Chromatographic Separation of Xanthine Derivatives on Single and Mixed-Template Imprinted Polymers

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We developed in the present study molecular imprinted polymers (MIPs), using single templates (pentoxifylline, caffeine and theophylline) and mixed-templates (pentoxifylline-caffeine, pentoxifylline-theophylline and caffeine-theophylline). The MIPs were prepared with methacrylic acid (MAA) as the monomer, ethylene glycol dimetharylate (EGDMA) as the crosslinker and 2,2'-azobis(isobutyronitrile) (AIBN) as the initiator. The obtained polymer particles (particle size after grinding was about 25-35 μm) were packed into a HPLC column (3.9 mm i.d. × 150 mm). The selectivity and chromatographic characteristics of the MIPs were studied using acetonitrile as the mobile phase at a flow rate of 0.8 mL/min. UV detector wavelength was set at 270 nm. Different single template MIPs showed different molecular recognitions to the templates and the structurally analogues, according to the rigidity and steric hindrance of the compounds. Recognition was improved on the mixed-template MIPs as a result of the cooperation or sum effect of the templates, whereas on the pentoxifylline-theophylline imprinted polymer, the highest selectivity and affinity were obtained. Separations of the test compounds on different polymers were also investigated.

Key Words : Molecular imprinted polymers (MIPs), Xanthine derivatives, Separation

Introduction

The technique of molecular imprinting consists of the self-assembly of a functional monomer and a template molecule in solution followed by the co-polymerization of the functional monomer and an excess of an appropriate crosslinking monomer. After removing the small molecule, the resulting network polymer exhibits a significantly higher affinity for the molecule used as the template than for similar molecules, including closely related isomers.[1-5] MIPs have been applied to chiral separation,[6,7] solid extraction,[8] biomimic sensor[9,10] and membrane separation.[11,12] MIPs can be prepared by both covalent and non-covalent methods, whereas the latter has been widely used in recent years because of the ease with which that method can be performed. The most successful non-covalent imprinting systems are based on commodity methacrylic monomers, such as MAA, because their carboxyl group is the most commonly hydrogen-bonding and acidic functional group in molecular imprinting when cross-linked with EGDMA.

MIPs have been shown to be useful as separation materials in the extraction of certain active components from herbs,[13] beverages and spiked human plasma.[14] This utility, which is based on their shape, size, and functionality selectivity, strong affinity on rebinding target compounds, the significantly low cost for preparation and the workability in organic solvents, calls for finding a proper template to improve their selectivity and affinity.

In the last few years, xanthines derivatives, including theophylline[15] and caffeine,[16] have become a group of templates of great interest in MIPs. It is the general case in MIPs that single compound is used as the template, mixed- or multi-biomolecule as the template is not reported. In the present study, both single template (pentoxifhylline, caffeine and theophylline) and mixed template (pentoxifhylline-caffeine, pentoxifylline-theophylline and caffeine-theophylline) were used and their chromatographic characteristics were investigated. Here we show that on one hand, the selectivity and affinity clearly related to the rigidity and steric hindrance of the template. And, on the other hand, combing together compounds structurally similar as the templates resulted in a cooperation or sum effect of the binding sites, affording stronger selectivity and affinity.

Experimental Section

Materials. Pentoxifylline, caffeine, theophylline, theobromine, MAA were obtained from Sigma (St Louis, MO, USA). AIBN was purchased from Junsei Chemical Co. Ltd. (Japan). EGDMA was obtained from Fluka (Buchs, Switzerland). All the above reagents were used directly without further treatment. Acetonitrile, chloroform, methanol were all of HPLC grade, bought from Duksan Pure Chemical Co. LTD. (Ansan, Korea). Acetic acid (analytical grade) was purchased from Oriental Chemical Industries (Incheon, Korea). Double distilled water was filtered with a 0.45 μm filter membrane before use.

Polymerization Preparation. The following were added to a 250 mL two-neck glass flask: 5 mmol of the monomer (MAA,), 30 mmol of the crosslinker (EGDMA,), 0.12 g of the initiator (AIBN), porogen and different templates, 1 mmol of pentoxifylline for P1, 1 mmol of caffeine for P2, 0.5 mmol of theophylline for P3, 0.5 mmol of pentoxifylline

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plus 0.5 mmol of caffeine for PC, 0.5 mmol of pentoxifylline plus 0.25 mmol of theophylline for PT and 0.5 mmol of caffeine plus 0.25 mmol of theophylline for CT, respectively. The reaction mixture was put in supersonic for 10 min, sparged with helium for 10 min to remove oxygen, then vacuumed for 10 min and sealed under vacuum. Polymerization was performed in a water bath for 24 hours with the temperature maintained at 60 °C. After the polymerization, the bulk polymer was taken out from the reaction flask and put into an oven. The dried polymer was grounded into particles and passed through a 35 μm sieve, small particles were removed by repeated sedimentations with water. By these procedures, particles of 25 μm-35 μm were collected. The dried particles were packed into a 3.9 mm i.d. × 150 mm Waters stainless steel column. Methanol/acetic acid = 90/10 (v/v) was first used as the mobile phase at a flow rate of 0.3 mL/min for 4 hours to remove the template, then only acetonitrile was used as the mobile phase for further chromatographic evaluation. Blank polymer was prepared following the same procedure as in the absence of template.

HPLC Application. Analysis was carried out by a liquid chromatography system consisting of a Waters 600s Multisolvent Delivery System and a Waters 616 pump (Waters, Milford, MA, USA), a detector of Waters 2487 Dual Absorbance (Waters, Milford, MA, USA) and a Rheodyne injection valve (20 μl sample loop). Millennium 3.2 (Waters, Milford, MA, USA) was used as the data acquisition system.

Chromatographic evaluation was performed using acetonitrile as the mobile phase. The wavelength was set at 270 nm. The capacity factor (k') was calculated as $(t-t_0)/t_0$, where t is the retention time of the compound, and t_0 the dead time of the column and determined by acetone as the marker.

Table 1. Capacity factors of pentoxifylline, caffeine, theophylline and theobromine on imprinted and blank polymers

Polymer	Template	Compound	Capacity factor (k')
P1	Pentoxifylline	Pentoxifylline	0.405
		Caffeine	0.580
		Theophylline	1.47
		Theobromine	1.83
P2	Caffeine	Pentoxifylline	0.329
		Caffeine	0.672
		Theophylline	1.59
		Theobromine	1.87
P3	Theophylline	Pentoxifylline	0.483
		Caffeine	0.736
		Theophylline	3.79
		Theobromine	2.40
PC	Pentoxifylline +caffeine	Pentoxifylline	0.363
		Caffeine	0.640
		Theophylline	1.51
		Theobromine	1.81
PT	Pentoxifylline +theophylline	Pentoxifylline	0.707
		Caffeine	1.05
		Theophylline	6.74
		Theobromine	3.89
CT	Caffeine +theophylline	Pentoxifylline	0.508
		Caffeine	0.874
		Theophylline	3.89
		Theobromine	2.46
P6	–	Pentoxifylline	0.243
		Caffeine	0.402
		Theophylline	1.22
		Theobromine	1.52

Results and Discussions

Single Template MIPs. First, the single compounds pentoxifylline, caffeine and theophylline were used as templates separately. The capacity factors (k') of the compounds are listed in Table 1. Though rather small difference existed in their structures, different imprinted polymers showed different selectivity for their templates. When pentoxifylline was used as the template, the templates capacity factor increased 67% compared with the blank polymer. A similar result was obtained for caffeine (caffeines capacity factor increased 67%) on the caffeine imprinted polymer. The theophylline imprinted polymer, on the other hand, showed a strong retention for theophylline (theophyllines capacity factor increased 210%). This difference can be discussed in term of the difference in the rigidity and steric hindrance of the template molecules. From Figure 1, the conclusion can be drawn that the steric hindrance of pentoxifylline is the strongest because a rather complex group is connected to N1, then caffeine and finally theophylline. For rigidity, theophylline is the strongest, followed by caffeine and then pentoxifylline. As is known, the rigid molecule tends to fasten to the recognition site and keep the recognition space. But for a given recognition site, the larger the steric hindrance of the molecule, the weaker the recognition.

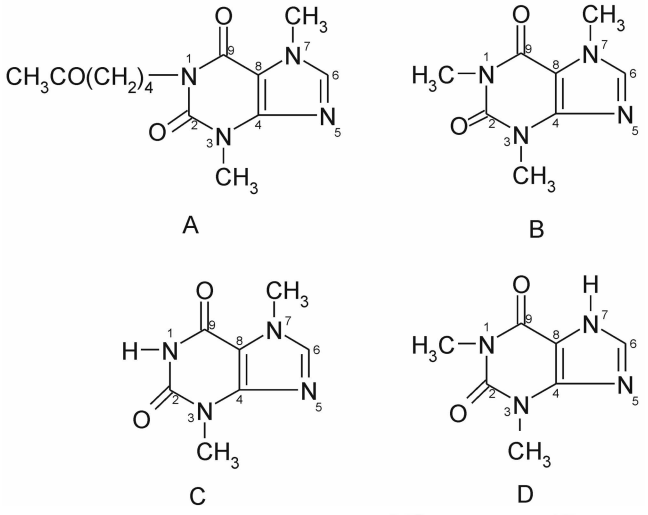

Figure 1. Molecular structure of pentoxifylline (A), caffeine (B), theobromine (C) and theophylline (D).

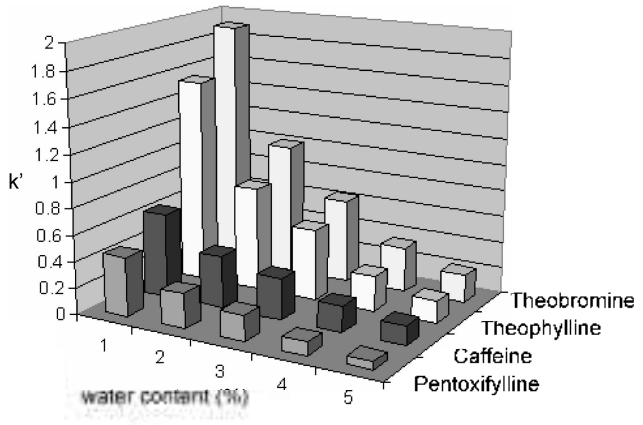

Figure 2. Effect of water content in the mobile phase on the capacity factors of P1.

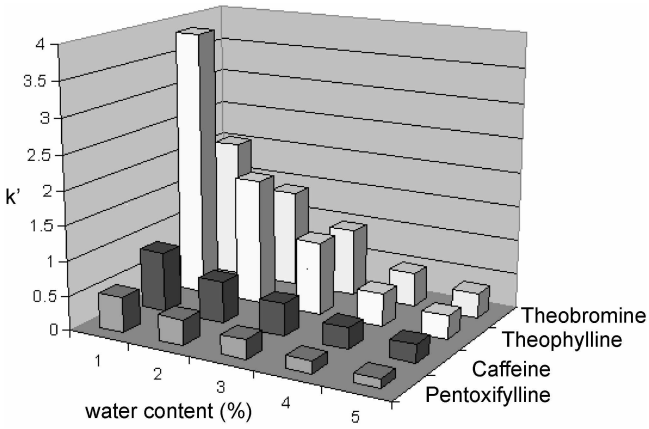

Figure 3. Effect of water content in the mobile phase on the capacity factors of P4

Beside this, hydrogen bonding may play a role in the molecular recognition of the MIPs, which can be seen from Figure 2. In Fig. 2 the effect of water content in mobile phase on the capacity factor is illustrated. An overall decrease in the capacity factors can be found when the water contents increases from 0-7% in the mobile phase. As water shows a stronger ability for hydrogen bonding, the addtion of water can interfere with the hydrogen binding interaction between the template (analogues) and the binding sites, which will decrease the retention of the template and the analogues.

Mixed-template MIPs. Three groups of mixed-template were used in this work: pentoxifylline-caffeine (PC), pentoxifylline-theophylline (PT) and caffeine-theophylline (CT). The chromatographic results are found in Table 1 and Figure 3. For the mixture template polymers, two groups involve the using of theophylline, and one is caffeine-theophylline, the other is pentoxifylline-theophylline. The capacity factors listed in Table 1 illustrates that caffeine-theophylline imprinted polymer shows a higher affinity for both the template and the analogues than the theophylline imprinted polymer, which shows the strongest affinity for the compounds among the single template polymers. But the

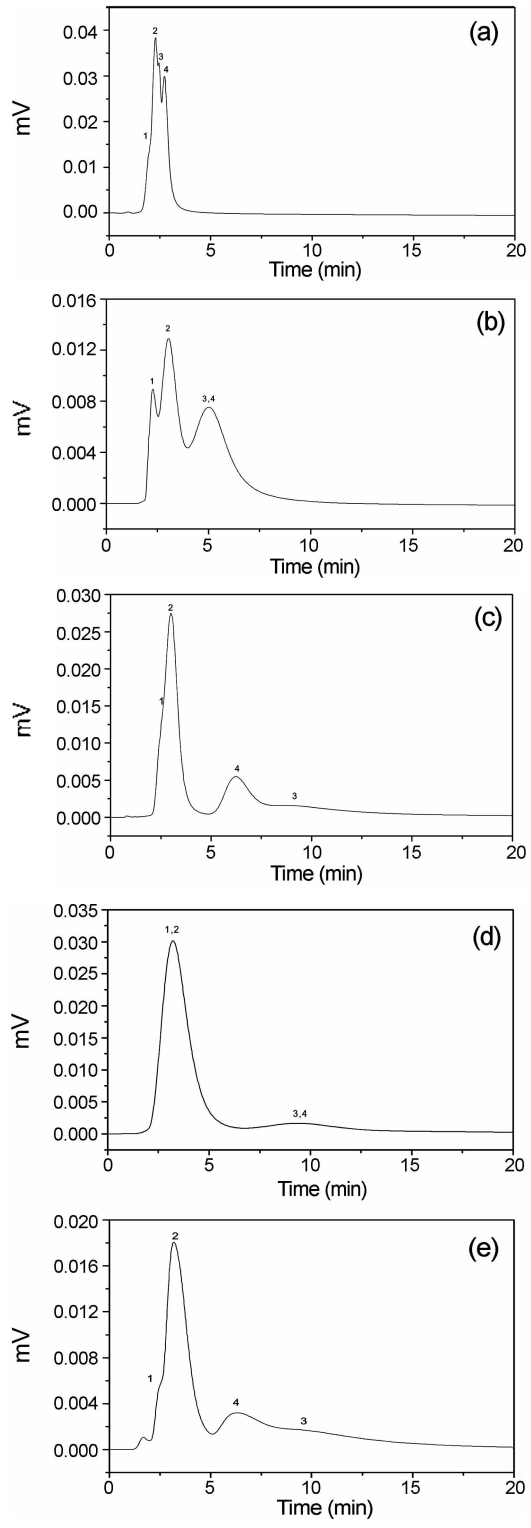

Figure 4. Chromatograms of the separation of four samples on blank and different imprinted polymers. (a) Blank polymer (b) P2 (c) P3 (d) PT (e) CT. Peaks: 1 = pentoxifylline, 2 = caffeine, 3 = theophylline, 4 = theobromine

increase is not that significant, which can be attributed to a sum effect of the binding sites. On the other hand, for pentoxifylline-theophylline imprinted polymer, the increase in affinity for the template and the analogues is significant,

which we regard it as a cooperation effect of the binding sites. From the molecular structure, theophylline shows the highest rigidity and pentoxifylline the lowest. As is known, a template with a high rigidity tends to fasten to the binding site, whereas the binding site formed by a template of lower rigidity shows higher flexibility. So in our work when the higher-rigidity theophylline and lower-rigidity pentoxifylline were mixted together as the template, a balance between the rigidity and flexibility of the binding sites was formed inside the polymer. This resulted in a cooperation effect and also higher accessibility to the binding sites, hence an increase in the affinity. Figure 3 shows that hydrogen bonding may also contribute to the molecular recognition on the mixed-template imprinted polymers.

Chromatographic Separation. Chromatographic separations of different polymers were carried out with acetonitrile as the mobile phase. Figure 4 illustrates the chromatograms of the separations. On the blank polymer, the four compounds were partly separated and the peaks overlap to a great degree. Improved separations were obtained on the imprinted polymer (Figs. 4b-4e), especially the caffeine-theophylline imprinted polymer. On the caffeine-theophylline imprinted polymer, good separation of caffeine, theophylline and theobromine were obtained and the separation between pentoxifylline and caffeine was also improved. From the chromatogram of pentoxifylline-theophylline imprinted polymer, though the highest retention was obtained, the peaks of pentoxifylline and caffeine, theophylline and theobromine overlap.

Conclusions

Through a protocol of combing structurally similar compounds as templates, an obvious cooperation, or sum effect, occurred in the present study, which resulted in an overall increased affinity and selectivity for the analogues. This technique can be useful for the preparation of solid phase extraction and separation materials with higher affinity.

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