Synthesis of Monolithic HPLC Stationary Phase with Self-Assembled Molecular Recognition Sites for 4-Aminophenol

Ersin Baykara,[1] Yeliz Tunc,[1] Kezban Ulubayram[1,2]

[1]*Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Hacettepe University, Ankara 06100, Turkey*
[2]*Graduate Department of Nanotechnology and Nanomedicine, Hacettepe University, Ankara 06100, Turkey*

Received 3 May 2010; accepted 28 February 2011
DOI 10.1002/app.34460
Published online 28 July 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: A molecularly imprinted polymer (MIP) monolith for selective recognition of 4-aminophenol (4-AMP) was prepared by *in situ* polymerization technique as high-performance liquid chromatography (HPLC) stationary phase. For this purpose, several 4-AMP imprinted monoliths were synthesized by using only methacrylic acid (MAA), acrylamide (AAM), or isobornyl methacrylate (IBMA) in the presence of high amount of crosslinker, ethylene glycol dimethacrylate (EDMA), and these polymeric monolith columns were connected to HPLC to evaluate their separation capabilities. By selection of appropriate functional monomer and optimization of polymerization conditions, MAA-based monolithic MIP showed good flow through properties, high selectivity to the templated molecule, and high resolution in the separation of paracetamol and its main impurity, 4-AMP. Besides, effective binding site density and dissociation constant of this monolith were estimated by using frontal chromatography and found as 7.95 μmol/g and 1.06 m*M*, respectively. Surface area of the same monolith was found as 23.48 m^2/g from multipoint BET analysis. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 493–501, 2012

Key words: monolith; molecular imprinting; *in situ* polymerization; 4-aminophenol; paracetamol; stationary phase

INTRODUCTION

The most exiting high-performance liquid chromatography (HPLC) column innovations in recent years have been the development of monoliths. Monoliths are columns that have the stationary phase composed of a continuous homogeneous phase instead of packed particles. The skeleton structures of monoliths, having interconnected macropores resulting in high porosity and permeability, let high flow rates applied at very low column back pressures and enhanced mass transfer rate compared with conventional packed columns. In addition, monolithic columns have advantages including easy of preparation, controllable preparation process, and high column efficiency. These unique properties have made monolithic columns very attractive in chromatographic separations. Excellent review on the monolithic columns in HPLC was written by Guiochon.[1] Although monolith concept can be traced back to 1960s,[2,3] the first work on application of porous silica rods for HPLC was published by Minakuchi et al. in 1996.[4] The pioneering works for the preparation of polymeric monoliths were performed by Hjerten et al.[5] and contributed much by the work of Svec and Frechet reported on *in situ* polymerization technique.[6] Combined with molecular imprinting, these studies supported the development of new generation of monoliths, which is called as molecularly imprinted polymers (MIPs) monolith. Recently, so many studies were conducted on the preparation of monolithic MIPs.[7–11]

Molecular imprinting, introduced by Wulff and Sarhan in 1972 which is known as "covalent approach,"[7] is a technique to create specific cavities (binding sites) complementary both functionally and structurally to template molecule. MIPs show specific recognition ability and selectivity to the targeted molecule by the help of their complementary cavities. MIPs are prepared by the polymerization of appropriate functional monomer(s) in the presence of template molecule and high amount of crosslinker. Functional monomer and the template molecule interact and form complex during the crosslinking reaction. Then imprinted template molecules are removed from the polymer matrix leaving recognition sites complementary to the template in terms of size, shape, and arrangement of the functional groups. Because of their specific recognition ability,

Correspondence to: K. Ulubayram (ukezban@hacettepe.edu.tr).

Contract grant sponsor: Hacettepe University, Scientific Research Unit (BAB); contract grant number: 07A301001.

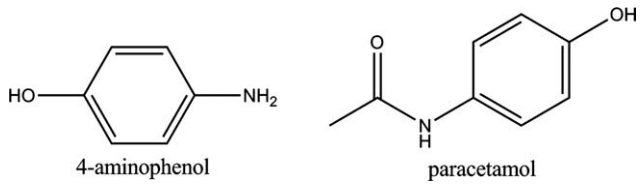

Figure 1 Structures of 4-AMP and paracetamol.

the major application area of MIPs is chromatographic separations, especially for the separation of structurally similar molecules, including challenged separations such as enantiomers.

The most widely used method for the preparation of MIPs is bulk polymerization in which crushing, grounding, and sieving are needed for chromatographic applications resulting in particles with irregular shape and size. Although other polymerization methods were developed, such as suspension[12] and precipitation polymerization,[13] to prepare spherical MIPs, they are still time consuming and low-yield producing methods. To eliminate the time consuming process, Matsui prepared MIP monolith by *in situ* polymerization technique.[10,14] This technique enabled the easy preparation of molecularly imprinted monolithic columns with good resolution and low back pressure.

Paracetamol (acetaminophen) is a widely used nonsteroid drug for the management of pain and as antipyresis. Under some improper conditions (heat and pH), it may degrade to 4-aminophenol (4-AMP) and acetic acid.[15] 4-AMP may exist as an impurity in the starting materials of paracetamol synthesis and may also form during the synthesis. In the studies with the use of animals, nephrotoxic effect of 4-AMP and its capability of causing methemoglobinemy were observed.[16] The amount of 4-AMP is limited to %0.005 (w/w) in the drug substance by the European, United States, British, and German Pharmacopoeias. Therefore, 4-AMP is one of the major impurities, which has to be detected in paracetamol. Because of the similar structures of these two molecules, the separation is quite challenging. Chromatographic methods have been widely applied to the separation of 4-AMP from paracetamol mostly by using silica-based columns. However, these methods are time consuming, and the silica-based columns have some disadvantages such as poor stability at extreme pH values.

In the present study, a new MIP monolith that has specific recognition sites for 4-AMP was prepared by using noncovalent approach.[17] The prepared monolith was successfully separated 4-AMP and paracetamol with good chromatographic and flow through properties. Methacrylic acid (MAA), acrylamide (AAM), or isobornyl methacrylate (IBMA) was used as functional monomer and ethylene glycol dimethacrylate (EDMA) as crosslinker. Nonimprinted monolith (NIP) was also prepared for control experiments. The preparation conditions were optimized. The retention behaviors and selective recognition abilities of resultant monoliths were examined. Their binding site densities, surface areas, and pore properties were determined. Furthermore, the monolith was directly used for the separation of 4-AMP and paracetamol in organic media.

EXPERIMENTAL

Chemicals

Methacrylic acid (MAA), acrylamide (AAM), isobornyl methacrylate (IBMA), ethylene glycol dimethacrylate (EDMA), 1-dodecanol, acetonitrile (ACN), methanol, and acetone were purchased from Sigma Aldrich (Steinheim, Germany). 2,2′-Azobis(2-methylpropionitrile) (AIBN) was supplied by Merck (Darmstadt, Germany). MAA, IBMA, and EDMA were purified by vacuum distillation to remove stabilizers prior to use. Benzyl alcohol was supplied by BDH Chemicals (Poole, England). Dimethylformamide (DMF), tetrahydrofuran (THF), and acetic acid were supplied by Fluka (Buchs, Switzerland). All solvents were analytical or HPLC grade. 4-AMP and paracetamol were also purchased from Sigma Aldrich (Steinheim, Germany), and their structures are given in Figure 1.

Thermal stability studies

Thermal stability studies of 4-AMP at 50°C and 60°C in different solvent mixtures were conducted as follows; 0.6-mg 4-AMP was dissolved in 12 mL of a mixture of different solvent–porogen pairs. While THF and DMF were used as potential solvents, 1-dodecanol and benzyl alcohol were used as potential porogens. 4-AMP was dissolved in a solvent/porogen mixture with different compositions starting from 1/2 to 1/5 (v/v). Then its UV spectra were continuously taken by a Shimatzu UV-1700 spectrophotometer coupled with a Shimatzu, CPS-240A temperature control unit (Kyoto, Japan) for 24 h at desired temperature with 1-h time intervals.

Preparation of 4-AMP imprinted polymer monoliths

4-AMP imprinted polymers were prepared by *in situ* polymerization technique as shown in Figure 2. General procedure was as follows: known amount of template molecule, 4-AMP, was dissolved in 2.1 mL of dodecanol/DMF solvent mixture; then monomer (0.75 mmol), crosslinker (EDMA), and 12.3-mg

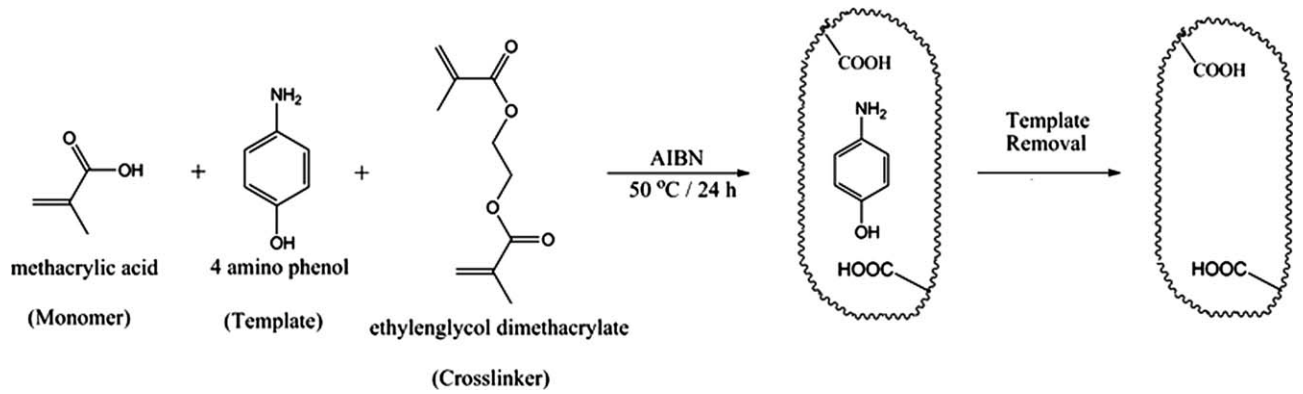

Figure 2 Schematic representation of 4-AMP imprinting process.

initiator, AIBN, were added to the mixture. The mixture was ultrasonicated for 5 min and deoxygenated by a stream of nitrogen for 10 min. After the solution was filled in an empty stainless steel HPLC column (150 × 3.9 mm i.d.) and sealed from both ends, it was placed in a vacuum oven and polymerized *in situ* by thermal initiation at 50°C for 24 h. After polymerization was completed, the column was connected to an HPLC pump and washed exhaustively with acetic acid/methanol (1/4) (v/v) to remove the template molecule, porogenic solvent, and unreacted monomers. Then acetic acid was washed away by flushing methanol through the column. The NIPs for control experiments were also prepared by the same procedure described earlier but in the absence of 4-AMP. As a first approach to optimize polymerization conditions, some parameters such as polymer composition, solvent–porogen ratio, template–monomer ratio, and monomer–crosslinker ratio were adjusted as listed in the Table I.

Chromatographic evaluation of monoliths

A Dionex Ultimate 3000 Analytical HPLC system composed of a LPG-3400A pump, a WPS-3000SL autosampler, a TCC-3000 column oven, and a DAD-3000 diode array detector was used (Sunnyvale, USA). After equilibrating the column, the mixture of 4-AMP and paracetamol (0.1 m*M* in each), prepared in mobile phase, was injected to the column and eluted with ACN as mobile phase at a flow rate of 1 mL/min at 25°C. Detection wavelength was 300 nm, and injection volume was 20 μL. Void volume was determined by the injection of acetone diluted by ACN (1 : 9). Each analysis was repeated for six times to ensure the chromatographic reproducibility.

The chromatographic parameters such as capacity factor, resolution, selectivity, and peak asymmetry were calculated.[18,19]

Capacity factors (k') were calculated by using the equation $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the

TABLE I
List of Prepared Polymeric Monoliths

Polymer	Functional monomer	Template–monomer ratio (mole : mole)	Monomer–crosslinker ratio (mole : mole)	Solvent–porogen ratio (v : v)
		Selection of functional monomer		
MIP_1	MAA	1 : 4	1 : 5	1 : 3
MIP_2	AAM	1 : 4	1 : 5	1 : 3
MIP_3	IBMA	1 : 4	1 : 5	1 : 3
		Optimization of solvent–porogen ratio		
MIP_1	MAA	1 : 4	1 : 5	1 : 3
MIP_4	MAA	1 : 4	1 : 5	1 : 4
		Optimization of template–monomer ratio		
MIP_5	MAA	1 : 2	1 : 5	1 : 3
MIP_1	MAA	1 : 4	1 : 5	1 : 3
MIP_6	MAA	1 : 6	1 : 5	1 : 3
		Optimization of monomer–crosslinker ratio		
MIP_7	MAA	1 : 6	1 : 4	1 : 3
MIP_6	MAA	1 : 6	1 : 5	1 : 3
MIP_8	MAA	1 : 6	1 : 6	1 : 3
		Control experiment		
NIP_7	MAA	Nonimprinted	1 : 4	1 : 3

retention times of the analyte and the void marker, respectively. Resolution (R_S) of 4-AMP and paracetamol peaks was calculated from the following equation:

$$R_S = 1.18[(t_2 - t_1)/(W_{\%50(2)} + W_{\%50(1)})],$$

where t_2 and t_1 are the retention times, and $W_{\%50(2)}$ and $W_{\%50(1)}$ are the peak widths at %50 peak height of 4-AMP and paracetamol, respectively. Selectivity (α) of the monoliths for 4-AMP against paracetamol was calculated by applying the equation $\alpha = (t_2 - t_0)/(t_1 - t_0)$. Peak asymmetry ($A$) values was also calculated by using the equation:

$$A = (\mathrm{RW}_{\%5} + \mathrm{LW}_{\%5})/2 * \mathrm{LW}_{\%5},$$

where $\mathrm{RW}_{\%5}$ and $\mathrm{LW}_{\%5}$ are the right and left peak widths of a given peak at %5 peak height.

Frontal chromatography

The binding properties of imprinted and NIPs were investigated by frontal chromatography method introduced into MIPs by Kempe and Mosbach.[20] This method enables the investigation of the interaction between the template and the polymer monolith and determination of the dissociation constant (K_D) and the effective number of binding sites (L_t). In frontal analysis, different concentrations of 4-AMP and paracetamol (0.1, 0.2, 0.5, and 1.0 mM) prepared in mobile phase were loaded to the column at 25°C. First, the column was connected to the HPLC and equilibrated with mobile phase at 300 nm. Then the column was disconnected from the system, the tube from the reservoir to the inlet of the monolithic column was filled with the mobile phase solution containing the lowest concentration of the analyte, and the column was reconnected to the system. After that, the solution was pumped to the column at a flow rate of 1 mL/min and simultaneously recording the signal at 300 nm, the breakthrough curve was obtained. In the following, the column was re-equilibrated, and the solution containing the next higher analyte concentration was pumped to the column. By this way, a breakthrough curve was obtained for each analyte solution, and the amount of bound analyte is found from breakthrough curves. The same procedure was also applied to acetone/ACN (5/95) solution, which was used as a void marker.

Binding isotherm of 4-AMP on both MIPs and NIPs columns was generated from breakthrough curves by using the following equation:

$$1/([A]_0(V - V_0)) = (K_D/([A]_0 L_t)) + 1/L_t$$

Using the equation above, effective number of binding sites (L_t) and dissociation constant (K_D) were calculated from the plot of $1/[A]_0$ $(V - V_0)$ versus $1/[A]_0$, where $[A]_0$ is the concentration of the analyte, and V and V_0 are elution volumes of the analyte and void volume, respectively. The number of effective binding sites was calculated from the intercept on the ordinate ($1/L_t$), and the dissociation constant was calculated from the intercept on the abscissa ($-1/K_D$).

Imprinting factor

Imprinting factor (IF) is used to determine whether or how much the imprinting process makes difference in MIPs.[21] It was calculated by using the equation $\mathrm{IF} = k'_{\mathrm{imprinted}}/k'_{\mathrm{nonimprinted}}$, where $k'_{\mathrm{imprinted}}$ and $k'_{\mathrm{nonimprinted}}$ are the capacity factors of 4-AMP for imprinted and nonimprinted monolithic columns, respectively.

Morphological analysis

The topographic morphologies of monolith samples were examined by a JEOL 6400 scanning electron microscope (SEM) (Tokyo, Japan). Micrographs of monoliths were obtained after being coated with gold. The surface area and mesopore properties were determined by a Quantachrome, Autosorb-1 surface area and pore size analyzer by applying nitrogen as adsorbant. BET and DFT-Monte-Carlo models were used for the evaluations of surface area and mesopore properties, respectively.

DISCUSSION

Selection of solvent–porogen pair

For a successful imprinting by noncovalent interactions, it is crucial to use solvents that have no or low hydrogen bonding capacity. Therefore, thermal stability of 4-AMP in various proper solvent and porogen mixtures was studied for 24 h. It was observed that 4-AMP decomposes very quickly at 60°C in any of the solvent/porogen mixtures. Thus, the temperature was reduced to 50°C, and it was seen that the 4-AMP is much more stable in mixtures involving DMF and 1-dodecanol than it is in other pairs. Therefore, DMF and 1-dodecanol were chosen as the solvent and as the porogen, respectively.

Selection of functional monomer

Selective recognition properties of MIPs are critically based on the strength and positioning of monomer–template interactions. Therefore, the selection of functional monomer to obtain MIP monolith with good molecular recognition properties is an important step. For this reason, a polar-acidic monomer,

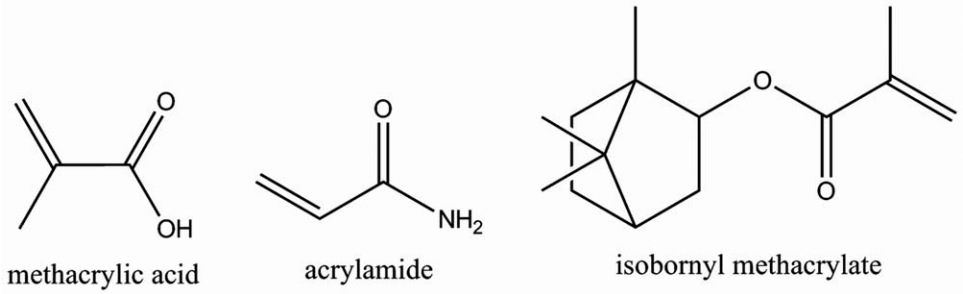

Figure 3 Chemical structures of functional monomers used.

MAA, a polar-neutral monomer, AAM, and an apolar monomer, IBMA, were tried individually in MIP monolith synthesis (Fig. 3). When MAA was used as the monomer, MIP_1 monolith exhibited good retention ($k' = 1.93$), while MIP_2 ($k' = 0.3$) and MIP_3 ($k' = 0.07$) monoliths had almost no retention to the template molecule. This result indicates that the stable complex was successfully formed between MAA and 4-AMP by the strong hydrogen bonding interactions as suggested in Figure 4. Since IBMA is an apolar monomer, it cannot interact with the polar template molecule, so no retention to 4-AMP ($k' = 0.07$) occurred as expected. However, it was very surprising that MIP monolith showed almost no retention ($k' = 0.3$) to the template molecule, when AAM was used as the functional monomer. The reason may come from that the amide group on the AAM monomer forms weak hydrogen bonds with the template molecule so that formed monomer–template complex does not have enough stability during imprinting process. Besides, for the basic template molecule ($pKa = 10.46$), the acidic MAA would be the best imprinting monomer.

Optimization of polymerization conditions

To prepare an MIP monolith with good chromatographic properties and low back pressure, some preparation conditions must be taken into account. In this study, solvent–porogen ratio, template–monomer ratio, and monomer–crosslink ratio were optimized as seen in Table I. Influence of these optimizations on chromatographic parameters was shown in Table II. When solvent–porogen ratio was increased from 1 : 3 (MIP_1) to 1 : 4 (MIP_4), column back pressure was reduced from 22 to 4 bar, and selectivity increased from 3.86 to 4.81. However, the resolution reduced to 1.27 as seen in Table II. Therefore, the ratio of 1 : 3 was evaluated as optimal.

In general, functional monomer is added in large excess to increase the possibility of surrounding the template molecules with functional monomers in noncovalent approach. Yet, increasing the functional monomer amount may also increase the nonspecific binding sites. On the other hand, low monomer–template ratio affords less than optimal complexation on account of insufficient functional monomer. Therefore, a proper template–monomer ratio has to be set. The influence of template–monomer ratio (1 : 2, 1 : 4, and 1 : 6) on the chromatographic parameters was evaluated. As shown in Table II, there is a tendency of increasing resolution but decreasing selectivity with decreasing template amount in the polymerization mixture. MIP_5 monolithic column, with template monomer ratio of 1 : 2, has the highest selectivity (5.59), but its resolution was very low (1.18). On the other hand, MIP_6 monolith, with the template : monomer ratio of 1 : 6 has the highest resolution (1.61). By also taking into account the sharpness of peaks, template : monomer ratio of 1 : 6 was chosen as the optimum ratio.

In molecular imprinting technology, the amount of crosslinker has a crucial role, which is not only to maintain the stability of recognition sites by forming rigid specific cavities for the template molecule but also to have some extent of flexibility to make the template molecules enter the cavities easily.[20] In this study, monomer–crosslinker ratios of 1 : 4 (MIP_7), 1 : 5 (MIP_6), and 1 : 6 (MIP_8) were examined. When the monomer–crosslinker ratio was 1 : 4, the retention time of 4-AMP was significantly increased to 9.1. This significant increase means that the required flexibility for template molecules to be able to enter the cavities easily was achieved. Thus, capacity factor, resolution, and selectivity values were also increased significantly to 4.1, 1.91, and 5.68, respectively. At higher crosslinker concentrations (MIP_6 and MIP_8), the recognition for the template molecule

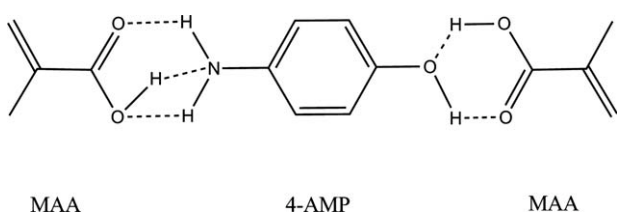

Figure 4 Suggested interactions between MAA and 4-AMP molecules in prepolymerization mixture.

TABLE II
Influence of Polymerization Conditions on Chromatographic Parameters

MIP	t_R (min)		k'		A		R_S	α
	4-AMP	Paracetamol	4-AMP	Paracetamol	4-AMP	Paracetamol		
Optimization of solvent–porogen ratio								
MIP_1	4.834	2.475	1.93	0.50	1.64	0.87	1.51	3.86
MIP_4	4.858	2.0	2.91	0.61	1.39	1.26	1.27	4.81
Optimization of template–monomer ratio								
MIP_5	5.82	2.396	2.53	0.45	1.55	1.32	1.18	5.59
MIP_1	4.834	2.475	1.93	0.50	1.64	0.87	1.51	3.86
MIP_6	4.667	2.562	1.72	0.49	2.17	0.89	1.61	3.49
Optimization of monomer–crosslinker ratio								
MIP_7	9.10	3.072	4.10	0.72	1.33	0.96	1.91	5.68
MIP_6	4.667	2.562	1.72	0.49	2.17	0.89	1.61	3.49
MIP_8	4.39	2.170	2.03	0.50	1.68	1.44	1.49	4.08

4-AMP decreased because of the difficulty of entering the cavities of rigid MIPs for the template molecule. The monolith that has the nearest asymmetry values to ideal case both for 4-AMP and paracetamol peaks is MIP_7 as seen in Table II, and the column back pressure was only 10 bar. In the view of these findings, it can easily be said that MIP_7 monolith has the best column efficiency when compared with other MIP monoliths.

Effect of molecular imprinting on recognition properties

To evaluate the effect of molecular imprinting, MIP_7 and its nonimprinted monolith (NIP_7) were compared. HPLC analysis showed that monolithic column of MIP_7 could successfully separate our two analytes, 4-AMP and paracetamol, with a resolution of 1.91 and with a selectivity of 5.68 [Fig. 5(a)]. While the retention time of paracetamol was only 3.072 on MIP_7 monolith, retention time of 4-AMP was 9.10, and the capacity factors of paracetamol and 4-AMP were 0.72 and 4.10, respectively. However, it was surprising that the NIP_7 monolith could also separate such two molecules [Fig. 5(b)] with similar structures and polarities showing some selectivity (3.47), but with a low resolution (1.30) (Table III). Probably, the difference between the elution times of 4-AMP and paracetamol comes from the strong hydrogen bonding interactions between 4-AMP and functional groups on the surface of polymer backbone of NIP_7 monolith as suggested in Figure 6(a). As figured out from the HPLC analysis, paracetamol molecules could not form such hydrogen bonding interactions with the functional groups on NIP_7 surface. Since 4-AMP and paracetamol differs structurally only on amide group, the difference should have come from this side. Intramolecular H-bonding of paracetamol molecule [Fig. 6(b)] prevents the formation of hydrogen bonding with the functional groups on polymer monolith during the elution. HPLC analyses carried on different column temperatures also support this idea. As seen in Figure 7, the capacity factors of 4-AMP and paracetamol decreased with increasing column temperature. While the decrease in capacity factor of 4-AMP was very significant, the capacity factor of paracetamol decreased slightly. By increasing the column temperature, the formation of hydrogen bonds was prevented. Therefore, it is expected that the effect of temperature on the capacity factor of the analyte having more functional groups capable of forming hydrogen bonds with the polymer would be more significant. In the light of these findings, it can be suggested that 4-AMP forms hydrogen bonds with the functional groups of polymer via both its amine and hydroxyl group, while paracetamol can form hydrogen bonds with the polymer only by its hydroxyl group because of intramolecular hydrogen bonding between the hydrogen on amine and the carbonyl group.

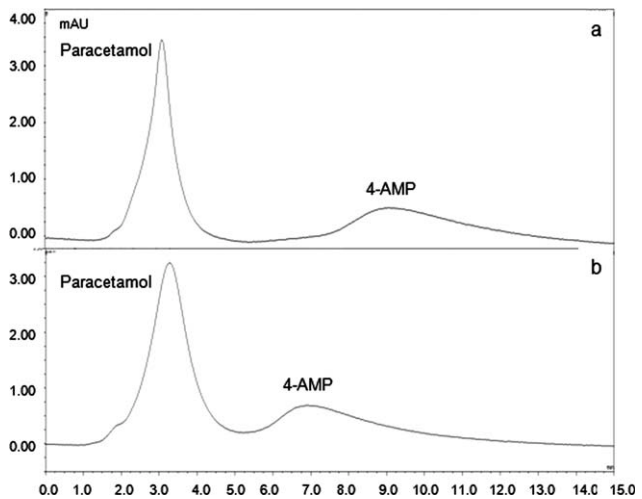

Figure 5 Separation of 4-AMP and paracetamol on (a) MIP_7 and (b) NIP_7 monolith. Chromatographic conditions: injection of 4-AMP and paracetamol mixture (0.1 m*M* in each), 1 mL/min mobile phase flow of ACN, detection at 300 nm.

TABLE III
Comparison of Chromatographic Properties of MIP_7 and NIP_7 Monoliths

Polymer	t_R (min)		k'		A_S		R_s	α
	4-AMP	P	4-AMP	P	4-AMP	P		
MIP_7	9.10	3.072	4.10	0.72	1.33	0.96	1.91	5.68
NIP_7	6.931	3.267	2.89	0.83	1.68	1.09	1.30	3.47

Despite nonimprinted column was achieved to separate 4-AMP and paracetamol, imprinted column shows a higher selectivity to 4-AMP as expected, and the IF was calculated as 1.42.

Effect of molecular imprinting on morphology of monoliths

The SEM microphotographs in Figure 8 showed that the existence of template did not change the macropore structure of the imprinted and nonimprinted polymers significantly. When surface areas of MIP_7 and its nonimprinted monolith (NIP_7) were compared, it was seen that the surface area of NIP_7 monolith (105 m^2/g) was about four times higher than those of MIP_7 (23.48 m^2/g). Mesopore size distributions of both monoliths were given in Figure 9. As seen, the main mesopore size was the same, which is 5 nm for MIP_7 and NIP_7. However, total pore volume of mesopores was calculated for MIP_7 and NIP_7 and found as 2.99×10^{-2} cc/g and 9.24×10^{-2} cc/g, respectively. Therefore, it can be said that the existence of template molecule in the polymerization mixture changes the mesopore structure of monolith in the direction of decreasing the amount of mesopores so that the surface area of MIP_7 monolith decreases. It was also observed that the mesopores of NIP_7 monolith were much more well defined than mesopores of MIP_7. While NIP_7 monolith has almost no mesopore bigger than 5 nm, MIP_7 monolith has some other mesopores reaching up to 30 nm. From this point, it can be suggested that size of specific cavities created by molecular imprinting technique were between 5 and 30 nm.

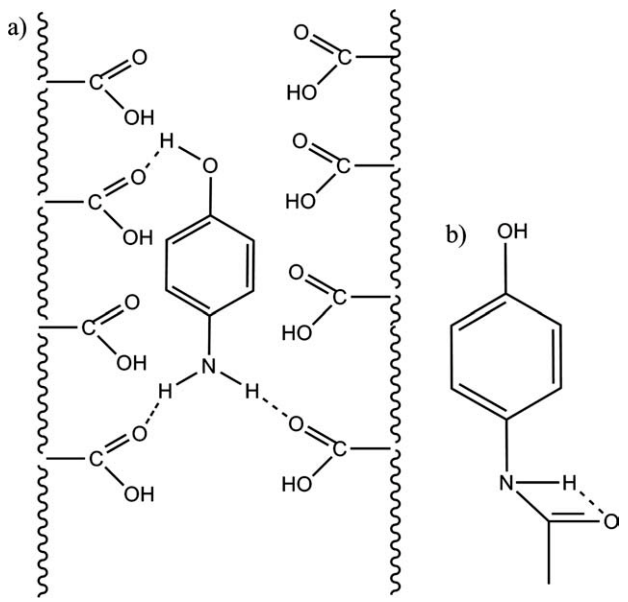

Figure 6 Suggested interactions of 4-AMP on the monolithic column (a) and intramolecular H-bonding of paracetamol (b).

Frontal chromatography results

Frontal chromatography is commonly used to investigate specific interactions. The dissociation constant and binding density for the interaction between the analyte and polymer can be determined by this method. To see the binding behavior of 4-AMP on both imprinted and nonimprinted columns, effective binding site densities of MIP_7 and NIP_7 for 4-AMP were calculated from the plot of $1/([A]_0\ (V-V_0))$ versus $1/[A]_0$ and found as 7.95 μmol/g for MIP_7 and 8.82 μmol/g for NIP_7 monoliths. Although the effective binding site density of NIP for 4-AMP was slightly higher than those of imprinted monolith, because of the fact that NIP_7 monolith has much more surface area than those of MIP_7 monolith, retention time of 4-AMP on MIP_7 column was higher than that it was on NIP_7 column, meaning that specific recognition sites were successfully created by molecular imprinting leading to binding sites with higher affinity to 4-AMP. The dissociation constants (K_D) of imprinted and NIPs for 4-AMP, calculated from the plot of $1/([A]_0\ (V-V_0))$ versus $1/[A]_0$, were 1.06 and 1.28 m*M*, respectively. These values also

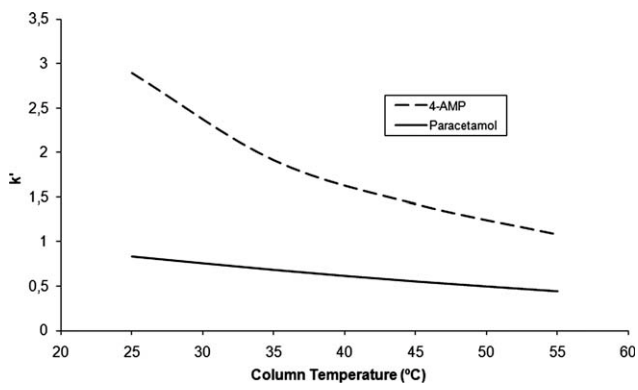

Figure 7 Effect of column temperature on retention times of 4-AMP and paracetamol on NIP_7 column.

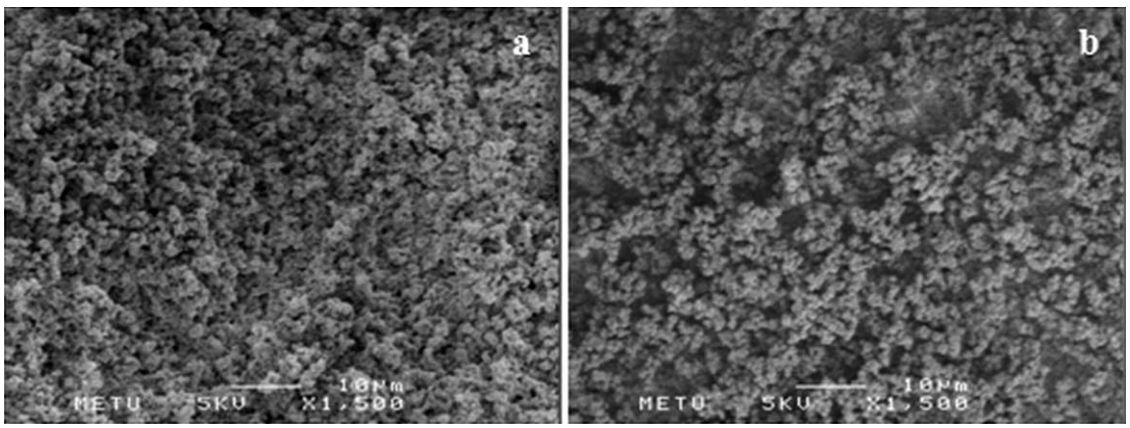

Figure 8 SEM images of (a) MIP_7 and (b) NIP_7 monolith.

prove the formation of specific binding sites showing higher affinity to the template molecule.

CONCLUSIONS

In this study, polymerization conditions and binding characteristics of 4-AMP imprinted polymers were optimized, and recognitive polymeric monolith not only with good flow-through properties but also high resolution and selectivity for chromatographic separation of structurally similar compounds was obtained. 4-AMP and paracetamol were completely resolved on prepared MIP monolith directly without a need of method development process. The prepared column can be used as a precolumn or enrichment column by further researches and also be an alternative to silica-based monolithic columns.

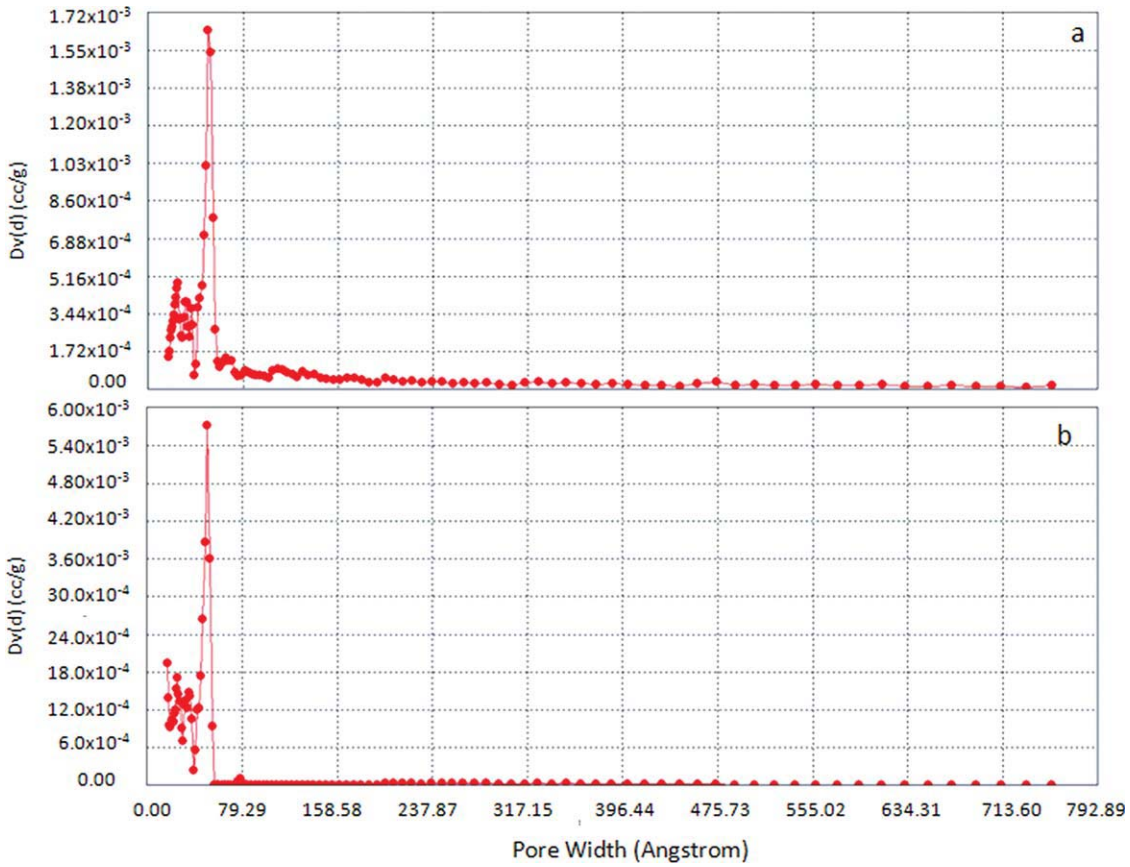

Figure 9 Mesopore size distribution of (a) MIP_7 and (b) NIP_7 monolith. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The authors thank Prof. Dr. Nursabah Basci (HU, Faculty of Pharmacy, Department of Analytical Chemistry) for helpful discussions.

References

1. Guiochon, G. J Chromatogr A 2007, 1168, 101.
2. Kubin, M.; Spacek, P.; Chromecek, R. Collect Czech Chem Commun 1967, 32, 3881.
3. Ross, W. D.; Jefferson, R. T. J Chrom Sci 1970, 8, 386.
4. Minakuchi, H.; Nakanishi, K.; Soga, N.; Ishizuka, N.; Tanaka, N. Anal Chem 1996, 68, 3498.
5. Hjerten, S.; Liao, J. L.; Zhang, R. J Chromatogr 1989, 473, 273.
6. Svec, F.; Frechet, J. M. Anal Chem 1992, 64, 820.
7. Wulff, G.; Sarhan, A. Angew Chem 1972, 84, 364.
8. Mayes, A. G.; Mosbach, K. Anal Chem 1996, 68, 3769.
9. Ye, L.; Weiss, R.; Mosbach, K. Macromolecules 2000, 33, 8239.
10. Matsui, J.; Kato, T.; Takeuchi, T.; Suzuki, M.; Yokoyama, K.; Tamiya, E.; Karube, I. Anal Chem 1993, 65, 2223.
11. Li, Z. Y.; Liu, Z. S.; Zhang, Q. W.; Duan, H. Q. Chin Chem Lett 2007, 18, 322.
12. Liu, X.; Ouyang, C.; Zhao, R.; Shangguan, D.; Chen, Y.; Liu, G. Anal Chim Acta 2006, 571, 235.
13. Ou, J.; Kong, L.; Pan, C.; Su, X.; Lei, X.; Zou, H. J Chromatogr A 2006, 1117, 163.
14. Lin, L. Q.; Li, Y. C.; Fu, Q.; He, L. C.; Zhang, J.; Zhang, Q. Q. Polymer 2006, 47, 3792.
15. Mohamed, F. A.; Abdullah, M. A.; Shammat, S. M. Talanta 1997, 44, 61.
16. Kaszuba, E. W.; Grzeskiewicz, M. W.; Fijalek, Z. J Pharm Biomed Anal 2003, 32, 1081.
17. Arshady, R.; Mosbach, K. Makromol Chem 1981, 182, 687.
18. Sellergren, B. In Molecularly Imprinted Polymers; Sellergren, B., Ed.; Elsevier Science B.V.: Amsterdam, 2001; Chapter 5.
19. Snyder, L. R.; Kirkland, J. J.; Dolan, J. W. Introduction to Modern Liquid Chromatography; Wiley: Hoboken, NJ, 2009.
20. Kempe, M.; Mosbach, K. Anal Lett 1991, 24, 1137.
21. Legido-Quigley, C.; Oxelbark, J.; De Lorenzi, E.; Zurutuza-Elorza, A.; Cormack, P. A. G. Anal Chim Act 2007, 591, 22.