Synthesis and Characterization of Functional Methacrylate Copolymers and Their Application in Molecular Imprinting

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ABSTRACT: Novel molecularly imprinted polymer systems have been designed that incorporate in a preformed polymer both specific recognition functions and cross-linkable groups. Copolymers of 2-methacryloylethyl methacrylate and methacrylic acid P(MAEMA-*co*-MAA) with different compositions have been synthesized and characterized. The living free radical polymerization of 2-(trimethylsilyloxy)ethyl methacrylate (HEMA-TMS) and *tert*-butyl methacrylate (tBMA) with different monomer feed ratios gives a series of random copolymers. These materials can then be further functionalized by methacryloylation of the pendant hydroxyl group and acid hydrolysis of *tert*-butyl groups. The resultant copolymers were characterized by ^{1}H NMR, FT-IR, DSC, and TGA. These copolymers can then be imprinted with a variety of chemical templates possessing complementary functionalities, forming strong noncovalent and specific binding interactions. These polymer−template matrices can then be readily cross-linked by appropriate posttreatment, such as irradiation or heating in the presence of initiators, either in the bulk or as a film deposited on a suitable substrate, to lock the recognition cavities. The templates can then be removed by thorough washing. Preliminary experiments reported here show that these copolymers are highly selective MIPs toward theophylline in comparison to comparable molecules with similar chemical structures such as caffeine and theobromine.

Introduction

Molecularly imprinted polymers (MIPs) are receiving increasing attention because of their potential applications in a variety of areas, such as separation, catalysis, chemical sensors, biology, and drug discovery.[1−5] The preparation of MIPs is usually divided into two broad categories, covalent and noncovalent, based on the template loading methodology. In both approaches, the MIPs are traditionally prepared by mixing together in a solvent the functional monomers, cross-linker, template, and initiator. After polymerization and cross-linking, usually initiated by heat or UV light, the resulting monolith is ground into a fine powder prior to the template being removed by cleavage or extraction, leaving the locked in structure vacated by the template. The main applications of MIPs are in chromatography and solid phase extraction (SPE). Unfortunately, this approach is not applicable in designing MIPs as chemical or biosensors where the formation of a membrane or film is preferred.[6,7]

Recently a new methodology to prepare MIPs has emerged, in which a polymer is synthesized first and then the MIP is fabricated from this polymer using a variety of methodologies.[8−12] This approach offers many advantages in applications such as sensors where easy processability is desired.[9] For example, Lim et al.[12] have reported the synthesis of an aromatic polyimide with an attached template molecule. After thermal cleavage, the polyimide film showed selective rebinding of the template without the need for additional cross-linking. Kanekiyo et al.[10,11] recently prepared an MIP from amylose functionalized with cross-linkable groups, which displayed selective rebinding capabilities toward bisphenol A as a function of pH. MIP films have also been prepared using a phase inversion method by Kobayashi et al.[8] However, it is generally believed that attaining highly selective MIPs requires the use of highly cross-linked materials in order to ensure the formation of rigid recognition cavities within the matrix.

We herein report the synthesis and characterization of a range of novel cross-linkable polymeric materials with dual characteristics and multiple functionalities, capable of the selective rebinding of selected molecular targets. This has been achieved through the use of multifunctional copolymers that possess both specific recognition groups and triggerable cross-linkable functionalities. This cross-linking activity is activated once the recognition event has taken place, locking in the structure. In this study we report on the results of preliminary investigations using these novel MIPs for the selective rebinding of theophylline as a model template material.

Experimental Section

tert-Butyl methacrylate (tBMA, 98%) and 2-(trimethylsilyloxy)ethyl methacrylate (HEMA-TMS, 96%) were obtained from Aldrich and purified by vacuum distillation before use. Chloroform (stabilized with 0.75% ethanol) was purchased from BDH and used as received. Dichloromathane (DCM, 99.5%, purchased form EMD) and triethylamine (TEA, 99%, purchased from Anachemia) were purified prior to use by distillation. *N,N,N,N,N*-Pentamethyldiethylenetriamine (PMDETA, 99%), methyl 2-bromopropionate (MBrP, 98%), methacrylic anhydride (MAAN, 94%), and trifluoroacetic acid (TFA, 99%) were purchased from Aldrich and used as received. 2,2′-Azobis(2,4-dimethylvaleronitrile) (V-65) was purchased from Wako Chemicals. All other reagents were purchased from Aldrich and used as received.

^{1}H NMR spectra were measured using a 400 MHz Varian Unity Inova spectrometer. FT-IR spectra were recorded on a

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Midac M1200-SP3 spectrophotometer. UV−vis absorption spectra were obtained on a Hewlett-Packard 8453 spectrophotometer. Molecular weights were determined using a gel permeation chromatograph (GPC) on a Waters model 515 HPLC pump equipped with three Waters μ-Styragel columns (porosity is 10^3, 10^4, and 10^5 Å, respectively, and bead sizes are all 15 μm) using THF as eluent at 35 °C with a flow rate of 1.00 mL/min. The system has two detectors: a Waters model 410 differential refractometer and a model 996 photodiode array detector. The columns were calibrated with polystyrene standards. The differential scanning calorimetry (DSC) analysis was performed under a nitrogen atmosphere (50 mL/min) using a TA Instruments DSC 2920 at heating rate of 10 °C/min, calibrated with the melting transition of indium. The onset temperature of the exothermic peak was determined by extrapolation of the resulting DSC curve. The thermal gravimetric analysis (TGA) was performed using a TA Instruments TGA 2950 at heating rate of 20 °C/min under a nitrogen atmosphere (50 mL/min).

Preparation of the Copolymer P(HEMA$_{0.5}$-*co*-tBMA$_{0.5}$) (PH1B1). In a typical synthesis of the copolymer PH1B1, CuBr (41.0 mg, 0.287 mmol) was introduced into a 100 mL round-bottom flask and sealed with rubber septa. The flask was then evacuated and flushed with nitrogen three times. The degassed monomers tBMA (7.45 mL, 45.9 mmol) and HEMA-TMS (10.0 mL, 45.9 mmol) along with DCB (18 mL), PMDETA (60.0 μL, 0.287 mmol), and MBrP (64.0 μL, 0.573 mmol) were then added, and the mixture was stirred at 80 °C for 16 h. Tetrahydrofuran (THF, 40 mL) was then added to dilute the polymer solution, which was then passed through a neutral activated alumina to remove the catalyst. HCl (6 N, 6 mL) was then added to the resultant solution, which was stirred again for another hour before precipitating the polymer in hexane. The polymer was collected by filtration and dried under vacuum, prior to being redissolved in 40 mL of THF and reprecipitated in water (1 L) to give the purified copolymer (yield: 9.8 g, 79%). Other copolymers, with different compositions, were prepared using the same procedure using different monomer feed ratios.

^{1}H NMR (400 MHz, DMSO) δ (ppm): 1.32 (s, −C(CH$_3$)$_3$), 3.87 and 3.55 (s, br, −CH$_2$CH$_2$−), 4.78 (s, br, −OH). FT-IR (diamond plate) ν (cm^{-1}): 842 (−C(CH$_3$)$_3$), 1718 (C=O), 3430 (−OH). GPC (THF, 35 °C): $M_w = 2.93 \times 10^4$ g/mol.

Preparation of P(MAEMA$_{0.5}$-*co*-tBMA$_{0.5}$) (PM1B1). The polymer PH1B1 (2.0 g, 7.4 mmol in terms of the −OH group) was dissolved in a mixture of DCM (14 mL) and TEA (2.6 mL, 18.7 mmol). MAAN (2.8 mL, 18.5 mmol) was then added dropwise with stirring. After stirring at room temperature for 4 h, the solution was concentrated by a rotary evaporator and diluted with THF (20 mL). The solution was then precipitated in hexane to yield the polymer. The resultant viscous solid was purified by redissolving in THF and precipitation again into hexane to give a white powder (yield: 2.2 g). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 1.32 (s, −C(CH$_3$)$_3$), 1.92 (s, =CCH$_3$), 4.10 and 4.27 (s, br, −CH$_2$CH$_2$−), 5.67 (s, CH$_2$=C), 6.08 (s, CH$_2$=C). FT-IR (diamond plate) ν (cm^{-1}): 842 (−C(CH$_3$)$_3$), 1641 (C=C), 1733 (C=O).

Preparation of Polymer P(MAEMA$_{0.5}$-*co*-MAA$_{0.5}$) (PM1M1). The polymer PM1B1 (2.2 g, 6.4 mmol in terms of *tert*-butyl group) in DCM (20 mL) was added to TFA (5 mL, 65 mmol), and the resulting solution stirred at room temperature for 4 h prior to being concentrated in a rotary evaporator. The polymer was then precipitated by pouring into a mixture of hexane and diethyl ether (200 mL, 50/50 v/v). The resulting white powder was filtered and dried under vacuum (yield: 1.7 g, 93%).

^{1}H NMR (400 MHz, DMSO) δ (ppm): 1.92 (s, =CCH$_3$), 4.10 and 4.31 (s, br, −CH$_2$CH$_2$−), 5.67 (s, CH$_2$=C), 6.04 (s, CH$_2$=C), 12.41 (s, br, −COOH). FT-IR (diamond plate) ν (cm^{-1}): 1638 (C=C), 1733 (C=O), 3300 (−COOH).

General Preparation Procedures of the Molecularly Imprinted Polymers (MIPs). A solution of the copolymer P(MAEMA$_{0.7}$-*co*-MAA$_{0.3}$) (PM7M3, 0.700 g, 1.28 mmol for −COOH) in 10 mL of chloroform was mixed with theophylline (114 mg, 0.63 mmol) and stirred at 55 °C for 0.5 h until the theophylline was completely dissolved. The solution was then concentrated to 6 mL and purged with nitrogen for 10 min prior to the addition of the initiator V-65 (14 mg, 0.056 mmol). The solution was then heated at 50 °C overnight to cross-link the copolymer. The resulting solid was finely ground with a mortar and pestle and thoroughly washed with methanol by stirring at RT for at least 1 h. This washing procedure was repeated five times with fresh solvent to ensure the complete removal of the theophylline template. The powder was then reground and dried in a vacuum (yield: 0.66 g, 94%). For the MIP prepared by P(MAEMA$_{0.9}$-*co*-MAA$_{0.1}$) (PM9M1), polymer (700 mg, 0.37 mmol for −COOH) and theophylline (34 mg, 0.19 mmol) were dissolved in chloroform (10 mL), and other procedure was the same as above. For control purposes, a nonimprinted polymer (NIP) was prepared by using the same procedure as above, except for the absence of the theophylline template. FT-IR (diamond plate) ν (cm^{-1}): 1733 (C=O), 3300 (−COOH).

The rebinding potential of theophylline and other similar molecules was then investigated using heterogeneous batch experiments with theophylline (or alternative) in chloroform at concentrations of 9.0×10^{-5} M. UV−vis spectrum measurements were used to measure the concentration of theophylline (or its analogues) in these solutions before and after the rebinding experiments. A standard calibration equation was established by measuring the intensity of the absorbance peak at 276 nm for different concentrations between 1×10^{-5} and 10×10^{-5} M. In a typical experiment, 5 mg of the MIP sample was mixed with a 10 g of solution of theophylline in chloroform. Following a suitable absorption time (3 h was found suitable for equilibrium measurements), the solution was filtered and the UV−vis spectrum measured. The matrix rebinding capability of the MIP was calculated by comparing the theophylline concentration in solution before and after absorption. Rebinding experiments using different theophylline concentrations in chloroform were studied in the same manner.

Results and Discussion

Preparation of the Copolymers of HEMA-TMS and tBMA. The procedure used for the copolymerization experiments are outlined in the upper part of Scheme 1. The copolymers with different compositions were prepared using an atom transfer radical polymerization using the following molar ratios of HEMA-TMS/tBMA: 30/70, 50/50, 70/30, and 90/10. The atom transfer radical polymerization of these individual monomers as well as those of the silyl protected HEMA and *tert*-butyl methacrylate (tBMA) have been reported previously.[13,14] In this work, we used CuBr/PMDETA as the catalyst system and methyl-2-bromopropionate (MBrP) as the initiator and carried out the copolymerization at 80 °C for 16 h. This reaction produced copolymers with controlled molecular mass and narrow polydispersity. In comparing the bulk polymerization with the solution polymerizations (batches 2a and 2b in Table 1), the copolymers prepared in dichlorobenzene had a narrower polydispersity, probably due to the lower polymerization rate in solution, as reported previously.[13] One of the major concerns regarding the copolymerization of monomers such as these by atom transfer radical polymerization (ATRP) is the structure of the resultant polymers (i.e., will the copolymers be block, graft, or random).[14] The structure can have an important impact on determining the suitability of these copolymers for use in molecular imprinting. The structures of the copolymers were therefore investigated by withdrawing aliquots during the polymerization process of the copolymer PH1B1. These materials were characterized by ^{1}H NMR spectroscopy as a function of time. Comparing the peak intensities of the *tert*-butyl proton in tBMA (1.29−1.48 ppm) and the ethylene proton in HEMA

Scheme 1. Preparation of Poly(2-hydroxylethyl methacrylate-*co*-*tert*-butyl methacrylate) and Postfunctionalization of the Copolymers

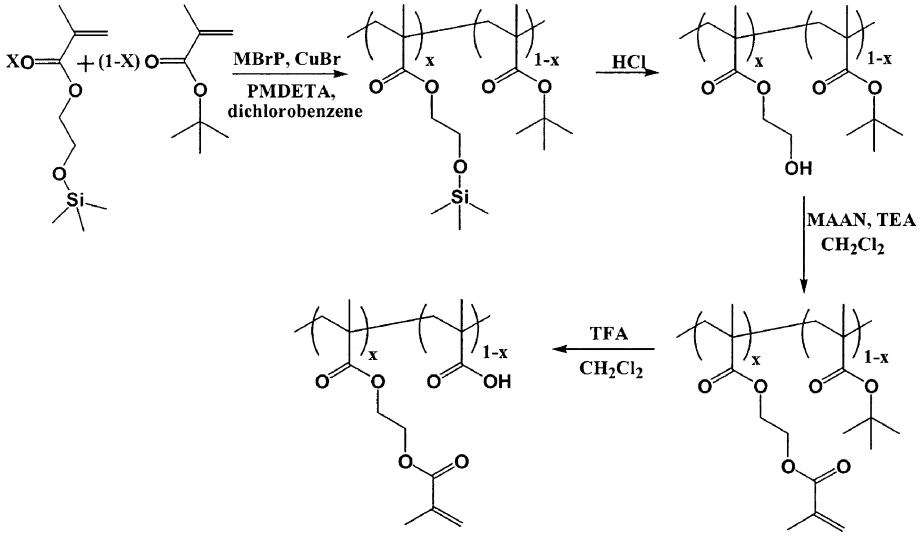


Table 1. Polymerization Conditions and the Structures of the Resultant Polymers

monomer ratio[a] (HEMA-TMS/tBMA)	batch	solvent[b]	HEMA[c] content (mol %)	M_w ($\times 10^{-4}$ g/mol)	M_n ($\times 10^{-4}$ g/mol)	M_w/M_n[d]
90:10	1	DCB	89.2			
70:30	2a	DCB	68.9	2.82	2.08	1.36
70:30	2b	bulk	69.2	2.83	1.82	1.55
50:50	3	DCB	48.7			
30:70	4	DCB	28.5			

[a] Molar ratio of monomers: MBrP:CuBr:PMDETA = 160:1:0.5:0.5. [b] Solvent to monomer: 1:1 volume ratio. [c] Determined by ^{1}H NMR spectra. [d] Measured by GPC in poly(2-trimethylsilyloxyethyl methacrylate-*co*-*tert*-butyl methacrylate) form with THF used as eluent.

(3.65−4.31 ppm) demonstrated that the tBMA content was usually between 0.48 and 0.51 when the conversion was between 30 and 80%. This indicates that the copolymers are random in nature and that the functional groups required for the recognition of the molecular templates and cross-linking of the polymer are evenly distributed along the polymer backbone.

High-T_g polymers are usually preferred for MIPs since they have higher polymer chain rigidity at ambient temperature, thereby offering higher selectivity and better affinity of the targeted molecules.[1−3] Consequently, *tert*-butyl methacrylate (tBMA) was selected as the functional monomer instead of the *tert*-butyl acrylate (tBA) since the methacrylic polymers have higher glass transition temperatures (T_g). For the other monomer, the silyl-protected HEMA instead of HEMA itself was used because this monomer possesses a similar reactivity in free radical polymerization as that of tBMA, thereby favoring the formation of a random copolymer structure.[13] Once the polymerization was complete, the silyl protection group was cleaved with dilute HCl in a THF solution. The polymerization conditions and the chemical structures of the copolymers produced in this study are summarized in Table 1. The structural compositions of the copolymers produced (as determined by ^{1}H NMR spectroscopy) closely match those expected on the basis of the feed ratio of the monomers used.

Postfunctionalization. The experimental procedure for the postfunctionalization of the copolymers is outlined in the lower part of Scheme 1. The first step was the methacryloylation of the pendant hydroxyl groups on the polymer chain. This reaction has been reported extensively as a way to prepare cross-linkable polymers.[15,16] Usually, the reaction is carried out in DMF using methacryloyl chloride or MAAN at elevated temperatures. However, using these conditions, the degree of methacryloylation is usually low and cross-linking side reaction is possible. Recently, Jae-Sun Koo et al.[17,18] reported a procedure for carrying out the reaction in dichloromethane using MAAN at room temperature, which gave high yields. Using this approach, the conversions approached 100% when the reaction was carried out in a mixture of purified DCM and TEA. The ^{1}H NMR spectrum of the precursor copolymer, P(HEMA-*co*-tBMA), is shown in Figure 1a while that of the methacrylated copolymer, P(MAEMA-*co*-tBMA), is shown in Figure 1b. From this figure it can be clearly seen that the proton peak at 4.78 ppm associated with the −OH in HEMA disappears completely while the methylene

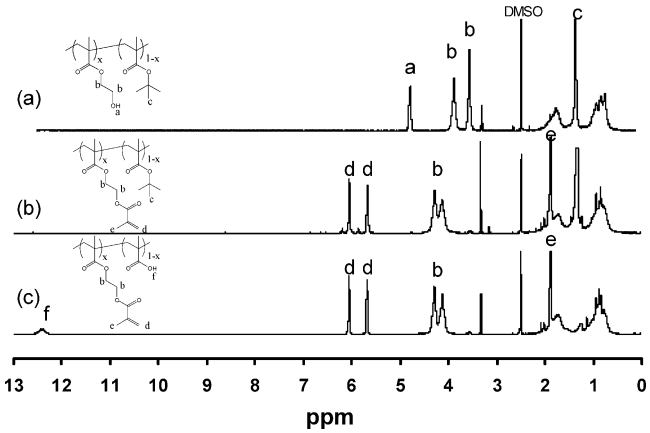


Figure 1. ^{1}H NMR spectra of the copolymers (a) poly(2-hydroxylethyl methacrylate$_{0.7}$-*co*-*tert*-butyl methacrylate$_{0.3}$), PH7B3; (b) poly(2-methacryloylethyl methacrylate$_{0.7}$-*co*-*tert*-butyl methacrylate$_{0.3}$), PM7B3; and (c) poly(2-methacryloylethyl methacrylate$_{0.7}$-*co*-methacrylic acid$_{0.3}$), PM7M3.

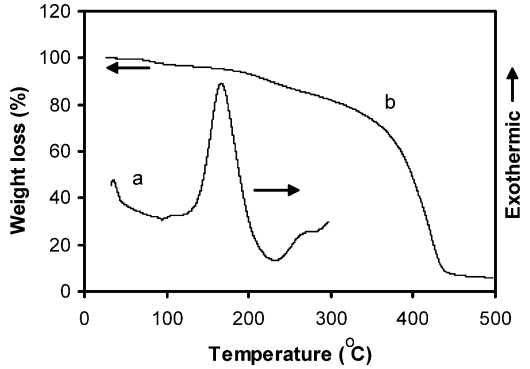


Figure 2. DSC (a) and TGA (b) thermograms of the copolymer sample poly(2-methacryloylethyl methacrylate$_{0.7}$-*co*-methacrylic acid$_{0.3}$), PM7M3.

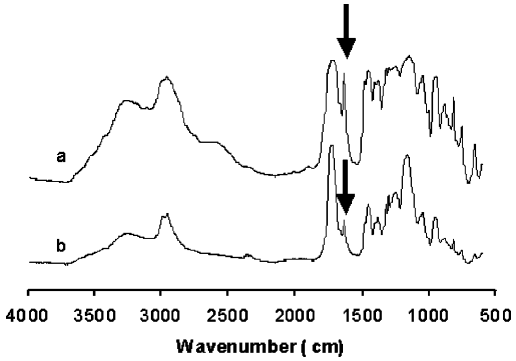


Figure 3. FT-IR spectra of powdered samples of the copolymer poly(2-methacryloylethyl methacrylate$_{0.7}$-*co*-methacrylic acid$_{0.3}$), PM7M3, before (a) and after (b) thermal cross-linking at 140 °C for 2 h.

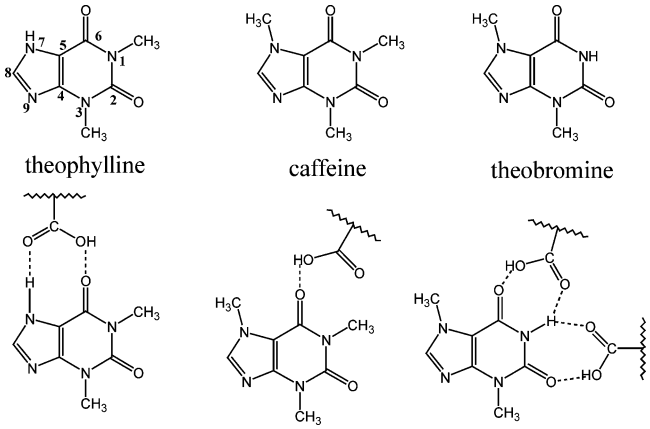
proton peaks shift from 3.87 and 3.55 ppm to 4.27 and 4.10 ppm. The full assignment of all new peaks is reported in Figure 1. This reaction was followed by the hydrolysis of the *tert*-butyl groups of the tBMA units, which was carried out over 4 h at room temperature in a mixture of DCM and TFA (25% v/v). The complete hydrolysis of the *tert*-butyl groups was confirmed by the disappearance of the peak at 1.32 ppm, associated with the nine protons on the *tert*-butyl group (Figure 1c). From Figure 1c it can be seen that the peaks associated with the vinyl groups at 6.04 and 5.67 ppm remain unchanged, indicating that the methacrylate double bonds in the MAEMA units were unaffected by this reaction. The fact that the copolymers PMAEMA-*co*-PMAA are soluble in organic solvents such as DMF and THF also indicates that no cross-linking occurred during these reactions.

Properties of the Copolymers. The stability of the copolymers was investigated by their thermal behavior in DSC and TGA analysis. Figure 2a shows the DSC curve of the copolymer PM7M3. The large exothermic peak starting around 140 °C appears to be attributable to the irreversible thermally induced cross-linking of the pendant methacrylate double bonds. The TGA curve in Figure 2b shows a slight weight loss beginning at around 100 °C, which should be dehydration of the absorbed water. The copolymer starts to decompose at around 200 °C.[17]

All of the polymers were readily cross-linked on heating with or without an initiator and could be photo-cross-linked by the use of an appropriate photoinitiator. The cross-linked copolymers were completely insoluble in common organic solvents. However, depending upon the degree of cross-linked functionality within the copolymer, a certain degree of swelling was observed in such solvents such as chloroform or THF. The cross-linking reaction was investigated by FT-IR spectroscopy, and a typical spectrum before and after cross-linking is shown in Figure 3. The disappearance of the peak at 1641 cm^{-1} associated with the carbon double bond can be used to follow the extent of the cross-linking reactions.[17,18] The powdered FT-IR spectrum of the copolymer before heating is shown in Figure 3a while Figure 3b shows the spectra after heating at 140 °C for 2 h. Clearly the peak at 1641 cm^{-1} decreases dramatically, indicating that alkene side chains are being consumed as a result of the heat-induced cross-linking.

Study of the Complex Formation before Cross-Linking: Solubility and ^{1}H NMR Measurements. The preparation of the MIP requires the formation of a stable complex between the functional groups of the

Scheme 2. Structure of Templates and Their Possible Interactions with the Carboxyl Groups



molecular template of interest and the complementary functional groups of the molecular imprinting copolymer prior to the rigidification reactions (cross-linking). In this study the interaction between the molecular template (theophylline) and −COOH groups in the MIP were investigated by ^{1}H NMR. The solubility of theophylline in chloroform was only about 3.6 mg/mL. The addition of the copolymer (PM7M3) to this chloroform solution, however, resulted in a significant increase in the solubility of theophylline. For example, the addition of 70 mg/mL of this copolymer allowed a solution with a theophylline concentration of 11.4 mg/mL to be obtained. Under these conditions a molar ratio between the theophylline and −COOH groups in the copolymer of about 1:2 was obtained. This observation demonstrates that important interactions are occurring between the template and the functional carboxylic acid groups of the copolymer as outlined in Scheme 2.

The choice of solvent was also important since it not only must dissolve the template as well as the copolymer but also should minimize its interactions with the functional groups involved in the recognition and binding process. Chloroform was the best solvent in this regard since it has a low hydrogen-bonding propensity, thereby only minimally interfering with the formation of the stable complex between the theophylline and the functional groups of the MIP.[1]

The interaction of the template with the functional group in the copolymer was therefore followed using ^{1}H NMR spectroscopy with $CDCl_3$ as the solvent. The chemical shift of the proton bound to oxygen is highly dependent on the solvent, temperature, and concentra-

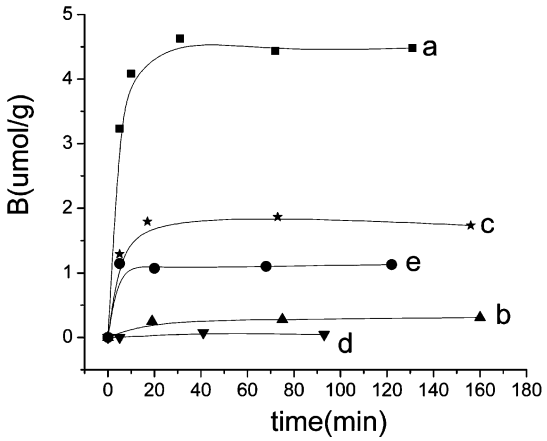


Figure 4. Time profiles of the binding amounts of (a) theophylline to MIP and (b) theophylline to control nonimprinted polymer (NIP) prepared by copolymer poly(2-methacryloylethyl methacrylate$_{0.7}$-*co*-methacrylic acid$_{0.3}$), PM7M3; (c) theophylline to MIP prepared with polymer poly(2-methacryloylethyl methacrylate$_{0.9}$-*co*-methacrylic acid$_{0.1}$), PM9M1; (d) caffeine; and (e) theobromine to MIP prepared by polymer poly(2-methacryloylethyl methacrylate$_{0.7}$-*co*-methacrylic acid$_{0.3}$), PM7M3. All the MIP was prepared using theophylline as template.

tion.[19] Consequently, the theophylline and copolymer solutions are prepared using a fixed copolymer concentration (120 mg/mL). In the case of the pure copolymer solution, the proton resonance of the COOH groups was located at 11.22 ppm. As the concentration of the theophylline in the solution increased to 40 mM (a 1:6 molar ratio to COOH groups), this peak moved to 11.30 ppm and then to 11.33 ppm in 82 mM (1:3 to COOH group) solution. This behavior suggests that the proton of the COOH group was participating in hydrogen bond formation with the template. In the case of the pure theophylline solution in $CDCl_3$, the peak at 12.64 ppm was attributed to the nitrogen bound proton at position 7. As the polymer concentration increases, this peak shifts slightly to a lower field, becomes broader, and finally disappears. This behavior has been attributed to the occurrence of a lower electron density around this proton due to increased hydrogen-bonding interactions with the COOH groups of the copolymer.

Preparation of the MIPs and Their Properties. Theophylline and its analogues (i.e., caffeine and theobromine) were selected as model molecules for this study since they have been widely used as molecular templates in several MIP systems.[20−23] As noted above, although pure theophylline is only slightly soluble in chloroform, its solubility is increased substantially in the presence of functionalized polymers. The molar ratio of the functional carboxyl group to the theophylline in these experiments was set at 2:1, since previous studies had indicated that such a molar ratio seemed to give the best results for the preparation of MIPs.[20,21] Cross-linking was carried out at 50 °C with V-65 as the initiator because of its low decomposition temperature. MIPs using caffeine and theobromine as the molecular template were also prepared using similar conditions. After cross-linking, the copolymer was ground to a fine powder prior to the template being extracted with methanol and acetone.

The rebinding experiments were carried out in chloroform solutions with template concentrations around 9.0×10^{-5} M. Figure 4a shows the rebinding of the theophylline to the MIP prepared by copolymer P(MAEMA$_{0.7}$-*co*-MAA$_{0.3}$) in chloroform as measured by UV−vis spectroscopy. At room temperature, the rebinding reaches a plateau after about 30 min, indicating that the imprinted sites are saturated with the theophylline. Compared with MIP prepared by phase inversion molecular imprinting[8] or by the monomer approach,[20] our MIP shows a faster binding kinetics. The reason is believed to be that our approach used relatively more solvent as porogen so our MIP shows a more porous structure. Comparing this MIP with the nonimprinted polymer (NIP) (Figure 4b), it is clear that a much higher rebinding capacity (∼15 times) is achieved with the MIP compared with the NIP. In the case of the MIP obtained with P(MAEMA$_{0.9}$-*co*-MAA$_{0.1}$) copolymer (Figure 4c) the theophylline rebinding capacity was about 3 times higher than that obtained with the NIP. These data would indicate that the cross-linking reaction, following the selective binding of the theophylline on the copolymers complementary functional groups, has resulted in the formation and preservation of specific recognition cavities within the matrix that can be subsequently reused for the rebinding of the molecular template. This result was further confirmed by analyzing the rebinding curves obtained with the caffeine and theobromine (Figure 4d,e), two chemicals with similar structures to those of theophilline (Scheme 2). For example, in the case of caffeine, the theophilline imprinted MIP showed almost no rebinding capacity. This result can be explained on the basis of the fact that the chemical structure of caffeine does not allow for the formation of strong hydrogen bonding, as depicted in Scheme 2. In this case cyclic double hydrogen bonding can occur when theophylline is used.[8] In the case of theobromine, a similar cyclic hydrogen bonding can occur with the COOH groups. This helps explain the intermediate rebinding behavior of this molecule in comparison to that obtained with theophylline and caffeine. Several MIPs were prepared using these various molecular templates to investigate the effect of the template on the rebinding selectivity and capacity of the resulting MIPs. A MIP using caffeine as the template showed absolutely no selectivity toward caffeine, theophylline, or theobromine. This lack of recognition behavior is presumably due to the weak hydrogen-bonding interactions that exist between the caffeine template and the COOH groups of the copolymer prior to the cross-linking reactions. Consequently, under these conditions nonselective cavities can be expected, an observation noted by others.[20] Meanwhile, the lack of success in preparing a MIP using theobromine as the molecular template was felt to be due to the limited solubility of the theobromine in chloroform that hindered the formation of the initial binding events prior to cross-linking.

To further investigate the molecular imprinting properties of these MIPs, the binding isotherms for the MIP and the NIP obtained using different theophylline concentrations were determined (Figure 5a). The rebinding capacity of theophylline to MIP can go up to 180 μmol/g compared with an expected capacity of 900 μmol/g at theophylline concentration of 0.02 M in chloroform solution. The MIP showed a higher rebinding capacity toward theophylline than the NIP, with the trend being more pronounced at lower theophylline concentrations. To better understand the data, the results were replotted using log F to log B scales and fitting the results to the Freundlich isotherm, $B(F) = aF^m$. In this equation B and F are the concentrations of

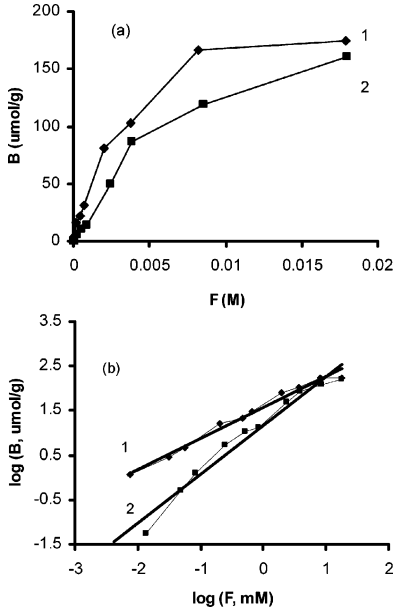


Figure 5. (a) Binding isotherms of theophylline and (b) with Freundlich fit to molecular imprinted polymers (1) and control nonimprinted polymer (2) prepared by poly(2-methacryloylethyl methacrylate$_{0.7}$-*co*-methacrylic acid$_{0.3}$), PM7M3, using theophylline as template.

the bound and free theophylline, while a and m are fitting parameters.[24,25] In the case of the MIP, the heterogeneity index m was 0.69 while that for the NIP was 1. This result is consistent with data reported by Shimizu,[24,25] who observed that MIPs contain a wide range of binding sites, each with different affinities. Meanwhile, the binding sites in the case of the NIP have more homogeneous binding abilities although with low affinities. From the data presented in Figure 5b, the median binding affinity K_0 ($K_0 = a^{1/m}$) for the MIP was 1.93 (μmol g^{-1} mM^{-1}), which was higher than that for the NIP (1.15). At the lower concentrations (<0.1 mM), the difference between the binding ability of the MIP and NIP is due to increased role of the high affinity sites, while at the higher concentrations (>10 mM), the two curves converge because of the predominant contribution of the low affinity binding sites. Compared with the MIPs prepared using the more traditional monomer approach,[20−23] the MIPs prepared from the copolymer reported in this study show reduced binding capacities. A possible explanation for this discrepancy may be due to the fact that the formation of a stable template−functional group complex is much easier with mobile monomers than with polymers in which the polymer chain backbone restricts freedom of motion.

However, considering the greater processability of polymeric materials in microfabrication processes used by the industry, the polymer approach has advantages. This is especially the case for the preparation of MIPs for applications such as chemical and biosensors, where the MIP is required as a membrane or film. In addition, these copolymer materials can also be used as novel cross-linkers in the preparation of other MIPs.[26,27]

Conclusions

A novel polymer approach for the preparation of MIP has been demonstrated. Random copolymers of PtBMA and PHEMA-TMS have been prepared using ATRP. The resulting polymers were subsequently functionalized using methacryloylation, followed by acid-catalyzed hydrolysis to produce polymers with a methacrylate double bond and a carboxyl group. These functionalities in the copolymers facilitated cross-linking and hydrogen-bonding abilities, respectively. The MIPs obtained showed greater selectivity for the molecular recognition template, theophylline, than toward other molecular analogues. Analysis of the binding abilities of the MIP using the Freundlich equation confirmed the molecular imprinting properties of these materials. While the rebinding capacity of these copolymers were lower than those achievable by the traditional monomer approach, the film-forming capabilities and stability of these novel copolymers may prove interesting in the formation of surface MIPs for sensing and detection purposes.

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