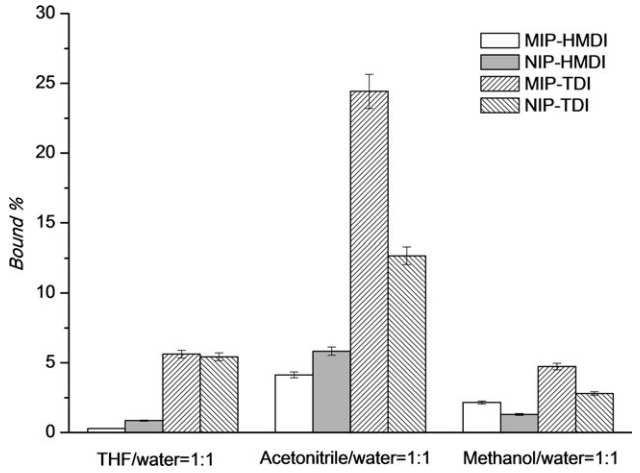

Figure 4. Adsorption of LCT by β-CD polymers in different organic solvent/water (v/v) media.

organic solvent in the aqueous solution was of crucial importance for the guest to stay in the hydrophobic cavity of β-CD. The adsorption differences among the three media were related to the distinct properties of the involved organic solvents. By comparison, THF was less polar than acetonitrile and methanol, with smaller values of both the dielectric constant and dipole moment; this led to the weak binding of LCT by the β-CD polymers. Although acetonitrile and methanol are similar in dielectric constant, the former's dipole moment is much larger than that of the latter. Additionally, acetonitrile is classified as a polar aprotic solvent, whereas methanol is a polar protic solvent. Therefore, the uptake of LCT by the β-CD polymers could have been significantly different between the acetonitrile/water and methanol/water mixtures. Probably, among the three kinds of media, the acetonitrile/water = 1:1 (v/v) mixture had the lowest impact on the interactions between LCT and the polymer matrices, and this resulted in the strongest adsorption of LCT by the β-CD polymers.

Concerning the two types of β-CD polymers, the TDI-based polymers showed much higher LCT uptakes than HMDI-based polymers in all three of the carrier solutions. Also, MIP–TDI could adsorb more LCT than the control polymer NIP–TDI, particularly in the aqueous medium of 50% (v/v) acetonitrile; this reflected the success of imprinting. By contrast, MIP–HMDI was rather less active than its reference NIP–HMDI, except in the solution of 50% (v/v) methanol. When binding studies were examined in the mixture of acetonitrile and water (1:1 v/v), the greater adsorbing activity of MIP–TDI compared to that of MIP–HMDI was attributed to the fact that the rigidity and spacer arm length of the TDI molecule was adequate for precisely immobilizing the location of the β-CD residues, which were preinvolved in the inclusion complexes with LCT at the sites of its hydrophobic terminals, whereas the hexamethylene chain in HMDI was too flexible to regulate these β-CD moieties.[29,40] Figure 5 depicts the presumable binding modes of LCT to MIP–HMDI and MIP–TDI. On the other hand, the intrinsic binding properties of the TDI-based polymer backbone were superior to those of the HMDI-based one; this might also have been associated with the nature of the crosslinking agents, the main difference between the two types of polymer backbones. Possibly, the crosslinker TDI with methyl–phenyl group promoted the overall adsorption of LCT in light of the proximity–compatibility principle. With regard to MIP–TDI, the presence of the LCT molecule led to the formation of ordered assemblies of β-CDs, which created specific interaction sites and further enhanced the pre-existing binding properties of the TDI-based polymer backbones. However, in the case of MIP–HMDI, a negative imprinting effect was even found, and this could be explained by the scarce generation of valid template-induced recognition sites or by the relatively easier access to random interaction sites on NIP–HMDI. These observations were in agreement with a previously reported perspective[41] that MIP synthesis did not improve the template uptake when adsorption by the blank polymer was too weak, whereas the MIP exhibited a significant imprinting effect under the condition that the polymer backbone had inherent binding properties toward the template.

Effect of Acetonitrile Content on Specific Recognition

According to the above-mentioned results, we preferred to use the TDI-based polymers in the following tests, and different acetonitrile–water mixtures were set as carrier solutions to evaluate the influence of the acetonitrile content on the specific recognition ability of MIP–TDI for LCT. Because of the solubility of LCT, the binding tests were carried out in various acetonitrile/water (4:8, 4:7, 4:6, 4:5, 5:5, and 6:5 v/v) mixtures with the initial guest concentration modified to 10 μM. As shown in Figure 6, the apparent adsorption of LCT on either MIP–TDI or NIP–TDI decreased dramatically with increasing content of acetonitrile in the range 33.3–54.5% v/v, whereas the amount of specific binding increased at first and then decreased. When exposed to mixtures of 36–40% v/v acetonitrile in water, MIP–TDI displayed a high specific binding to LCT, with a ΔBound% values of greater than 24%. It was clear that different acetonitrile contents remarkably affected the responses of the polymers toward LCT.

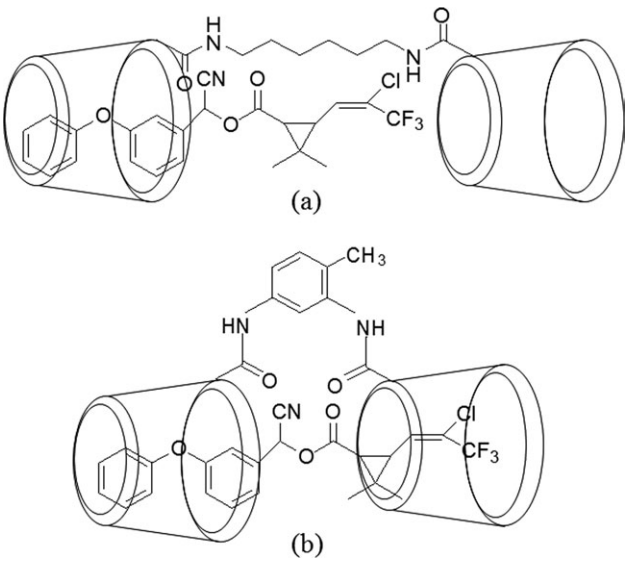

Figure 5. Possible binding modes of LCT to (a) MIP–HMDI and (b) MIP–TDI.

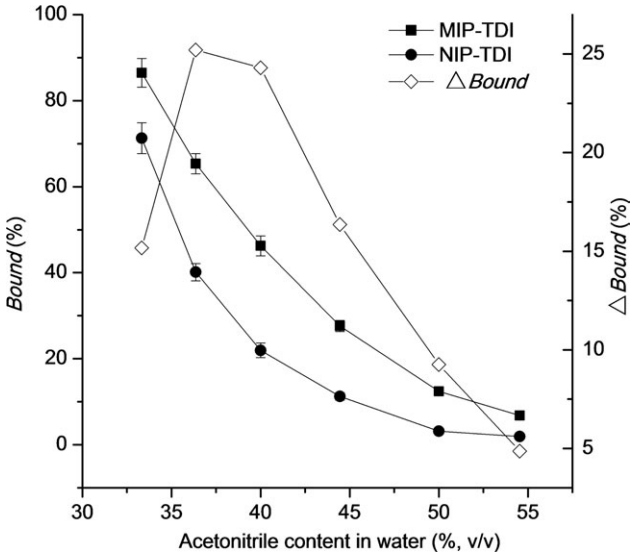

Figure 6. Adsorption of LCT by MIP–TDI/NIP–TDI in acetonitrile–water mixtures. The value of ΔBound% was obtained by the subtraction of the mean of Bound%$_{NIP-TDI}$ from that of Bound%$_{MIP-TDI}$.

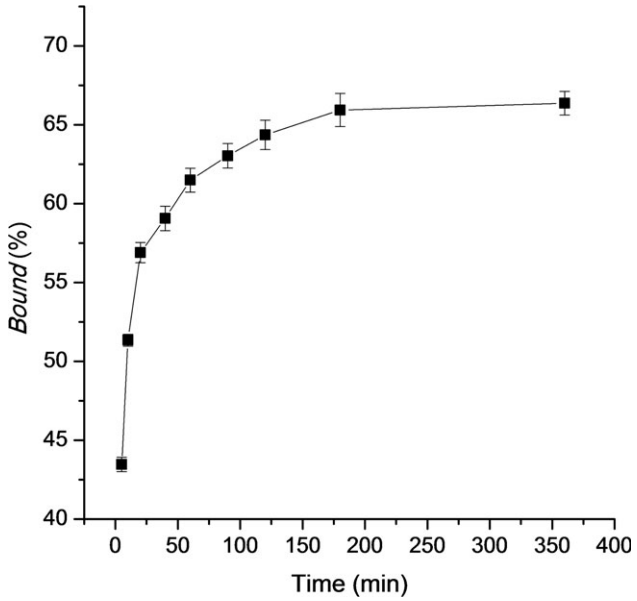

Figure 7. Kinetic curve of LCT bound by MIP–TDI in the 4:7 (v/v) mixture of acetonitrile/water.

These results could be rationalized by the binding nature that the hydrophobicity of β-CD cavity facilitated the inclusion of nonpolar compounds in the aqueous media or high polar solvent. Although the hydrogen bonding between the carbonyl group of LCT and the OH residue of β-CD might have also contributed to the formation of the β-CD inclusion complex with LCT in DMSO (for imprinting), it could be easily disturbed by water. Hence, the hydrophobic effect was the main force forming the inclusion complex in aqueous media (for binding). Along with the increasing water content (i.e., the increasing polarity of the aqueous media), the hydrophobic effect was highlighted so that more and more nonpolar template molecules could be driven into the matrix of the β-CD polymers; this led to the strong adsorption by both the imprinted and nonimprinted polymers (i.e., the high nonspecific binding) in the solutions with a high proportion of water. To reduce the nonspecific uptake, organic solvents (e.g., acetonitrile) are often used as polar modifiers to select an optimal medium where the specific guest binding induced by the imprinting process is highest. As a result, the strongest specific interaction between LCT and MIP–TDI occurred in the solutions with moderate polarity. In view of the trade-off between the total amount of apparent adsorption and the specific portion, the 4:7 v/v acetonitrile/water mixture was chosen as the appropriate binding solution for further studies.

Adsorption Kinetics

To investigate the time-dependent adsorption kinetics of MIP–TDI, binding tests were performed at various incubation times (5, 10, 20, 40, 60, 90, 120, 180, and 360 min) in the 4:7 v/v acetonitrile/water mixture containing LCT concentration at 10 μM (Figure 7). As the incubation time increased, the uptake amount of LCT increased sharply in the first 20 min and then gradually increased to saturation, reaching the final equilibrium after 3 h. The adsorption reaction was so rapid that more than three-quarters of the equilibrium amount was bound within the first 10 min. Such high mass-transfer kinetics were principally ascribed to the mesopores in MIP–TDI, which gave the polymer network good permeability. Consequently, 3 h was adopted as the incubation time in the next binding tests.

Overall, the fast adsorption kinetics of the β-CD-based MIP could be considered as a prominent advantage, in contrast to the traditional bulk MIPs with highly crosslinked matrices and large mass-transfer resistances.

Equilibrium Adsorption Isotherms

Figure 8 depicts the equilibrium adsorption isotherms of LCT on MIP–TDI and NIP–TDI. Because of the limited solubility of

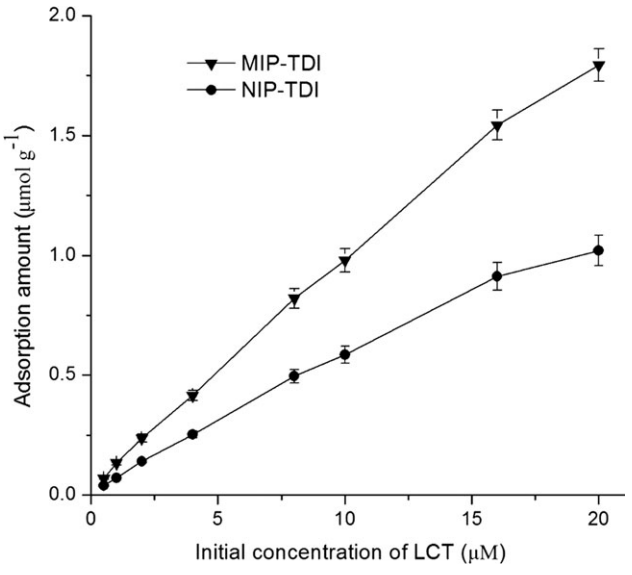

Figure 8. Equilibrium adsorption isotherms of LCT on MIP–TDI and NIP–TDI.

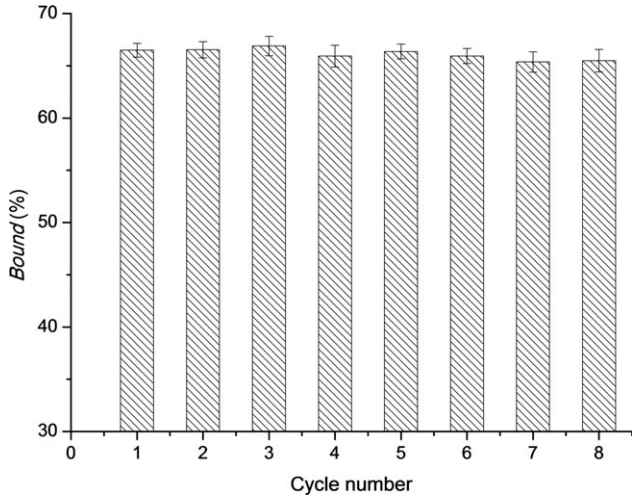

Figure 9. Adsorption of LCT by MIP–TDI in eight sequential binding–regeneration cycles.

LCT in the carrier solution and its residue level in real samples, the binding experiment was conducted at an initial guest concentration of 0.5–20 μM. Clearly, the greater amount of LCT bound by MIP–TDI compared to NIP–TDI proved the imprinting efficiency again. We also observed that in the tested concentration range, the adsorption amount (i.e., n) of LCT by either MIP–TDI or NIP–TDI increased with increasing initial concentration of LCT, but it did not show a tendency toward saturation. This could be explained by the low initial concentration of LCT used and the relatively large binding capacity of the β-CD polymers. It seemed that this β-CD-based MIP would be a potential adsorption material for the enrichment of LCT residues in aqueous media.

Reusability

The reusability of artificial receptors is a key factor in estimating their potential application values. In this study, the binding activity of MIP–TDI for LCT was repeatedly measured after each adsorption–desorption cycle under the same test conditions. As shown in Figure 9, it was clear that MIP–TDI could be reused at least eight times without a significant loss in the adsorption capacity for LCT; this suggested that the robust β-CD-based MIP would be promising for regeneration in practical applications (e.g., sensors).

Cross-Selectivity Study

To shed light on the specific binding sites created by the template-directed imprinting, LCT's analogues (seven common pyrethroids) and other representative pesticides were used to investigate the cross-selectivity of MIP–TDI in the same binding solution. The results are listed in Table II. It was revealed that the polymers had significantly distinct recognition capabilities to different types of pesticides in the solution of acetonitrile and water (4:7 v/v). Above all, MIP–TDI exhibited a higher adsorption of other pyrethroids (except bifenthrin) than the template LCT, but the portion of nonspecific binding was comparatively larger, even greater than 80% for deltamethrin, cypermethrin, cyfluthrin, and permethrin. Possibly, in this aqueous medium, the polymer matrix (β-CDs crosslinked by TDI) intrinsically preferred the pyrethroids with bigger hydrophobic terminals (bromine or chlorine). Also, the polymers had some affinity (21–36% bound) to triazophos and chlorpyrifos, whose structures contained two hydrophobic residues (an alkyl group and aromatic ring), whereas relatively polar and small pesticides (atrazine and carbofuran) could not be well adsorbed by the β-CD polymers (bound $<$ 10%).

Table II. Cross-Selectivity of MIP–TDI to Different Kinds of Pesticides[a]

Pesticide		log P[b]	Bound%		K (mL/g)		IF
			MIP–TDI	NIP–TDI	MIP–TDI	NIP–TDI	
Type II pyrethroids	LCT	6.90	66.99	40.78	304.41	103.28	2.95
	Esfenvalerate	6.24	72.57	58.23	396.92	209.09	1.90
	Fenpropathrin	6.04	69.63	55.61	343.91	187.94	1.83
	Cyfluthrin	6.00	89.12	82.06	1228.34	686.04	1.79
	Cypermethrin	5.30	91.72	86.65	1661.61	973.2	1.71
	Deltamethrin	4.60	96.19	94.13	3815.45	2415.88	1.58
Type I pyrethroids	Bifenthrin	6.60	59.14	52.19	217.13	163.73	1.33
	Permethrin	6.10	86.35	80.63	948.84	624.59	1.52
Other common pesticides	Chlorpyrifos	4.70	35.75	25.91	83.47	52.45	1.59
	Fipronil	3.75	11.11	8.64	18.75	14.19	1.32
	Triazophos	3.55	21.44	29.88	40.93	63.93	0.64
	Atrazine	2.70	4.31	10.00	6.75	16.67	0.41
	Carbofuran	1.80	3.88	7.56	6.05	12.26	0.49

[a]A batch-binding experiment for each guest was performed in an acetonitrile/water (4:7 v/v) mixture at a concentration of 10 μM, with an incubation time of 3 h. The average data of triplicate measurements were used for calculation of Bound%, K (mL/g), and IF.
[b]These data were cited from 'IUPAC Global Availability of Information on Agrochemicals' at http://sitem.herts.ac.uk/aeru/iupac/index.htm.

Judging from the values of IF, MIP–TDI exhibited a greater specific recognition for the template itself (IF ≈ 3) than any other guest tested; this implied the satisfactory formation of template–complementary binding sites during the process of imprinting. In general, it seemed that the pesticide with a higher log *P* (partition coefficient in n-octanol/water) could be more selectively bound by MIP–TDI (i.e., the higher IF); this indicated the contribution of the hydrophobic effect. However, the type I pyrethroids (without α-cyano moieties) bifenthrin and permethrin were not in line with this tendency because they were of relatively high heterology to the template LCT, particularly for bifenthrin, whose structure contained 2-methyl–3-phenylbenzyl instead of α-cyano-3-phenoxybenzyl. Therefore, MIP-TDI displayed a smaller IF to them than to other five type II pyrethroids (with α-cyano moieties), which were spatially homologous to LCT. Moreover, the guests with terminal groups similar to those of the template LCT promoted their selective adsorption on MIP–TDI. For instance, IF values from chlorpyrifos and fipronil (both containing halogens) were higher than those from triazophos, even though the polymer matrix had less affinity to fipronil than to triazophos. Overall, we inferred that the cross-selective binding was mainly governed by the stereo-shape effect inherent in MIP–TDI.

From the results obtained, we concluded that the main driving force of the strong guest binding on MIP–TDI was a hydrophobic effect for the formation of an inclusion complex with the *β*-CDs, and shape selectivity was also responsible for the delicate molecular recognition of LCT and its analogues by the *β*-CD-based MIP–TDI.

Comparison with Other Reports on Pyrethroid MIPs

The notable class-selective recognition of the LCT-imprinted *β*-CD polymer for other pyrethroids was primarily derived from the nonspecific hydrophobic effect. Similarly, Vonderheide et al.[23] described that the applicability of permethrin-imprinted bulk MIP to the extraction of cyfluthrin and cypermethrin was almost equivalent; this was supported by the fact that the best selective MIP interactions were established from the hydrophobic imprinting approach involving divinylbenzene. Recently, Shi et al.[26] developed traditional MIP-packed cartridges, which showed group selectivity and a good enrichment capability targeted to six pyrethroids at trace levels in aquaculture seawater. These results are certainly associated with the fact that the members of the pyrethroid family have a common core structure.

However, in three other studies of MIP-based sensors for the determination of LCT,[24] deltamethrin,[25] and fenvalerate,[22] respectively, each assay had a very high selectivity to the corresponding analyte without significant interference from other common pyrethroids. Probably, such a high selectivity and sensitivity of those MIP-based assays was by virtue of the involved surface-imprinting approach and was also due to the sensor's direct detection mode based on fluorescence quenching[24,25] or capacitance changes[22] caused by the template analyte getting into the recognition sites.

To summarize, the selectivity behaviors of the pyrethroid–MIPs obtained by bulk imprinting differed from those obtained via surface imprinting. Inspired by this finding, we propose that different strategies for the molecular imprinting of pyrethroids should be rationally adopted according to various practical purposes. In cases of multitarget screening or determination, class-selective MIPs from bulk imprinting are favorable in applications, such as solid-phase extraction and chromatographic separation, whereas highly selective MIPs based on surface imprinting may be preferred for the direct quantification of a single analyte.

Note that the objective of this study was to make use of CD inclusion interaction for the molecular imprinting for pyrethroids, with only a simple and traditional method for MIP preparation (bulk polymerization) and evaluation (batchwise binding operation). However, nonspecific adsorption is inevitable for *β*-CD-based MIPs under aqueous conditions. Thereby, the imprinting efficiency and specific binding performance of pyrethroid–MIPs may be further improved if a combination of *β*-CD and other kinds of functional monomers (e.g., vinyl monomer/methacrylic acid[26]) is taken into account for the imprinting, even in collaboration with metal ions to enhance interactions between the monomers and template molecules.[42] Apart from LCT-imprinted polymers, the use of other pyrethroid analytes of interest as templates in the preparation of corresponding *β*-CD-based MIPs and a further comparison of their cross-selectivity need to be researched to systematically investigate the template-dependent recognition mechanism of MIPs and thus provide reasonable guidance toward their various applications in real sample analysis.

CONCLUSIONS

In this study, *β*-CDs were crosslinked by diisocyanates to prepare polymeric receptors for LCT. The results of equilibrium batch-binding tests confirm that MIP–TDI gave strong specific binding toward LCT in acetonitrile/water mixtures with moderate polarity. The guest adsorption reached equilibrium quickly, and the artificial receptor could be recycled eight times at least; this was advantageous for further application of the *β*-CD-based MIP. Moreover, an extensive cross-selectivity study manifested that both CD inclusion interactions (mainly hydrophobic effects) and space–structure matching contributed to the efficient recognition of LCT and its analogues by MIP–TDI in aqueous media. This study demonstrated that *β*-CD acted as a promising functional monomer to prepare high-performance materials for the fast enrichment and/or separation of pyrethroids in aqueous media.

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