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ORIGINAL PAPER

Synthesis of molecularly imprinted polymers via ring-opening metathesis polymerization for solid-phase extraction of bisphenol A

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Abstract The use of molecularly imprinted polymers (MIPs) prepared by ring-opening metathesis polymerization (ROMP) for bisphenol A (BPA) was reported in this article. The resulting MIPs have high imprinting and adsorption capacities, and can be used for separation and determination of BPA in environmental water samples. The successful application of ROMP in the molecular imprinting field is described here. For the first time, two cross-linkers (dicyclopentadiene and 2,5-norbornadiene) and two Grubbs catalysts (first and second generation) were investigated to compare their effects on the binding performance of MIPs. The ROMP technique is able to create the imprinted polymers within 1 h under mild conditions. Furthermore, it can provide MIPs with obvious imprinting effects towards the template, very fast template rebinding kinetics, high binding capacity and appreciable selectivity over structurally related compounds. The adsorption process for MIPs in this study can be completed within 45 min, which is much faster than that of bulk MIPs synthesized by traditional free-radical polymerization. The resulting imprinting polymer was evaluated for its use as a sorbent support in an off-line solid-phase extraction approach to recover BPA from diluted aqueous samples. The optimized extraction protocol resulted in a reliable MISPE method suitable for selective extraction and preconcentration of BPA from tap water, human urine and liquid milk samples. This article demonstrates the practical feasibility of the MIPs prepared via ROMP as solid-phase extraction materials.

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Introduction

Molecular imprinting is a technique to create recognition sites in the polymer matrix. A molecularly imprinted polymer (MIP) is synthesized by co-polymerization of functional monomers and cross-linkers in the presence of template molecules [1]. After a highly cross-linked polymer has been formed, the template molecules are removed, leaving recognition sites in the polymer matrix. These recognition sites are chemically and sterically complementary to the template molecules [2–4].

So far, the most popular method for preparation of MIPs is traditional free-radical polymerization due to its tolerance for a wide range of functional groups and template structures. However, it is very hard to control chain propagation and termination during the traditional radical polymerization processes. Side reactions such as termination/chain-transfer reactions will happen during polymerization, potentially causing the functional monomers and cross-linkers to irregularly co-polymerize, resulting in heterogeneous binding sites toward the target molecules. The presence of heterogeneity within the network structures of the MIPs would have a significant negative impact on the binding sites created inside them, which might be responsible for some of the inherent drawbacks of the MIPs such as the relatively low affinity, broad site heterogeneity, and slow kinetics [5]. If we can have better control over the polymerization progress, we can expect to have MIPs with better molecular recognition performance.

Recently, some controlled/"living" polymerization methods have been introduced to prepare MIPs in a controlled manner. Many living polymerization systems for MIPs have been reported, including nitroxide-mediated polymerization [5], reversible addition-fragmentation chain transfer polymerization [6], iniferter-induced polymerization [7], and atom transfer radical polymerization [8]. They have attracted significant attention for providing simple and robust routes to the synthesis of well-defined polymers, leading to homogeneous polymer networks with a narrow distribution of the network chain length in comparison with that of the highly cross-linked microdomains in the heterogeneous polymer networks prepared via traditional free-radical polymerization [9, 10].

Ring-opening metathesis polymerization (ROMP) [11] is another controlled/"living" polymerization method. It represents a chain-growth polymerization process where strained cyclic olefins are converted to polymeric materials (see Fig. 1 for an illustrative example). The mechanism of ROMP is based on the olefin metathesis: a unique metal-mediated double carbon–carbon bond exchange process [12, 13]. Like other olefin metathesis reactions, ROMP reactions are reversible and equilibrium-controlled, and the position of the equilibrium (monomer vs. polymer) can be predicted by considering the thermodynamics of the polymerization [11]. ROMP reaction not only enables synthesis of well-defined polymers with low polydispersities but also allows stoichiometric design of the polymers, such as controlled chain length and the buildup of block copolymers [14, 15]. ROMP is also an applicable technique for creating polymeric materials with rigid structure and high surface area [16], and for controlling the shape, size, and porosity of the products. These optimized products have been used successfully as stationary phase for chromatography [17–20].

There have been only a few attempts to prepared MIPs via ROMP. Steinke and co-workers [21, 22] had synthesized stereoselective MIPs via ring-opening metathesis polymerization, in essentially, quantitative yield. Enholm and co-workers [23] found that the ROMP method not only improved the binding properties of the polymers but also increased the selectivity.

Bisphenol A (BPA), a known endocrine disrupter [24, 25], is the monomer used for the production of polycarbonate plastics and epoxy resins, such as baby bottles, foodstuff containers, and dental sealants [26, 27]. It can be released into the environment and easily migrate into the human body to produce adverse effects on health. To protect consumers from exposure to BPA residues in harmful concentrations, method development for the selective extraction and determination of BPA from complex matrix is necessary.

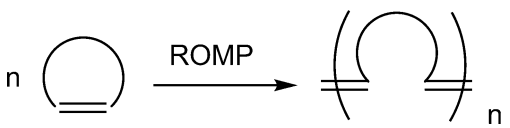

Fig. 1 A generalized example of a ring-opening metathesis polymerization (*ROMP*) reaction

In this article, the successful application of ROMP in the molecular imprinting field is described. For the first time, two Grubbs catalysts (first and second generation) and two cross-linkers (dicyclopentadiene and 2,5-norbornadiene) were investigated to compare their effects on the binding performance of MIPs. The relationship between the polymerization process and the binding properties of MIPs were studied. Several parameters such as template rebinding properties, surface areas, rebinding kinetics and selectivity of the resulted MIPs were examined to investigate the efficiency and selectivity of the molecular imprinting. The MIPs can be applied as a sorbent for the selective extraction and preconcentration of BPA from large volumes of aqueous samples, such as tap water, human urine, and liquid milk.

Experimental

Materials and method

First-generation Grubbs catalyst, second-generation Grubbs catalyst, BPA, and 2,5-norbornadiene were purchased from Sigma-Aldrich. Dicyclopentadiene (DCPD) and acryloyl chloride were purchased from Alfa Aesar. Dichloromethane was freshly distilled from CaH_2 prior to use. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Other solvents and reagents were used as received. Silica column chromatography was carried out using 200~300 mesh silica gel provided by Qingdao Haiyang Chemical Co. Ltd.

Proton and carbon NMR spectra were recorded on a Bruker AMX-400 (^{1}H 400 MHz, ^{13}C 100 MHz) instrument. Chemical shifts (δ) are quoted in parts per million, referenced to residual solvent. IR spectra were recorded on a Nicolet Magna-IR 750 FTIR spectrometer. All chromatographic analysis were performed using an HP 1100 Series LC system equipped with a quaternary pump, a column compartment, a vacuum degasser and a UV–Vis detector. Instrumental parameters were controlled by Hewlett-Packard ChemStation software. The analytical column was packed with Diamonsil ODS RP-18, 5 μm (25 cm× 4.6-mm i.d.) from Dikama. BPA analyses were performed at 25 C using CH_3CN/water (30/70, *v*/*v*) as the mobile phase. The flow rate was 1.0 mL/min. The UV detection wavelength was 278 nm.

Synthesis of template-I

Norborn-2-ene-5-carboxylic acid chloride was firstly synthesized according to the published procedure [28]. A solution of norborn-2-ene-5-caroboxylic acid chloride (6 mL, 39.3 mmol) was added dropwise to a cooled solution (in ice-bath) containing BPA (4.22 g, 18.5 mmol) and triethylamine (4.52 g, 44.3 mmol) in dry THF (100 mL). The reaction solution was kept at room temperature and stirred overnight. Then, the solvent was removed, and the residue was dissolved in ethyl acetate. The organics was washed with water, dried, and evaporated to yield the crude product. The crude product was purified by a silica gel column (petroleum ether: ethyl acetate=1:9, *v*/*v*). Removal of the solvent in vacuo gave 3.78 g (yield, 44%) white solid. IR (KBr, cm^{-1}): 2960 bs, 1727, 1510 s, 1453 s, 1351 m, 1316 m, 1219 s, 1183 s, 1079 s. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=7.23 (m, 4H), 6.94 (m, 4H), 6.24 (dxd, 2H), 6.12 (dxd, 2H), 3.2 (m, 2H), 2.97 (bs, 4H), 1.2–1.7 (14H), (multiplet aliphatic positions of norbornene end group and–CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 151.3, 147.8, 138.1, 135.6, 132.1, 120.7, 49.6, 46.5, 45.8, 43.3, 42.4, 30.9. Elemental analysis calcd. for $C_{31}H_{32}O_4$ (M_w=468.23): C 79.46, H 6.88; found C 79.64, H 6.93 (Fig. 2).

Preparation of MIPs

BPA-imprinted polymers (MIP) and non-imprinted polymers (NIP) were prepared using either first- or second-generation Grubbs catalyst (Table 1). The monomers were dissolved in 6 mL dry dichloromethane and the mixture was purged with a gentle flow of Ar for 10 min. Then, a solution of Grubbs catalyst (0.4 mol% in dry dichloromethane (200 μl)) was added. The ampoule was sealed under vacuum. The reaction temperature and reaction time were shown in Table 1. After polymerization, the polymer monolith was crushed into small particles. The powder was suspended in dichloromethane and ethylvinyl ether was added to remove any excess of monomers and Grubbs catalyst.

To remove the covalently linked BPA, the ground polymer was suspended in 1 mol/L NaOH with THF and water (*v*/*v*, 10:1) as solvent and refluxed overnight. Then, the suspensions were added to an excess of diluted hydrochloric acid. The products were filtered and washed with water. The polymer was Soxhlet-extracted with methanol and then with CH_2Cl_2 for 4 h, and finally dried in vacuo. The amount of BPA in the hydrolysate and washing solutions (the filtrate) from each preparation were determined by HPLC.

Measuring the monomers conversion

After the respective polymerization time, 2 mL of dichloromethane was added to each vial. Overnight, the amount of dissolved un-reacted monomers was determined. The un-reacted template-I was determined by UV–Vis spectrometer. The resulting polymer was dried in vacuo, and weighed, the un-reacted cross-linkers were calculated by the following equation: un-reacted cross-linkers=(total weight of the template-I and cross-linkers)−(weight of the resulting polymer)−(weight of the un-reacted template-I). The conversion of monomers was calculated as $(1-M/M_0)\times 100\%$, where M and M_0 are the measured and the started un-reacted amounts of the studied monomers, respectively.

Batch rebinding experiments

A series of BPA solutions with different concentrations were prepared in CH_2Cl_2. Two-milliliter aliquots of each solution were incubated with 20 mg polymer particles for 6 h. The mixtures were filtrated and the BPA concentration

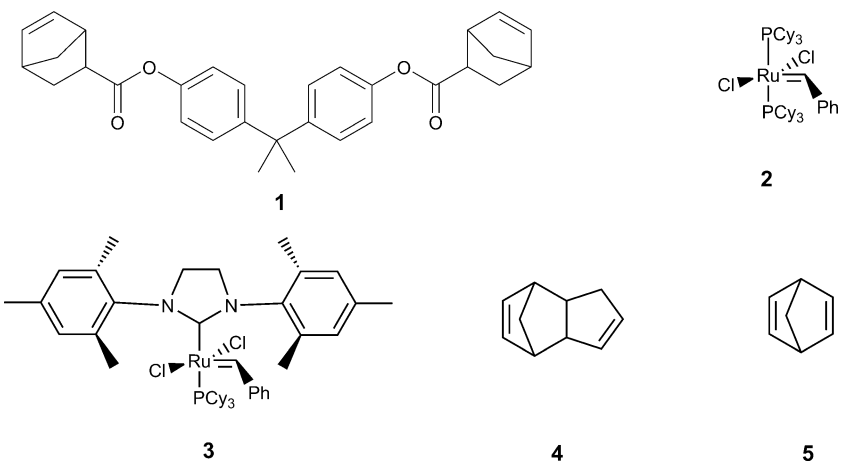

Fig. 2 Structure of template, catalysts and cross-linkers used in this study: (*1*) template-I; (*2*) first-generation Grubbs catalyst; (*3*) second-generation Grubbs catalyst; (*4*) Dicyclopentadiene; (*5*) 2,5-norbornadiene

Table 1 Preparation and characterization of molecularly imprinted polymers

Polymer	Grubbs catalyst	Monomer (mmol)			Polymerization temperature (°C)	Polymerization Time (h)	Surface area (m^2/g)
		Template-I	DCPD	2,5-Norbornadiene			
NIP1	1st	0	15.7	0	60	20	103
MIP1	1st	1.2	15.7	0	60	20	96
NIP2	1st	0	0	15.7	60	20	162
MIP2	1st	1.2	0	15.7	60	20	149
NIP3	2nd	0	15.7	0	Room temperature	1	154
MIP3	2nd	1.2	15.7	0	Room temperature	1	138
NIP4	2nd	0	0	15.7	Room temperature	1	190
MIP4	2nd	1.2	0	15.7	Room temperature	1	176

in the filtrate was measured by HPLC. All of the measurements were made in triplicate. The amount of absorbed BPA was calculated from the following formula:

$$Q = \frac{(c_0 - c)V}{M}$$

where Q is the amount of BPA adsorbed onto a unit mass of polymer (μmol/g); c_0 and c are the concentrations of BPA in the initial and equilibrium solutions, respectively (mmol/L); V is the volume of the solutions treated (mL); and M is the mass of polymer (g).

Their imprinting factors (IF), defined as the ratio of the maximum binding capacity of the MIP to that of the non-imprinted reference polymer, IF=Q_{max}(MIP)/Q_{max}(NIP).

The Scatchard analysis was carried out using Scatchard equation [29]:

$$\frac{Q}{C_{eq}} = \frac{(Q_{max} - Q)}{K_d}$$

Where Q and Q_{max} are the amount of BPA bound to a unit mass of polymer and the maximum adsorption capacity of a unit mass of polymer, respectively (μmol/g); C_{eq} is the concentration of BPA in equilibrium solutions (mmol/L); and K_d (mol/L) is the dissociation constant for the template BPA to the polymer.

Binding kinetic experiments

Twenty milligrams of imprinted polymers were added to the 2 mL BPA solutions (4.0 mmol/L) in dichloromethane. The mixtures were incubated in a shaken bed at 25 ºC for 15 min, 30 min, 45 min, 1 h, 1.5 h and 2 h, respectively, and then filtrated. The amounts of analytes adsorbed by the polymer particles were determined by measuring the residual analytes in the filtrate using HPLC.

Competitive adsorption test

Adsorption and competitive recognition studies were performed with hydroquinone (HQ), *p*-nitrophenol (*p*-NP), bisphenol F (BPF), and diethylstilbestrol (DES). Two milliliters of CH_2Cl_2 solution containing 1 mmol/L of each compound was incubated with 20 mg MIP or 20 mg NIP for 1 h. The mixtures were filtrated and the analytes in the filtrate were measured by HPLC. Chromatographic separation of these compounds was performed using an HP 1100 Series LC system equipped with a quaternary pump, a column compartment, a vacuum degasser and a UV–Vis detector. A Diamonsil ODS RP-18 column (25 cm×4.6 mm I.D., 5 μm) from Dikama was used for the determination of analytes. The mobile phase was a mixture of acetonitrile, water, and acetic acid (50/49/1). The flow rate was 0.6 mL/min. The UV detection wavelength was 254 nm. All of the measurements were made in triplicate.

Preparation of MISPE cartridges

For preparation of MISPE cartridge, an amount of 200 mg of dried imprinted polymer particles MIP4 was slurry-packed into the SPE cartridges (6.0 mL) in methanol. One PTFE frit was placed above the polymer sorbent bed, and one below. Before use, the MISPE column was washed with 20 mL of a methanol/acetic acid mixture (9:1, *v*/*v*) and 20 mL of methanol consecutively and repeatedly until no BPA was detected, and then it was conditioned with 5 mL of loading solvent. For comparison, a blank SPE cartridge was prepared in the same way but packed with the non-imprinted polymer NIP4.

Analysis of real sample

Tap water samples were obtained from our laboratory and analyzed immediately after sampling. One hundred microliters of the BPA standard solution (10 or 100 μg/mL) was

added to 100 mL tap water sample, and then filtered through a 0.45-μm PTFE syringe, before being applied to the MISPE column.

One hundred microliters of the BPA standard solution (10 or 100 μg/mL) was added to 2 mL of newly collected human urine, followed by diluting with purified water to 100 mL. The urine samples were centrifuged for 20 min at 10,000 rpm and then filtered through a 0.20-μm filter. The filtrate was diluted with purified water to 100 mL, and stored at −20 C until analysis was performed, with the minimum possible delay.

One hundred microliters of the BPA standard solution (10 or 100 μg/mL) was added to 2 mL of liquid milk, followed by extraction with 5 mL acetonitrile and then centrifugation for 20 min at 10,000 rpm at 0°C. The supernatant was filtered through a 0.20 μm filter, and then diluted with purified water to 100 mL.

Results and discussion

Preparation of the polymer

In contrast to free-radical polymerization, ROMP represents a metal-catalyzed polymerization system. First- and second-generation Grubbs catalysts were selected because of their well-established reactivity and remarkable tolerance toward most functional groups in metathesis reactions [30–32]. In order to maximize the compatibility of the template monomer and the catalyst, the imprinted polymer was synthesized following the covalent approach [33] in which the template molecular was linked with 2-norbornene before polymerization. DCPD and 2,5-norbornadiene were investigated as the cross-linkers. After polymerization, ethylvinyl ether was added in order to ensure a clean removal of the ruthenium core from the polymer. Then the polymer was Soxhlet-extracted with dichloromethane.

To synthesize the imprinted polymers by the covalent approach, hydrolysis of the ester groups was required to remove the templates and generate the recognition sites. Here, hydrolysis was realized by overnight reflux with 1 mol/L NaOH in a mixture of THF and H_2O. After hydrolysis, the templates were removed from the polymer, leaving –COOH functional groups that were able to interact with the BPA during the rebinding process. The amount of BPA recovered from the hydrolysate was measured to determine the percentage of accessible binding sites, which was calculated to about 70~85%.

Co-monomers conversion studies

After polymerization, the un-reacted monomers in the MIPs were dissolved in dichloromethane and the amount of un-reacted monomers was determined. Figure 3 shows the calculated time conversion plots of template-I, DCPD, 2,5-norbornadiene in MIPs. Figure 3a shows the time conversion plots of three monomers in MIPs in the presence of first-generation Grubbs catalyst. The polymerization rates for the two cross-linkers and template-I are not equal. The full conversion of 2,5-norbornadiene occurs much faster than DCPD conversion and template-I conversion. The full conversion of DCPD occurs slightly faster than the template-I conversion. Figure 3b shows that the conversion processes of these three monomers were done at almost the same speed in the presence of second-generation Grubbs catalyst.

Because of the different polymerization rates of these monomers, the composition of polymers changes with polymerization time. These changes in the polymer composition with time may be the cause of the differences in the template rebinding properties of polymers. Figure 4 shows the imprinting factor of MIPs against polymerization time. As shown in Fig.4, the imprinting factor increased with time first, and then reached a plateau. For MIP1 and MIP2, the full conversion of monomers can be achieved in 2 h, but the highest imprinting factor can only be achieved after 20 h. For MIP3 and MIP4, the full conversion of monomers can be achieved in 20 min, but the highest imprinting factor can only be achieved after 1 h. Before the full conversion of monomers is reached, it is not surprising that the imprinting factor increased because more templates-I are built into the polymer.

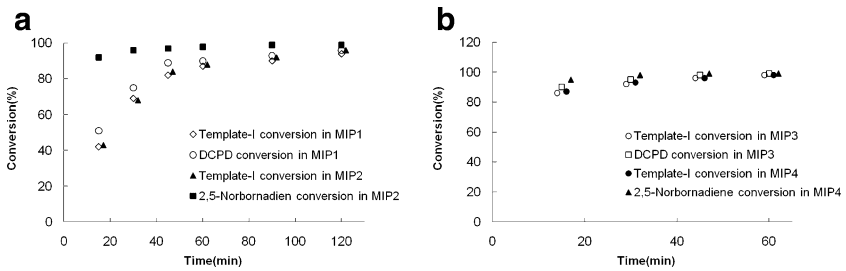

Fig. 3 Conversion of template-I, DCPD and 2,5-norbornadiene in MIPs vs. time. **a** For better visibility the points have been shifted by 2 min to the right (template-I conversion in MIP2); **b** For better visibility, the points have been shifted by 1 min to the left (template-I conversion in MIP3) or to the right (template-I conversion in MIP4) or 2 min to the right (2,5-Norbornadiene conversion in MIP4)

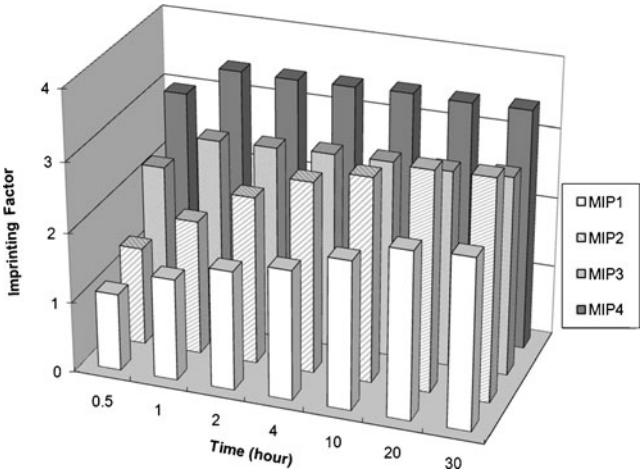

Fig. 4 Imprinting factor of MIPs vs polymerization time

One interesting phenomena is that the imprinting factor increased with polymerization time even after the full monomer conversion was reached. It is probably because of the extra time needed to form the rigid structure of the function polymer via cross-linking network. The cross-linkers and template-I are all bifunctional monomers, so they will disappear from solution when one of their two double bonds reacts with the polymer chain while the other double bound remains intact for further reaction. The remaining second double bond may react in two ways: by making cross-linking with cross-linkers or by binding the template. The reactions of the second double bond are crucial to form the cross-linking of the copolymer network which keeps the imprinting cavities stable after the removal of BPA.

Choice of catalysts and cross-linkers

The choice of the appropriate catalyst and cross-linker represents an important step in order to create a good efficient imprinting system. Two catalysts and two cross-linkers were investigated in this study. The monomer conversion experiments and rebinding experiments were employed in order to study the performance of the catalysts and cross-linkers.

First- or second-generation Grubbs catalysts were studied here. The second-generation Grubbs catalyst has a higher activity than first-generation Grubbs catalyst [34–36]. In the presence of first-generation Grubbs catalyst, it was impossible to obtain the co-polymerization of template-I and DCPD with monomer conversion of more than 85% at room temperature. Therefore, the polymerization temperature for first-generation Grubbs catalyst was set at 60 C. As shown in Fig. 3, the co-polymerization of template-I and the cross-linker can be achieved in the presence of first- or second-generation Grubbs catalyst. Full co-monomers conversion can be achieved by carrying out the polymerization in the presence of first-generation Grubbs catalyst at 60 ºC for 2 h, leading to imprinted polymer in 97% yield. The preparation of MIPs using second-generation Grubbs catalyst provided a cross-linked polymer insoluble in dichloromethane in less than 5 min. The 99% yield can be achieved at room temperature in 20 min. As shown in Fig. 4, MIP4 had higher imprinting factor than MIP2 did, while MIP3 had higher imprinting factor than MIP1 did. It means that the MIPs prepared by the second-generation Grubbs catalyst had higher IF than the MIPs prepared by first-generation Grubbs catalyst. The second-generation Grubbs catalyst exhibits more efficiency than first-generation Grubbs catalyst, allowing not only a faster polymerization but also a higher imprinting factor.

Since the nature of the cross-linking has great impact on the specificity of MIPs [37], the polymers were prepared in parallel with two cross-linkers. Both 2,5-norbornadiene and DCPD were chosen as cross-linkers in the current study. It is the first time that the influence of the two cross-linkers on the specificity of MIPs prepared via ROMP is compared.

As shown in Fig. 4, the imprinting factor of MIPs prepared by 2,5-norbronadiene was higher than that of MIPs prepared by DCPD. 2,5-Norbornadiene exhibited enough reactivity to get high percentage cross-linking, while DCPD did not exhibit the desired cross-linking properties. The cross-linking ability of DCPD was lower than that of 2,5-norbornadiene [38]. And DCPD did not exhibit the desired cross-linking properties, implying that some cavities in the DCPD-based imprinted polymers were destroyed after the removal of BPA. This agreed with previous studies, where the cross-linking of DCPC was found to occur only if induced thermally at high monomer concentration [39–41].

Binding properties of the polymers

To investigate the recognition properties of the MIP for the analytes, different initial concentrations of BPA were applied in equilibrium rebinding experiments. The unhydrolyzed imprinted polymers and the non-imprinted polymer were used as control samples to measure non-specific BPA adsorption outside the cavities.

According to the above experiments, the resulting binding isotherms for BPA to MIPs (before and after hydrolysis) and NIPs are shown in Fig. 5. The imprinted hydrolyzed polymers bound much more BPA than the corresponding imprinted unhydrolyzed polymers and non-imprinted polymers did. The difference in BPA binding before and after hydrolysis indicated that the imprinted sites had much higher BPA affinity, resulting from a combination of hydrogen bond interaction and the complementary fit of BPA in the cavities. Figure 5 shows that the non-specific

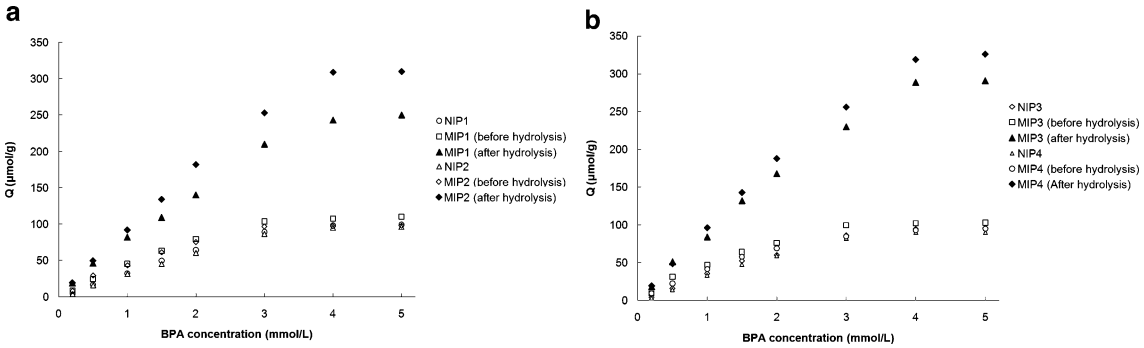

Fig. 5 Binding isotherms of BPA to the MIPs (before and after hydrolysis) and NIPs

BPA binding to non-imprinted polymer was slightly lower than that with unhydrolyzed imprinted polymers. However, BPA binding in the specific cavities of the hydrolyzed imprinted polymers was still predominant. These results confirmed the presence of selective binding sites created by the template in the obtained MIPs and thus confirmed the successful molecular imprinting processes via ROMP. In addition, the MIPs prepared in the presence of second-generation Grubbs catalyst are proved to bind more BPA than that those prepared in the presence of first-generation Grubbs catalyst. The MIPs prepared with 2,5-norbornadiene proved to bind more BPA than that prepared with DCPD.

To get more insight into the binding characteristics of the MIPs prepared via different catalysts and cross-linkers, they were further studied with Scatchard analysis [29], from which some isothermal binding parameters of the MIPs such as the binding dissociation constant K_d and apparent maximum number of binding sites could be obtained. The Scatchard plot of one representative MIP (MIP4) is shown in Fig. 6a. The Scatchard plot of MIP4 actually consists of two linear parts with different slopes, suggesting that at least two classes of heterogeneous binding sites for BPA are formed in the MIP. From the slopes and intercepts of the two lines, the values of K_d and B_{max} for the higher affinity binding site were found to be 5.9×10^{-5} mol/L and 512.3 μmol/g, respectively, while the values of K_d and B_{max} for the lower affinity binding site were 1.1×10^{-3} mol/L and 183.4 μmol/g, respectively. In comparison, Fig. 6b indicates that there is only one kind of binding sties in the non-imprinted polymer NIP4. K_d and Q_{max} of the NIP4 were calculated to be 1.4×10^{-3} mol/Land 135.4 μmol/g, respectively.

It is known that the surface properties of the MIPs/NIPs have significant influence on their binding properties. Therefore, the surface areas of the obtained MIPs/NIPs were also characterized by performing nitrogen adsorption experiments using the BET model. As shown in Table 1, the cross-linked polymers prepared using the two catalysts had quite different surface area, i.e., the polymer initiated by second-generation Grubbs catalyst had a surface area about 20~50% higher than the polymer initiated by Grubbs catalyst first-generation Grubbs catalyst. The 2,5-norborna-diene-based polymers showed higher surface area than DCPD-based polymer due to their different cross-linking properties. Higher surface area can contain more functional groups, thus the interaction between polymer and templates will be increased accordingly.

Kinetic binding studies

The study of binding kinetics can provide the information on the time required to reach equilibrium. Kinetics experiments were performed by adding BPA to the MIPs and monitoring the concentration of unbound BPA at different intervals. As shown in Fig. 7, all the MIPs and NIPs prepared via ROMP in this study can reach their binding equilibriums in about 45 min, indicating quite fast binding processes.

In previous studies, it takes 10 h for BPA bound on the MIP by free-radical polymerization to reach binding equilibrium

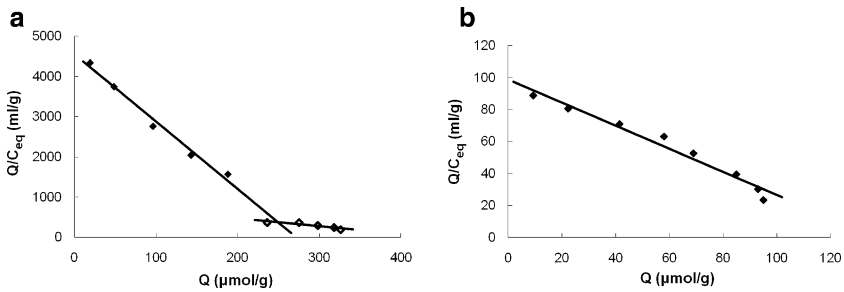

Fig. 6 Scatchard plots of the MIP4 (**a**) and NIP4 (**b**) prepared via ROMP with BPA as the template

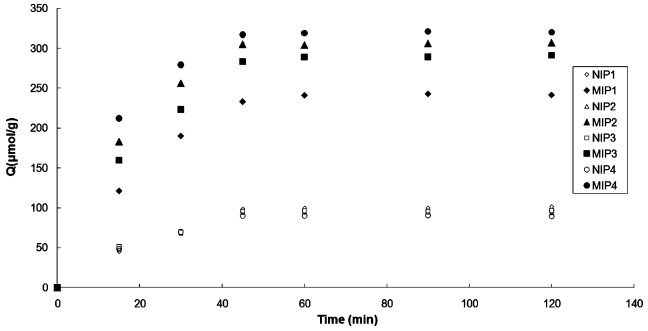

Fig. 7 Kinetic binding profiles for the BPA by MIPs and NIPs

[42], while it takes 3 h for cholic acid bound on the MIP prepared by radical polymerization to reach binding equilibrium [43]. In Enholm's study [23], the template bound on its MIP synthesized by ROMP conditions only needs 20 min to reach equilibrium. In our study, the binding equilibrium of MIP synthesized by ROMP can be reached within 45 min. This suggested that the binding kinetics of the MIPs synthesized by ROMP were remarkably fast and were even much faster than that of traditional bulk MIPs synthesized by free-radical polymerization

Selectivity of the polymers

Due to the high imprinting factor of MIP4, the MIP4 and NIP4 were selected to do further analysis. The selective adsorption of the MIP4 was evaluated using BPA and several other structurally related compounds (Fig. 8), such as HQ, *p*-NP, BPF, and diethylstilbestrol (DES). A solution containing 1 mmol/L of each compound was incubated with 20 mg MIP4 or 20 mg NIP4 at room temperature. The amounts of compounds adsorbed on the polymers were measured and shown in Fig. 9. It can be seen that the MIPs have the highest affinity for BPA. Also, high cross-binding selectivity was observed for bisphenol F because its structure is very similar to that of BPA, so it is possible that they also fit the cavities and form hydrogen bonds with the –COOH groups of the MIP. The MIP4 shows lower affinity for diethylstilbestrol than BPA because the molecular size of DES is a little bigger than that of BPA and the distance between the two phenol groups in the DES is longer than that in BPA. On the other hand, the molecular sizes of hydroquinone and *p*-nitrophenol are much smaller than that of BPA. These molecules can also enter into the cavities of the MIP and form hydrogen bonds. However, due to the lack of spatial complementarities, their adsorption capacities on the MIP are much lower than that of BPA. This suggests that the apparent binding behavior to MIP4 results from highly shape-selective binding cavities generated in the ROMP based cross-linked polymer matrix. The shape-selective binding sites can recognize mismatched structures between the templates and the reference compounds.

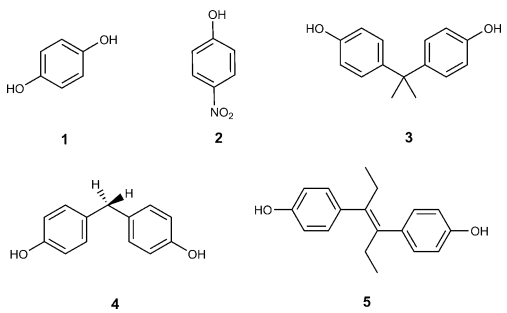

Fig. 8 Chemical structures of the compounds used in this work. (*1*) hydroquinone (HQ); (2) *p*-nitrophenol (*p*-NP); (*3*) bisphenol A; (*4*) bisphenol F (BPF); (*5*) diethylstilbestrol (DES)

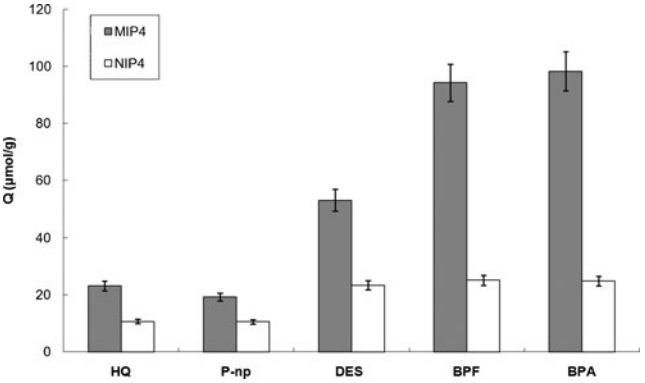

Fig. 9 Cross-binding reactivity of the MIP4 and NIP4: BPA and its analogues adsorbed by MIP4 and NIP4

MISPE

MISPE extraction procedure

The performance of the BPA-imprinted polymer for solid-phase extraction of aqueous samples was evaluated in off-line mode. The SPE process was optimized and the performance of the imprinted polymers for the extraction of BPA was compared with that of the non-imprinted polymers. First of

Table 2 The effect of the washing conditions on the recovery of BPA in washing fractions for the MISPE and NISPE columns

Percentage of methanol in dichloromethane (%)	Mean recoveries of BPA in the washing fraction (RSD)(%)	
	MISPE	NISPE
0	0.6 (2.1)	39.4 (3.9)
2	0.7 (3.3)	62.4 (4.2)
4	2.1 (1.9)	73.8 (6.2)
5	3.3 (2.6)	92.4 (5.7)
6	3.8 (3.1)	96.7 (5.9)
10	10.1 (4.2)	98.2 (6.7)

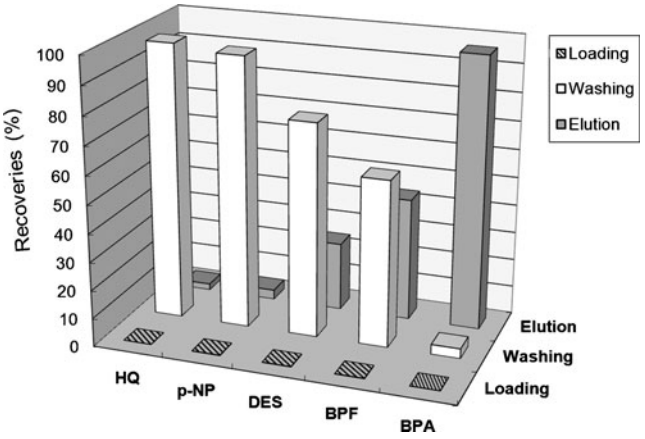

Fig. 10 Recoveries of selected BPA and its analogues from 100 mL of 100 μg/L spiked water after washing and elution step

all, the conditioning and the loading steps were optimized. A conditioning step consisting of passing 5 mL of purified water was applied. After conditioning, a volume of 100 mL of purified water, spiked at 10 μg/L concentration levels of BPA, were loaded on the MIP4- or NIP4-packed cartridges at a flow rate of 1.5 mL/min.

Under aqueous loading conditions, the analytes in the sample were retained on the MIP based on the non-specific hydrophobic interaction. The washing step was included to remove the non-specifically bound BPA from the MIP. Several solvents such as dichloromethane, methanol, and dichloromethane/methanol solution were tested as the washing solution. Table 2 shows the recoveries of BPA in washing fractions with different volume ratios of methanol in dichloromethane for the MISPE and NISPE columns. From Table 2, we can see that dichloromethane is not an effective washing solvent with recoveries of BPA lower than 40% for both MIP and NIP. The addition of methanol to dichloromethane effectively clean-up BPA from NIPs cartridge. At a final methanol concentration of 6% (*v*/*v*) in dichloromethane, BPA was completely cleaned up from NIP cartridge, while BPA specifically bound on the MIPs was retained. Therefore, in this study, dichloromethane/methanol (94:6, *v*/*v*) was used as washing solution.

Due to its high polar activity, methanol has often been used to elute bound template molecules from MISPE columns. In this study, we found that 3 mL of methanol was sufficient to elute all of the BPA specifically bound on the MISPE cartridge.

Specificity of the MISPE

Selectivity of the MISPE was evaluated with BPA and its analogues including HQ, *p*-NP, BPF and DES. One hundred milliliters of purified water spiked 100 μg/L BPA and its analogues were loaded on the MISPE column. The recoveries of these compounds were calculated. As shown in Fig. 10, different recoveries of these compounds were obtained, where the template has the largest recovery, followed by BPF, DES, *p*-NP, and HQ. The results obtained shows highly selective binding affinity for BPA, and the adsorption of BPA is due to imprinted binding sites and not due to non-specific binding.

MISPE application on environmental water samples

To further investigate the potential of this MIP for selective extraction of BPA in complex samples, tap water, urine sample, and liquid milk spiked with BPA were used to simulate the complex matrixes in real samples. The spiking concentration was set at two levels, 10 and 100 μg/L.

For each sample, a 100-mL with BPA spiking was loaded onto the MISPE column. Limits of detection (LOD, signal-to-noise=3) and recoveries of BPA from different spiked samples were shown in Table 3. For the spiked tap water, LOD of BPA obtained was 22 ng/L, and recoveries of BPA were between 94.3% and 95.2%, which are similar to those obtained with purified water spiked BPA. For urine and milk samples with complex matrixes, although LODs of BPA increased to 53 and 380 ng/L, respectively, recoveries were not seriously reduces. For human urine sample, the recoveries of BPA were between 85.6% and 89.8%. For liquid milk samples, the recoveries of BPA were between 74.8% and 81.3%. Because of the special selectivity of BPA, the MISPE combining with HPLC determination could be applied for selective and sensitive determination of traces BPA in complicated samples.

Conclusion

The successful application of ROMP in the molecular imprinting field is demonstrated. For the first time, two Grubbs catalysts (first and second generation) and two

Table 3 Average recovery, reproducibility and limit of detection of BPA from different spiked samples (the number of the repetition for each data is 3)

Samples	LOD (ng/L, *S*/*N*=3)	10 μg/L		100 μg/L	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Spiked tap water	22	94.3	5.3	95.2	3.7
Spiked human urine	53	85.6	6.4	89.8	7.2
Spiked liquid milk	380	74.8	8.9	81.3	8.3

cross-linkers (dicyclopentadiene and 2,5-norbornadiene) were investigated to study their effects on the binding performance of MIPs. The relationship between the polymerization process and the binding properties of MIPs were studied here. 2,5-Norbornadiene was applied as a cross-linker to prepare imprinted polymer for the first time. We found that 2,5-norbornadiene-based imprinted polymer has higher binding capacity than the dicyclopentadiene-based imprinted polymer. Compared with first-generation Grubbs catalyst, the second-generation Grubbs catalyst exhibits higher efficiency; it allowed not only a faster polymerization, but also better binding properties. The ROMP technique has proven to be able to create the imprinted polymer within 1 h under mild conditions, and provide MIPs with obvious imprinting effects towards the template, very fast template rebinding kinetics, high BPA adsorption capacity and appreciable selectivity over structurally related compounds. The adsorption process for MIPs in this study could be completed within 45 min, which was much faster than that of bulk MIPs synthesized by traditional free-radical polymerization.

The MIP was successful applied as adsorbent for extraction of BPA. Under the optimized SPE conditions, selective extraction of BPA from standard mixture aqueous sample was feasible with the MIP cartridge. Finally, the MIP cartridge was successfully used for selective extraction of BPA spiked in tap water, human urine, and liquid milk samples and satisfactory recoveries were obtained. The high extraction efficiency of MISPE from different complex matrices suggested that the proposed method could thus be a promising alternative for assaying complex large-volume samples.

With the development of new catalysts [44, 45], the catalysts can not only be used with a large number of functional groups, but also be used in the aqueous solution under mild conditions. The ROMP technique will be useful to prepare MIPs for some light-/heat-sensitive compounds and many unstable biomolecules like enzymes, hormones, and polypeptides.

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