Molecular Imprinting

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Efficient One-Pot Synthesis of Water-Compatible Molecularly Imprinted Polymer Microspheres by Facile RAFT Precipitation Polymerization**

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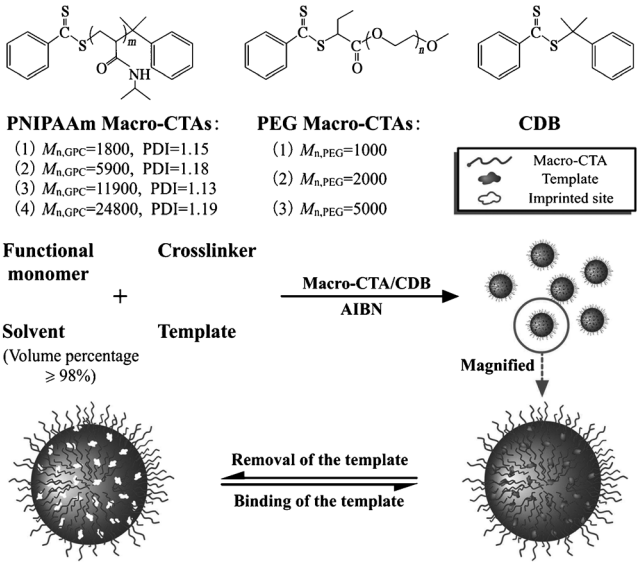
Molecular imprinting is a versatile and straightforward method for the preparation of polymer receptors with tailor-made recognition sites.[1,2] Despite the tremendous progress made in this field, many challenges still remain to be addressed. In particular, it has been shown that the presently developed molecularly imprinted polymers (MIPs) are normally only organic solvent compatible and they mostly fail to show specific template bindings in pure aqueous solutions, thus significantly limiting their practical applications in the field of biotechnology.[2b] Although some approaches, which either use specifically designed functional monomers[3] or apply the conventional imprinting protocol,[4] have been developed for the preparation of MIPs with molecular recognition ability under aqueous conditions, versatile approaches for the preparation of MIPs that are applicable in pure aqueous environments are still rare.

Herein, we report a new and efficient one-pot approach to obtain pure-water-compatible and narrowly dispersed MIP microspheres with surface-grafted hydrophilic polymer brushes by facile reversible addition/fragmentation chain-transfer (RAFT) precipitation polymerization (RAFTPP),[5] mediated by hydrophilic macromolecular chain-transfer agents (Macro-CTAs; Scheme 1). The presence of hydrophilic polymer brushes on MIP microspheres significantly improved their surface hydrophilicity and dramatically reduced their hydrophobic interactions with template molecules in pure aqueous media, thus leading to their water compatibility.[5b,c] The easy availability of many different hydrophilic Macro-CTAs (by either RAFT polymerization of hydrophilic monomers or hydrophilic polymer end group modification),[6] together with the versatility of RAFTPP for the controlled preparation of MIP microspheres,[5] makes this strategy highly applicable for the design of hydrophilic and water-compatible MIPs. Two strategies have been developed



Scheme 1. Chemical structures of the utilized RAFT agents (including hydrophilic Macro-CTAs and CDB) and the schematic protocol for the one-pot preparation of water-compatible MIP microspheres by RAFT precipitation polymerization.

for the synthesis of water-compatible MIPs by improving their surface hydrophilicity; these strategies involve the use of a hydrophilic comonomer,[7] functional monomer,[8] or cross-linker[9] in the molecular imprinting process, and the post-modification of the preformed MIPs.[10,11] Although simple in principle, the former strategy either requires time-consuming optimization of MIP formulation components[7,12] or can only be applied in some special systems.[8,9] In comparison, the latter strategy, which involves the surface grafting of hydrophilic polymer layers, has proven highly attractive because it not only significantly improves the MIPs' surface hydrophilicity, but also provides a protective layer to prevent protein molecules from blocking their imprinting sites in biological solutions.[10] Very recently, we have successfully prepared pure-water-compatible MIP microspheres by the controlled grafting of hydrophilic polymer layers onto the preformed MIP particles.[5b,c] Compared with this two-step approach, the new strategy presented herein allows the more efficient controlled synthesis of pure-water-compatible MIP microspheres with surface-grafted hydrophilic polymer brushes by a one-pot RAFTPP method.

To show proof-of-principle for our strategy, a model noncovalent molecular imprinting system was chosen because

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RAFT = reversible addition/fragmentation chain transfer.

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of its versatility in generating MIPs; this system uses 2,4-dichlorophenoxyacetic acid (2,4-D), 4-vinylpyridine (4-VP), ethylene glycol dimethacrylate (EGDMA), and a mixture of methanol and water (4:1 v/v) as the template, functional monomer, crosslinker, and porogenic solvent, respectively. RAFTPP was carried out to prepare 2,4-D-imprinted polymers using azobisisobutyronitrile (AIBN) as the initiator in the presence of the appropriate chain-transfer agent (i.e., RAFT agent) and a large amount of porogenic solvent ($\geq$98% of the total reaction volumes);[5] in this system all the reactants were compatible with both the RAFT polymerization and molecular imprinting processes and 4-VP could form hydrophobic interactions and ionic bonds with 2,4-D in polar solvents.[13]

To evaluate the scope of our strategy and demonstrate its general applicability for the preparation of water-compatible MIPs, a series of hydrophilic Macro-CTAs with different chemical structures and molecular weights (M_n) were prepared and used as the RAFT agents for RAFTPP; these Macro-CTAs included poly(*N*-isopropylacrylamide) (PNIPAAm) and PEG Macro-CTAs (Scheme 1). A rapid screen of the polymerization conditions revealed that the use of soley Macro-CTAs in RAFTPP led to either irregular MIP and control polymer (CP) particle aggregates or MIP and CP microspheres with broad size distributions (not shown), while RAFTPP mediated with a mixture of one Macro-CTA and a normal RAFT agent (i.e., cumyl dithiobenzoate; CDB) provided narrowly dispersed MIP and CP microspheres (Figure 1 b, c, e, f, Table S2 in the Supporting Information).

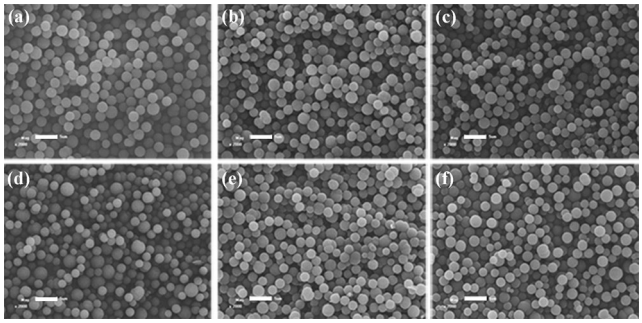

Figure 1. SEM images of the ungrafted MIP (a) and CP (d) microspheres, the MIP (b) and CP (e) microspheres grafted with PNIPAAm brushes (M_n = 11 900), and the MIP (c) and CP (f) microspheres grafted with PEG brushes (M_n = 2000). The scale bar is 5 μm in the above images.

The cause is not very clear at this stage, and further investigation is ongoing to explain this phenomenon. In the case of the RAFTPP mediated with a mixture of a Macro-CTA and CDB, the Macro-CTAs acted as both the cochain-transfer agents and steric stabilizers, thus leading to MIP and CP particles with surface-grafted hydrophilic polymer brushes.[14] The ungrafted MIP and CP microspheres were also prepared by RAFTPP using only CDB as the RAFT agent, and these microspheres were used as the control to the grafted ones in the following studies. The SEM results showed that the ungrafted and grafted MIP and CP microspheres had number-average diameters (D_n) around 2 μm and polydispersity indices of less than 1.1 (Figure 1, Table S2). Note that although hydrophilic Macro-CTA-mediated RAFTPP (or namely RAFT dispersion polymerization) has been studied for the preparation of uncrosslinked and lightly crosslinked nanometer and submicrometer polymer particles with surface-grafted hydrophilic polymer brushes,[14] the findings we report herein represent, to our knowledge, the first successful example of the generation of either highly crosslinked spherical polymer or MIP particles in the micrometer size range with surface-grafted hydrophilic polymer brushes by this method.

The above-obtained MIP and CP microspheres were then characterized with FTIR, as well as with static contact angle and water dispersion experiments. The presence of the characteristic peaks of the amide I band (1674 cm^{-1}, C=O stretching) and the amide II band (1530 cm^{-1}, N–H stretching) in the FTIR spectra of the MIP and CP microspheres that were prepared by RAFTPP mediated by a mixture of CDB and PNIPAAm Macro-CTAs (Figure S2 in the Supporting Information),[15] together with the significantly reduced static water contact angles of the grafted MIP and CP microspheres, and their better dispersion in pure water in comparison with the ungrafted ones (Figure 2, Figure S3 and Table S2),

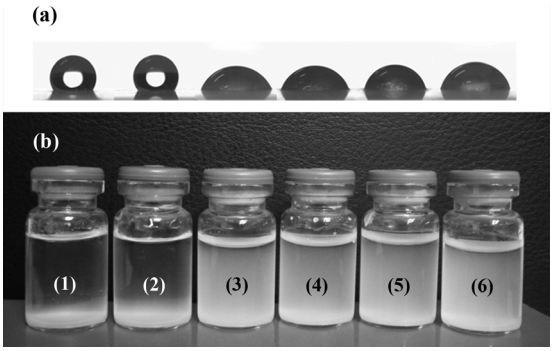

Figure 2. The profiles of a water drop on the films of the ungrafted and grafted MIP and CP microspheres (a) and their dispersion photographs in pure water (1 mg mL^{-1}) at 25 °C after settling down for 1.5 h (b). The samples located from left to right in the above two figures are the ungrafted MIP (1) and CP (2) microspheres, the MIP (3) and CP (4) microspheres grafted with PNIPAAm brushes (M_n = 11 900), and the MIP (5) and CP (6) microspheres grafted with PEG brushes (M_n = 2000).

strongly verified the successful grafting of hydrophilic polymer brushes. In addition, the rather similar grafting levels of the hydrophilic polymer brushes on the grafted MIP and corresponding CP microspheres were also revealed by their FTIR spectra and very similar static water contact angles.

With the ungrafted and grafted MIP microspheres and the corresponding CP microspheres in hand, we started to study their equilibrium binding properties in an organic-solvent-rich medium (i.e., methanol/water (4:1 v/v)). As shown in Figure 3 a and Figure S4, both the ungrafted and grafted MIPs proved to bind more template than their corresponding CPs. For example, in a dilute solution of 2,4-D in methanol/water (4:1 v/v), while 16 mg mL^{-1} of the ungrafted MIP, the MIP

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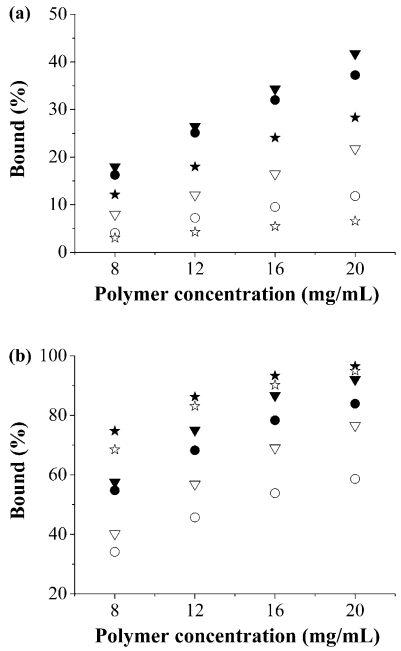

Figure 3. Equilibrium bindings of 2,4-D on different amounts of the ungrafted (star) and grafted (with PNIPAAm brushes ($M_n = 11\,900$, circle) or PEG brushes ($M_n = 2000$, triangle)) MIP (filled symbols) and CP (open symbols) microspheres in solution (0.08 mM) in methanol/water (4:1 v/v) (a) and in pure water (b) at 25 °C, respectively.

ure 3b). This, together with the obvious selectivity of the grafted MIPs towards the template in pure water (Figure S6, Table S3), reveals the high efficiency of this one-pot synthetic strategy for the preparation of pure-water-compatible MIPs.

The effect of the molecular weights of the utilized hydrophilic Macro-CTAs (i.e., the chain length of the grafted polymer brushes) on the equilibrium template binding properties of the resulting MIP and CP microspheres was also studied (Figure 4, Figure S4). It can be seen clearly that

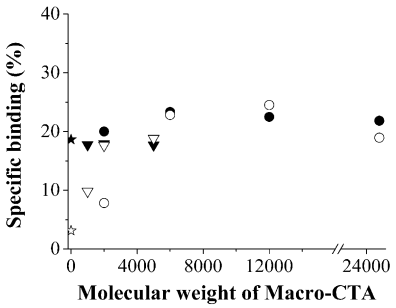

Figure 4. Specific template bindings on the ungrafted MIP microspheres (i.e., M_n of the Macro-CTA = 0; star), the MIP microspheres grafted with PNIPAAm brushes ($M_n = 1800$, 5900, 11 900, and 24 800; circle), and those grafted with PEG brushes ($M_n = 1000$, 2000, and 5000; triangle) in a 2,4-D solution (0.08 mM) in methanol/water (4:1 v/v; filled symbols) and in pure water (open symbols) at 25 °C, respectively (polymer concentration: 16 mg mL^{-1}).

grafted with PNIPAAm brushes ($M_n = 11\,900$), and the MIP grafted with PEG brushes ($M_n = 2000$) bound 24, 32, and 34 % of the template, respectively, an equivalent amount of the corresponding controls bound only 5, 10, and 16 %, respectively. This, together with the high selectivity of the MIPs towards the template (Figure S5), suggests the presence of specific binding sites in both the ungrafted and grafted MIPs. If we simply define the specific template binding as the binding difference between the MIP and its CP,[16] the specific template binding values of the grafted MIPs were found to be rather close to that of the ungrafted MIP, thus demonstrating that the addition of hydrophilic Macro-CTAs into the molecular imprinting systems had negligible influence on the formation of specific binding sites.

We then performed the equilibrium binding experiments in a pure aqueous solution system. It has been well established that the water incompatibility of MIPs is mainly due to their hydrophobically driven nonspecific template binding in the aqueous media; this nonspecific template binding depends on the hydrophobicity of the template molecules and the exposed MIP surfaces.[7] As expected, the specific template bindings of the ungrafted MIP almost completely disappeared in pure aqueous solution and both the ungrafted MIP and CP exhibited rather high binding capacities (Figure 3b), mainly because of their high surface hydrophobicity. In sharp contrast, the grafted MIPs showed obvious specific template bindings in pure aqueous media as a result of their largely improved surface hydrophilicity by the grafting of hydrophilic polymer brushes,[5b,c,17] thus leading to their reduced nonspecific template bindings and pure-water compatibility (Figwhile the specific template bindings of the MIP microspheres in methanol/water (4:1 v/v) were relatively independent of the molecular weights of the Macro-CTAs, they increased dramatically in the pure aqueous media when the chain length of Macro-CTAs was increased in the low molecular weight range and then leveled off at a molecular weight of ≥ 2000 for PEG Macro-CTAs and ≥ 5900 for PNIPAAm Macro-CTAs. The above results suggest that the chain length of the hydrophilic polymer brushes had a significant influence on the water compatibility of the grafted MIP microspheres and only those polymer brushes with high enough molecular weights could act as an efficient hydrophilic protective shield for the MIP microspheres.[5c] Nevertheless, the grafted MIPs prepared with hydrophilic Macro-CTAs of different chemical structures and a wide range of molecular weights showed excellent pure-water-compatible template binding properties (i.e., their specific template bindings in pure water were almost the same with those in methanol/water (4:1 v/v)),[18] thus indicating the general applicability of our strategy.

In conclusion, we have demonstrated for the first time a facile and highly efficient one-pot approach to obtain narrowly dispersed pure-water-compatible MIP microspheres by using hydrophilic Macro-CTA-mediated RAFTPP. The obtained MIP microspheres showed significantly enhanced surface hydrophilicity and excellent template recognition ability in pure aqueous solutions. The addition of hydrophilic Macro-CTAs into the molecular imprinting systems proved to have negligible influence on the formation of specific binding sites. The general applicability of the strategy was confirmed by the successful generation of pure-water-compatible MIPs

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with hydrophilic Macro-CTAs of different chemical structures and molecular weights. In view of the easy availability of a wide range of hydrophilic Macro-CTAs and the versatility of RAFTPP, we believe the present method represents a promising way to develop advanced MIP microspheres with enormous potential in such applications as biotechnology and bioanalytical chemistry.

Experimental Section

The detailed synthetic procedures for the hydrophilic PNIPAAm and PEG Macro-CTAs, the ungrafted MIP and CP microspheres, and the MIP and CP microspheres grafted with PNIPAAm and PEG brushes are described in the Supporting Information.

For details on GPC, SEM, and FTIR characterization as well as the static contact angle, dispersion, equilibrium template binding, and competitive binding experiments, see the Supporting Information.

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[15] Note that the characteristic FTIR absorption peaks of PEG chains were overlapped with those of the poly(4-VP-*co*-EGDMA) cores in the MIP and CP microspheres grafted with PEG brushes.

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[18] A slight decrease in the specific template binding of the MIP microspheres grafted with PNIPAAm brushes ($M_n = 24800$) in pure water could be attributed to the relatively low Macro-CTA efficiency during the RAFTPP process owing to its too high molecular weight,[14b] which might lead to MIP microspheres with a lower grafting density of polymer brushes, as revealed by their relatively higher static water contact angles in comparison with those grafted with shorter PNIPAAm brushes (see Table S2, entries 5–10).

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