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Narrowly Dispersed Molecularly Imprinted Polymer Microspheres with Photo- and Thermo-Responsive Template Binding Properties in Pure Aqueous Media by RAFT Polymerization

Abstract
The facile and controlled synthesis of narrowly dispersed molecularly imprinted polymer (MIP) microspheres with both photo- and thermo-responsive template binding properties in pure aqueous media is described. Narrowly dispersed "living" core polymer microspheres with surface-immobilized dithioester groups were firstly prepared via reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization (RAFTPP). The polymer microspheres were then successively grafted with an azobenzene (azo)-containing MIP layer and thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) brushes via surface-initiated RAFT polymerization to provide the desired product. The successful grafting of the azo-containing MIP layer and PNIPAAm brushes was confirmed by Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and static contact angle experiments. The attachment of an azo-containing MIP layer onto the "living" core polymer beads with a narrow size distribution allows the direct generation of narrowly dispersed photoresponsive core-shell MIP microspheres. Moreover, the introduction of PNIPAAm brushes onto the core-shell MIP microspheres has been shown to significantly improve their surface hydrophilicity leading to pure water-compatibility. Additionally, this modification confers thermo-responsive template binding properties upon the microspheres.

Keywords
Molecular imprinting, • Polymer microspheres • Water-compatible • Photoresponsive • Thermo-responsive • RAFT polymerization

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1. Introduction

Stimuli-responsive molecularly imprinted polymers (MIPs) have recently attracted a large degree of attention because they represent a new generation of intelligent and self-regulated artificial receptors and have shown great potential in many applications [1]. Thus far, MIPs responsive towards different stimuli such as temperature, pH, or light have been developed by the addition of quantified levels of specific (co)monomers (e.g., *N*-isopropylacrylamide (NIPAAm), acrylic acid, or azobenzene (azo) monomer) into molecular imprinting systems [1-5]. Among these systems, photoresponsive materials exhibit unique advantages over systems that rely on other stimuli because light stimulus can be imposed instantly and delivered in specific amounts with high accuracy [1].

Photoresponsive MIPs have been mainly prepared by introducing a functional azo moiety into the selective binding sites of MIPs [1,4,6-11]. The configuration of the azo groups can be regulated by light illumination, which causes a marked alteration of the spatial arrangement of the binding functionalities in the binding sites, thus leading to a significant change in the strength of the host-guest interactions. Despite previous reports demonstrating the promising potential of such photoresponsive azo-containing MIPs in smart separation and assays as well as efficient drug delivery, photoresponsive MIPs with molecular recognition ability in aqueous media are still rare, which greatly limits their practical applications. Lam and coworkers described the preparation of water-compatible photoresponsive MIP hydrogels for the photoregulated release and uptake of pharmaceuticals in aqueous media using a water-soluble azo functional monomer 4-((4-methacryloyloxy) phenylazo)benzenesulfonic acid [10]. However, the bulk hydrogel physical format in addition to the irregular shapes and relatively large sizes (normally tens of micrometers in diameter after time-consuming and laborious grinding and sieving of the bulk MIP hydrogels) employed are inappropriate for such applications as smart binding assays and drug delivery because the best physical format for such purposes is spherical

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beads [12,13]. Moreover, the binding sites inside MIP particles of relatively large sizes are inaccessible, thereby significantly lowering the template loading capacities of the MIP particles. *Therefore, the development of photoresponsive azo-containing spherical MIP particles having water-compatible template binding properties and micrometer dimensions remains an important goal.* In addition, although some reports exist on stimuli-responsive MIPs towards two different stimuli including temperature, pH values, or salt concentrations [14,15], the synthesis of photoresponsive MIPs that can respond to other external stimuli remains a challenging task; such advanced MIPs are highly desirable in many applications [16].

In this paper, we describe a facile, general, and highly efficient approach to obtain narrowly dispersed azo-containing MIP microspheres with both photo- and thermo-responsive binding properties in pure aqueous media. Narrowly dispersed "living" core polymer microspheres with surface-immobilized dithioester groups via reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization (RAFTPP) are first prepared followed by successive surface-grafting of an azo-containing MIP layer and thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) brushes via surface-initiated RAFT polymerization (Scheme 1). The chemical structures, morphologies, particle sizes, and surface hydrophilicity of the obtained MIP microspheres as well as their equilibrium binding properties in both acetonitrile and pure water under different conditions (including in the dark, under UV light irradiation, and different temperatures) were investigated in detail.

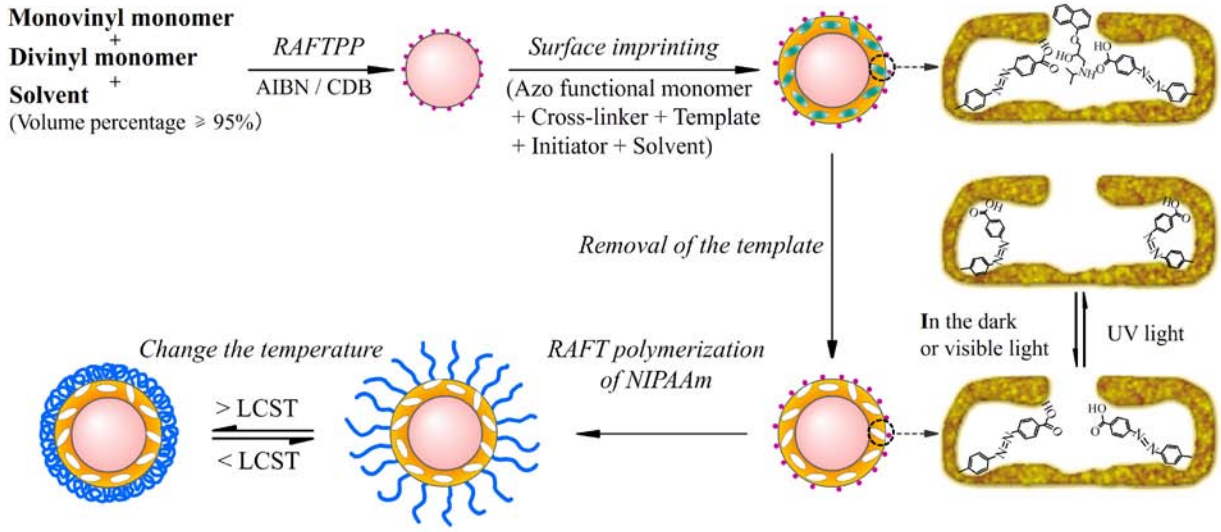
2. Experimental

2.1 Materials

4-Vinylpyridine (4-VP, Alfa Aesar, 96%), ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%), and *N,N*-dimethylformamide (DMF, Tianjin Jiangtian Chemicals, Analytical grade (AR)) were purified by distillation under vacuum. Methanol (Tianjin Jiangtian Chemicals, AR) was distilled prior to use. Tetrahydrofuran (THF, Tianjin Jiangtian Chemicals, 99%) was refluxed over sodium and then distilled. Acetonitrile (Tianjin Concord Chemicals, China, AR) was refluxed over calcium hydride (CaH_2) and then distilled. *N*-Isopropylacrylamide (NIPAAm, Acros, 99%) was recrystallized from hexane prior to use. Azobisisobutyronitrile (AIBN, Chemical Plant of Nankai University, AR) was recrystallized from ethanol before being used. Cumyl dithiobenzoate (CDB) was prepared according to a literature procedure [17]. 4-((4-Methacryloyloxy)phenylazo) benzoic acid (MPABA) was prepared following our previously reported procedure (Scheme S1) [18]. (±)-Propranolol hydrochloride (Alfa Aesar, 99%) was converted into its free base form (Scheme S2) before use following a previously reported procedure [19]. Atenolol (National Institute for the Control of Pharmaceutical and Biological Products, China, Chemical reference substance, Scheme S2) was dried at 105°C for 3 h before use. All the other reagents were commercially available and used as received.

2.1.1 Preparation of Narrowly Dispersed "Living" Core Polymer Microspheres with Surface-Immobilized Dithioester Groups.

Polymer microspheres with surface-immobilized dithioester groups were prepared via RAFTPP according to the following procedure: 4-VP (0.611 mmol), EGDMA (2.445 mmol), CDB (0.165 mmol), AIBN (0.055 mmol), and a mixture of methanol and water (4/1 v/v, 60 mL) were sequentially added to a one-neck round-bottom flask (100 mL). A clear purple reaction mixture was obtained after stirring for 10 min at room temperature. The reaction mixture was then purged with argon for 30 min, sealed, and submerged in an oil bath. The oil bath was heated from room temperature to 70°C over 2 h and the polymerization was conducted at 70°C for 24 h with continuous stirring. The resulting



Scheme 1. Synthesis of narrowly dispersed MIP microspheres with both photo- and thermo-responsive template binding properties in pure aqueous media by RAFT polymerization and their stimuli-responsive behavior.

polymer particles were collected by filtration and subsequently washed with methanol three times. After being dried at 40°C under vacuum for 48 h, a light pink powder was obtained (61%).

2.1.2 Preparation of Propranolol-Imprinted Azo-Containing Core-Shell Polymer (i.e., Core-Shell MIP (CS-MIP)) Microspheres.

Surface-initiated RAFT polymerization was utilized to prepare propranolol-imprinted azo-containing core-shell polymer microspheres using the above-obtained narrowly dispersed "living" core polymer microspheres with surface-immobilized dithioester groups as the immobilized RAFT agent, AIBN as the initiator, propranolol as the template, MPABA as the azo functional monomer, and EGDMA as the cross-linker according to the following procedure: MPABA (1.05 mmol), propranolol (1.05 mmol), and a mixture of THF and acetonitrile (1/2 v/v, 150 mL) were added into a one-neck round-bottom flask (250 mL). A clear solution was obtained after stirring at room temperature for 30 min. The solution was subsequently stored at 4°C in the dark overnight to allow the formation of the MPABA-propranolol complex. The "living" core polymer microspheres with surface-immobilized dithioester groups (150 mg), EGDMA (4.62 mmol), and AIBN (0.015 mmol) were then added into the above solution. The obtained reaction mixture was degassed in an ultrasonic bath for 5 min, purged with argon for 30 min in an ice bath, and then sealed. The polymerization was carried out at 50°C for 2 h and then at 60°C for 22 h in an oil bath in the dark with continuous stirring. The resulting polymer particles were collected by centrifugation and washed thoroughly with methanol/acetic acid (9/1 v/v) and methanol successively. After being dried at 40°C for 48 h under vacuum, orange-yellow azo-containing core-shell MIP microspheres (i.e., CS-MIP microspheres) were obtained.

The corresponding core-shell non-imprinted or control polymer (CP) (i.e., core-shell CP (CS-CP)) microspheres (orange-yellow color) were prepared and purified under identical conditions in the absence of a template.

It is worth mentioning that low levels of minute particles were generated in the above polymerization systems during the surface imprinting processes. These particles could be completely removed by low-speed centrifugation (1000 rpm) of the resulting polymerization solutions.

2.1.3 Preparation of CS-MIP/CS-CP Microspheres Grafted with PNIPAAm Brushes.

The propranolol-imprinted/non-imprinted core-shell polymer microspheres grafted with PNIPAAm brushes (i.e., the grafted CS-MIP/CS-CP microspheres) were prepared via surface-initiated RAFT polymerization of NIPAAm using the above-obtained (ungrafted) CS-MIP/CS-CP microspheres as the immobilized RAFT agent according to the following procedure: The ungrafted CS-MIP/CS-CP microspheres (50 mg), NIPAAm (13.27 mmol), CDB (0.01 mmol), AIBN (0.003 mmol), and DMF (2.5 mL) were successively added into a two-neck round-bottom flask (25 mL). After being degassed with five freeze-pump-thaw cycles, the flask was sealed and then immersed in a thermostatted oil bath at 70°C and stirred for 24 h. The resulting polymer products were collected by centrifugation and thoroughly washed with methanol until no white sediment was detectable when ether was added into the washing solutions. The products were then dried at 30°C under vacuum to constant weights, affording orange-yellow grafted CS-MIP and CS-CP microspheres with a weight increase of 11% in comparison to the corresponding ungrafted materials.

The addition of CDB into the above polymerization systems also led to the generation of free PNIPAAm in the reaction solutions [20], which were isolated by precipitating the supernatant solutions (after the centrifugation of the reaction mixtures) into pentane, filtering, and then drying at 30°C under vacuum for 48 h, affording light pink polymer products.

2.2 Characterization

Fourier transform infrared (FT-IR) spectra of the "living" core polymer microspheres, ungrafted, and grafted CS-MIP/CS-CP microspheres were recorded with a Nicolet Magna-560 FT-IR spectrometer.

The morphologies, particle sizes, and size distributions of the "living" core polymer microspheres, ungrafted, and grafted CS-MIP/CS-CP microspheres were characterized with a scanning electron microscope (SEM, Shimadzu SS-550). All SEM size data reflect the averages of ~200 particles (they represent all the particles in one representative area in the SEM image), which are calculated using the following formulas [21]:

$$D_n = \sum_{i=1}^{k} n_i D_i / \sum_{i=1}^{k} n_i ; \quad D_w = \sum_{i=1}^{k} n_i D_i^4 / \sum_{i=1}^{k} n_i D_i^3 ; \quad U = D_w / D_n$$

where D_n is the number-average diameter, D_w denotes the weight-average diameter, k represents the total number of the measured particles, D_i is the diameter of the measured microspheres, n_i is the number of the microspheres with a diameter D_i, and U the size distribution index.

The molecular weights and polydispersity indices (PDIs) of the free polymers generated in the polymerization solutions during the surface-initiated RAFT polymerization of NIPAAm (due to the addition of sacrificial RAFT agent) were determined using a gel permeation chromatograph (GPC) equipped with an Agilent 1200 series manual injector, an Agilent 1200 high-performance liquid chromatography (HPLC) pump, an Agilent 1200 refractive index detector, and three Waters UltraStyragel columns with 5K-600K, 500-30K, and 100-10K molecular ranges (the temperature of the column oven was 35°C). THF was used as the eluent at a flow rate of 1 mL/min, and the calibration curve was obtained using polystyrene (PS) standards.

The polymer films of the "living" core polymer microspheres and the ungrafted/grafted CS-MIP/CS-CP microspheres were prepared by casting their suspension solutions in acetonitrile (10 mg/mL, after ultrasonic dispersion) on clean glass surfaces. After the solvent was allowed to evaporate at ambient temperature overnight, a KRÜSS FM40 Easy Drop contact angle

equipment (Germany) was utilized to determine their static water contact angles. Two measurements were taken across each sample, with the average being used for analysis.

Equilibrium binding experiments were performed by incubating a propranolol solution in acetonitrile (0.5 mL, 0.05 mM) or in pure water (0.5 mL, 0.05 mM) with different amounts (0.38 mg, 0.5 mg, 0.63 mg, and 0.75 mg) of the ungrafted or grafted CS-MIP/CS-CP microspheres at 25°C for 16 h. After centrifugation (12000 rpm, 10 min), the amounts of the template remaining in the supernatants were quantified using HPLC (Scientific System Inc., USA) equipped with a UV-vis detector, from which the amounts of the template bound to the ungrafted and grafted CS-MIP/CS-CP microspheres could be obtained. The wavelength used for the determination of propranolol was 293 nm. A mixture of acetonitrile and 0.4% aqueous solution of triethylamine (7/3 v/v) was used as the mobile phase at a flow rate of 1 mL/min. The retention time of the template was 10 min. The amounts of the bound template are expressed as percentage values (i.e., Bound (%), the ratio of the bound template to the original template in the solutions) or as mg template per gram of polymer microspheres.

The binding selectivity of the ungrafted and grafted CS-MIP/CS-CP microspheres was evaluated by measuring their competitive binding capacities towards propranolol and a structurally related compound, atenolol, as follows: 0.5 mg of ungrafted or grafted CS-MIP/CS-CP microspheres were incubated with 0.5 mL of a mixed solution of propranolol and atenolol in acetonitrile or in pure water ($C_{\text{propranolol or atenolol}}$ = 0.05 mM) at 25°C for 16 h and the amounts of propranolol and atenolol bound to the ungrafted and grafted CS-MIP/CS-CP microspheres were quantified by HPLC. The wavelength used for the determination of the mixed solution of propranolol and atenolol was 275 nm. A mixture of acetonitrile and 0.4% aqueous solution of triethylamine (7/3 v/v) was used as the mobile phase at a flow rate of 1 mL/min. The retention times of propranolol and atenolol were 9 and 4 min, respectively.

The "imprinting-induced promotion of binding" (IPB) has proven to be a useful parameter for evaluating the MIPs' selectivity because the difference in the intrinsic nonspecific bindings of the MIPs towards different analytes is normalized [22,23]. IPB can be defined by the following equation:

$$\text{IPB (\%)} = [(B_{\text{MIP}} - B_{\text{CP}})/B_{\text{CP}}] \times 100\%$$

where B_{MIP} and B_{CP} are the equilibrium bindings of the studied MIP and its corresponding CP towards an analyte, respectively. The larger the IPB value of the MIP towards the analyte, the better the selectivity of the MIP.

Studies on the photoregulated release and uptake of the template propranolol and atenolol by the grafted CS-MIP microspheres were performed by alternately switching on and off the UV light irradiation of the mixtures of the grafted CS-MIP microspheres and a mixed pure aqueous solution of propranolol and atenolol as follows: A series of samples were prepared by adding 0.5 mg of the grafted CS-MIP microspheres and 0.5 mL of a mixed aqueous solution of propranolol and atenolol (0.05 mM) into plastic Eppendorf tubes (2 mL), respectively, which were subsequently sealed and placed in an incubator equipped with a 365 nm UV lamp (16 W). After incubation at 25°C in the dark for 10 h, one sample was removed from the incubator and centrifugated. HPLC was used to determine the amounts of the analytes bound by the grafted CS-MIP microspheres. The UV light was then switched on in the incubator to irradiate the remaining samples immediately after the first sample was removed. After 6 h of UV light irradiation with incubation of the samples at 25°C, the UV light was switched off and a second sample was removed and the level of analyte binding was similarly determined. The remaining samples were then incubated at 25°C in the dark for another 18 h, and a third sample was withdrawn and analyzed. The UV light was then switched on again to irradiate the remaining samples at 25°C immediately after the third sample was removed. The above photoswitching cycles (i.e., UV light on for 6 h and off for 18 h alternately) were repeated until all the other samples were analyzed.

The photoregulated release and uptake of the analytes by the grafted CS-CP microspheres were similarly studied to provide control results for the grafted CS-MIP microspheres.

Temperature-dependent equilibrium binding experiments were also performed by incubating a propranolol solution in pure water (0.5 mL, 0.05 mM) with the grafted CS-MIP/CS-CP microspheres (0.5 mg) at a series of different temperatures for 16 h. After centrifugation, the amounts of the template remaining in the supernatants were quantified with HPLC.

In addition to the above photo- and thermo-responsive template binding experiments in pure aqueous solutions, those in the template solutions in "regular" water (which was taken from Xinkai Lake in Nankai University on May 30, 2012) were also performed to demonstrate the applicability of the grafted CS-MIP microspheres in common water.

All the above binding analyses were performed in duplicate and the mean values were used.

3. Results and Discussion

3.1 Synthesis and Characterization of Narrowly Dispersed Azo-Containing Core-Shell MIP Microspheres with Surface-Grafted PNIPAAm Brushes

Narrowly dispersed "living" core polymer microspheres were prepared via RAFTPP of 4-vinylpyridine (4-VP) and ethylene glycol dimethacrylate (EGDMA) with cumyl dithiobenzoate (CDB) as the RAFT agent and azobisisobutyronitrile (AIBN) as the initiator in a large amount of methanol/water (4/1 v/v), following our previously reported procedure which was modified with respect to reactant composition, polymerization temperature and polymerization procedure [23,24]. The polymerization was carried out using a one-pot approach and relatively high yields of polymer particles were readily obtained. The resulting polymer beads had a light pink color, suggesting the presence of dithioester groups.

The obtained narrowly dispersed "living" core polymer microspheres were then used as both the supporting beads and immobilized RAFT agent for the subsequent surface-imprinting process, which was realized via surface-initiated RAFT polymerization with 4-((4-methacryloyloxy)phenylazo)benzoic acid (MPABA) (Scheme S1 in the Supporting Information), propranolol

(a sympatholytic non-selective β-blocker, Scheme S2), EGDMA, AIBN, and a mixture of tetrahydrofuran and acetonitrile (1/2 v/v) as the functional monomer, template, cross-linker, initiator, and porogenic solvent, respectively (Scheme 1). The polymerization was carried out firstly at 50°C for 2 h and then at 60°C for 22 h in an oil bath in the dark with stirring. After low-speed centrifugation (1000 rpm) of the resulting reaction mixture (in order to remove low amounts of small particles generated during the surface imprinting processes) and thorough washing with methanol/acetic acid (9/1 v/v) and methanol successively, azo-containing core-shell MIP (i.e., CS-MIP) microspheres were obtained. The corresponding core-shell non-imprinted polymer (or control polymer, CP) (i.e., CS-CP) microspheres were also prepared similarly by omitting the template during the polymerization process. Both the CS-MIP and CS-CP particles were orange-yellow in color, suggesting the successful grafting of the azo-containing polymer layer onto the core polymer microspheres. The living nature of surface-initiated RAFT polymerization should provide "living" CS-MIP/CS-CP particles with dithioester groups on their their surfaces. Surface-initiated RAFT polymerization of NIPAAm was further carried out to prepare CS-MIP/CS-CP microspheres grafted with PNIPAAm brushes (i.e., grafted CS-MIP/CS-CP microspheres) using the above-obtained ungrafted (or unmodified) CS-MIP/CS-CP microspheres as the immobilized RAFT agent, CDB as the sacrificial RAFT agent, AIBN as the initiator, and *N,N*-dimethylformamide (DMF) as the solvent. The addition of a certain amount of CDB into the reaction systems not only increased the control over the polymerization, but also helped to characterize the molecular weights and polydispersities of the grafted polymer brushes [20]. A weight increase of 11% was observed for both the CS-MIP and CS-CP microspheres after their surface modification, demonstrating the successful grafting of polymer brushes. It is important to stress that the increased weights of the grafted CS-MIP/CS-CP particles should mainly stem from the surface-grafted polymer brushes since the rather high cross-linking densities (~ 80%) of the CS-MIP/CS-CP particles would prevent them from swelling in the reaction media and only allow the occurrence of surface polymerization, as reported by Tirelli and coworkers [25] and also by us [26,27].

The above-obtained "living" core polymer microspheres, ungrafted, and grafted CS-MIP/CS-CP microspheres were firstly characterized by SEM. The results revealed narrowly dispersed spherical particles with number-average diameters (D_n) being 2.602, 3.206, 3.172, 3.227, and 3.195 μm, respectively, and size distribution indices (U) all ≤ 1.06 (Figure 1, Table 1). In addition,

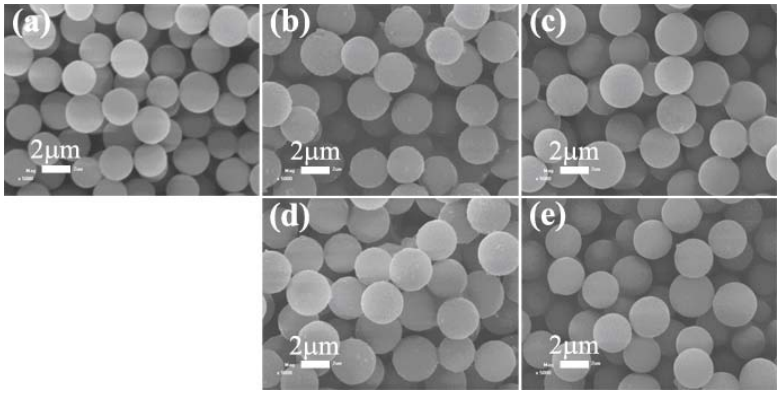


Figure 1. SEM images of the "living" core polymer microspheres (a), the ungrafted CS-MIP (b)/CS-CP (c) microspheres, and the grafted CS-MIP (d)/CS-CP (e) microspheres. The scale bar is 2 μm in the above images.

Table 1. Characterization data for the "living" core polymer microspheres and also ungrafted and grafted CS-MIP/CS-CP microspheres.

Sample	Color	D_n (μm)[a]	U [a]	L (nm)[b]	$M_{n,GPC}$ [c]	PDI[c]	Contact angle (°)[d]
Core polymer microspheres	Light pink	2.602	1.06	-	-	-	115.3±1.9
Ungrafted CS-MIP	Orange-yellow	3.206	1.02	302	-	-	120.8±2.5
Ungrafted CS-CP	Orange-yellow	3.172	1.02	285	-	-	124.5±2.4
Grafted CS-MIP	Orange-yellow	3.227	1.02	11	26700	1.4	69.4±2.1
Grafted CS-CP	Orange-yellow	3.195	1.02	12	25400	1.5	67.9±1.8

[a] *D_n and U refer to the number-average diameter and size distribution index of the polymer microspheres, respectively.*
[b] *L is the thickness of the azo-containing MIP/CP layer for the ungrafted CS-MIP/CS-CP microspheres or that of the polymer brush layer for the grafted CS-MIP/CS-CP microspheres, which is equal to $\Delta D_n/2$ (where ΔD_n is the increased diameter after each modification step).*
[c] *The number-average molecular weights ($M_{n,GPC}$) and polydispersity indices (PDI) of the free PNIPAAm (generated in the polymerization solutions during the surface-initiated RAFT polymerization of NIPAAm due to the addition of sacrificial RAFT agent) were determined by gel permeation chromatography (GPC) with THF as the mobile phase and polystyrene as standards.*
[d] *The static water contact angles of the polymer films.*

the D_n values of the polymer microspheres increased following both the surface imprinting and polymer brushes-grafting processes, indicating the successful grafting of both the azo-containing MIP/CP layer and hydrophilic polymer brushes.

Figure 2 shows the FT-IR spectra of the obtained polymer microspheres. Absorbances from the core poly(4-VP-*co*-EGDMA) microspheres such as those characteristic of the bonded EGDMA [i.e., 1710 (C=O stretching) and 1233/1131 cm^{-1} (C-O-C stretching)] and those corresponding to the C=N stretching (1578 and 1540 cm^{-1}) and C=C stretching (1436 cm^{-1}) from the bonded 4-VP can be clearly identified. In addition the CS-MIP and CS-CP microspheres exhibit the characteristic broad peak of the carboxylic acid group (~3295 cm^{-1}, O-H stretching) of the bonded azo monomer MPABA, which suggests a successful grafting of the azo-containing MIP/CP layer onto the core polymer microspheres after the surface imprinting process. Moreover, some new peaks characteristic of PNIPAAm (i.e., the amide I band (1657 cm^{-1}, C=O stretching) and amide II band (1517 cm^{-1}, N-H stretching)) can also be discerned in the FT-IR spectra of the grafted CS-MIP/CS-CP microspheres prepared by the surface-initiated RAFT polymerization of NIPAAm. These observations indicate that the hydrophilic polymer brushes were successfully attached onto the CS-MIP/CS-CP microspheres after the polymer brushes-grafting process. Furthermore, all the above polymer microspheres display the characteristic peaks of "living" dithioester groups ~1029 cm^{-1} (i.e., C=S stretching).

It is generally accepted that the molecular weights and polydispersities of the free polymers generated in surface-initiated RAFT polymerization systems (due to the addition of the sacrificial chain transfer agent) can be utilized to represent those of the grafted polymer brushes [20]. Therefore, the free polymers obtained in our study were characterized by GPC; the number-average molecular weights ($M_{n,GPC}$) of the polymer brushes grafted on the CS-MIP and CS-CP microspheres were found to be 26700 and 25400, respectively, and their polydispersity indices were 1.4 and 1.5, respectively (Table 1). The rather similar molecular weights of the grafted polymer brushes on the CS-MIP and CS-CP microspheres indicated that the surface-initiated RAFT polymerization happened in a controlled manner.

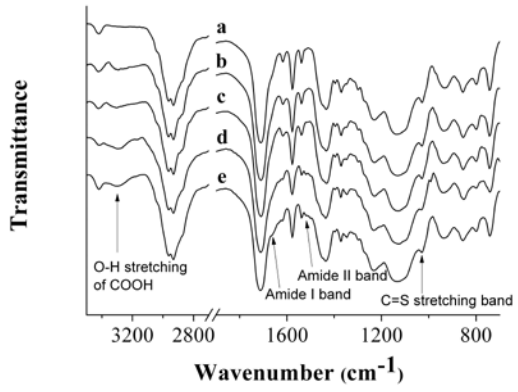


Figure 2. FT-IR spectra of the "living" core polymer microspheres (a), the ungrafted CS-MIP (b)/CS-CP (c) microspheres, and the grafted CS-MIP (d)/CS-CP (e) microspheres.

It has been well established that surface-grafting of hydrophilic polymer brushes is a highly efficient approach to improve the surface hydrophilicity of materials. The surface hydrophilicity of the "living" core polymer microspheres, ungrafted, and grafted CS-MIP/CS-CP microspheres were investigated by conducting water contact angle experiments [21,23,26]. It can be seen clearly from Table 1 that the grafted CS-MIP and CS-CP films displayed much lower static water contact angles than the films of the "living" core polymer microspheres and the ungrafted CS-MIP/CS-CP microspheres, demonstrating the successful grafting of hydrophilic polymer brushes on the modified CS-MIP/CS-CP microspheres. In addition, both the ungrafted and grafted CS-MIP films exhibited similar hydrophilicity to the corresponding CS-CP materials, as revealed by their close static water contact angles.

3.2 Equilibrium Template Binding Properties of Ungrafted and Grafted CS-MIP/CS-CP Microspheres

With the ungrafted and grafted CS-MIP/CS-CP microspheres in hand, we studied their equilibrium template binding properties in acetonitrile. As shown in Figure 3a (or Figure S1a in the supporting information), both the ungrafted and grafted CS-MIPs bound more template than their corresponding CS-CPs, suggesting the presence of specific binding sites in both the ungrafted and grafted CS-MIPs. In addition, both the grafted CS-MIP and CS-CP were found to bind less template than their corresponding ungrafted materials over a range of polymer concentrations, which again verified the occurrence of surface modification for the grafted CS-MIP/CS-CP microspheres.

Equilibrium template binding experiments were then carried out in pure water at ambient temperature. It has been well demonstrated that the water-incompatibility of MIPs is mainly due to their hydrophobically driven nonspecific template bindings in aqueous media, which depends on the hydrophobicity of the template molecules and the exposed MIP surfaces [28]. As expected, the specific template bindings (i.e., the template binding difference between the MIP and its CP [29]) of the ungrafted CS-MIP almost completely disappeared in pure aqueous media and both the ungrafted CS-MIP and CS-CP exhibited rather high binding capacities (Figure 3b), mainly due to their high surface hydrophobicity. In sharp contrast, the grafted CS-MIP showed obvious specific template bindings in pure aqueous solutions as a consequence of their largely improved surface hydrophilicity. This demonstrates the high efficiency of the hydrophilic polymer brushes-grafting strategy for the preparation of pure water-compatible MIPs, which is in agreement with previously reported results [26,27].

The binding selectivity of the ungrafted and grafted CS-MIPs/CS-CPs was studied by measuring their competitive binding capacities towards propranolol and the structurally related atenolol (Scheme S2) in both acetonitrile and pure water in order to confirm that the higher template binding capacities of the ungrafted and grafted CS-MIPs (in comparison with the corresponding CS-CPs) observed in Figure 3a (Figure S1a) and

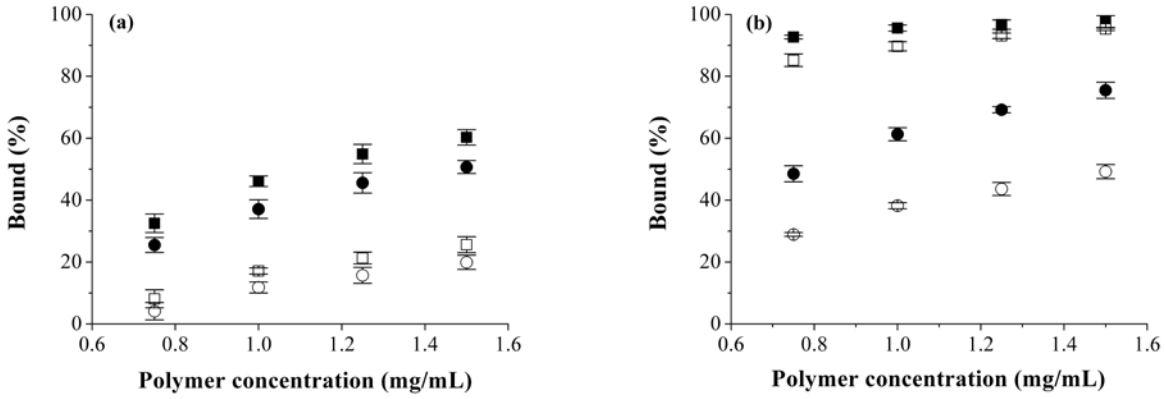


Figure 3. Equilibrium bindings (as percentage values) of propranolol on different amounts of the ungrafted (square) and grafted (circle) CS-MIP (filled symbols)/CS-CP (open symbols) microspheres in its solution (0.05 mM) in acetonitrile (a) and in pure water (b) at 25°C, respectively (the binding analyses were performed in duplicate).

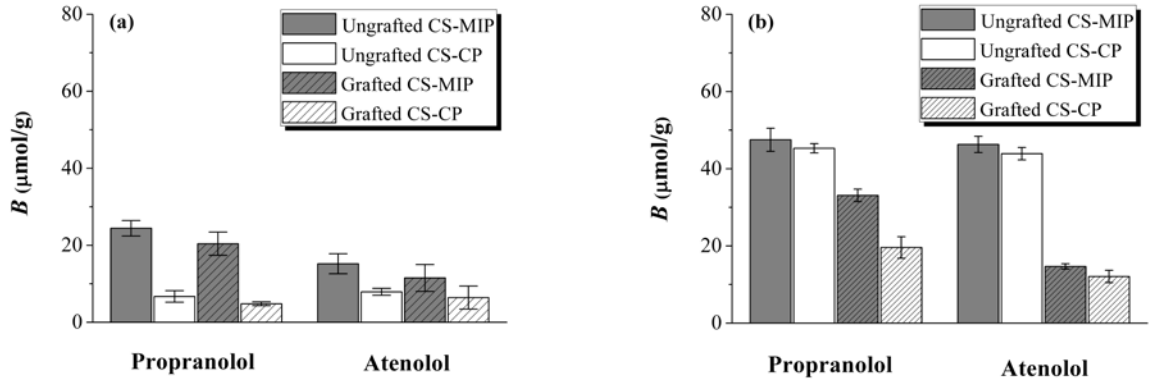


Figure 4. Selective bindings of the ungrafted and grafted CS-MIP/CS-CP microspheres towards propranolol and atenolol in their mixed solution ($C_{Propranolol\ or\ atenolol}$ = 0.05 mM) in acetonitrile (a) and in pure water (b), respectively (polymer concentration: 1 mg/mL) (the binding analyses were performed in duplicate).

those of the grafted CS-MIP observed in Figure 3b (Figure S1b) were due to the specific binding sites created by molecular imprinting. Figures 4a and b show the selective (or competitive) bindings of the ungrafted and grafted CS-MIPs/CS-CPs towards propranolol and atenolol in their mixed solution in acetonitrile and in pure water, respectively, from which the "imprinting-induced promotion of binding" (IPB) data of the ungrafted and grafted CS-MIPs/CS-CPs towards propranolol and atenolol in their mixed solutions in acetonitrile and in pure water were derived. The data is summarized in Table 2 [22,23]. It can be seen clearly that while both the ungrafted and grafted CS-MIP microspheres showed obvious specificity towards propranolol in acetonitrile solution, only the grafted CS-MIP exhibited obvious specificity towards propranolol in pure aqueous solution and no specificity towards propranolol was observed for the ungrafted CS-MIP under pure aqueous conditions.

3.3 Photo- and Thermo-Responsive Binding Properties of Grafted CS-MIP Microspheres in Aqueous Media

The water-compatible photoresponsive template binding properties of the grafted CS-MIP microspheres were confirmed by their photoregulated release and uptake of propranolol in pure aqueous solutions. As can be seen clearly from Figure 5a (or Figure S2a), the equilibrium template binding capacities of the grafted CS-MIP microspheres decreased upon exposure to 365 nm UV light, suggesting that the UV light irradiation led to the obvious release of the template from the grafted CS-MIP microspheres into the aqueous solution. The subsequent thermal back-isomerization of the above system in the dark led to re-uptake of the template by the grafted CS-MIP microspheres, resulting in an increase in the equilibrium template binding capacity. Repeating the photoswitching cycles (i.e., UV light on for 6 h and off for 18 h alternately) led to the release and uptake of propranolol in quantities very similar to those of the previous cycles, which clearly demonstrated the reversibility of the binding site configuration and substrate affinity in the course of photoswitching of azo chromophores in water (Scheme 1). It is worth noting that the photoregulated release and uptake of propranolol were also observed for the grafted CS-CP microspheres under the repetitive photoswitching conditions, but in the opposite direction to that of the grafted CS-MIP microspheres. For example, the equilibrium bindings of

the grafted CS-CP microspheres towards propranolol increased from 40.3 to 46.6% upon the UV light irradiation, while it dropped back to 39.8% upon switching off the UV light. This behavior is not yet fully understood; it may be that the polarity difference between the *trans*- and *cis*-azo moieties on the surfaces of the grafted CS-CP microspheres under the photoswitching conditions [30], leads to their increased nonspecific template bindings upon UV light irradiation [11]. To exclude the influence of these photoinduced nonspecific template binding changes, the dependence of the specific template bindings of the grafted CS-MIP microspheres on photoswitching conditions are also shown (Figure 5b, Figure S2b), demonstrating the photoregulated release and uptake of the template by the grafted CS-MIP microspheres more clearly. The grafted CS-MIP microspheres also exhibited some degree of photoregulated release and uptake for the structural analogue of the template (i.e., atenolol) but to a significantly lower extent than that of propranolol under similar experimental conditions (Figures 5a and b, Figures S2a and b). This indicates that the binding sites in the grafted CS-MIP microspheres possess a specific affinity for the template propranolol. Based on the above results, it can be concluded that the grafted CS-MIP microspheres indeed show obvious photoresponsive template binding properties in pure aqueous solutions and the affinity of their binding sites towards the template can be easily tuned by simple photoswitching.

PNIPAAm is a well-known thermo-responsive polymer and it can undergo a conformation change from a hydrated (coiled and soluble) to a dehydrated (collapsed and insoluble) state in water around its lower critical solution temperature (LCST~32°C) [31]. Therefore, it can be envisaged that the CS-MIP/CS-CP microspheres grafted with PNIPAAm brushes would be responsive towards the temperatures of the aqueous solutions and would exhibit thermo-responsive template binding properties. The results presented in Figures 6 (Figure S3) support this hypothesis. It can be seen clearly from Figure 6a (Figure S3a) that the template binding capacities of the grafted CS-CP microspheres increased measurably with increasing solution temperatures from 20 to 30°C. This increase might be attributed to the increased hydrophobicity of the grafted CS-CP particle surfaces with the polymer brushes changing from

Table 2. Selective binding properties of the ungrafted and grafted CS-MIPs/CS-CPs towards propranolol and atenolol in their mixed solutions in acetonitrile and in pure water, respectively.

Analyte/ solvent	Ungrafted CS-MIP/CS-CP			Grafted CS-MIP/CS-CP		
	$B_{\text{Ungrafted CS-MIP}}$ (μmol/g) [a]	$B_{\text{Ungrafted CS-CP}}$ (μmol/g) [a]	IPB (%) [b]	$B_{\text{Grafted CS-MIP}}$ (μmol/g) [a]	$B_{\text{Grafted CS-CP}}$ (μmol/g) [a]	IPB (%) [b]
Propranolol/ acetonitrile	24.4	6.7	264.2	20.4	4.8	325.0
Atenolol/ acetonitrile	15.2	7.9	92.4	11.5	6.4	79.7
Propranolol/ water	47.5	45.3	4.9	33.1	19.6	68.9
Atenolol/ water	46.3	43.9	5.5	14.7	12.1	21.5

[a] $B_{\text{Ungrafted CS-MIP}}/B_{\text{Ungrafted CS-CP}}$ and $B_{\text{Grafted CS-MIP}}/B_{\text{Grafted CS-CP}}$ are the equilibrium binding capacities of the ungrafted CS-MIP/CS-CP and grafted materials towards propranolol and atenolol in their mixed solutions ($C_{\text{propranolol or atenolol}}$ = 0.05 mM), respectively (polymer concentration: 1 mg/mL), which are identical to those shown in Figure 4.
[b] IPB refers to the "imprinting-induced promotion of binding" value of the studied CS-MIPs, which can be calculated using the following equation: IPB (%) = $[(B_{MIP} - B_{CP})/B_{CP}] \times 100\%$.

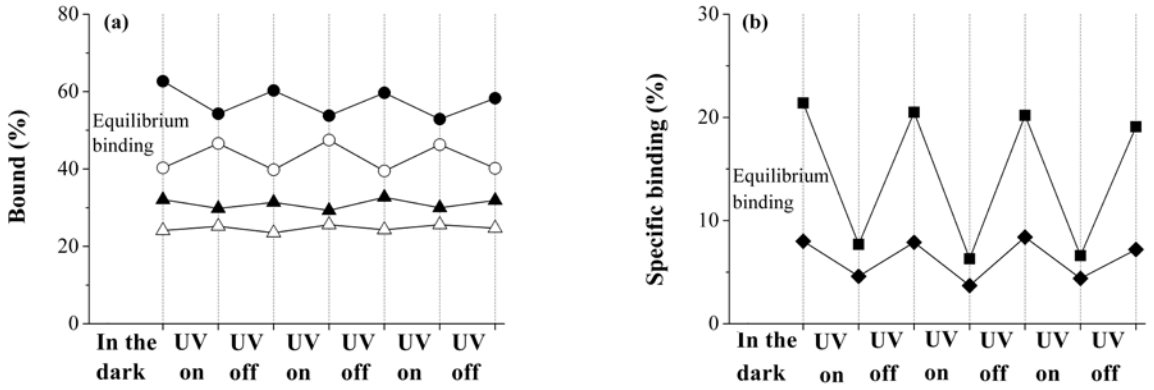


Figure 5. (a) Photoregulated release and uptake (as percentage values) of propranolol (circle) and atenolol (triangle) by the grafted CS-MIP (filled symbols)/CS-CP (open symbols) microspheres in their mixed pure aqueous solutions under photoswitching conditions (UV light on for 6 h and off for 18 h alternately at 25°C); (b) Photoresponsive specific analyte (propranolol (square) and atenolol (diamond)) bindings (as percentage values) of the grafted CS-MIP microspheres in the mixed pure aqueous solutions of propranolol and atenolol under photoswitching conditions: Polymer concentration = 1.0 mg/mL, $C_{\text{propranolol or atenolol}}$ = 0.05 mM.

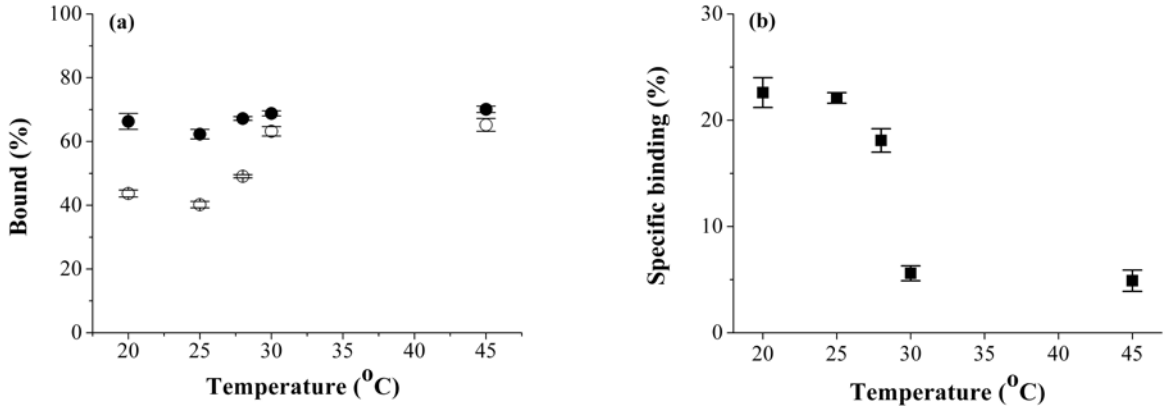


Figure 6. (a) Temperature-dependent equilibrium bindings (as the percentage values) of the grafted CS-MIP (filled circle)/CS-CP (open circle) microspheres towards propranolol in pure water; (b) Temperature-dependent specific bindings (as percentage values) of the grafted CS-MIP microspheres towards propranolol in pure water: Polymer concentration = 1.0 mg/mL, Cpropranolol = 0.05 mM (the binding analyses were performed in duplicate).

the hydrophilic soluble state to hydrophobic insoluble state following the temperature changes, thus leading to their higher nonspecific template bindings as the solution temperature increases. In comparison, the template binding capacities of the grafted CS-MIP microspheres remained more or less unchanged as the solution temperatures increased from 20 to 30°C. The above results demonstrate that the specific template bindings of the grafted CS-MIP microspheres (which could be derived by subtracting the binding capacities of the grafted CS-CP from those of the grafted CS-MIP) decreased with increasing solution temperatures (Figure 6b, Figure S3b). This drop off in binding capacity may be due to the collapse of the polymer brushes at higher temperatures, thus resulting in the blocking of the imprinted binding sites in the grafted CS-MIP microspheres (Scheme 1), as reported by the group of Hoffman for a protein system [32].

To demonstrate the applicability of the grafted CS-MIP microspheres in regular water (which was taken from Xinkai Lake in Nankai University on May 30, 2012), photo- and thermo-responsive binding experiments in the template solutions were also performed. The experimental results revealed that the grafted CS-MIP microspheres exhibited obvious photo- and thermo-responsive template binding properties and the template binding capacities of the grafted MIP microspheres could be easily tuned by simple photoswitching and temperature change (Figures S4, S5, S6, and S7).

4. Conclusions

We have demonstrated a facile, general, and highly efficient approach to obtain narrowly dispersed, water-compatible, and dual stimuli-responsive MIP microspheres by the successive grafting of an azo-containing MIP layer and thermo-responsive hydrophilic polymer brushes onto the preformed "living" core polymer microspheres through surface-initiated RAFT polymerization. The resulting grafted core-shell MIP microspheres exhibited significant photo- and thermo-responsive template binding properties in pure aqueous solutions. The versatility of RAFTPP for the direct synthesis of "living" core polymer microspheres [23,24,26,27,33,34] and surface-initiated RAFT polymerization for the controlled growth of uniform MIP/polymer brush layers with adjustable thickness as well as the easy availability of many different stimuli-responsive hydrophilic polymer brushes, lead us to conclude that the present methodology represents a promising protocol for the development of water-compatible MIP microspheres possessing various dual or even multiple stimuli-responsive template binding properties. We are inclined to believe that the advanced functional and smart MIPs thus fabricated may have great potential in such applications as stimuli-responsive drug delivery and bioanalytical chemistry.

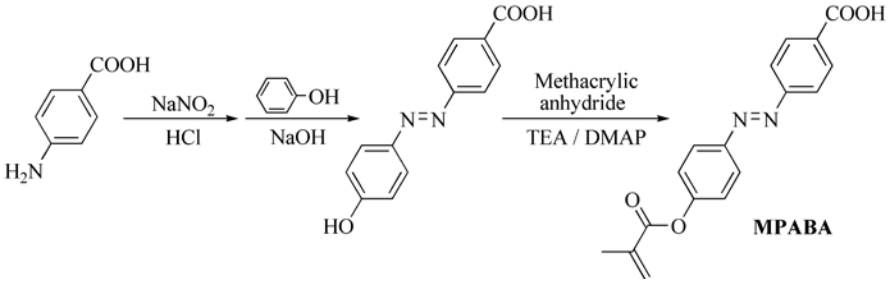
Supporting Information

Synthetic route to azobenzene functional monomer MPABA and chemical structures of propranolol and atenolol as well as the analyte binding figures of the ungrafted and grafted CS-MIP/CS-CP microspheres.

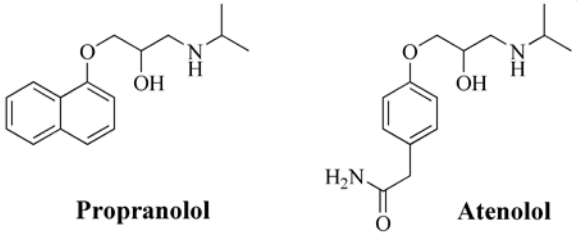
Acknowledgements

We thank the financial supports from National Natural Science Foundation of China (20974048, 21174067), Natural Science Foundation of Tianjin (11JCYBJC01500), a supporting program for New Century Excellent Talents (Ministry of Education) (NCET-07-0462), and the project sponsored by SRF for ROCS, SEM.

Supporting Information



Scheme S1. *Synthetic route to azobenzene functional monomer 4-((4-methacryloyloxy)phenylazo)benzoic acid (MPABA).*



Scheme S2. *The chemical structures of propranolol and atenolol.*

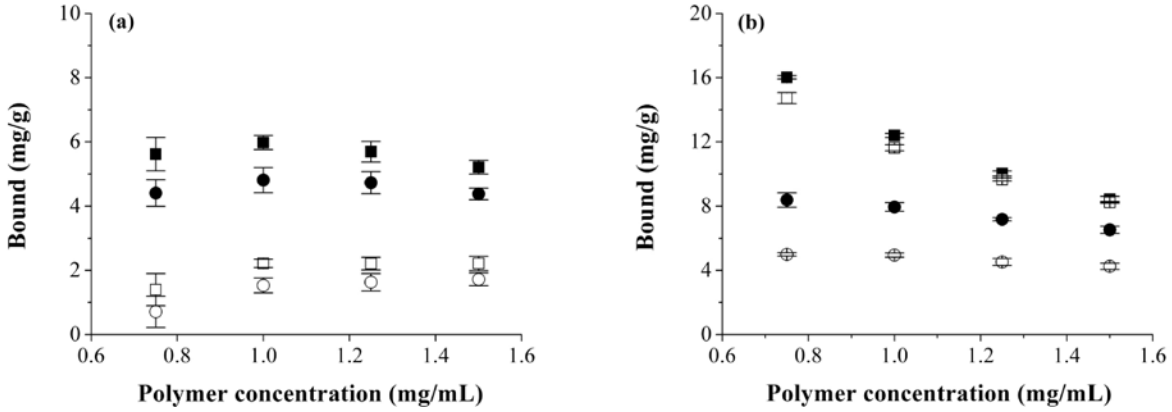


Figure S1. *Equilibrium bindings (as mg template per gram polymer microspheres) of propranolol on different amounts of the ungrafted (square) and grafted (circle) CS-MIP (filled symbols)/CS-CP (open symbols) microspheres in its solution (0.05 mM) in acetonitrile (a) and in pure water (b) at 25°C, respectively (the binding analyses were performed in duplicate).*

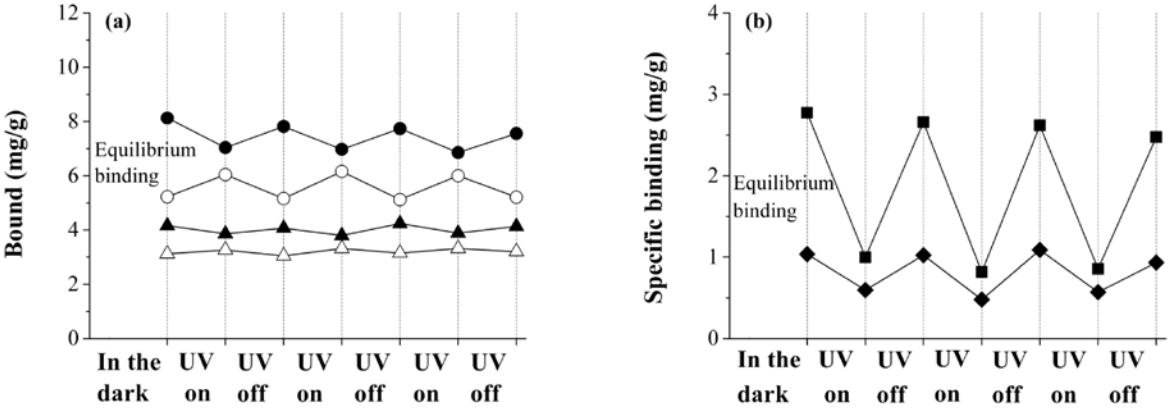


Figure S2. *(a) Photoregulated release and uptake (as mg template per gram polymer microspheres) of propranolol (circle) and atenolol (triangle) by the grafted CS-MIP (filled symbols)/CS-CP (open symbols) microspheres in their mixed pure aqueous solutions under photoswitching conditions (UV light on for 6 h and off for 18 h alternately at 25°C); (b) Photoresponsive specific analyte (propranolol (square) and atenolol (diamond)) bindings (as mg template per gram polymer microspheres) of the grafted CS-MIP microspheres in the mixed pure aqueous solutions of propranolol and atenolol under photoswitching conditions: Polymer concentration = 1.0 mg/mL, $C_{propranolol\ or\ atenolol}$ = 0.05 mM.*

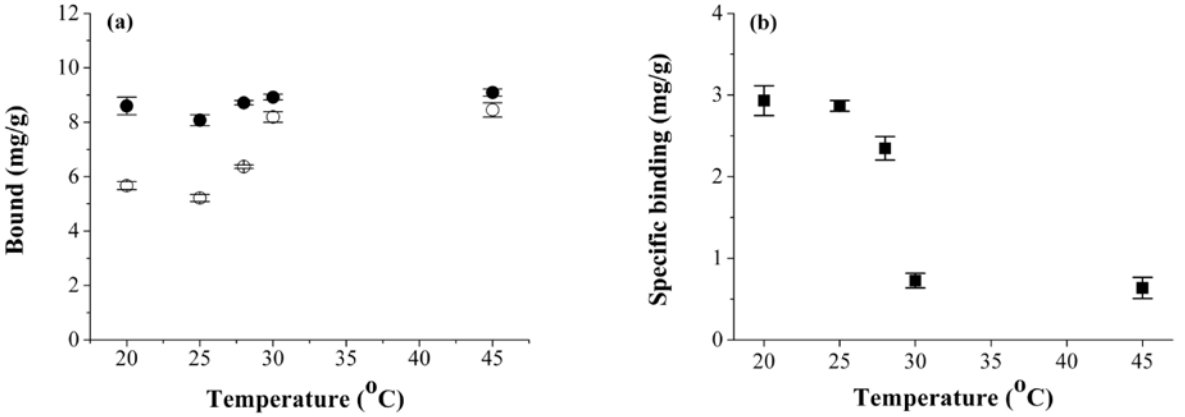


Figure S3. *(a) Temperature-dependent equilibrium bindings (as mg template per gram polymer microspheres) of the grafted CS-MIP (filled circle)/ CS-CP (open circle) microspheres towards propranolol in pure water; (b) Temperature-dependent specific bindings (as mg template per gram polymer microspheres) of the grafted CS-MIP microspheres towards propranolol in pure water: Polymer concentration = 1.0 mg/mL, $C_{propranolol}$ = 0.05 mM (the binding analyses were performed in duplicate).*

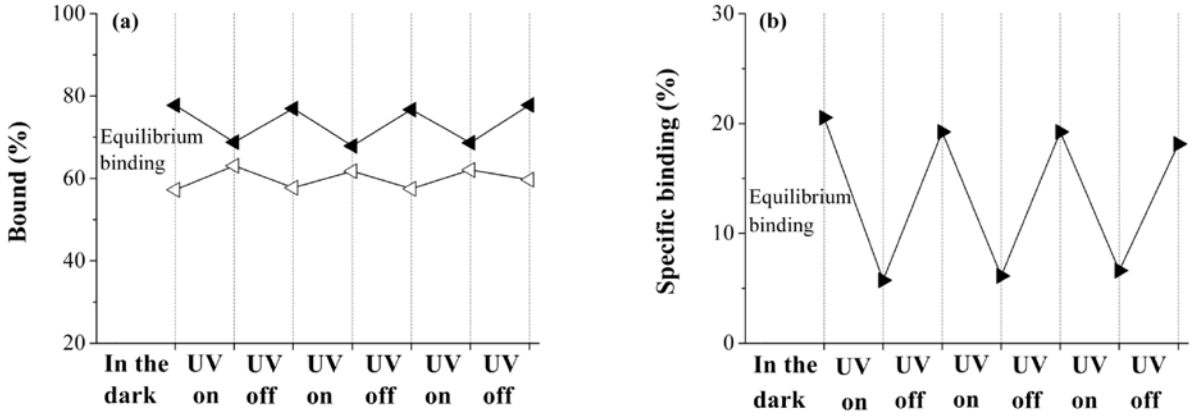


Figure S4. *(a) Photoregulated release and uptake (as percentage values) of propranolol by the grafted CS-MIP (filled symbols)/CS-CP (open symbols) microspheres in its solutions in real water under photoswitching conditions (UV light on for 6 h and off for 18 h alternately at 25°C); (b) Photoresponsive specific template bindings (as percentage values) of the grafted CS-MIP microspheres in the solutions of propranolol in real water under photoswitching conditions: Polymer concentration = 1.0 mg/mL, $C_{propranolol}$ = 0.05 mM.*

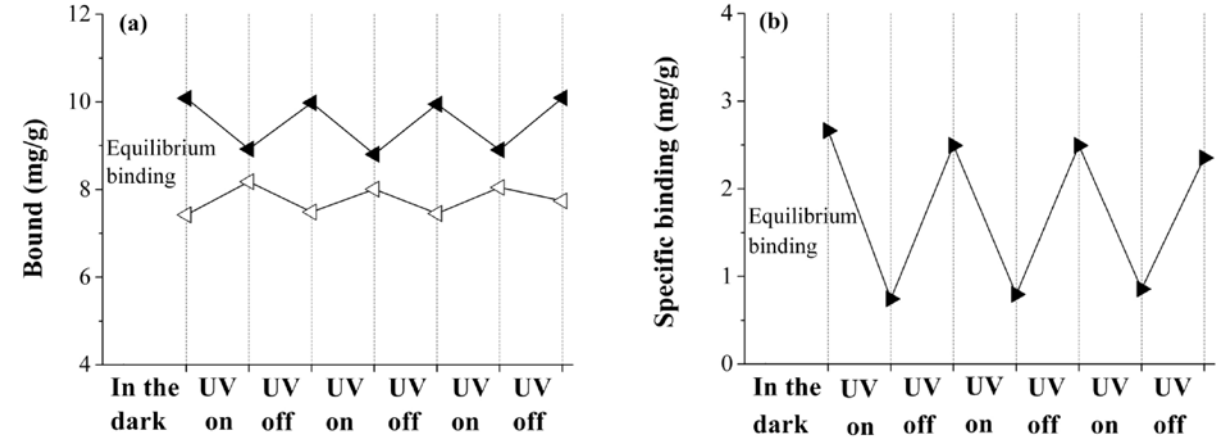


Figure S5. *(a) Photoregulated release and uptake (as mg template per gram polymer microspheres) of propranolol by the grafted CS-MIP (filled symbols)/CS-CP (open symbols) microspheres in its solutions in real water under photoswitching conditions (UV light on for 6 h and off for 18 h alternately at 25°C); (b) Photoresponsive specific template bindings (as mg template per gram polymer microspheres) of the grafted CS-MIP microspheres in the solutions of propranolol in real water under photoswitching conditions: Polymer concentration = 1.0 mg/mL, $C_{propranolol}$ = 0.05 mM.*

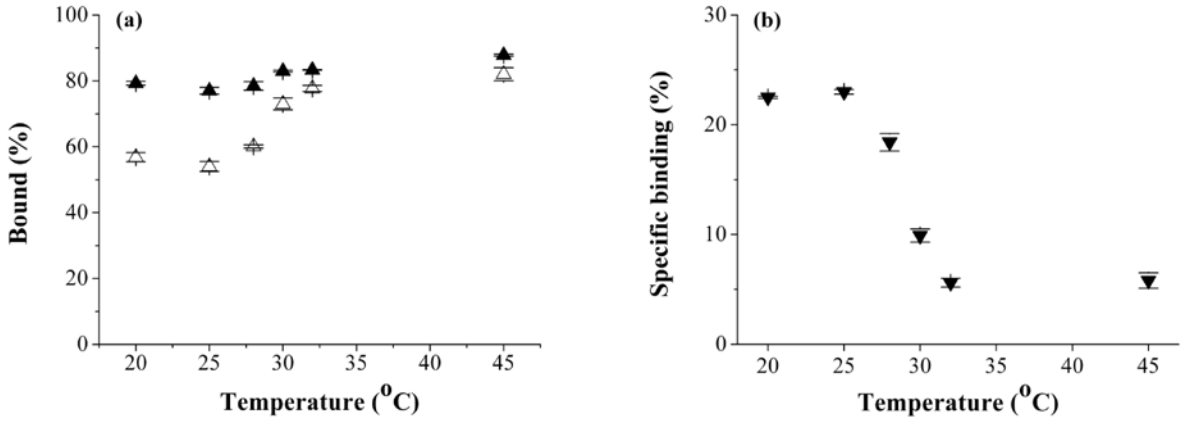


Figure S6. *(a) Temperature-dependent equilibrium bindings (as percentage values) of the grafted CS-MIP (filled triangle)/CS-CP (open triangle) microspheres towards propranolol in real water; (b) Temperature-dependent specific bindings (as percentage values) of the grafted CS-MIP microspheres towards propranolol in real water: Polymer concentration = 1.0 mg/mL, $C_{propranolol}$ = 0.05 mM (the binding analyses were performed in duplicate).*

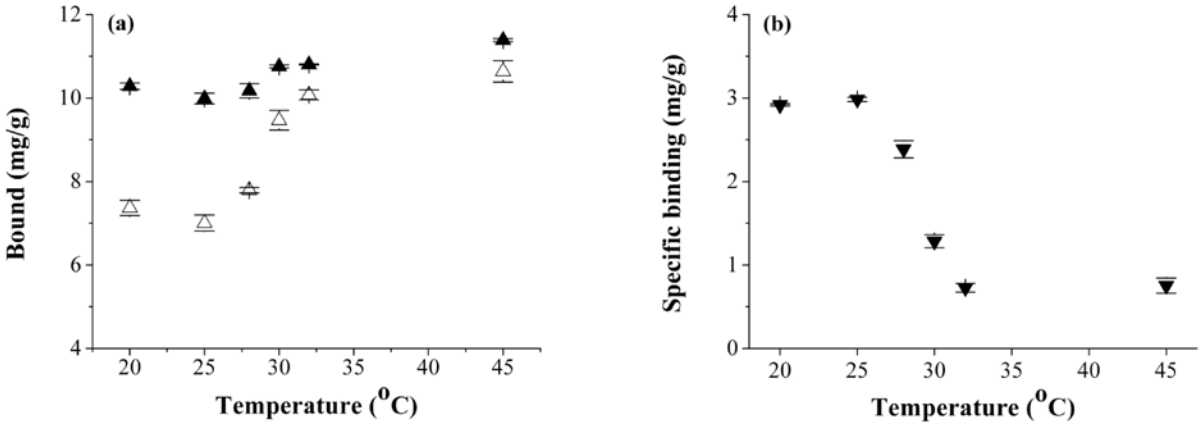


Figure S7. *(a) Temperature-dependent equilibrium bindings (as mg template per gram polymer microspheres) of the grafted CS-MIP (filled triangle)/ CS-CP (open triangle) microspheres towards propranolol in real water; (b) Temperature-dependent specific bindings (as mg template per gram polymer microspheres) of the grafted CS-MIP microspheres towards propranolol in real water: Polymer concentration = 1.0 mg/mL, $C_{propranolol}$ = 0.05 mM (the binding analyses were performed in duplicate).*

References

[1] Puoci F., Iemma F., Picci N., Stimuli-responsive molecularly imprinted polymers for drug delivery: A review, Curr. Drug Deliv., 2008, 5, 85-96.

[2] Oya T., Enoki T., Grosberg A. Yu., Masamune S., Sakiyama T., Takeoka Y., Tanaka K., Wang G., Yilmaz Y., Feld M. S., Dasari R., Tanaka T., Reversible molecular adsorption based on multiple-point interaction by shrinkable gels, Science, 1999, 286, 1543-1545.

[3] Kanekiyo Y., Naganawa R., Tao H., pH-responsive molecularly imprinted polymers, Angew. Chem. Int. Ed., 2003, 42, 3014-3016.

[4] Gong C., Lam M. H. W., Yu H., The fabrication of a photoresponsive molecularly imprinted polymer for the photoregulated uptake and release of caffeine, Adv. Funct. Mater., 2006, 16, 1759-1767.

[5] Chen Z., Xu L., Liang Y., Zhao M., pH-sensitive water-soluble nanospheric imprinted hydrogels prepared as horseradish peroxidase mimetic enzymes, Adv. Mater., 2010, 22, 1488-1492.

[6] Minoura N., Idei K., Rachkov A., Uzawa H., Matsuda K., Molecularly imprinted polymer membranes with photoregulated template binding, Chem. Mater., 2003, 15, 4703-4704.

[7] Minoura N., Idei K., Rachkov A., Choi Y., Ogiso M., Matsuda K., Preparation of azobenzene-containing polymer membranes that function in photoregulated molecular recognition, Macromolecules, 2004, 37, 9571-9576.

[8] Gomy C., Schmitzer R., Synthesis and photoresponsive properties of a molecularly imprinted polymer, Org. Lett., 2007, 9, 3865-3868.

[9] Takeuchi T., Akeda K., Murakami S., Shinmori H., Inoue S., Lee W., Hishiya T., Photoresponsive porphyrin-imprinted polymers prepared using a novel functional monomer having diaminopyridine and azobenzene moieties, Org. Biomol. Chem., 2007, 5, 2368-2374.

[10] Gong C., Wong K. L., Lam M. H. W., Photoresponsive molecularly imprinted hydrogels for the photoregulated release and uptake of pharmaceuticals in the aqueous media, Chem. Mater., 2008, 20, 1353-1358.

[11] Fang L., Chen S., Zhang Y., Zhang H., Azobenzene-containing molecularly imprinted polymer microspheres with photoresponsive template binding properties, J. Mater. Chem., 2011, 21, 2320-2329.

[12] Puoci F., Iemma F., Muzzalupo R., Spizzirri U. G., Trombio S., Cassano R., Picci N., Spherical molecularly imprinted polymers (SMIPs) via a novel precipitation polymerization in the controlled delivery of sulfasalazine, Macromol. Biosci., 2004, 4, 22-26.

[13] Yoshimatsu K., Reimhult K., Krozer A., Mosbach K., Sode K., Ye L., Uniform molecularly imprinted microspheres and nanoparticles prepared by precipitation polymerization: The control of particle size suitable for different analytical applications, Anal. Chim. Acta, 2007, 584, 112-121.

[14] Demirel G., özçetin G., Turan E., çaykara T., pH/temperature-sensitive imprinted ionic poly(*N-tert*-butylacrylamide-*co*-acrylamide/maleic acid) hydrogels for bovine serum albumin, Macromol. Biosci., 2005, 5, 1032-1037.

[15] Hua Z., Chen Z., Li Y., Zhao M., Thermosensitive and salt-sensitive molecularly imprinted hydrogel for bovine serum albumin, Langmuir, 2008, 24, 5773-5780.

[16] Stuart M. A. C., Huck W. T. S., Genzer J., Müller M., Ober C., Stamm M., Sukhorukov G. B., Szleifer I., Tsukruk V. V., Urban M., Winnik F., Zauscher S., Luzinov I., Minko S., Emerging applications of stimuli-responsive polymer materials, Nat. Mater., 2010, 9, 101-113.

[17] Le T. P., Moad G., Rizzardo E., Thang S. H., PCT Int. Appl. WO 98/01478; Chem. Abstr. 1998, 128, 115390.

[18] Li X., Wen R., Zhang Y., Zhu L., Zhang B., Zhang H., Photoresponsive side-chain liquid crystalline polymers with an easily cross-linkable azobenzene mesogen, J. Mater. Chem., 2009, 19, 236-245.

[19] Schweitz L., Spégel P., Nilsson S., Molecularly imprinted microparticles for capillary electrochromatographic enantiomer separation of propranolol, Analyst, 2000, 125, 1899-1901.

[20] Rowe M. D., Hammer B. A. G., Boyes S. G., Synthesis of surface-initiated stimuli-responsive diblock copolymer brushes utilizing a combination of ATRP and RAFT polymerization, Macromolecules, 2008, 41, 4147-4157.

[21] Jiang J., Zhang Y., Guo X., Zhang H., Narrow or monodisperse, highly crosslinked, and “living” polymer microspheres by atom transfer radical precipitation polymerization, Macromolecules, 2011, 44, 5893-5904.

[22] Hishiya T., Shibata M., Kakazu M., Asanuma H., Komiyama M., Molecularly imprinted cyclodextrins as selective receptors for steroids, Macromolecules, 1999, 32, 2265-2269.

[23] Pan G., Zhang Y., Ma Y., Li C., Zhang H., Efficient one-pot synthesis of water-compatible molecularly imprinted polymer microspheres by facile RAFT precipitation polymerization, Angew. Chem. Int. Ed., 2011, 50, 11731-11734.

[24] Pan G., Zu B., Guo X., Zhang Y., Li C., Zhang H., Preparation of molecularly imprinted polymer microspheres via reversible addition-fragmentation chain transfer precipitation polymerization, Polymer, 2009, 50, 2819-2825.

[25] Bontempo D., Tirelli N., Feldman K., Masci G., Crescenzi V., Hubbell J. A., Atom transfer radical polymerization as a tool for surface functionalization, Adv. Mater., 2002, 14, 1239-1241.

[26] Pan G., Zhang Y., Guo X., Li C., Zhang H., An efficient approach to obtaining water-compatible and stimuli-responsive molecularly imprinted polymers by the facile surface-grafting of functional polymer brushes via RAFT polymerization, Biosens. Bioelectron., 2010, 26, 976-982.

[27] Pan G., Ma Y., Zhang Y., Guo X., Li C., Zhang H., Controlled synthesis of water-compatible molecularly imprinted polymer microspheres with ultrathin hydrophilic polymer shells via

surface-initiated reversible addition-fragmentation chain transfer polymerization, Soft Matter, 2011, 7, 8428-8439.

[28] Dirion B., Cobb Z., Schillinger E., Andersson L. I., Sellergren B., Water-compatible molecularly imprinted polymers obtained via high-throughput synthesis and experimental design, J. Am. Chem. Soc., 2003, 125, 15101-15109.

[29] Boonpangrak S., Whitcombe M. J., Prachayasittikul V., Mosbach K., Ye L., Preparation of molecularly imprinted polymers using nitroxide-mediated living radical polymerization, Biosens. Bioelectron., 2006, 22, 349-354.

[30] Wang Y., Xu H., Zhang X., Tuning the amphiphilicity of building blocks: Controlled self-assembly and disassembly for functional supramolecular materials, Adv. Mater., 2009, 21, 2849-2864.

[31] Schild H. G., Poly(*N*-isopropylacrylamide): Experiment, theory and application, Prog. Polym. Sci., 1992, 17, 163-249.

[32] Stayton P. S., Shimoboji T., Long C., Chilkoti A., Chen G., Harris J. M., Hoffman A. S., Control of protein-ligand recognition using a stimuli-responsive polymer, Nature, 1995, 378, 472-474.

[33] Xu S., Li J., Chen L., Molecularly imprinted polymers by reversible addition–fragmentation chain transfer precipitation polymerization for preconcentration of atrazine in food matrices, Talanta, 2011, 85, 282-289.

[34] Chen L., Xu S., Li J., Recent advances in molecular imprinting technology: current status, challenges and highlighted applications, Chem. Soc. Rev., 2011, 40, 2922-2942.