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One-Pot Synthesis of Hydrophilic Molecularly Imprinted Nanoparticles

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ABSTRACT: Molecularly imprinted nanoparticles were prepared using a simple distillation precipitation polymerization method. In this work, propranolol-imprinted nanoparticles were synthesized using different cross-linkers combined with methacrylic acid (MAA) as a functional monomer in refluxing acetonitrile. Using the new synthetic method, uniform prorpanolol-imprinted nanoparticles were obtained in less than 3 h, which is in contrast to the long reaction time (24 h) required in previous precipitation polymerization. The new synthetic method also provides a convenient means to prepare core−shell structured nanoparticles, where the core contains imprinted binding sites and the shell has more hydrophilic characteristics. Molecular recognition properties of the imprinted nanoparticles were studied through equilibrium binding experiments. For nanoparticles containing *N,N′*-methylenebis(acrylamide) cross-linker, the residual C═C bonds remaining in the nanoparticles were utilized to immobilize a fluorescent compound, 1-pyrenemethylamine, through nondestructive Michael addition reaction. The fluorescent modification did not deteriorate the molecular recognition property of the nanoparticles, suggesting that the Michael addition reaction took place only in nonselective sites. Using a similar Michael addition reaction, we anticipate that the residual C═C bonds in the acrylamide moiety can be utilized to introduce other reporter molecules into molecularly imprinted nanoparticles or to immobilize such nanoparticles on amine- or thiol-functionalized surfaces to develop different chemical sensors. The hydrophilic shell of the imprinted core−shell nanoparticles shall provide effective screening to prevent nonspecific adsorption of, e.g., proteins while allowing small target molecules to enter the imprinted sites in the more hydrophobic core, thereby potentially useful for extraction of small organic molecules from complex biological samples.

Introduction

Molecularly imprinted polymer nanostructures have attracted great interests in recent years because of their outstanding molecular recognition properties and high stability.[1,2] Potential applications of imprinted nanoparticles have been reported in recent literature.[3−9] The methods commonly used to prepare imprinted nanoparticles include emulsion polymerization,[10,11] miniemulsion polymerization,[12] microemulsion polymerization,[13] and precipitation polymerization.[14] More recently, uniform imprinted nanoparticles have been prepared using self-assembly of well-defined block copolymers as starting materials, followed by cross-linking reaction to create imprinted binding sites under special solvent conditions.[15] Surface-initiated atom transfer radical polymerization (ATRP) and reversible addition−fragmentation chain transfer (RAFT) polymerization have also been used to prepare imprinted shell on different nanoparticle cores.[16,17] Among the different methods presently used to prepare imprinted nanoparticles, precipitation polymerization has unique advantages because of its simplicity and general applicability for different molecular imprinting systems.[18]

In traditional precipitation polymerization, cross-linking reactions are carried out in near theta solvents either in a static reactor or under a gentle agitation provided by a rotating bottle.[19,20] The experimental setup is efficient to produce small amount (e.g., from 0.5 to 10 g) of MIP nanoparticles but not appropriate for large-scale (above kilogram level) preparations. To achieve a narrow size distribution of imprinted nanoparticles and microspheres, in precipitation polymerization the monomer concentration has to be kept very low prior to particle nucleation. This has led to the long reaction time (e.g., 24 h) required to achieve high particle yield (above 80%). For more efficient preparation of nanoparticles without molecular imprinting, a modified precipitation polymerization method has recently been developed by Huang and co-workers, allowing uniform nanoparticles and microspheres to be conveniently synthesized within a short time (typically 2−3 h). With the modified method the cross-linking polymerization was carried out in neat acetonitrile under refluxing conditions, with half of the reaction solvent being distilled off in the later phase of the polymerization. The method itself is accordingly named distillation precipitation polymerization (DPP).[21] Compared to traditional precipitation polymerization, DPP appears to be more suitable for large-scale production of uniform cross-linked polymer beads, since the refluxing solvent can provide efficient mixing of the reaction components, and the reaction temperature can be easily controlled during the whole reaction process.

It is intriguing for us to investigate whether DPP can be utilized to synthesize molecularly imprinted polymer beads, particularly hydrophilic nanoparticles that are more suitable for bioanalytical applications under aqueous conditions. Despite the apparently higher reaction temperature that may affect template-functional monomer complex, the possibility of synthesizing MIP beads quickly and obtaining more uniform and smaller MIP beads is highly attractive. In this work we used a well-established template, propranolol, as a model to study direct synthesis of hydrophilic imprinted nanoparticles using DPP. In addition to simple MIP nanoparticles, more complex core−shell structures composed of imprinted hydrophobic core and nonimprinted

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hydrophilic shell were also prepared using one-pot DPP synthesis. The obtained nanoparticles were characterized by scanning electron microscope and dynamic light scattering to reveal their uniformity in particle size and morphology. The molecular binding selectivity was tested through equilibrium binding analysis using both liquid scintillation counting and HPLC-MS/MS. For imprinted polyacrylamide nanoparticles, we also attempted to introduce fluorescent reporters into MIP nanoparticles by means of simple Michael addition reaction between residual C=C bonds in nanoparticles and a fluorescent amine, 1-pyrenemethylamine. Because of the large molecular size of the fluorescent amine, the imprinted sites were kept intact during the chemical modification.

Experimental Section

Materials. Acetonitrile (99.7%) and azobis(isobutyronitrile) (AIBN, 98%) were purchased from Merck (Darmstadt, Germany). AIBN was recrystallized from methanol before use. Methacrylic acid (MAA, 98.5%) from ACROS (Geel, Belgium) and trimethylolpropane trimethacrylate (TRIM, technical grade) from Aldrich (Dorset, UK) were used as received. Acrylamide was purchased from ICN (Irvine, CA) and *N,N′*-methylenebis(acrylamide) (MBA, ≥98.0%) from Fluka (Dorset, UK). (*R,S*)-Propranolol hydrochloride (99%), (*S*)-propranolol hydrochloride (99%), and (*R*)-propranolol hydrochloride (99%) supplied by Fluka (Dorset, UK) were converted into free base form before use. Atenolol (98%) and pindolol (98%) were purchased from Sigma (Gillingham, UK) and used as received. (*S*)-[4-^{3}H]-Propranolol (specific activity 555 GBq mmol^{-1}, 66.7 μM solution in ethanol) was purchased from NEN Life Science Products Inc. (Boston, MA). The fluorescent amine 1-pyrenemethylamine hydrochloride (95%) was purchased from Sigma-Aldrich.

Imprinted Poly(MAA-*co*-MBA) Nanoparticles. Propranolol-imprinted poly(MAA-*co*-MBA) nanoparticles (mip(MBA)) were synthesized using DPP in a round-bottom flask connected to a Dean−Stark receiver. The experimental setup is schematically shown in the Supporting Information (Figure S1). In the round-bottom flask equipped with a magnetic stir bar, (*R,S*)-propranolol (299 mg, 1.15 mmol), MAA (105 mg, 1.22 mmol), MBA (755 mg, 4.90 mmol), and AIBN (17 mg, 0.104 mmol) were dissolved in 80 mL of acetonitrile. The solution was purged with nitrogen for 15 min before the flask was connected to the Dean−Stark receiver, submerged in an oil bath, and heated to 80 °C for 20 min under stirring. The oil bath temperature was then increased to ∼115 °C to keep the reaction to proceed under reflux for 30 min. Thereafter, half of the reaction solvent (40 mL) was removed in 1.5 h through the Dean−Stark receiver, before the reaction was stopped by removing the oil bath. After the polymerization, the nanoparticles were collected by centrifugation. The template was removed by extraction with methanol:acetic acid (10:1, v:v) repeatedly until no template could be detected in washing solvent by the UV−vis spectrophotometer. To change the washing solvent, ultracentrifugation was used between two extraction steps. The nanoparticles were finally washed with acetone and dried in a vacuum chamber. A nonimprinted reference polymer (ref(MBA)) was synthesized and treated under the same conditions, except that no template was added during the DPP reaction.

Surface Modification of Poly(MAA-*co*-MBA) Nanoparticles by Michael Addition Reaction. The poly(MAA-*co*-MBA) nanoparticles (200 mg of mip(MBA) or ref(MBA)) were dispersed in 50 mL of *N,N′*-dimethylformamide (DMF). After addition of 250 mg of 1-pyrenemethylamine hydrochloride, the mixture was incubated at 20 °C for 48 h. A rocking table was used to provide gentle mixing. After the incubation, the nanoparticles were collected by centrifugation and washed repeatedly with methanol:acetic acid (10:1, v:v) to remove the excess 1-pyrenemethylamine, until no fluorescent dye could be detected in the washing solvent with a fluorescence spectrophotometer. The nanoparticles were finally washed in acetone and dried in a vacuum chamber.

Imprinted Poly(MAA-*co*-TRIM) Nanoparticles. Propranolol-imprinted poly(MAA-*co*-TRIM) nanoparticles (mip(TRIM)) were synthesized using the same procedure as described above. Briefly, (*R,S*)-propranolol (137.5 mg, 0.53 mmol), MAA (111.1 μL, 1.31 mmol), TRIM (645 μL, 2.02 mmol), and AIBN (15.9 mg, 2 wt % relative to the monomers) were dissolved in 80 mL of acetonitrile in a round-bottom flask equipped with a magnetic stir bar. The solution was gently purged with nitrogen for 10 min. The flask was then connected to a Dean−Stark apparatus composed of a Liebig condenser and receiver (Figure S1). The flask was submerged in an oil bath and heated to 80 °C for 20 min under stirring. Then the oil bath was heated to 115 °C, and the reaction system was kept under reflux for 30 min. Under this condition the reaction temperature in the round-bottom flask was 83−84 °C. Thereafter, the solvent was distilled out from the reaction system, and the reaction was stopped after 40 mL of acetonitrile was removed within 1.5 h. After the polymerization, the particles were collected by centrifugation. A nonimprinted polymer, ref(TRIM), was synthesized in the same way except that no propranolol was added. The template (propranolol) was extracted by washing the nanoparticles repeatedly in 30 mL of methanol:acetic acid (10:1, v:v), until no template could be detected from the washing solvent using a UV−vis spectrophotometer. The nanoparticles were finally washed with acetone and dried in a vacuum chamber. The reference polymer ref(TRIM) was washed and treated in the same way as mip(TRIM).

Imprinted Core−Shell Nanoparticles. Propranolol-imprinted core−shell nanoparticles (mipCS(TRIM/Am)) were synthesized as the following: Propranolol-imprinted poly(MAA-*co*-TRIM) core was first synthesized using the same DPP procedure as described above, except that the reaction mixture (composed of (*R,S*)-propranolol (137.5 mg, 0.53 mmol), MAA (111.1 μL, 1.31 mmol), TRIM (645 μL, 2.02 mmol), and AIBN (15.9 mg, 2 wt % relative to the monomers) dissolved in 80 mL of acetonitrile) was refluxed for 1 h to give the propranolol-imprinted core particles. Thereafter, a solution of acrylamide (298 mg, 4.20 mmol), MBA (100 mg, 649 μmol), and AIBN (8 mg) dissolved in 40 mL of acetonitrile was slowly added into the round-bottom flask through a dropping funnel with a pressure equalizing arm, while 40 mL of the reaction solvent was removed at the same speed through the Dean−Stark receiver within 1.5 h. After the polymerization, the particles were collected by centrifugation and treated in the same way as described above to remove the template. A nonimprinted reference core−shell polymer (refCS(TRIM/Am)) was synthesized in the same way as mipCS(TRIM/Am), except that no propranolol was added during the synthesis of the core particles.

Dynamic Light Scattering. The hydrodynamic size of the polymer particles was measured by dynamic light scattering (DLS). A Zetasizer Nano ZS instrument equipped with a software package DTS Ver. 5.03 (Malvern Instruments Ltd., Worcestershire, UK) was used with the CONTIN algorithm. Polymer particles (2 mg) were suspended in acetonitrile (1 mL), sonicated in a benchtop ultrasonic cleaner for 20 min, and then diluted to a final concentration of 20 μg mL^{-1} prior to the measurement. The solvent used was prefiltered through a 0.2 μm PTFE HPLC-filter (Sun SRi, Rockwood, TN) to remove any particulates. The DLS measurement was carried out at 21 °C. Data reported are the hydrodynamic size distribution by intensity.

Equilibrium Binding Analysis. *Radioligand Binding Analysis.* In a series of polypropylene microcentrifuge tubes, increasing amounts of polymer particles were suspended in 1 mL of acetonitrile or a mixture of 25 mM citrate buffer (pH 6.0): acetonitrile (50:50, v:v). After addition of (*S*)-[4-^{3}H]-propranolol (246 fmol), the mixture was incubated overnight at 20 °C on a

rocking table. After the incubation, samples were centrifuged at 14 000 rpm for 10 min. Supernatant (500 μL) was taken from each microcentrifuge tube and mixed with 10 mL of scintillation liquid (Ecosint A, National Diagnostics, Atlanta, GA). The radioactivity was measured using a model 1219 Rackbeta β-radiation counter from LKB Wallac (Sollentuna, Sweden). The amount of labeled (*S*)-propranolol bound to polymer particles was calculated by subtraction of the free fraction from the total amount added. Data are mean values from triplicate samples.

Chiral selectivity of the (*S*)-propranolol imprinted sites in the nanoparticles was evaluated by radioligand displacement analysis. The radioligand binding experiments as described above was performed in the presence of excess of either (*R*)- or (*S*)-propranolol, which competed with the tritium-labeled (*S*)-propranolol for binding to the limited number of high-affinity sites in the nanoparticles. The displacement data are mean values obtained from triplicate samples.

Cross-Recognition of Structurally Similar β-Blockers. To investigate the binding selectivity of the particles for structurally similar β-blockers, a cross-recognition experiment was performed by incubating the particles together with propranolol, atenolol, and pindolol simultaneously. In polypropylene microcentrifuge tubes, polymer particles (5 mg) was mixed with a 1 mL solution of propranolol, atenolol, and pindolol dissolved in either acetonitrile or a mixture of 10 mM ammonium acetate buffer (pH 6.7):acetonitrile (50:50, v:v). The initial concentration of each test compound was fixed at 34 μM. The tubes were incubated overnight on a rocking table. The samples were then centrifuged at 13 000 rpm for 30 min, and the supernatant was filtered through a 0.2 μm PTFE HPLC-filter (Sun SRi, Rockwood, TN) before HPLC-MS/MS quantification. Data are mean values of triplicate determinations.

Analytical Quantification by HPLC-MS/MS. Analyte concentration in the cross-recognition studies was determined by HPLC-MS/MS analysis. A SunFire C_{18} (3.5 μm, 2.1 mm $\times$ 50 mm) column mounted on a Waters 2695 separation module was used to separate the three β-blockers. Mobile phase A: 0.1% formic acid in water; B: methanol. The test compounds were separated in 3 min under isocratic elution (A:B = 65:35, v:v) using a flow rate of 0.3 mL min^{-1} and monitored using a Waters Quattro micro API mass spectrometer (triple quadrupole). The following MRM monitoring for quantification of individual compounds was used: ES+ 249 > 116 (pindolol), ES+ 260 > 116 (propranolol), ES+ 267 > 116 (atenolol). Data were acquired with the Masslynx 4.0 software from Waters.

Results and Discussion

One-Pot Synthesis of Molecularly Imprinted Polymer Nanoparticles. In contrast to the traditional precipitation polymerization method that required long reaction time, the synthetic conditions used in the present DPP produced uniform imprinted nanoparticles in less than 3 h. The refluxing solvent provided efficient mixing for the reaction components and at the same time kept the reaction system under an oxygen-free environment. The experimental setup used in this work made it very convenient to achieve a good control upon the rate of the solvent removal, as well as the continuous addition of new reagents. As shown in Figure 1, the two types of propranolol-imprinted nanoparticles, mip(MBA) and mip(TRIM), have very uniform physical shape and apparently a narrow size distribution. All the polymer particles obtained by the present DPP have diameters less than 1 μm. The size of the poly(MAA-*co*-MBA) particles are in general larger than the poly(MAA-*co*-TRIM) particles, which may be attributed to the different types of cross-linking monomers used. The hydrodynamic sizes of the polymer particles measured by DLS are summarized in Table 1. As seen, the imprinted polymers have somehow smaller particle size than the corresponding nonimprinted reference particles. This phenomenon has been observed in

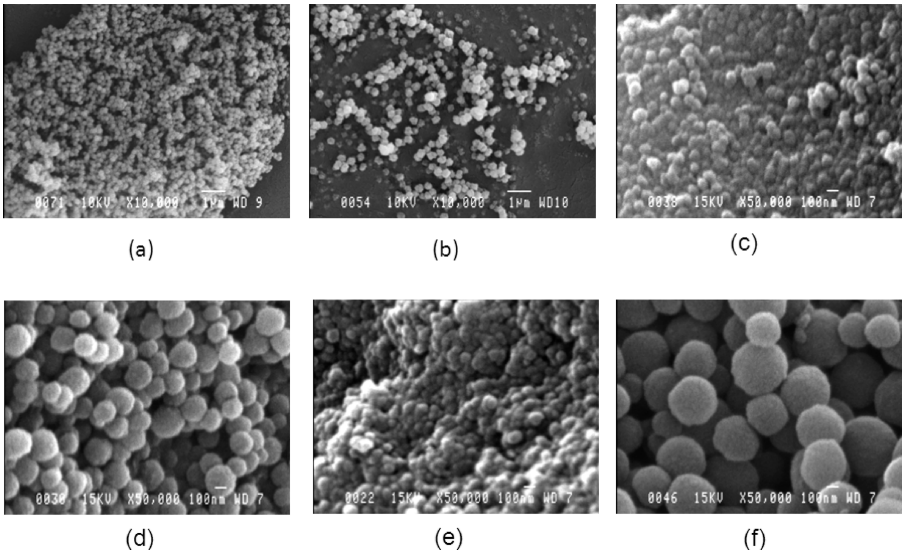


Figure 1. SEM images of nanoparticles synthesized by DPP: (a) mip(MBA), (b) ref(MBA), (c) mip(TRIM), (d) ref(TRIM), (e) mipCS(TRIM/Am), and (f) refCS(TRIM/Am).

Table 1. Hydrodynamic Size of Polymer Nanoparticles Determined by DLS

entry	polymer	type of nanoparticles	diameter (nm)	polydispersity index (PDI)
1	mip(MBA)	imprinted poly(MAA-*co*-MBA)	261	0.038
2	ref(MBA)	nonimprinted poly(MAA-*co*-MBA)	540	0.11
3	mip(TRIM)	imprinted poly(MAA-*co*-TRIM) core	82.0	0.088
4	ref(TRIM)	nonimprinted poly(MAA-*co*-TRIM) core	216	0.034
5	mipCS(TRIM/Am)	imprinted poly(MAA-*co*-TRIM) core/poly(Am-*co*-MBA) shell	130	0.16
6	refCS(TRIM/Am)	nonimprinted poly(MAA-*co*-TRIM) core/poly(Am-*co*-MBA) shell	447	0.078

previous precipitation polymerization systems involving propranolol as template.[20] The reduction of particle size for the imprinted polymer was proposed to be caused by the template compound, which somehow altered the solubility of the growing polymer chain by forming template−polymer complexes, thereby influencing the nucleation and growth process of the cross-linked particles. For the more hydrophobic poly(MAA-*co*-TRIM) particles, the hydrodynamic diameter of the imprinted nanoparticles (82 nm) prepared by DPP is much smaller than the particles prepared under standard precipitation polymerization conditions.[20] In separate synthesis experiments, we confirmed that the solvent removal during the polymerization had no influence on the particle size, since the nanoparticles prepared under simple refluxing condition (without removing acetonitrile) had essentially the same size as if half of acetonitrile was removed. Obviously, the higher reaction temperature used in this work (83−84 °C) has a profound impact on the particle size. In our previous work we also noticed that for poly(MAA-*co*-TRIM) particles prepared by precipitation polymerization, when the reaction temperature was changed from 4 °C (with UV initiation) to 60 °C, the particle diameter decreased from several micrometers to hundreds of nanometers, and the size distribution of the obtained nanoparticles also decreased significantly.[20,22]

With the present experimental setup for DPP, we obtained core−shell nanoparticles comprised of a hydrophobic core containing imprinted binding sites and a loosely cross-linked hydrophilic shell. The core−shell nanoparticles were easily achieved by simple one-pot synthesis without interrupting the polymerization process. The hydrophilic shell was grafted to the core particles through copolymerization of the residual C=C bonds remaining in the core with the hydrophilic monomers added in the second phase of the reaction (Scheme 1). Compared to the core particles prepared under similar reaction condition, the size of the core−shell nanoparticles is almost 2 times as large. The size distribution curves for all the core−shell particles are shifted to larger sizes, indicating that virtually all the core particles have been coated with the hydrophilic shell, and the formation of secondary poly(AAm-*co*-MBA) particles under the present reaction condition is negligible (Figure 2).

The improved hydrophilicity of the core−shell nanoparticles was tested by mixing the different nanoparticles with water and observing the colloidal stability of the samples. For the imprinted polymers, it was difficult to disperse the core particles (mip(TRIM)) in pure water due to its hydrophobic characteristics that led to strong particle aggregation. The imprinted core−shell nanoparticles (mipCS(TRIM/Am)) could however be easily dispersed in water to give stable nanoparticle dispersion. The colloidal status of mipCS(TRIM/Am) maintained unchanged even after the sample was left standing at ambient temperature for 5 days (see Supporting Information, Figure S2).

Nondestructive Surface Modification of Imprinted Nanoparticles. For highly cross-linked polymer particles, residual C=C bonds are often observed because these vinyl groups have much reduced mobility after the gel point and are not accessible to react with other remaining active free radicals in the system. If the remaining C=C bonds are from acrylate- or acrylamide-based cross-linkers, they can be treated with Michael addition reagents (organic amine or thiol compounds) to introduce new functions into imprinted polymers conveniently. To maintain the molecular binding selectivity, such surface modification should be limited to nonspecific sites in order to keep the high affinity sites intact. This nondestructive modification may be achieved by using an organic amine or thiol compound that has a molecular

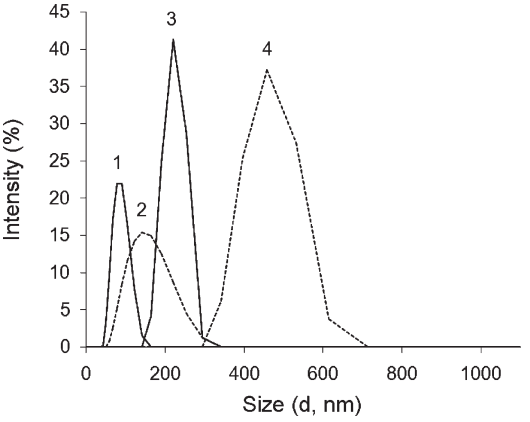
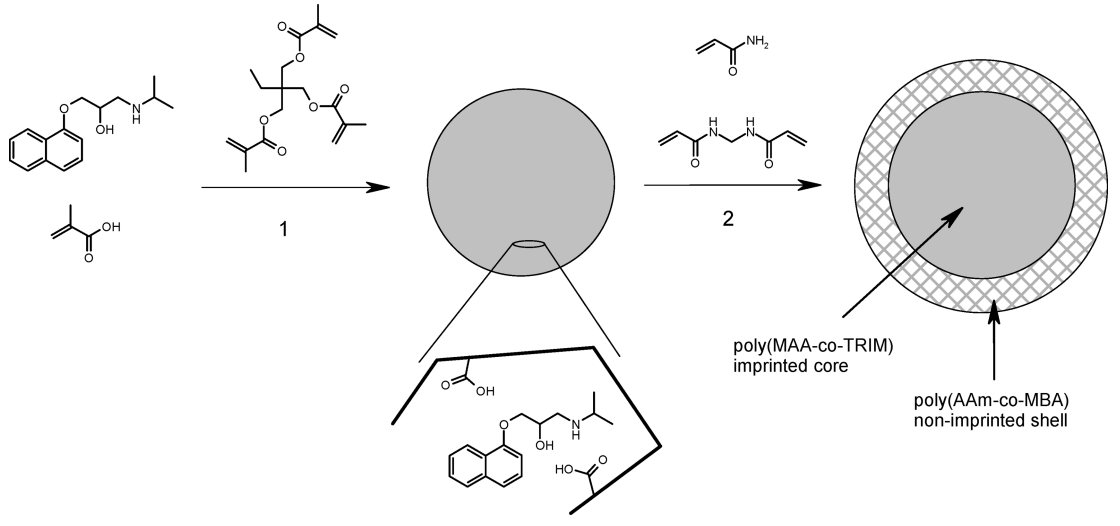


Figure 2. Distribution of particle size measured by DLS: (1) mip(TRIM), (2) mipCS(TRIM/Am), (3) ref(TRIM), and (4) refCS(TRIM/Am).

Scheme 1. One-Pot Synthesis of Propranolol-Imprinted Core−Shell Nanoparticles[a]



[a] Step 1: propranolol-imprinted sites are generated by cross-linking polymerization between MAA and TRIM in the presence of the molecular template. Step 2: formation of hydrophilic shell by subsequent copolymerization with AAm and MBA.

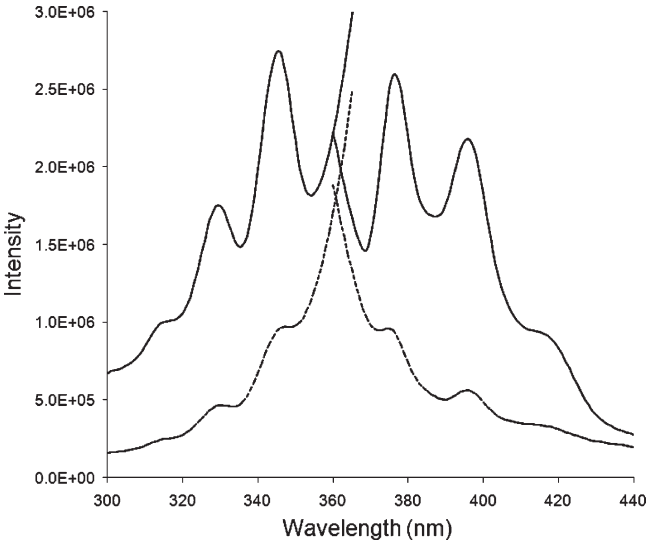


Figure 3. Fluorescence excitation (λ_{em} = 376 nm) and emission (λ_{ex} = 345 nm) spectra of modified mip(MBA) (solid lines) and ref(MBA) (dotted lines).

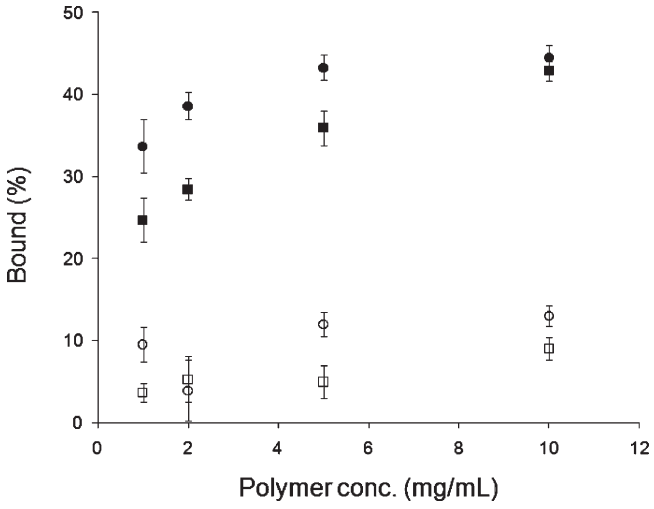


Figure 4. Uptake of labeled (*S*)-propranolol (246 pM) by different amount of polymer nanoparticles in acetonitrile: mip(MBA) (●), ref(MBA) (○), modified mip(MBA) (■), and modified ref(MBA) (□).

size larger than the original template, so that the Michael reagent can be excluded from the best imprinted sites. In this way the chemical modification will take place only on nonspecific surfaces. To test this hypothesis, we used a fluorescent amine, 1-pyrenemethylamine, to modify the poly(MAA-*co*-MBA) nanoparticles through Michael addition reaction and evaluated the effect of the chemical modification on the binding property of the imprinted nanoparticles.

The presence of residual C=C bonds in mip(MBA) and ref(MBA) was first confirmed by FT-IR analysis of the nanoparticles. Both mip(MBA) and ref(MBA) have characteristic IR absorption bands at 3050 cm^{-1} (C−H stretching in alkene), between 1675 and 1640 cm^{-1} (C=C stretching), and 984 cm^{-1} (C−H out-of-plane bending in alkene) (see Supporting Information, Figure S3). The IR signal intensities indicate that the amount of residual C=C bonds remaining in the imprinted and the nonimprinted polymers were comparable. These C=C bonds were treated with excess of 1-pyrenemethylamine to introduce fluorescent moieties to the polymer nanoparticles. The modified nanoparticles obtained were washed thoroughly with solvent before their fluorescent excitation and emission spectra were studied. While the surface modified imprinted nanoparticles displayed strong pyrene fluorescence emission, the modified reference nanoparticles gave much weaker fluorescence signal (Figure 3). The low signal intensity suggested that the Michael addition reaction with the reference particles was much less effective than with the imprinted nanoparticles, even though the FT-IR results indicated that the amount of residual C=C bonds in the two types of polymers were comparable. It seems that the C=C bonds in the reference polymer is less accessible to the fluorescent amine. Indeed, titration of the nanoparticles with $KMnO_4$ revealed that the accessible C=C bonds in the imprinted nanoparticles (19.3 μmol/g) were much higher than in the reference nanoparticles (7.5 μmol/g) (see Supporting Information), which can explain the lower fluorescent signal recorded from the modified ref(MBA) nanoparticles. To investigate whether the different availability of the reactive C=C bonds is caused by the difference in polymer swelling, we also measured the apparent solvent uptake by mip(MBA) and ref(MBA) (see Supporting Information). The solvent uptake by the imprinted polymer (mip(MBA)) was 1.32 mL/g for acetonitrile and 1.31 mL/g for DMF, and the solvent uptake by the reference polymer (ref(MBA)) was 1.24 mL/g for acetonitrile and 1.21 mL/g for DMF, respectively. The imprinted polymer displayed apparently higher swelling than the reference polymer in both acetonitrile and DMF. Combining the results of FT-IR and solvent uptake, we suggest that it is the higher swelling of mip(MBA) in DMF that makes its residual C=C bonds more accessible for fluorescent modification through the Michael addition reaction.

The successful surface modification of the imprinted nanoparticles suggest that similar Michael addition reactions based on organic amine or thiol may be utilized to introduce other new functions into MIP particles or to allow MIP nanoparticles to be immobilized on transducer surfaces precoated with organic amines or thiols. The latter possibility should be specially useful for fabrication of nanoparticle-based chemical sensors.

The necessity of using large Michael reagent for nondestructive modification was demonstrated by carrying out similar modification reactions using benzylamine and ethanolamine as Michael donors (see Supporting Information). In this case the smaller organic amine reacted nonselectively with all the accessible C=C bonds in mip(MBA) and ref(MBA). As a result, the specific molecular binding, calculated as the ratio of propranolol uptake between the modified mip(MBA) and the modified ref(MBA), decreased significantly from 4.75 (modified with 1-pyrenemethylamine) to 1.18 (modified with benzylamine) and 1.49 (modified with ethanolamine) (see Supporting Information, Table S1).

Molecular Recognition Properties of Imprinted Nanoparticles. As shown in Figure 4, the imprinted poly(MAA-*co*-MBA) nanoparticles displayed clearly much higher propranolol binding in comparison with the nonimprinted reference polymer in acetonitrile. With a polymer concentration of 5 mg/mL, propranolol uptake by the imprinted polymer is ~4 times of that by the reference polymer, suggesting that specific binding sites have been successfully generated during the imprinting reaction. The propranolol uptake by the present imprinted poly(MAA-*co*-MBA) nanoparticles (46% uptake by 10 mg nanoparticles) is lower than previously developed imprinted poly(MAA-*co*-TRIM) nanoparticles (65% uptake by 2 mg nanoparticles),[20] partly because a different cross-linker, MBA, was used in the

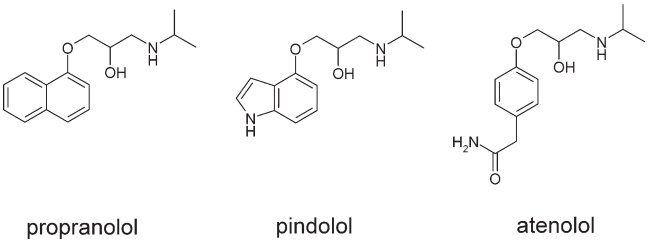


Figure 5. Chemical structures of the test compounds.

present preparation. The present higher reaction temperature is also responsible for the reduced binding affinity of the polymer particles. Nevertheless, the short reaction time required (< 3 h) for particle synthesis is a definite advantage in comparison with the long reaction time (24 h) used in previous precipitation polymerization processes. The use of MBA as cross-linker also resulted in more hydrophilic imprinted nanoparticles, which should have better compatibility with aqueous samples.

In order to confirm that the higher propranolol binding with the imprinted nanoparticles was due to the imprinted sites rather than their smaller particle size (which lead to apparently larger surface area), we also tested the cross-selectivity of mip(MBA) for three structurally related compounds: propranolol, pindolol, and atenolol, all belonging to the same family of therapeutic β-blockers (Figure 5). The three β-blockers were incubated simultaneously with mip(MBA) or ref(MBA) in acetonitrile, and their equilibrium binding to mip(MBA) and ref(MBA) was measured by HPLC-MS/MS analysis. Figure 5 shows that all the three β-blockers contain the same isopropylaminoethanol moiety, capable of interacting with both mip(MBA) and ref(MBA) through hydrogen bond interaction. Nevertheless, the biggest difference of uptake between the imprinted and the reference polymer was displayed by the original template, propranolol (Figure 6). Pindolol displayed a similar binding pattern but with slightly reduced selectivity, due to the minimal structural alteration in its aromatic ring. The β-blocker atenolol has more profound change around its aromatic ring and therefore displayed no selectivity between the imprinted and the reference polymers.

The obvious difference of propranolol uptake by mip(MBA) displayed in Figures 4 and 6 deserves further discussion. In Figure 4, the results were obtained from radioligand binding analysis using very low concentration of propranolol. Here the binding was preferentially determined by the partitioning of propranolol between polymer and solvent. To reach high percentage of binding for low concentration analyte, very high affinity sites were required. In Figure 6, the results were obtained from much higher concentration of β-blockers (total concentration at 102 μM, which was about 400 000 times of the radioligand used in Figure 4). Under this condition the many weak binding sites could also contribute to analyte binding, leading to greatly increased propranol uptake. The involvement of the abundant weak binding sites, on the other hand, caused the selectivity of binding to be reduced.

Template binding with the imprinted poly(MAA-*co*-MBA) under aqueous conditions was also tested. As seen in Figure 7, as soon as buffer was added into the incubation solvent, specific binding of propranolol as judged from the difference of propranolol uptake between the imprinted and the reference polymer disappeared. This result is different from our previous imprinted poly(MAA-*co*-TRIM) nanoparticles, which retained specific propranolol binding in acetonitril containing as high as 80% citrate buffer.[20] In

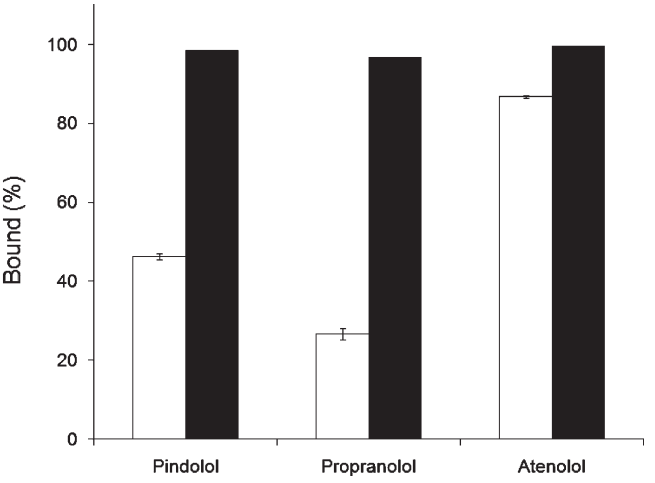


Figure 6. Uptake of the three β-blockers by mip(MBA) (filled bar) and ref(MBA) (empty bar) in acetonitrile. Initial concentration of each test compounds: 34 μM; polymer concentration: 5 mg/mL.

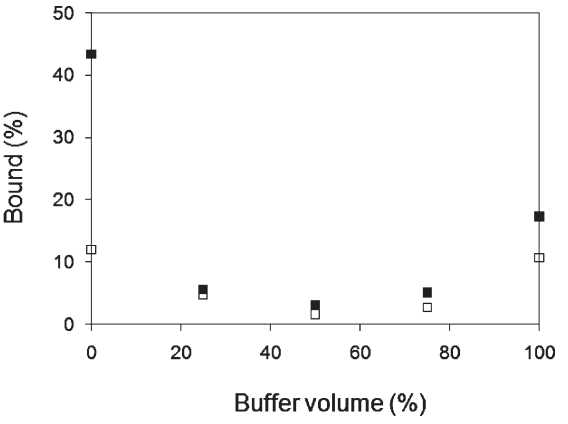


Figure 7. Uptake of labeled (*S*)-propranolol (246 pM) by mip(MBA) (■) and ref(MBA) (□) under different solvent conditions. Polymer concentration: 10 mg/mL.

the presence of buffer, the reduced propranolol binding with mip(MBA) may be explained by the reduced hydrophobic interaction between the imprinted sites and the naphthalene moiety of propranolol, since the cross-linker MBA used to synthesize the present mip(MBA) can not provide the same well-defined hydrophobic interaction as the more hydrophobic cross-linker used in the previous studies.

When new TRIM-based nanoparticles were synthesized using the present DPP method, equilibrium binding results confirmed that the imprinted polymer (mip(TRIM)) remained specific propranolol binding in both neat acetonitrile and acetonitrile containing 50% citrate buffer. At a polymer concentration of 2 mg/mL, mip(TRIM) was able to bind ~32% of the radioisotope-labeled propranolol in acetonitrile: citrate buffer. This value is somehow lower than that obtained with the previous particles prepared by traditional precipitation polymerization.[20] Apparently, the higher reaction temperature used is the main cause of the reduced propranolol affinity. Nevertheless, the difference between the imprinted and the reference nanoparticles remained significant (Figure 8a). The imprinted chiral selective sites are clearly seen from simple displacement experiments carried out in acetonitrile:buffer; i.e., the nonlabeled (*S*)-propranolol effectively reduced binding of the labeled (S)-propranolol by 49%, as compared to the slight reduction

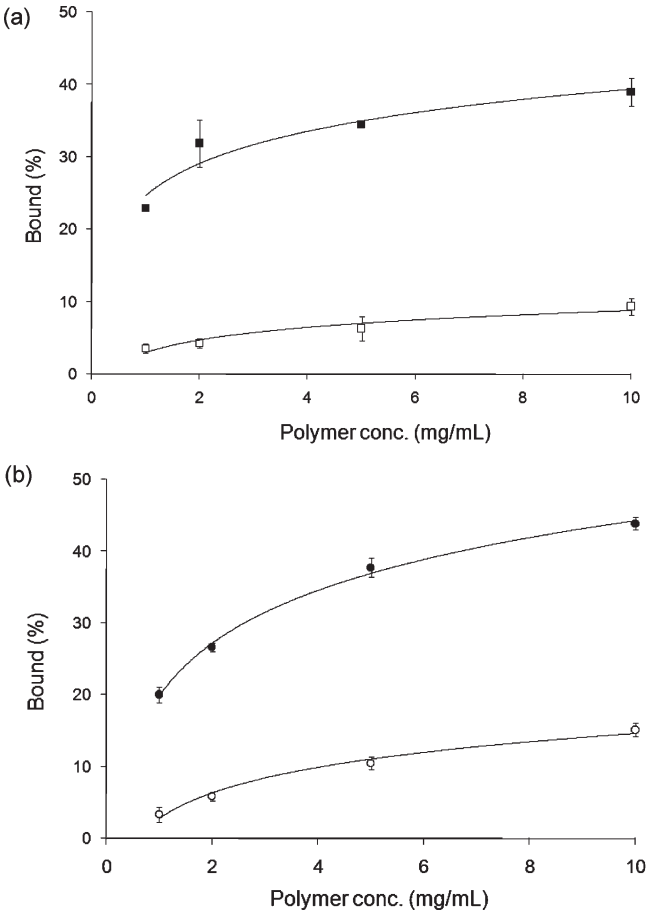


Figure 8. Uptake of labeled (*S*)-propranolol (246 pM) by different amount of polymer nanoparticles in 25 mM citrate buffer (pH 6.0): acetonitrile (50:50): (a) mip(TRIM) (■) and ref(TRIM) (□); (b) mipCS-(TRIM/Am) (●) and refCS(TRIM/Am) (○).

of radioligand binding (by 23%) caused by the nonlabeled (*R*)-propranolol (Figure 9).

The hydrophilic core−shell particles prepared by one-pot DPP maintained high specific binding in acetonitrile:buffer. As shown in Figure 8b, within the polymer concentration tested, uptake of low level of propranolol by mipCS(TRIM/Am) was 3−4-fold of by the nonimprinted refCS(TRIM/Am). As seen from the competitive radioligand binding results (Figure 9), the nonlabeled (*S*)-propranolol is much more effective than (*R*)-propranolol to displace the bound labeled (*S*)-propranolol, indicating that the chiral imprinted sites in the core of mipCS(TRIM/Am) are kept intact and remain accessible to template recognition. Clearly, the grafting of the hydrophilic polyacrylamide shell did not destroy the selective molecular recognition capability of the imprinted core−shell particles.

Selectivity of mip(TRIM) and mipCS(TRIM/Am) for the structurally related β-blockers was further tested in acetonitrile:buffer. The three β-blockers used previously were incubated with the core and core−shell nanoparticles, and the uptake of each individual β-blockers was analyzed by HPLC-MS/MS. From Figure 10, the difference of uptake between the imprinted and the reference core particles are the largest for the original template, propranolol. Again, pindolol displayed similar binding pattern but with slightly reduced selectivity due to the minimal structural alternation of the aromatic ring. The imprinted core particles have no specific binding for atenolol because of its more profound change around its aromatic ring. For the core−shell

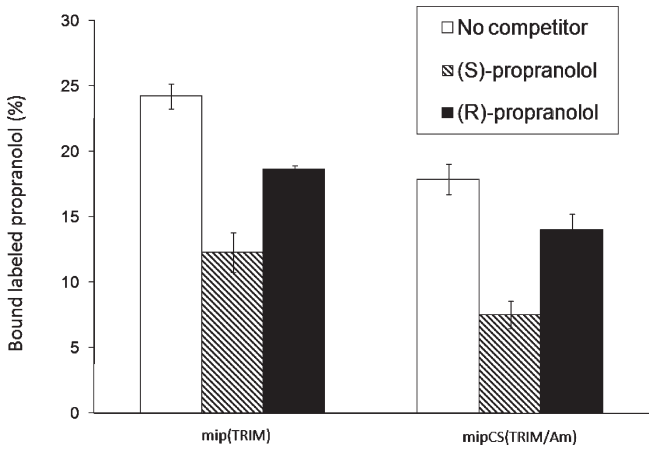


Figure 9. Uptake of labeled (*S*)-propranolol (246 pM) by 2 mg of mip(TRIM) or mipCS(TRIM/Am), with or without 34 μM nonlabeled (*S*)-propranolol or (*R*)-propranolol in 25 mM citrate buffer (pH 6.0): acetonitrile (50:50).

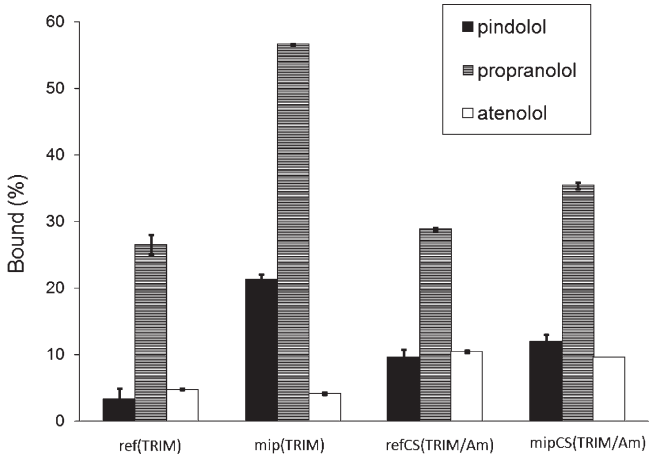


Figure 10. Uptake of the three β-blockers by the TRIM-based nanoparticles in 25 mM citrate buffer (pH 6.0):acetonitrile (50:50). Initial concentration of each test compound: 34 μM; polymer concentration: 5 mg/mL.

nanoparticles, the additional polyacrylamide shell has some effect on analyte binding. While the uptake of propranolol has decreased after grafting the polyacrylamide shell, the difference between mipCS(TRIM/Am) and ref(TRIM/Am) is still clearly seen. On the other hand, the difference of uptake of pindolol and atenolol has completely disappeared, accompanied by an increased binding to both the imprinted and the nonimprinted core−shell particles. In contrast to radioligand binding analysis which showed clearly the specific binding at very low analyte concentration (Figure 8), the competitive binding at high analyte concentration (Figure 10) involved the participation of mainly the more abundant low affinity sites. The involvement of the weak binding sites caused the selectivity to be reduced. The reduced propranolol binding with mipCS(TRIM/Am) can also be explained by the polyacrylamide shell that did not contribute to specific target binding.

The new imprinted core−shell nanoparticles display very desirable target molecule recognition in modified aqueous buffer, especially at low concentration level of target molecules. For analysis of biological samples, the hydrophilic shell of the particles is expected to provide effective screening to prevent protein adsorption while allowing small target molecules to reach the imprinted sites inside the core.

Although dynamic protein adsorption on more hydrophobic nanoparticles (e.g., synthesized from *N*-isopropylacrylamide and *N*-*tert*-butylacrylamide) was found to be complex and dependent on many parameters including particle surface characteristics and chemical composition, size, and protein identity,[23] protein-resistant surfaces based on hydrophilic polyacrylamide gels (similar to our polyacrylamide layer on the core−shell particles) are well-documented in the literature.[24,25] It is hopeful that such imprinted core−shell particles can be more easily used to extract target analytes from biological samples for routine bioanalysis. Further investigation in this direction will be carried out in our future studies.

Conclusion

For the first time, a convenient one-pot distillation precipitation polymerization method has been used to synthesize molecularly imprinted nanoparticles. Hydrophilic MIP nanoparticles were synthesized directly using MBA as cross-linker. The imprinted nanoparticles displayed high specific propranolol binding in polar organic solvent but showed increased nonspecific binding under mainly aqueous conditions. The residual C=C bonds in the imprinted hydrophilic nanoparticles can be utilized to realize nondestructive surface modification using simple Michael addition reactions. This feasibility was confirmed by immobilizing a fluorescent compound to the imprinted nanoparticles while maintaining the specific molecular recognition of the modified nanoparticles. Similar Michael addition reaction may be used for other purposes, e.g., to introduce new functions into MIP nanoparticles or to facilitate nanoparticle immobilization on transducer surfaces.

To maintain target specific binding in aqueous solvent, core−shell nanoparticles were further synthesized, where the hydrophobic core of the nanoparticles was formed using TRIM as cross-linker embedding imprinted molecular recognition sites, and the hydrophilic shell made from acrylamide provides good water compatibility. The core−shell nanoparticles maintained good molecular recognition property in modified buffer especially for recognition of low level of target compounds. The new hydrophilic MIP core−shell nanoparticles are very promising for application with biological samples because the loosely cross-linked hydrophilic shell is expected to reduce nonspecific protein adsorption yet allowing small organic molecules to enter the specific sites inside the core.

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Supporting Information Available: Experimental setup for nanoparticle synthesis, colloidal stability of core−shell nanoparticles, FT-IR spectra, titration of unreacted C=C bonds, test of solvent uptake, and surface modification with small Michael reagents. This material is available free of charge via the Internet at http://pubs.acs.org.

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