Preparation of Molecularly Imprinted Polymer Films on Monodisperse Macromolecular Beads

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ABSTRACT: A new and facile technique for preparing homogeneous and highly selective molecularly imprinted polymers (MIPs) films on porous monodisperse poly-(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) beads ($P_{GMA\text{-}EDMA}$ beads) has been described: before polymerization, initiator was immobilized on the $P_{GMA\text{-}EDMA}$ beads' surface by chemical reactivity; then functional monomers and crosslinkers were initiated by the surface-bound initiator and copolymerized to form MIPs films on the $P_{GMA\text{-}EDMA}$ beads' surface in the presence of template molecules. The resulting beads were analyzed by FTIR spectroscopy and X-ray photoelectron spectra. The effects of the initiator amount and copolymerization time on the content of MIPs, and MIPs contents on morphologies and pore properties of the resulting beads were investigated. The results show that the content of MIPs can be adjusted by changing initiator amount or by controlling copolymerization time. The binding experiments indicate that, at lower grafting level, with the increase of MIPs content, the adsorption and recognition capabilities of the resulting beads enhance. When MIPs content increase to 16.75%, the highest adsorption and recognition capabilities are obtained. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2620–2627, 2009

Key words: molecular imprinting films; high performance polymers; beads; molecular recognition; naproxen

INTRODUCTION

Molecularly imprinted polymers (MIPs) have been applied as selective sorbents in analytical techniques, including liquid chromatography,[1–3] capillary electrochromatography,[4] solid-phase extraction,[5] and "immunoassay."[6,7] Synthesis of MIPs is a process in which functional monomers and crosslinkers are copolymerized in the presence of template molecules. After polymerizing, the functional groups are held in place by the highly crosslinked polymer structure, so the template molecules are encased within a rigid cavity. The extraction of the template molecules results in formation of cavities whose size, shape, and chemical functionality complements the template molecules in the polymer. These cavities (usually called binding sites) can be used to selectively bind template molecules.[8]

Configurations of MIPs, which have been produced include polymer monoliths,[9] polymer beads,[10] and membranes.[11] When MIPs are used as chromatographic media, especially as high-performance liquid chromatography (HPLC) packing materials, spherical and monodisperse beads were especially desirable. In recent years, several methods, such as core-shell emulsion polymerization,[12,13] suspension polymerization,[14] seed swelling and polymerization,[15,16] and precipitation polymerization[17] have been introduced to prepare MIPs beads with a narrow size distribution. However, the accessibility of the binding sites of these beads prepared with these techniques is poor, for most binding sites are buried in these beads.[18,19] Some times this can result in long response time when the materials are assessed as recognition elements in chemical sensors and broad, asymmetric peaks when they are assessed as HPLC packing materials. These problems can be overcome by surface imprinting technique, where MIPs are grafted on preformed support beads.[20,21] In most of the reports, the grafting is carried out by the polymerization of the unsaturated vinyl groups on the surface of preformed support beads along with the monomers by free-radical copolymerization (initiator exists in solution).[22,23] With this process, the thickness and uniformity of the grafted MIPs films are difficult to control. Sellergren and coworkers[24] used silica particles containing surface-bound free-radical initiators as supports to graft MIPs films. This technique offers a means of fine tuning the MIPs layer thickness, but the silica-based packings are less stable under a high-pH condition (they can only be used in the pH range from 2 to 8[25]). In most

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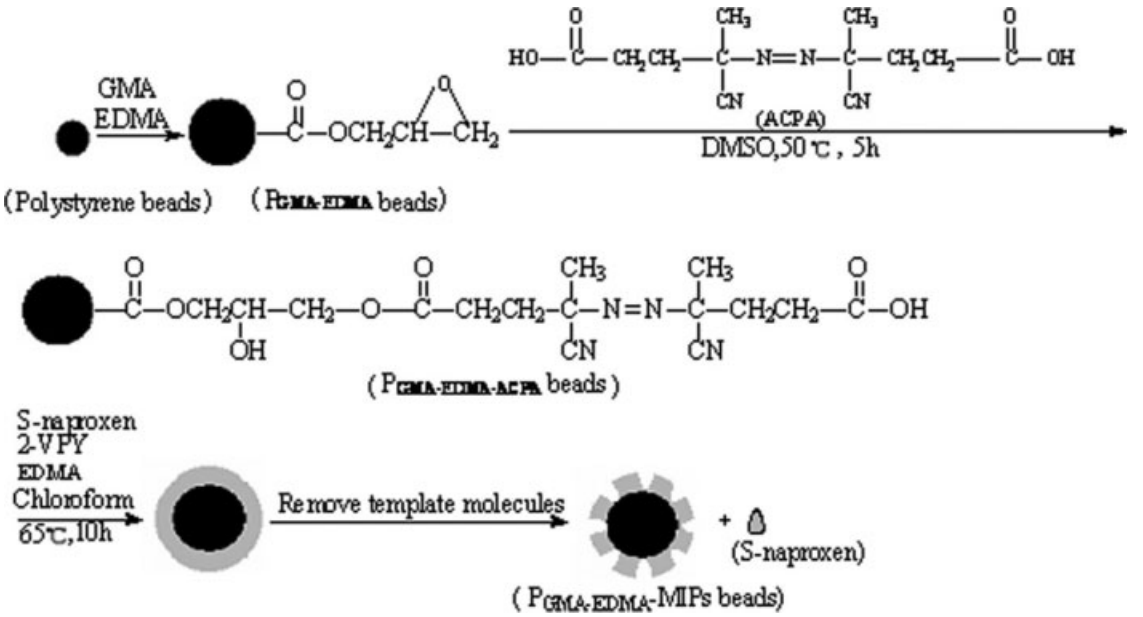

Figure 1 Schematic presentation of the preparation of MIPs films on monodisperse macromolecular beads.

cases, polymer-based packings can be used in the pH range from 1 to 14.[26]

In this study, we investigate a possible route to graft MIPs films on the surface of the porous monodisperse macromolecular beads. The route includes three steps (Fig. 1). First, the monodisperse porous poly-(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) ($P_{GMA\text{-}EDMA}$) beads with epoxy group on their surfaces were synthesized by a single-step swelling and polymerization method. Second, initiator was immobilized on the $P_{GMA\text{-}EDMA}$ beads' surface by chemical reactivity. Finally, functional monomers and crosslinkers were initiated and copolymerized to form MIPs films on the $P_{GMA\text{-}EDMA}$ beads' surface in the presence of template molecules. Following by this way, a homogeneous MIPs films can be easily prepared, and the content of the grafted MIPs can be easily fine tuned. In this study, a chiral drug, *S*-naproxen, was used as the template molecule, which will allow the grafted MIPs to perform the competitively binding experiments with its enantiomer.

EXPERIMENTAL

Materials

Ethylene glycol dimethacrylate (EDMA) was obtained from Tiantai Yunkai Chemical Reagent Co (Shandong, China) and was extracted for three times with 10% aqueous sodium hydroxide and distilled water, and then dried with anhydrous magnesium sulfate. 2-vinylpyridine (2-VPY) was purchased from Fluka (Buchs, Switzerland) and was used after vacuum distillation. Styrene was a product of Linfeng Chemical Reagent Co (Shanghai, China) and was purified by distillation under vacuum. Glycidyl methacrylate was supplied by Guangzhou Shuangjian Co. (Guangzhou, China). 4, 4′-azobis (4-cyanopentanoic acid) (ACPA) was purchased from Fluka (Buchs, Switzerland). *S*-naproxen and *R*-naproxen were bought from Xianju Chetou Pharmacy Plant (Zhejiang, China). Azobisisobutyronitrile (AIBN), dibutylphthalate, sodium dodecyl sulfonate (SDS), polyvinyl alcohol (PVA) and other chemicals and solvents were analytical grade and were used without further purification. Scanning electron micrographs (SEMs) were carried on a XL-30 ESEM scanning electron microscope (Holland). Pore diameter and specific surface areas were measured by Micromeritics ASAP 2010M Gas Adsorption Analyzer (USA). The concentration of *S*-naproxen and *R*-naproxen in the solution was determined using a U-301 UV–Vis spectrophotometer (Japan). *X*-ray photoelectron spectra (XPS) were obtained with a PHI-1600 ESCA X-ray photoelectron energy dispersive spectroscopy (USA). IR spectra were recorded in the form of clean KBr pellets using a Vector-33 FTIR spectrophotometer (Germany). Elemental analyses were obtained using a CHN-O-RAPID elemental analyzer (Germany).

Preparation of monodisperse $P_{GMA\text{-}EDMA}$ beads

The monodisperse porous $P_{GMA\text{-}EDMA}$ beads were synthesized by a single-step swelling and polymerization method using polystyrene beads as seed beads. Dispersed polystyrene beads (0.38 g) were prepared according to the method reported by Pain et al.[27] and 10 mL 1.0% aqueous PVA (w/w) solution were placed in a 250-mL flask, and the mixture was stirred slowly by a mechanical stirrer. Then

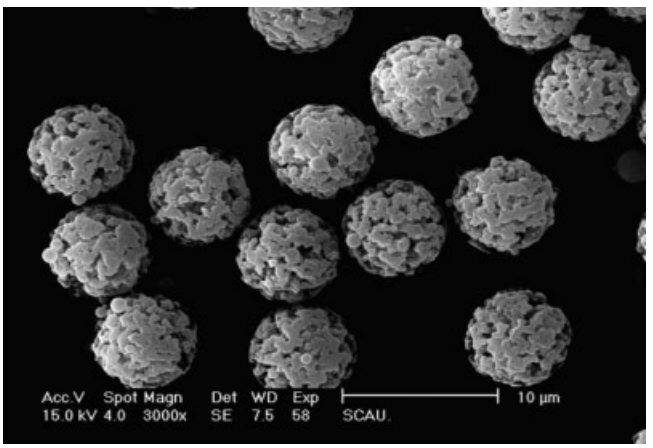

Figure 2 Scanning electron micrograph of the $P_{GMA\text{-}EDMA}$ beads (×3000).

8.6 g of a mixture consisting of GMA and EDMA with different ratios, 9.0 g chloroform, 2.9 g dibutyl phthalate, and 0.3 g AIBN were added into 75 mL aqueous solution of 0.1% (w/w) SDS and 1.0% (w/w) PVA, and then emulsified under ultrasonic conditions until the size of oil drops reached, at most 0.5 μm (observed by optical microscope). The emulsion was then added into the dispersion solution of the polystyrene beads. The mixture was stirred for 10 h at room temperature to make all the emulsified organic phase be sorbed by the polystyrene beads. Then, the mixture was degassed by purging with nitrogen for 20 min, and the polymerization was carried out at 70°C for 24 h with continuously stirring. The resulting beads were washed repeatedly with water and methanol, and then extracted with tetrahydrofuran in a Soxhlet apparatus for removing polystyrene. Figure 2 shows the scanning electron micrograph of the $P_{GMA\text{-}EDMA}$ beads.

Preparation of MIPs films on $P_{GMA\text{-}EDMA}$ beads

Immobilization of the initiator ACPA on $P_{GMA\text{-}EDMA}$ beads was carried out as follows: into a 50 mL three-necked flask, 2.0 g $P_{GMA\text{-}EDMA}$ beads, 35 mL DMSO, 0.5 g ACPA, and 8 mL pyridine were added. The reaction mixture was stirred at 50°C. After reaction, the obtained beads were washed with methanol and dried under vacuum at room temperature.

ACPA-modified $P_{GMA\text{-}EDMA}$ beads (0.5 g) were added into a solution of *S*-naproxen (0.23 g), 2-VPY (0.42 g), and EDMA (3.96 g) dissolved in 10 g chloroform. Before grafting, the suspension was saturated with nitrogen for 20 min. The grafting was carried out at 65°C for 10 h with continuous stirring. After grafting, the beads were extracted with methanol for 24 h in a Soxhlet apparatus and dried in air to obtain thin MIPs films on $P_{GMA\text{-}EDMA}$ beads ($P_{GMA\text{-}EDMA}$-MIPs beads).

Binding experiments of $P_{GMA\text{-}EDMA}$-MIPs beads

In a 25 mL conical flask, 30.0 mg of $P_{GMA\text{-}EDMA}$-MIPs beads were mixed with 5.0 mL acetonitrile solution containing 0.01 mmol *S*-naproxen or 0.01 mmol *R*-naproxen. The conical flask was oscillated at room temperature for 12 h. Then the mixture was transferred into a centrifuge tube and centrifuged for 5 min, and the concentration of free *S*-naproxen or *R*-naproxen in the solution was determined by using UV–Vis spectrophotometer at λ_{max}. The adsorption amount of the $P_{GMA\text{-}EDMA}$-MIPs beads, Q, was calculated by subtracting free substrate concentration from initiative substrate concentration. The static distribution coefficient K_D and the separation factor α are defined as:

$$K_D = C_P/C_S$$

where C_P is the number of molecules bound per gram of $P_{GMA\text{-}EDMA}$-MIPs beads (μmol g^{-1}) and C_S is the concentration of substrate in solution (mmol L^{-1}) and

$$\alpha = K_{Di}/K_{Dj}$$

where K_{Di} and K_{Dj} are the K_D values of *S*-naproxen and *R*-naproxen, respectively.

RESULTS AND DISCUSSION

Immobilization of ACPA on $P_{GMA\text{-}EDMA}$ beads

Immobilization of ACPA can be confirmed by the contrast of infrared spectra of the $P_{GMA\text{-}EDMA}$ beads and the ACPA-modified $P_{GMA\text{-}EDMA}$ beads (see Fig. 3). In the infrared spectrum of the ACPA-modified $P_{GMA\text{-}EDMA}$ beads, a new absorption observed at 1586 cm^{-1}, can be assigned to N=N bond of azo groups. On the other hand, before the modification, the relative values of the intensities of the absorption peaks at 909 cm^{-1} and 850 cm^{-1}, compared with the intensity of the absorption peak of the carbonyl groups at 1732 cm^{-1}, are of about 30.03% and 28.37%, respectively. Whereas, after the modification, the relative values, respectively, decrease to 24.26% and 23.77%, indicating that the epoxy group content in the $P_{GMA\text{-}EDMA}$ beads decreases after the ACPA modification. So, it can be concluded that immobilization of ACPA on $P_{GMA\text{-}EDMA}$ beads was achieved by the reaction of epoxy groups with ACPA in DMSO at 50°C using pyridine as catalyst. It is not necessary to take into account the decomposition of ACPA during the reaction because the halflives of ACPA is reported to be about 10,000 min.[28]

The $P_{GMA\text{-}EDMA}$ beads with different amount of epoxy groups were prepared by adjusting the ratio of GMA to EDMA. The effects of both the epoxy

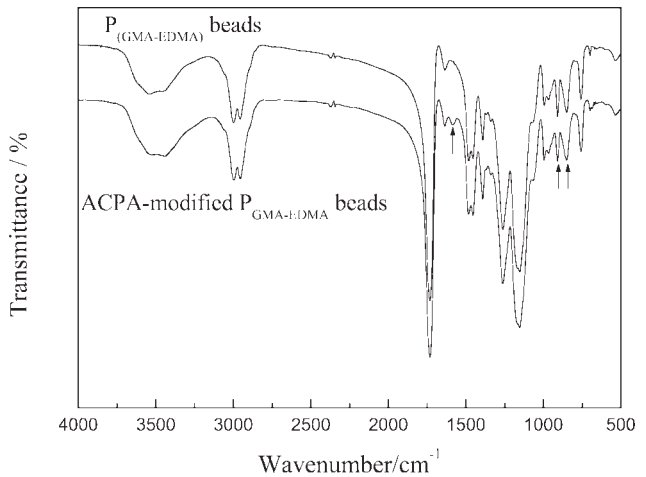

Figure 3 IR spectra of the $P_{GMA\text{-}EDMA}$ beads and the ACPA-modified $P_{GMA\text{-}EDMA}$ beads.

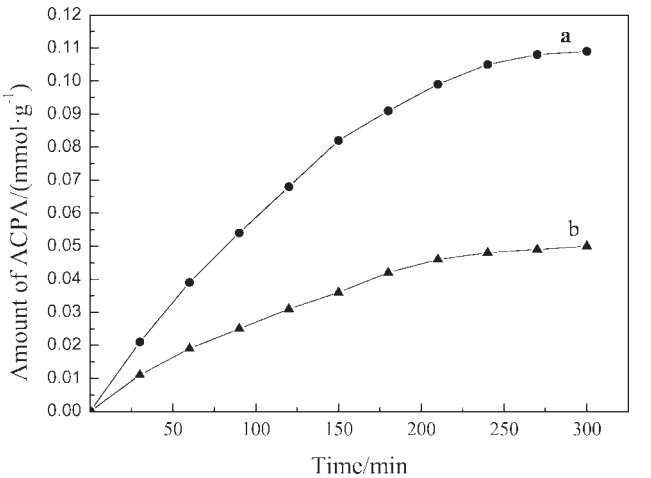

Figure 4 Effect of reaction time on the amounts of ACPA immobilized on the $P_{GMA\text{-}EDMA}$ beads (The epoxy groups amount of $P_{GMA\text{-}EDMA}$ bead: (a) = 1.76 mmol g^{-1}; (b) = 0.74 mmol g^{-1}).

group amount of $P_{GMA\text{-}EDMA}$ beads and reaction time on the immobilization of ACPA were investigated (Table I and Fig. 4). Table I shows that the ACPA amount increases with the increase of epoxy group amount of the $P_{GMA\text{-}EDMA}$ beads. Figure 4 shows that the amount of ACPA increases continuously throughout the initial 4 h reaction time. The above results indicate that the amount of ACPA immobilized on the $P_{GMA\text{-}EDMA}$ beads can be controlled by the reaction time or by adjusting the epoxy group amount of $P_{GMA\text{-}EDMA}$ beads.

Spectroscopic analysis of $P_{GMA\text{-}EDMA}$-MIPs beads

The $P_{GMA\text{-}EDMA}$-MIPs beads were analyzed with infrared (IR) and XPS techniques. The presence of pyridine ring is evident from the characteristic peak at 1571 cm^{-1} and 1592 cm^{-1} in the IR spectrum of $P_{GMA\text{-}EDMA}$-MIPs beads using 2-VPY as functional monomer, as shown in Figure 5.

XPS was used to analyze the chemical nature of the surfaces of $P_{GMA\text{-}EDMA}$-MIPs beads. The advantages of the technique include its element specificity and sensitivity to surface chemical compositions. The observable elemental composition of the $P_{GMA\text{-}EDMA}$ beads comprises only carbon and oxygen. Accordingly, the XPS spectrum of the $P_{GMA\text{-}EDMA}$ beads shown in Figure 6(a) contains only O (1s) and C (1s) peaks at 532 and 285 eV, respectively. The XPS spectrum of the $P_{GMA\text{-}EDMA}$-MIPs beads using 2-VPY as functional monomer [Fig. 6(b)] shows a N (1s) peak at 399 eV, in addition to the O (1s) and C (1s) peaks.

TABLE I
Effects of Epoxy Group Amount on the Amounts of ACPA Immobilized on the $P_{GMA\text{-}EDMA}$ Beads

Sr no	Ratio of GMA to EDMA	Amount of epoxy groups[a] (mmol g^{-1})	Amount of ACPA[b] (mmol g^{-1})
1	1 : 0.7	2.32	0.12
2	1 : 1	1.76	0.11
3	1 : 3	0.74	0.05
4	1 : 6	0.39	0.03

[a] Determined as follows: The $P_{GMA\text{-}EDMA}$ beads were dispersed in 0.1 mol L^{-1} tetraethylammonium bromide in acetic acid solution and titrated with 0.1 mol L^{-1} perchloric acid solution until the crystal violet indicator changed to blue–green.
[b] Determined by elemental analysis of nitrogen.

Effects of ACPA amount and copolymerization time on grafting

The grafting is carried out by copolymerization of functional monomers and crosslinkers via the initiation of ACPA immobilized on $P_{GMA\text{-}EDMA}$ beads, so

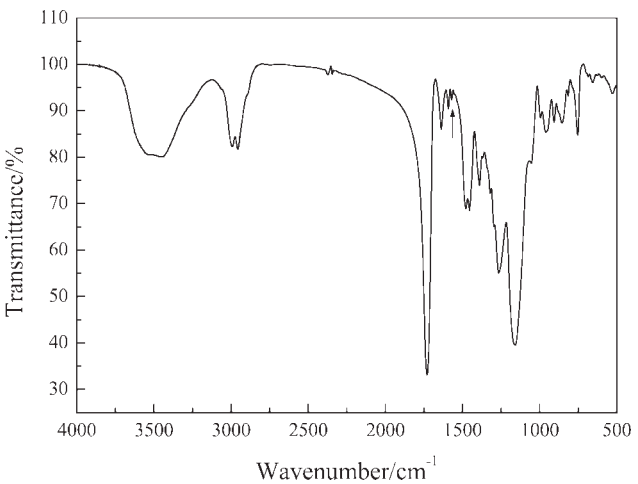

Figure 5 IR spectrum of the $P_{GMA\text{-}EDMA}$-MIPs beads using 2-VPY as functional monomers.

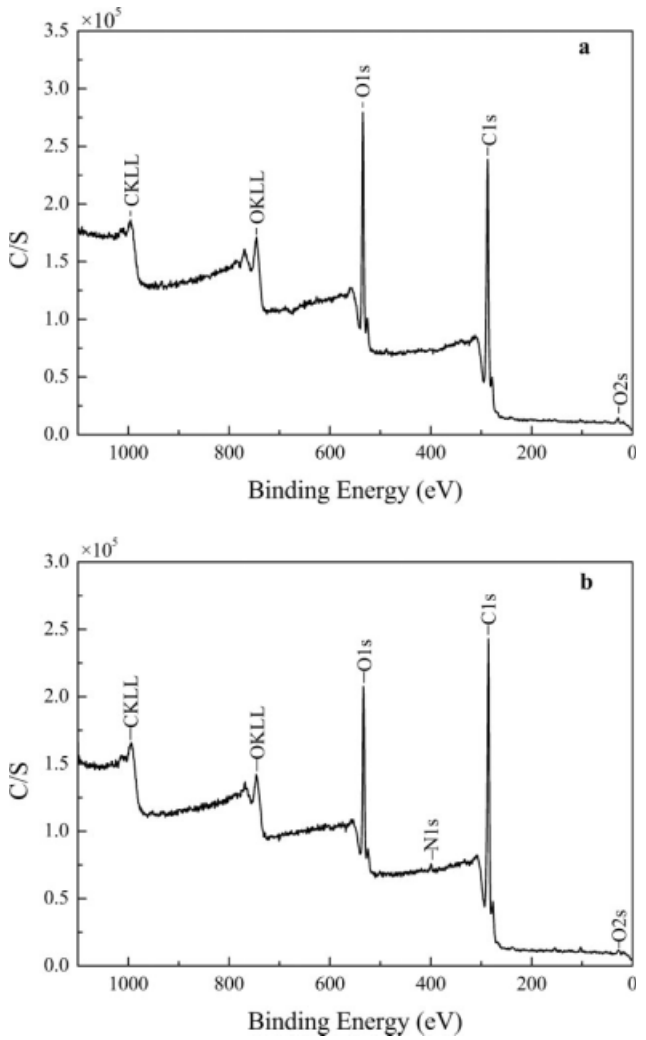

Figure 6 XPS spectrum of the $P_{GMA\text{-}EDMA}$ beads (a) and the $P_{GMA\text{-}EDMA}$-MIPs beads using 2-VPY as functional monomers (b).

ACPA amount can influence grafting. To investigate the influence of ACPA amount on grafting, the $P_{GMA\text{-}EDMA}$ beads with a different ACPA amount, prepared with the same $P_{GMA\text{-}EDMA}$ beads but different reaction time, were used as support beads to graft MIPs. The contents of MIPs grafted were calculated from the differences of weight of the beads before and after grafting:

$$\text{Content of MIPs}\ (\%) = \frac{P_{GMA\text{-}EDMA} - \text{MIPs beads(g)} - P_{GMA\text{-}EDMA}\text{beads (g)}}{P_{GMA\text{-}EDMA}\text{beads (g)}} \times 100$$

With the increase of ACPA amount, more functional monomers and crosslinkers are initiated and copolymerized on the surface of $P_{GMA\text{-}EDMA}$ beads, which leads to the increase of MIPs content (Fig. 7). With the increase of ACPA amount, the grafting rate

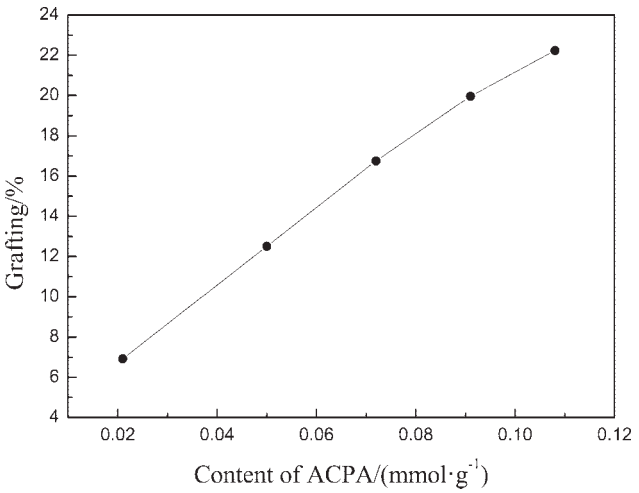

Figure 7 Effect of ACPA amount on the contents of MIPs (reaction time = 10 h).

becomes more rapid, and the grafting time needed for a fixed MIPs content becomes shorter (Fig. 8). But the ACPA amount should not be too high, otherwise the grafting rate will be too rapid, resulting in the difficulty to control the MIPs content. Moreover, high ACPA amount can make the beads attach to each other. It also can be seen from Figure 8 that, the grafting time extends, the MIPs content increases, but the changing extent becomes unconspicuous. The above results indicate that the MIPs content can be fine tuned by changing the ACPA amount or by controlling grafting time.

Effects of MIPs content on morphologies and pore properties of the $P_{GMA\text{-}EDMA}$-MIPs beads

Scanning electron micrographs shown in Figure 9 reveal significant changes in the surface morphology of $P_{GMA\text{-}EDMA}$-MIPs beads containing different MIPs

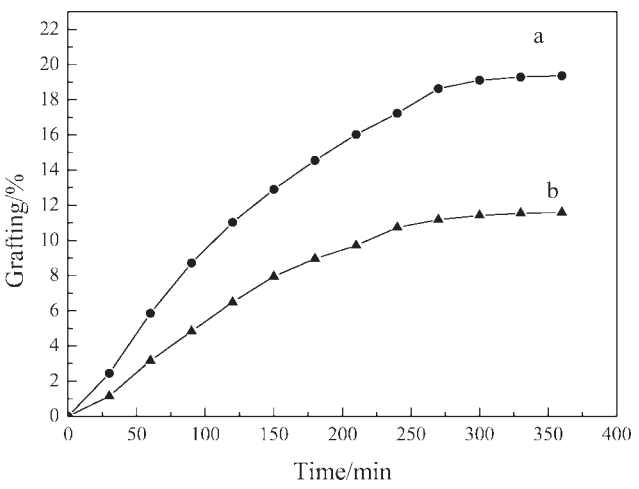

Figure 8 Effect of copolymerization time on the contents of MIPs (The amounts of ACPA immobilized on $P_{GMA\text{-}EDMA}$ beads: (a) = 0.091 mmol g^{-1}; (b) = 0.050 mmol g^{-1}).

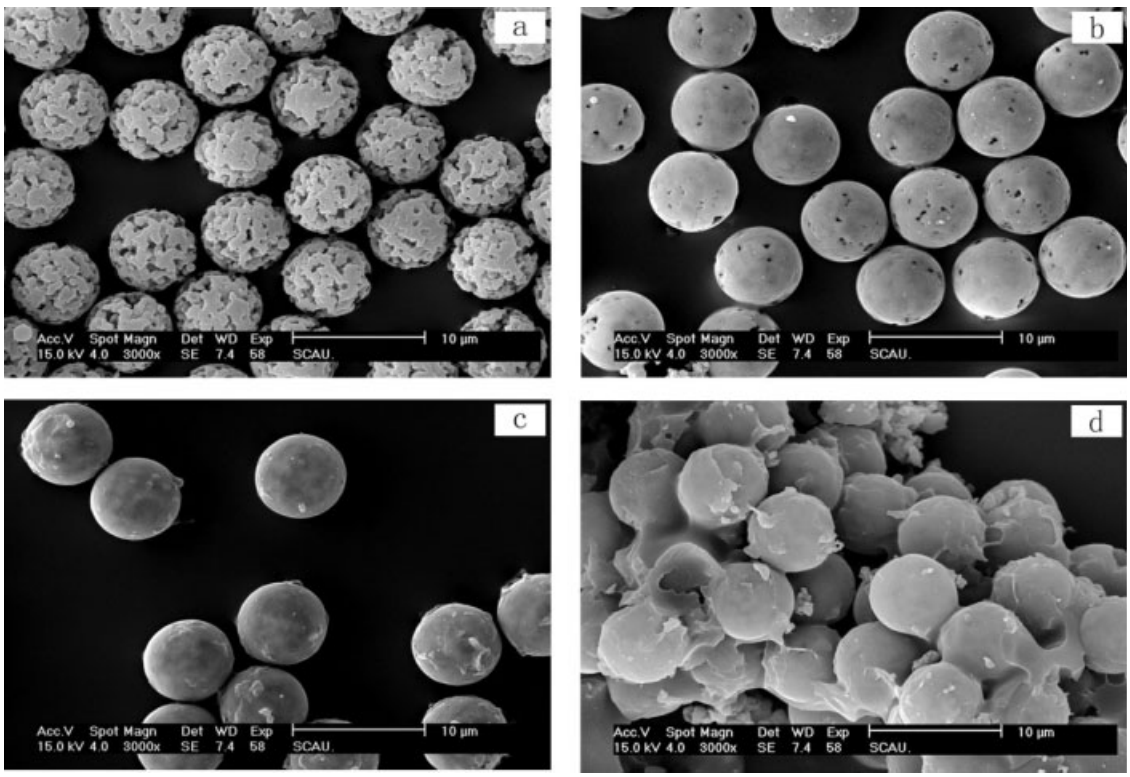

Figure 9 Scanning electron micrographs of the $P_{GMA\text{-}EDMA}$-MIPs beads with different MIPs content (×3000): (a) 12.51%; (b) 19.96%; (c) 22.24%; and (d) 26.03%.

content. When the content of MIPs is lower than 12.51, the grafted beads have porous structure, and its surface morphology is the same as that of $P_{GMA\text{-}EDMA}$ beads (Fig. 2). This indicates that MIPs are evenly grafted on the $P_{GMA\text{-}EDMA}$ beads. When the MIPs content increases to 19.96%, it is clearly seen that the beads' surface is covered by MIPs, and the number of pores on $P_{GMA\text{-}EDMA}$-MIPs beads decreases obviously. When the MIPs content increases to 22.24%, porous structure disappears. At higher grafting levels, the beads attach to each other.

The pore properties of both the $P_{GMA\text{-}EDMA}$ beads and the $P_{GMA\text{-}EDMA}$-MIPs beads with different MIPs content were characterized by BET nitrogen adsorption analysis, and the results are listed in Table II. It can be seen that grafting MIPs onto $P_{GMA\text{-}EDMA}$ beads makes the mean pore size and specific surface areas decrease, which could be due to the fact that the fixation of MIPs films in their original pores by grafting. At lower MIPs content (<16.75%), the changing extent of specific surface areas is smaller. But when the MIPs content is up to 19.96%, the grafted MIPs may partly block the original pores, and make the specific surface areas of the beads decrease obviously.

Effects of MIPs content on adsorption and recognition capabilities of the $P_{GMA\text{-}EDMA}$-MIPs beads

In this work, *R*-naproxen, the enantiomer of template molecule, was chosen as competitor to evaluate the binding specificity of the $P_{GMA\text{-}EDMA}$-MIPs beads. The amount of *S*-naproxen and *R*-naproxen bound to the $P_{GMA\text{-}EDMA}$-MIPs beads with different MIPs content was determined by equilibrium binding experiments, and this data are plotted in Figure 10. The recognition capability of the $P_{GMA\text{-}EDMA}$-MIPs beads to template molecule was estimated by separation factor (Fig. 11). It can be seen that the

TABLE II
Pore Properties of the $P_{GMA\text{-}EDMA}$ Beads and the $P_{GMA\text{-}EDMA}$-MIPs Beads

		$P_{GMA\text{-}EDMA}$-MIPs beads containing different MIPs content			
	$P_{GMA\text{-}EDMA}$ beads	6.92%	12.51%	16.75%	19.96%
Mean pore size (nm)	8.09	6.77	5.83	5.11	4.75
BET surface area ($m^2\ g^{-1}$)	120.99	111.14	99.75	85.08	51.53

$P_{GMA\text{-}EDMA}$-MIPs beads exhibit strong adsorption and recognition capabilities to *S*-naproxen. The separation of MIPs to the template molecules may be realized mainly through the adsorption and recognition of their accessible binding sites to the template molecules. The adsorption and recognition capacities of MIPs enhance with the increase of the number of accessible binding sites.[29–31] At lower grafting levels, with the increase of MIPs content, the number of accessible binding sites increases, thus the adsorption and recognition capabilities enhance. The highest adsorption and recognition capabilities are obtained when MIPs content increases to 16.75%, and the adsorption amount and the separation factor is up to 72.03 μmol g^{-1} and 2.39, respectively, at a substrate concentration of 2.0 mmol L^{-1}. However, with the further increase of the MIPs content, the specific surface areas of the $P_{GMA\text{-}EDMA}$-MIPs beads decreases obviously, thus the number of accessible binding sites decreases accordingly, which leads to the decrease of the adsorption and recognition capacities.[29]

CONCLUSIONS

A new and facile technique for preparing homogeneous and highly selective MIPs films on the porous monodisperse $P_{GMA\text{-}EDMA}$ beads' surface has been described. By the approach, it can be easily realized to produce polymer films with different MIPs content, which may satisfy various requirements for practical applications. The approach also has the advantage to prepare MIPs films using a variety of solvents, functional monomers, crosslinkers and template molecules for a wide variety of applications. In our laboratory, we have prepared different MIPs films by the approach using methacrylic acid, acrylamide or 2-VPY as functional monomers, EDMA or trimethylolpropane trimethacrylate as crosslinkers. Because the morphology of the resulting beads is mainly determined by the $P_{GMA\text{-}EDMA}$ beads, and the $P_{GMA\text{-}EDMA}$ beads' pore sizes and particles sizes can be easily controlled by changing synthesis conditions,[26,29] which gives access to monodisperse, spherical imprinted particles with different pore sizes and particles sizes. Moreover, MIPs films prepared by surface grafting using $P_{GMA\text{-}EDMA}$ beads as supports can be used in a wide pH range from 1 to 14.

The strong adsorption and recognition capabilities of the MIPs films grafted on the porous monodisperse $P_{GMA\text{-}EDMA}$ beads' surface to template molecules together with their simple and inexpensive preparation provides a good basis for practical application of MIPs.

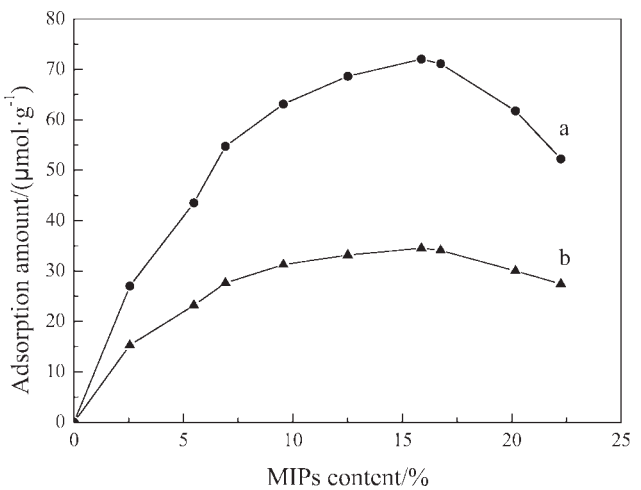

Figure 10 Adsorption amount of the $P_{GMA\text{-}EDMA}$-MIPs beads for different substrate: (a) *S*-naproxen; (b) *R*-naproxen.

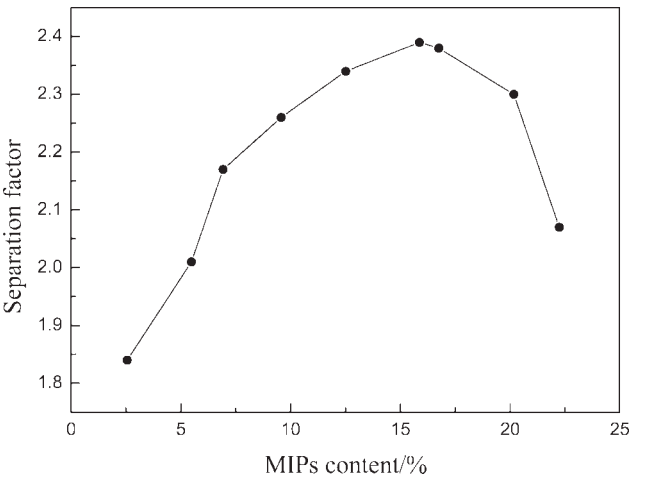

Figure 11 Effect of MIPs content on recognition capability.

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