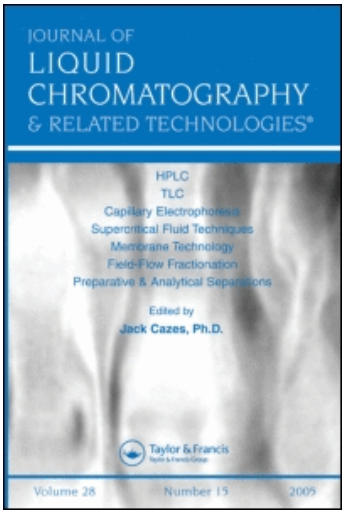

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Yan, Hongyuan and Row, Kyung Ho(2006) 'Characteristic and Molecular Recognition Mechanism of Theophylline Monolithic Molecularly Imprinted Polymer', Journal of Liquid Chromatography & Related Technologies, 29: 10, 1393 — 1404
To link to this Article: DOI: 10.1080/10826070600674778
URL: http://dx.doi.org/10.1080/10826070600674778

Journal of Liquid Chromatography & Related Technologies®, 29: 1393–1404, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600674778

Characteristic and Molecular Recognition Mechanism of Theophylline Monolithic Molecularly Imprinted Polymer

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Abstract: A monolithic molecularly imprinted polymer (MIP) with specific recognition ability for theophylline was prepared by in-situ therm polymerization, using acrylamide as a functional monomer, ethylene glycol dimethacrylate as a cross-linking agent, toluene and dodecanol as porogenic solvents and 2,2′-azobisisobutyronitrile as initiator. Scanning electron microscopy and an accelerated surface area and porosimetry system were used to identify the structural features of the MIP. The results show that the large through-pore structure allows mobile phase to flow through the MIP column with a low back pressure, and the other pores lead to the molecular recognition. Some chromatographic conditions such as the composition of the mobile phase, flow rate, and temperature were characterized and illustrated by a homologous series of xanthine derivatives, theophylline and caffeine. Effects of molecular recognition were also discussed and the possible recognition mechanisms were hydrogen bonding and hydrophobic interactions between the theophylline molecule and the MIP.

Keywords: Monolithic column, Molecularly imprinting polymer, In-situ polymerization, Theophylline

INTRODUCTION

Molecular imprinting is a rapidly developing technique for the preparation of polymers having specific molecular recognition properties. Much of the literature available on the subject frequently underlines the 'biomimetic' properties

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exhibited by these imprinted polymers with the substrate selective mechanisms being analogous to that of natural entities, such as antibodies and enzymes.[1–3] MIP can be called artificial antibodies or receptors. Since they are stable, easy to prepare, and inexpensive, they can be an attractive alternative or complement to natural antibodies and receptors. In recent years, MIP technology matured to a valuable complementary concept for biological recognition, with increased applicability in chiral separation,[4,5] solid extraction,[6] biomimic sensor,[7] and membrane separation.[8] However, it also becomes evident that the development of improved MIP technology with enhanced recognition properties requires further research into understanding the governing mechanisms of generating selectivity in MIP in molecular recognition.

Traditionally, the molecularly imprinted polymer were prepared by bulk, grinding the resulting polymer block into particles, and sieving the particles into the desired size ranges. Such ground and sieved particles have been packed into conventional HPLC columns. Although, the process of bulk polymerization is simple, the resulting polymer must be crushed, ground, and sieved to obtain the appropriate particle size, which is tedious and time consuming. Since only a portion of polymer can be used as packing material, this method suffered high consumption of the template molecules. In addition, the resulting polymer particles are polydisperse both in shape and size, which also has a negative impact on chromatographic performance.[9] UniformLy sized and monodispersed particles had been made by the suspension polymerization and the seed polymerization or multi-step swelling process.[10–12] Regular molecularly imprinted microspheres have been prepared by suspension polymerization and multi-step swelling polymerization methods. Although particles obtained using this technique are comparatively monodisperse in size and shape and well suited for chromatographic applications, however, fairly complicated procedures and reaction conditions are required, and the aqueous suspensions used in this technique could interfere with the imprinting and, thus, lead to a decrease in selectivity.

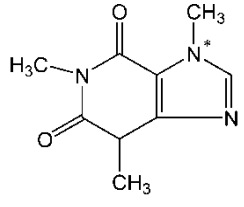
The monolithic molecularly imprinted technology represents a novel method for preparation of stationary phases.[13] These monoliths were prepared by a simple, one step, in-situ, free-radical polymerization "molding" process directly within the chromatographic column, without the tedious procedures of the grinding, sieving, and column packing. The monolithic molecularly imprinted technology has attracted significant interest because of their ease of preparation, high selectivity and sensitivity, high reproducibility, and rapid mass transport.[14–17] These features allow for an efficient and fast separation of especially large biomolecules. However, the prepared MIP often suffers from high back pressures and low efficiency, which results in their poor application and practical separation. Moreover, there is still a distinct lack of systematic investigation of fabrication of a monolithic MIP column. In addition, new prepared procedures need to be developed for various different materials due to their special structure.

This work describes the preparation of the monolithic MIP column with a specific recognition for theophylline by in-situ thermal polymerization. Separation characteristics such as template molecule, mobile phase composition, flow rate, and temperature on the retention and separations were investigated, and separation mechanism was also discussed. This method is very simple compared with the conventional procedure and its macroporous structure has excellent separation properties.

EXPERIMENTAL

Chemicals

Caffeine and theophylline were obtained from Sigma (ST Louis, MO, USA). The structures of these molecules are shown in Figure 1. Acrylamide (AM) from Duksan Pure Chemical Co., Inc.(Korea) was distilled prior to use. Ethylene glycol dimethacrylate (EDMA) from Tokyo Kasei Kogyo Co., LTD (Tokyo, Japan) was extracted with $2\,\mathrm{mol}\cdot\mathrm{L}^{-1}$ NaOH solution and water and dried over anhydroxide magnesium sulfate. α,α'-Azobis (isobutyronitrile) (AIBN) was the product of Junsei Chemical Co., Ltd. (Japan) and was recrystallized prior to use. Toluene was purchased from Oriental Chemical Industries (Japan). Dodecyl alcohol, acetonitrile, chloroform, and methanol are all of HPLC grade and 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 an 0.45 μm filter membrane before use.



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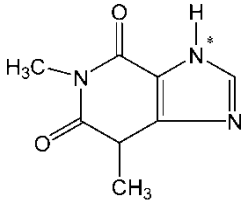

(2)

Figure 1. Molecular structure of caffeine (1) and theophylline (2).

Preparation of Monolithic MIP Column

The stationary phase was directly prepared by in-situ polymerization within the confines of a stainless steel chromatographic column (100 mm × 3.2 mm I.D.). The schematic illustrations of the imprint formation and molecular recognition processes were shown in Figure 2. The optimized polymerization mixture composed of an 0.25 mmol template molecule, 1.2 mmol acrylamide, 4.8 mmol ethylene glycol dimethacrylate (EDMA), and 0.021 g α,α'-Azobis (isobutyronitrile) (AIBN) was dissolved in the porogenic solvents (0.60 mL toluene and 1.68 mL dodecanol). The mixed solution was sonicated for 10 min and sparged with helium for 5 min to remove oxygen. The stainless steel tube, sealed at the bottom, was filled with the above polymerized mixture and then sealed at the top. The polymerization was performed in a water bath with the temperature maintained at 45°C for 12 h. After the polymerization, the seals were removed; the column was connected to HPLC pump and washed with tetrahydrofuran and methanol/acetic acid (80 : 20% v/v), respectively, to remove the porogenic solvents and the template molecules until a stable baseline was achieved. A non imprinted blank monolithic column (without addition of the template molecule) was prepared and treated in an identical manner.

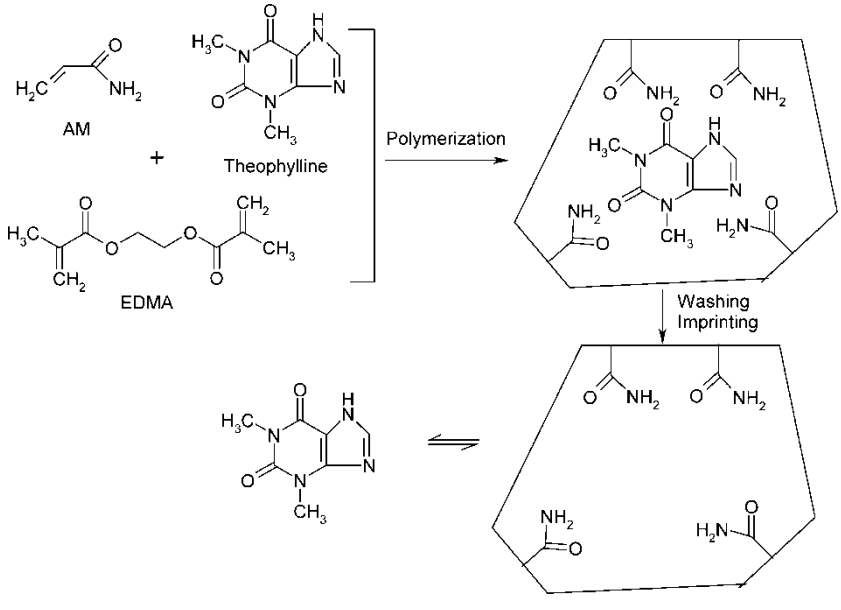

Figure 2. Schematic illustrations of the imprint formation and molecular recognition processes.

High Performance Liquid Chromatography

Separation characteristics of the monolithic MIP column were analyzed by a liquid chromatography system containing Waters 600s Multisolvent Delivery System and a Waters 616 pump (Waters, Milford, MA, USA), Waters 2487 Dual Absorbance UV detector (Waters, Milford, MA, USA), and Rheodyne injection valve (20 μL sample loop). The Millennium 3.2 software (Waters, Milford, MA, USA) was used as the data acquisition system. Acetonitrile was used as mobile phase, UV wavelength at 270 nm.

The separation factor (α) was determined by the following equation:

$$\alpha = k_2/k_1 \tag{1}$$

Where k_2 is the retention factor of the theophylline and k_1 is the retention factor of the caffeine. The retention factor was determined by:

$$k = (t_M - t_0)/t_0 \tag{2}$$

Where t_M is the retention time of the solute and t_0 is void time of the column. All the procedures were carried out at room temperature.

Characterization of Monolithic Molecularly Imprinted Polymer

After the chromatographic experiments had been completed, the column was washed with methanol/acetic acid (4 : 1 v/v) for 30 min. The column fitting was removed and the monolith polymer inside the column was pushed out of the tube using the pressure of the methanol mobile phase at a flow-rate of 4 mL/min. The cylindrical monolith was dried under 50°C for 12 h and cut into pieces with a razor blade. The polymer pore size and distribution were determined by nitrogen adsorption on a ASAP 2000 accelerated surface area and porosimetry system (micromeritics, USA), and the pore properties and microscopic analysis of the monolith was carried out in an S-4200 Scanning Electron Microscopy (Hitachi, Japan) at 3.0 kV.

RESULTS AND DISCUSSION

Morphological Characteristics of Theophylline Imprinted Monolith

Morphological analysis, including pore analysis and SEM analysis of the polymers were investigated in this experiment. It can be seen from the SEM image (Figure 3) that there are many macropores and flow through channels inlaid in the network skeleton of the theophylline imprinted monolith. These macropores and channels allowed mobile phase to flow through the monolith with low flow resistance, and thus, enables fast mass transfer of the solutes. The low backpressure allows their operation at higher flow

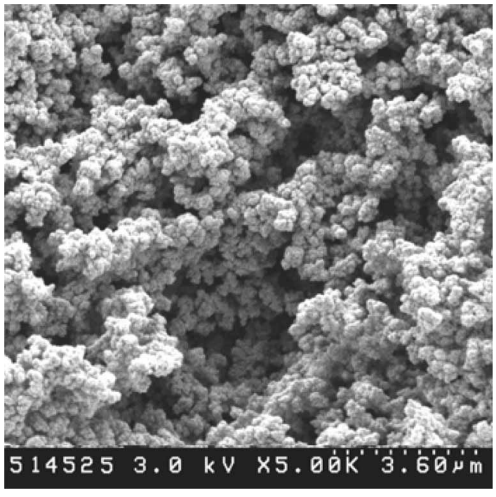

Figure 3. SEM of the monolithic MIP columns.

rates. The relationship of backpressure versus flow rate on the monolithic MIP showed that even at the high flow rate of 3.0 mL/min, the backpressure was only 7.85 MPa. In contrast, the backpressure of the packed column was relatively much higher over the whole range of flow rate, due to the irregular shape and non uniform sizes of packed particles. Nitrogen adsorption experiments were performed and the specific surface areas (A), specific pore volumes (V), and average pore diameter (dp) of theophylline-MIP were obtained. Results indicate the specific surface areas; specific pore volumes and average pore diameter were 218 m^2/g, 0.509 cm^3/g and 130 nm for the monolithic MIP.

The good morphologic properties for the monolithic MIP were more likely originated from the polymerization process. There are at least three factors that should be taken into account: the polymerization temperature, the composition of the prepolymerized solution, and the porogenic solvent. Temperature is regarded as the most convenient variable to adjust the pore size distribution of macroporous media, because it does not result in changes in the composition of the reaction mixture. It was believed that temperature could affect the polymer morphology in different ways, e.g., generating free radicals and forming cross linked nuclei, due to complexity of the phase separation. The relatively high temperature had a negative impact on the complex stability, which reduced the reproducibility of the monolithic stationary phases and produced high column pressure drops. Variations in the monomer/crosslinker ratio not only produce different porous structures but also lead to imprinted polymers with different compositions. A higher content of crosslinker results in more highly crosslinked MIP. Higher crosslinking favors the rigidity to preserve the structure of the cavity after

splitting off the template. The porogenic solvents also play an important role in the morphology of the molecularly imprinted monolith in terms of specific surface area and pore size. The porogenic solvents should produce large pores to assure good flow through properties of the resultant MIP, and it governs the strength of non-covalent interactions in addition to its influence on the polymer morphology.

Evaluation of the Monolithic Imprinted Polymer

Compared to a packed column, in which high efficiency and high speed are mutually exclusive, they do not permit the mobile phases to flow through the adsorbent with low flow resistance at high flow rates, the obvious advantage of monolithic columns is their porous, highly interconnected network structure, which supports the formation of a network of channels and provides the large surface area needed to achieve sufficient capacity. Compared with our previous work[18] of the bulk MIP, that shows broad and conjoint peaks of the caffeine and theophylline due to the low mass transfer originating from irregularly shaped and sized particles in the bulk MIP column, Figure 4 shows that baseline separation of theophylline and caffeine is achieved on monolithic MIP columns with more sharper and symmetrical peaks. At the same time, the continuous and porous structure of monolithic MIP allows for operation at higher flow rates with relatively low backpressures. The influence of flow rate on separation efficiency was measured in the range of 0.53 mL/min, and the results show that the dependency of separation efficiency on flow rate is small and the separation factor only changes from 4.35 to 4.07 when the flow rate change from 0.5 to 3 mL/min. These experiments indicate that the monolithic MIP has specific recognition for the theophylline molecule even at higher flow rates.

Influence of the Mobile Phase Composition on the Separation

The effect of mobile phase composition on the recognition properties was investigated using methanol, water, and acetonitrile as mobile phase. Caffeine and theophylline can't be separated if water is used as the mobile phase, and the best separation was obtained by using acetonitrile as the mobile phase. The effects of polar additives in the mobile phase were also evaluated with the mixtures of acetonitrile-acetic acid as the mobile phase. From Figure 5 we could see that with the increase of the solvent polarity in the mobile phase, the retention factors of caffeine and theophylline all decreased, the k_1 value of caffeine changes slightly, and the k_2 value of theophylline changes quickly with the changing of the proportion of the acetic acid. The retention factor of thephylline decreased from 3.52 to 1.34 with 4% (v/v) acetic acid in the mobile phase. When only acetonitrile was used as the mobile phase, the best retention factor was attained. The

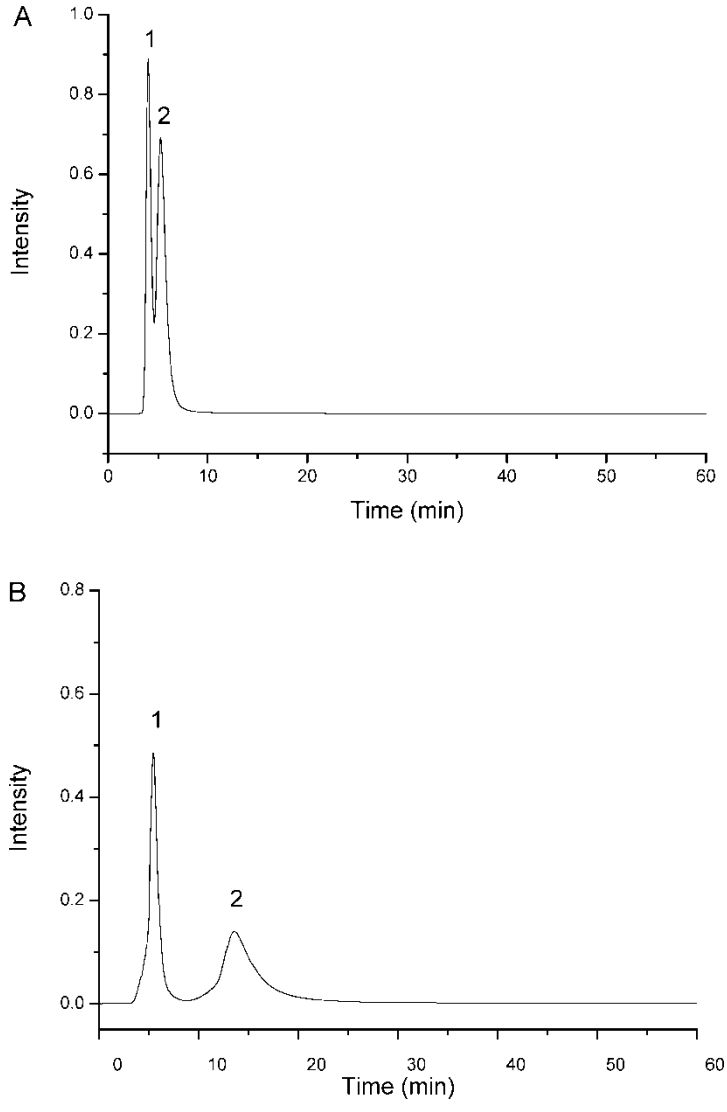

Figure 4. Chromatograms of caffeine and theophylline on monolithic MIP column and blank column. Determination condition: mobile phase: acetonitrile, flow rate: 0.7 mL/min, detection wavelength: 270 nm, peak 1: caffeine, peak 2: theophylline.

retention factor decreased with the increase of acetic acid proportion in the mobile phase, indicating that polar additives can interfere with the hydrogen bonds interactions between the matrix in MIP and the functional group of the analytes. The results imply that the hydrogen bonding interaction and hydrophobic interaction can play an important role in the retention and separation.

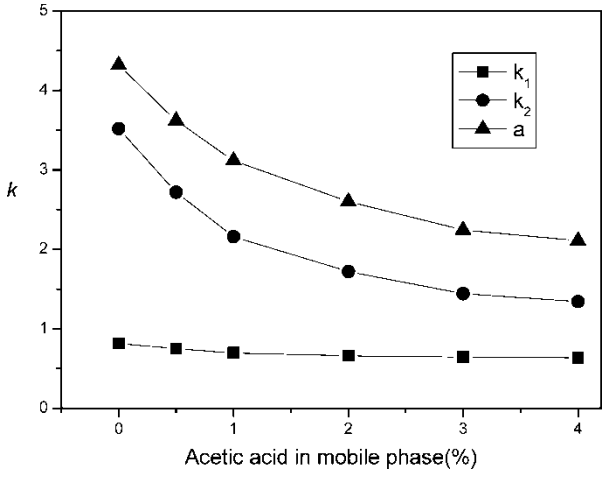

Figure 5. Effect of mobile phase composition on the retention factor. Determination condition: mobile phase: acetonitrile, flow rate: 0.7 mL/min, detection wavelength: 270 nm, k_1: the retention factor of caffeine, k_2: the retention factor of theophylline, a: separation factor of caffeine and theophylline.

Influence of Temperature on the Separation

The effects of different temperature changes from 20°C to 50°C on the separation were also investigated in this paper (Figure 6). The results showed that under higher temperature, the retention time of caffeine only slightly changed, but the retention time of theophylline changed much faster than the caffeine. The retention factor of theophylline decreased with increasing temperature because the adsorption of the analytes to the substrate weakened with increasing temperature, allowing the analytes to migrate faster through the monolithic column. This means the hydrogen bonding interaction and hydrophobic interaction between the template and polymer weakened with increasing temperature. Furthermore, the separation factors decreased with increasing elution temperature, due to higher temperature decreasing the interaction between the theophylline and the polymers more than the interaction between caffeine molecule and the polymers. Therefore, a lower temperature will lead to a higher sepatation.

Effect of Molecular Recognition

The imprinting process is commonly believed to result in the formation of shape complementary microcavities with defined spatial arrangement of functional groups. MIP can recognize the template molecule by the binding sites in

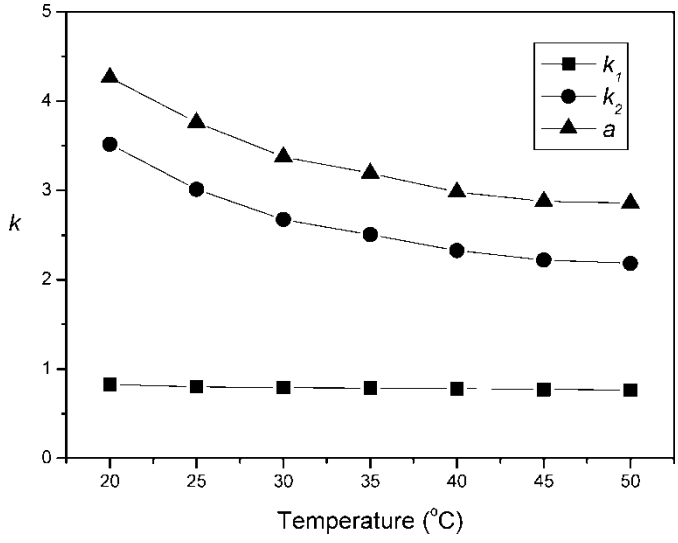

Figure 6. Effect of different temperatures on the separation factor and retention factor. Determination condition: mobile phase: acetonitrile, flow rate: 0.7 mL/min, detection wavelength: 270 nm, k_1: the retention factor of caffeine, k_2: the retention factor of theophylline, a: separation factor of caffeine and theophylline.

the cavities. Everything which affects the formation and stability micro-cavities will influence the molecular recognition. The main affects include:

1. Stability of the monomer template assembles. The functional monomers must strongly interact with the template prior to and during polymerization to achieve a high yield of imprinted binding sites. The templates offering multiple site of interaction for the functional monomer are likely to yield binding sites of higher specificity and affinity for the template. Moreover, the more monomer template rigidity, the more recognition ability could be obtained. From the structure of caffeine and theophylline, we could see that active hydrogen existed on N* in theophylline and the amino group in theophylline could form a hydrogen bond by the active hydrogen on N* with the monomer and produce specific sites. Because the amide group has stronger hydrogen binding ability than that of methacrylic acid in polar solvents, acrylamide was chosen as the functional monomer.
2. Site stability, integrity, and accessibility: For the formation of defined recognition sites, the structural integrity of the monomer template assemblies has to be preserved during polymerization to allow the functional groups to be fixed in space in a stable arrangement complementary to the template. This is achieved by the use of a high level of crosslinking. Higher crosslinking can favor the rigidity to preserve the structure of the cavity after splitting off the template.

3. Medium dependence in the rebinding to MIP: The rebinding to MIP is strongly dependent on the medium. For predictions of the optimum medium for rebinding, factors related to template structure, as well as to polymer structure and morphology, have to be considered. In this experiment, the best separation was obtained by using acetonitrile as mobile phase. Acetonitrile is a suitable mobile phase solvent, presumably due to its weak hydrogen bonding capacity and, thus, limited ability to compete for the hydrogen bonding sites on the template or the binding sites. Furthermore, it solvates the polymer backbone well and it is polar enough to dissolve a large number of compounds.

CONCLUSION

Theophylline monolithic MIP was successfully prepared by using in-situ therm polymerization and specific recognition ability for theophylline was obtained. The dependency of separation efficiency on the flow rate is extremely small and hydrogen bonding interaction plays an important role in the retention and separation. Moreover, effects of molecular recognition conditions were also discussed. The study results presented here have substantiated the significant research interest in monolithic MIP columns, compared with conventional particle columns and bulk MIP columns due to their ease of preparation, high separation efficiency, and rapid mass transport.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support of the center for Advanced Bioseparation Technology, Inha University.

REFERENCES

1. Owens, P.K.; Karlsson, L.; Lutz, E.S.M.; Andersson, L.I. Molecular Imprinting for Bio- and Pharmaceutical Analysis. Trends Anal. Chem. **1999**, *18*, 146–154.
2. Vlatakis, G.; Andersson, L.I.; Miller, R.; Mosbach, K. Drug assay using antibody mimics made by molecular imprinting. Nature **1993**, *361*, 645–647.
3. Peter, S.; Schweitz, L.; Nilsson, S. Molecularly imprinted polymers in capillary electrochromatography: recent developments and future trends. Electrophoresis **2003**, *24*, 3892–3899.
4. Huang, X.D.; Zou, H.F.; Chen, X.M.; Luo, Q.Z.; Kong, L. Molecularly imprinted monolithic stationary phases for liquid chromatographic separation of enantiomers and diastereomers. J. Chromatogr. A. **2003**, *984*, 273–282.
5. Matsui, J.; Nicholls, I.A.; Takeuchi, T. Molecular recognition in cinchona alkaloid molecular imprinted polymer rods. Anal. Chim. Acta. **1998**, *365*, 89–93.

6. Sellergren, B. Polymer and template-related factors influencing the efficiency in molecularly imprinted solid-phase extractions. Trends Anal. Chem. **1999**, *18*, 164–174.
7. Kriz, O.; Ramstrom, O.; Mosbach, K. Molecular imprinting: new possibilities for sensor technology. Anal. Chem. **1997**, *69*, 345A–349A.
8. Yoshikawa, M.; Fujisawa, T.; Izumi, J.; Kitao, T.; Sakamoto, S. Molecularly imprinted polymeric membranes involving tetrapeptide EQKL derivatives as chiral-recognition sites toward amino acids. Anal. Chim. Acta **1998**, *365*, 59–67.
9. Sellergren, B.; Shea, K.J. Origin of peak asymmetry and the effect of temperature on solute retention in enantiomer separations on imprinted chiral stationary phases. J. Chromatogr. A **1995**, *690*, 29–39.
10. Mayees, A.G.; Mosbach, K. Molecularly Imprinted Polymer Beads: Suspension Polymerization Using a Liquid Perfluorocarbon as the Dispersing Phase. Anal. Chem. **1996**, *68*, 3769–3774.
11. Hosoya, K.; Yoshizako, K.; Shirasu, Y.; Kimata, K.; Araki, T.; Tanaka, N.; Haginaka, J. Molecularly imprinted uniform-size polymer-based stationary phase for high-performance liquid chromatography, Structural contribution of cross-linked polymer network on specific molecular recognition. J. Chromatogr. A **1996**, *728*, 139–147.
12. Haginaka, J.; Kagawa, C. Uniformly sized molecularly imprinted polymer for d-chlorpheniramine. Evaluation of retention and molecular recognition properties in an aqueous mobile phase. J. Chromatogr. A **2002**, *948*, 77–84.
13. Liu, H.Y.; Row, K.H.; Yang, G.L. Monolithic Molecularly Imprinted Columns for Chromatographic Separation. Chromatogr. **2005**, *61*, 429–432.
14. Yan, H.Y.; Jin, L.M.; Row, K.H. Special Selectivity of Molecular Imprinted Monolithic Stationary Phase. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 3147–3155.
15. Liu, Z.S.; Xu, Y.L.; Yan, C.; Gao, R.Y. Preparation and characterization of molecularly imprinted monolithic column based on 4-hydroxybenzoic acid for the molecular recognition in capillary electrochromatography. Anal. Chim. Acta **2004**, *523*, 243–250.
16. Huang, X.D.; Qin, F.; Chen, X.M.; Liu, Y.Q.; Zou, H.F. Short columns with molecularly imprinted monolithic stationary phases for rapid separation of diastereomers and enantiomers. J. Chromatogr. B **2004**, *804*, 13–18.
17. Yin, J.F.; Yang, G.L.; Chen, Y. Rapid and efficient chiral separation of nateglinide and its L-enantiomer on monolithic molecularly imprinted polymers. J. Chromatogr. A **2005**, *1090*, 68–75.
18. Wang, D.X.; Hong, S.P.; Row, K.H. Chromatographic Separation of Xanthine Derivatives on Single and Mixed-Template Imprinted Polymers. Bull. Korean Chem. Soc. **2004**, *25*, 357–360.

Received December 24, 2005
Accepted January 26, 2006
Manuscript 6806