Studies on the Preparation and Properties of Inorganic Molecularly Imprinted Polymer (MIP) Based on Tetraethoxysilane and Silane Coupling Agents

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ABSTRACT: Inorganic molecularly imprinted polymer (MIP) based on tetraethoxysilane (TEOS), methyl triethoxysilane (MTEOS), and phenyl triethoxysilane (PTEOS) by sol-gel process has been developed. The MIP's preparation conditions, H_2O/Si molar ratios (R), template removal procedures, calcination temperatures, and quantity of ammonium hydroxide were investigated. The competition experiments of the MIP for template (caffeine, CAF) and analogue (theophylline, TH) were analyzed by high-performance liquid chromatography (HPLC). The results showed that the Ad_{CAF} decreased with an increase of the H_2O/Si molar ratios, but the selectivity (α) increased with an increase of the H_2O/Si molar ratios in the MIP. In addition, in a comparison of the procedures for removing the template, calcination obtained better efficiency and higher selectivity than extraction. The optimum adsorption and selectivity of MIP were obtained with $R = 10$ and the template was removed by calcination at 600°C. Moreover, the selectivity of the MIP (283.9) was greater than the nonimprinted polymer (2.45) under optimum preparation conditions. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 3994–3999, 2009

Key words: molecular imprinting; molecular recognition; high performance liquid chromatography; adsorption; selectivity

INTRODUCTION

Molecular recognition is a phenomenon that can be visualized as the preferential binding of a molecule to a "receptor" with high selectivity over its close structural analogues. This concept has been translated elegantly into the technology of molecular imprinting, which allows specific recognition sites to be formed in synthetic polymers through the use of various templates.[1–3] Molecular imprinting of synthetic polymers with a target molecule can be done if the target presents as a template or imprint molecule during the polymerization. The monomers carry certain functional groups, which can interact with one another as well as be arranged around the template, "frozen" into position by polymerization with a high degree of cross-linking in the presence of a porogenic molecularly imprinted polymer (MIP).[4,5] Imprinting may be achieved by three methods: noncovalent, sacrificial spacer, and covalent. Noncovalent imprinting relies on the ability of the template molecule to produce one or more strong intermolecular noncovalent interactions with the functional monomer (e.g., H-bonding, electrostatic or π–π interactions). Removal of the template results in a cavity complementary in size, shape, and functionality to the template molecule. In covalent imprinting, a prepolymerization template-monomer covalent bond is formed.

The technique of molecular imprinting, which may be traced back to the early 1970s, has received much attention in recent years.[6] Tangbin et al.[7] prepared the MIPs which used theophylline as template and methylacrylic acid as functional monomer by self-assembly. They discussed the monomer-template ratio to study the effects on the selectivity, and the MIP separated the theophylline and caffeine successfully. Kyung et al.[8] developed the biodegradable MIPs based on poly(ε-caprolactone). The theophylline-imprinted polymer showed higher binding capacity for theophylline compared with the nonimprinted polymer, and also showed selectivity for theophylline over caffeine. Keith et al.[9] used methacrylic acid or 2-vinylpyridine to prepare MIPs which for solid phase extraction (SPE). The SPE column was successfully applied for the quantitative determination of caffeine in a soft drink (Red Bull™). Hongyuan et al.[10] prepared the monolithic MIP column by a simple, *in situ* free-radical polymerization "molding" process directly within the

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chromatographic column. The results showed that the theophylline-imprinted monolithic column has high selectivity to theophylline (analog was caffeine). Molecular imprinted polymers have been used as chromatographic stationary phase, artificial antibodies, catalysts, sensors and drug assay tools.[11–17]

Many common MIPs are synthesized by organic methods, involving the polymerization of functional monomers and a cross-linking monomer such as acrylamide, acrylicacid, and ethylene glycol dimethacrylate. In this study, our laboratory synthesizes a novel inorganic MIP by the sol-gel process. Sol-gel process is a technique wherein inorganic (siloxane) based polymers can be formed through acid or base-catalyzed hydrolysis and the condensation of a series of silane monomers. The process can produce extremely versatile materials and has a wide range of application in analytical chemistry.[18–20]

In this study, the template—caffeine (1,3,7-trimethylxanthine)—is a natural alkaloid exerting many physiological effects such as stimulation of the central nervous system, diuresis, and gastric acid secretion. It is widely distributed in plant products and beverages and its quantification is mainly of pharmaceutical and alimentary concern.[21]

The main propose of this article is to prepare a series of the MIPs based on tetraethoxysilane (TEOS) and silane coupling agents and to investigate systemically their competition experiments of the MIP for template and analogue. In addition, the effects of H_2O/Si molar ratios, removing template ways, and NH_4OH amounts on the MIP were also studied.

EXPERIMENTAL

Materials

TEOS (Shin-Etsu Chemical, Tokyo, Japan), Methyl triethoxysilane (MTEOS) (Shin-Etsu Chemical), and Phenyl triethoxysilane (PTEOS) (Lancaster Synthesis, Lancashire, UK) as monomers were used as received. Caffeine (Lancaster Synthesis) as a template was used as received. Theophylline (SIGMA Chemical, St. Louis, MO) as a structure analogous molecule was used as received. Ammonium hydroxide (TEDIA, Fairfield) as an auxiliary agent of reaction was used as received.

Preparation of inorganic MIP

The 0.002 mol caffeine was dissolved completely with various deionized water and the solution was stirred at room temperature. To this solution, 0.236 mol TEOS, 0.006 mol MTEOS, and 0.008 mol PTEOS were added. This precursor was stirred at room temperature for 1 day. The pH was then adjusted using various ammonium hydroxides and reacted for 4 days. Then, the monoliths were ground and sieved to obtain particles sized between 38 and 53 μm. The template molecule was removed by calcination with various temperatures or extraction with methanol and the MIPs were purged with deionized water. A nonimprinted polymer (NIP) was prepared in parallel and under identical conditions but in the absence of the template.

High-performance liquid chromatography (HPLC) analysis of MIP

Both the caffeine and the structurally analogous molecule (theophylline) were dissolved in water to produce a standard solution which contained 100 ppm of caffeine and theophylline respectively. The 200 mg MIP was added in 10 mL standard solution and adsorbed at room temperature for 30 min to complete the adsorption. Then, the MIP solution mixtures were centrifuged to obtain the residual solution. The above solution was injected into high-performance liquid chromatography (HPLC) to investigate the residual concentration of caffeine and theophylline. The adsorption and selectivity (α) of MIP were determined by competition adsorption experiment; the quantity of absorption (Ad_{CAF} and Ad_{TH}) of MIP was obtained by the difference between the original concentration (100 ppm) and the residual concentration. The selectivity (α) was obtained by comparison the adsorption of the template (Ad_{CAF}) and analogue (Ad_{TH}) on MIP. The selectivity was defined as $\alpha = Ad_{CAF}/Ad_{TH}$. HPLC analysis was performed by a Waters 510 HPLC pump equipped with a SFD UV/vis Detector S32109 (273 nm). Samples were analyzed on a 150 mm $\times$ 4.6 mm 5_ Hypersil HS C18 column at room temperature with a flow rate of 1.0 mL/min (mobile phase, water:methanol:acetic acid = 69 : 28 : 3, v/v).

Accelerated surface area and porosimetry (ASAP)

BET surface area and pore structure characteristics were determined by a nitrogen adsorption–desorption isotherm used for analysis. Porosity measurements were performed with a Micromeritics ASAP 2010 analyzer. All samples were degassed at 120°C for 24 h before measurement. BET surface area and pore parameters were measured with a 55-point full analysis at cryogenic temperature (77.35 K). The micropore surface area and micropore pore volume were obtained by *t*-plot analysis with the Harkins–Jura equation.

Fourier transform infrared spectroscopy (FTIR) analysis of MIP

Fourier transform infrared spectra were recorded from pressed KBr pellets containing ~1% of the MIP

TABLE I
Preparation Conditions of the Inorganic Molecularly Imprinted Polymer

Sample codes	TEOS (mole)	MTEOS (mole)	PTEOS (mole)	H_2O (mole)	Caffeine (mole)
MR5	0.236	0.006	0.008	1.25	0.002
MR8	0.236	0.006	0.008	2.0	0.002
MR10	0.236	0.006	0.008	2.5	0.002
NR10[a]	0.236	0.006	0.008	2.5	0

[a] NR10: nonimprinted polymer, without the caffeine as template.

and NIP, respectively, using a JASCO FT/IR-410 spectrophotomer (Cork, Ireland).

Thermogravimetric analysis (TGA) of MIP

The thermal property was examined by a Perkin–Elmer TGA-7 apparatus (MA) at a temperature ranging from 50 to 900°C in air.

RESULTS AND DISCUSSION

Characterizations of the inorganic MIP

The preparation conditions of the MIP with various feed compositions are shown in Table I. MR5, MR8, and MR10 represent 5, 8, and 10 of the H2O/Si molar ratios in the sol-gel mixture, respectively. NR10 represents the nonimprinted polymer. The sol–gel process with many hydroxyl groups was produced after the hydrolysis of silicon alkoxide. These hydroxyl groups promoted the interaction (hydrogen-bond) between the imprinted molecule and the sol-gel matrix. Moreover, the PTEOS contained an aromatic ring facilitation and formed a possible π–π interaction with the template. During the preparation of MIP, the template (caffeine) was incorporated into an inorganic matrix. The imprinted cavities and specific recognition sites of MIP were then formed after the template was removed.

Effect of the H_2O/Si molar ratios(*R*) on MIP

The fundamental properties of the MIP with various H_2O/Si molar ratios (*R*) are shown in Table II. The results indicated that the competition experiment of the MIP for adsorption of template (Ad_{CAF}) and analogue (Ad_{TH}) decreased with an increase of the *R*, but the selectivity ($\alpha = Ad_{CAF}/Ad_{TH}$) increased with an increase of the *R* in the MIP. The BET surface area of MIP showed that the MR5 (668.3 m^2/g) was greater than MR10 (504.7 m^2/g). Moreover, the micropore volume of MIP showed that the MR5 (0.068 m^2/g) was greater than MR10 (0.014 m^2/g). The average pore diameter also increased with an increase of the *R* in the MIP. These results showed that the MIP had lesser water content (H_2O/Si molar ratios) to cause higher Ad_{CAF}, and greater BET surface area during the competition experiment and nitrogen adsorption–desorption isotherms. This was due to the quantity of water played an important role in the sol-gel process. The water did not only hydrolyze the reactant, but was likewise a product in the condensation reaction and played the third role of porogen. A greater quantity of water will result in bigger pores and a porous material with a looser structure, but it may cause the structure to collapse and reduce the volume of pores in the MIP. On the other hand, the molecule size of the caffeine was about 1 nm. The template cannot enter the specific recognition sites when the pore size inside the MIP is smaller than 1 nm, hence the small amount of micropore (pore size <2 nm) will increase the opportunity for recognition sites to bind with the template. Therefore, a greater BET surface area was obtained by the higher adsorption and a smaller micropore volume was obtained by the higher selectivity. Furthermore, it was proven that a greater quantity of water will result in a bigger average pore diameter.

In addition, a comparison of the imprinted (MR10) and nonimprinted (NR10) polymers showed that the adsorption of the MR10 was smaller than the NR10, but the selectivity of the MR10 was greater than the

TABLE II
Fundamental Properties of MIP Prepared by Various *R* Ratios

Sample codes	Ad_{CAF} (μmol/g)	Ad_{TH} (μmol/g)	α	BET surface area (m^2/g)	Micropore volume (cm^3/g)	Average pore diameter (nm)
MR5	2.67	0.69	3.87	688.34	0.068	2.16
MR8	2.12	0.42	5.09	610.32	0.030	2.32
MR10	1.88	0.30	6.23	574.85	0.014	2.66
NR10	13.68	8.62	1.59	723.12	0.078	1.97

In this table samples removed template by calcination at 600°C.

TABLE III
Fundamental Properties of MIP Removed Template by Extraction or Calcinations at 600°C

Sample codes	Removal method	Ad_{CAF} (μmol/g)	α	BET surface Area (m^2/g)	Micropore volume (cm^3/g)	Ave. pore diameter (nm)
MR10-N	Nonremoval	n.d.	n.d.	717.89	0.041	3.20
MR10-E	Extraction	10.86	1.96	879.90	0.039	3.80
MR10-C	Calcination	1.88	6.23	574.85	0.014	2.66

n.d., no data.

NR10. This was because the NR10 provided a larger BET surface area; obviously, a larger surface area results in a higher adsorption. At the same time, the adsorption of the MIP was rather small; even so, the selectivity of MIP was greater than the NIP. This result indicated that the MIP had the recognition ability.

Effect of the diverse ways of removing the template

The fundamental properties of the MIP removed template by extraction or calcination are shown in Table III. The diverse ways of removing the template were carried out on the MR10 sample. MR10-N, MR10-E, and MR10-C represent the template nonremoved, extraction, and calcination of MIP by diverse ways, respectively. The results shown in Table III reveal that the MIP's Ad_{CAF} in MR10-E (10.86 μmol/g) was greater than that of MR10-C (1.88 μmol/g). Furthermore, the MIP's α in MR10-E (1.96) was smaller than that of MR10-C (6.23). Additionally, the BET surface area of MIP were showed in the order of MR10-E > MR10-N > MR10-C. These results indicated that the higher Ad_{CAF} through its removal by extraction; furthermore, the MR10-N without removing the template could not adsorb the template. According to the above sections, it could be found that the smaller micropore volume could raise the α. The average pore diameter of MIP obtained by calcination (2.66 nm) was smaller than that by extraction (3.08 nm). This was due to hydroxyl groups continuing its condensation reaction while calcination occurs about 200°C. In addition, the methyl groups of MTEOS will continue to decompose because it will become hydroxyl groups, and react with each other during calcination. Through these processes, it is believed that removing the template by calcination will easily form a dense network structure (smaller pore diameter) of the MIP. Similar research in our laboratory has been observed.[22]

In order to investigate this hypothesis, the FTIR spectra of the nonremoved template, extraction, and calcination are shown in Figure 1, respectively. Almost similar FTIR spectra were exhibited for the nonremoved and the extraction. This result indicated that the functional groups of MIP structure were not significantly affected by the extraction process. For calcination, characteristic absorption peaks of $Si{-}CH_3$ at 780–880 cm^{-1} disappeared. This result indicated that the methyl groups will continue to decompose because it will become hydroxyl groups at 400°C. Therefore, the template that was removed by calcination obtained high selectivity even though the Ad_{CAF} was sacrificed. Consequently, the contention has been proved significant by the above accounts.

Effect of the calcination temperatures on MIP

Based on the results and descriptions in the above sections, greater efficiency and high selectivity of MIP by calcination were obtained. The results of removing the template by various calcination temperatures are shown in Table IV. The results indicated that the competition experiment of the MIP for Ad_{CAF} and Ad_{TH} was decreased with an increase of the calcination temperature, but the α was increased with an increase of the calcination temperature in the MIP, especially at 600°C.

The TGA diagram of the nonimprinted polymer is shown in Figure 2. The weight of the template

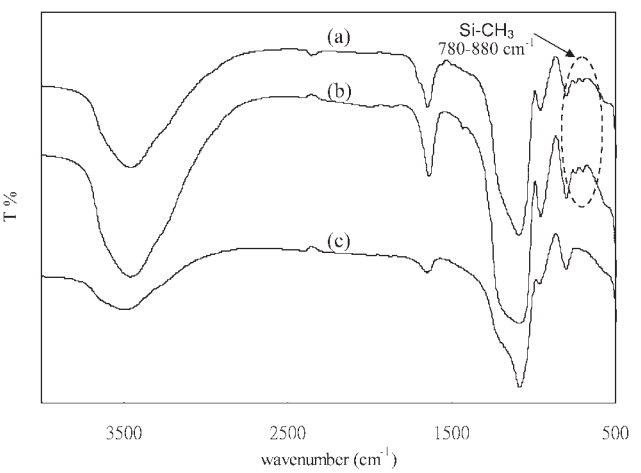

Figure 1 The FTIR spectra of the MIP removed template by nonremoval (a), extraction (b), and calcination at 600°C (c).

TABLE IV
Characterizations of MIP Removed Template by Various Calcination Temperatures

Sample codes	Calcination temperatures (°C)	Ad_{CAF} (μmol/g)	Ad_{TH} (μmol/g)	α
WR5		7.53	2.86	2.64
WR8	400	10.18	4.20	2.42
WR10		13.25	6.05	2.19
WR5		7.78	3.08	2.52
WR8	500	7.57	2.85	2.65
WR10		4.32	1.61	2.68
WR5		2.67	0.69	3.87
WR8	600	2.12	0.42	5.09
WR10		1.88	0.30	6.23

included in the imprinted matrices (0.81 wt %) was very small; therefore, the TGA diagrams of the imprinted (MIP) and nonimprinted (NIP) polymers were almost the same and overlapping. The TGA diagram of NIP which we only showed can be estimated clearly and to prove why we stopped the calcination at 600°C. In Figure 2, the results exhibite a weight loss of ~7% at 645°C. The aromatic ring of PTEOS was decomposed at 645°C, but the MIP networks still did not collapse at this temperature.[22] Therefore, aside from the hydrogen bond, the π–π interaction can also be used to bond with the template under 600°C. The MIP will therefore possess the recognition ability to improve selectivity. The optimum calcination temperature at 600°C was obtained as described in the above sections combined with this result.

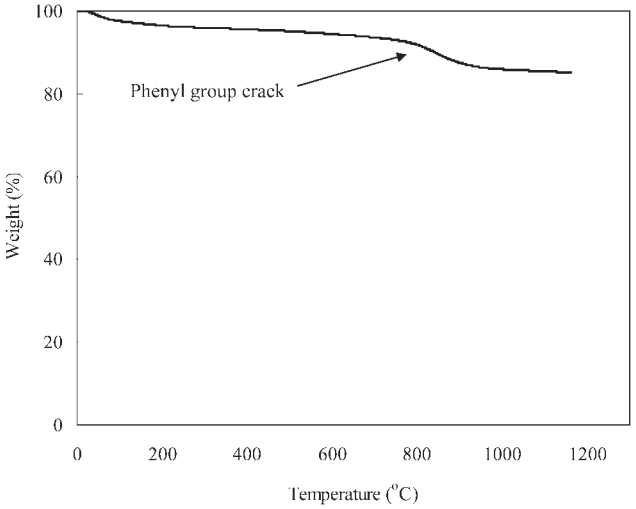

Figure 2 TGA diagram of nonimprinted polymer.

Effect of the quantity of ammonium hydroxide on MIP

In the sol-gel process, the rate of hydrolysis is fast compared with that of condensation in acidic (pH ≤ 2) conditions; however, in neutral or basic (pH ≥ 7) conditions, the rate of condensation is faster than that of hydrolysis. Under the weak acidic condition of pH = 4.2, the rates of hydrolysis and condensation are approximately equal.[23,24] During our study, ammonium hydroxide was used to control pH; therefore, the rate of hydrolysis and condensation was controlled. In this section, the conditions of preparation were fixed at $R = 10$ and the calcination temperature at 600°C. The fundamental properties of the MIP prepared with various quantities of ammonium hydroxide are shown in Table V. Ammonium hydroxide was added at 40, 80, 120, 160, and 200 μL, respectively, and dispersed in the sol-gel processes. MR10-40, MR10-80, MR10-120, MR10-160, and MR10-200 represent the 40, 80, 120, 160, and 200 μL of ammonium hydroxide dispersed in the MIP, respectively. The results shown in Table V indicate that the gelation time of MIP decreased with an increasing quantity of ammonium hydroxide. This was due to the rate of condensation being faster than that of hydrolysis under the basic (pH ≥ 7) conditions. Faster condensation means that the reaction rate was also faster. In this section, the larger quantity of ammonium hydroxide increased the condensation and pH values; hence, the reaction rate was raised which caused a faster gelation time. In other words, a decreasing gelation time implied an increasing rate of condensation; therefore, increasing pH resulted in an increasing reaction rate. In addition, the results show

TABLE V
Fundamental Properties of MIP Prepared by Various Quantities of Ammonium Hydroxide

Sample codes	Quantity of NH_4OH (μL)	pH (added NH_4OH)	Gelation time (min)	Ad_{CAF} (μmol/g)	Ad_{TH} (μmol/g)	α
MR10-40	40	4–5	900	2.84	0.01	283.9
MR10-80	80	5–6	300	3.72	0.63	5.87
MR10-120	120	6–7	30	3.92	1.29	3.04
MR10-160	160	7–8	10	4.50	2.29	1.97
MR10-200	200	8–9	1	4.98	2.42	2.06
NR10-40[a]	40	4–5	900	8.88	3.62	2.45

[a] NR10-40: nonimprinted polymer, without the caffeine as template.

that the α decreased with an increasing quantity of ammonium hydroxide. This was because the rate of condensation was so rapid that more nonhydrolysis $-OCH_2CH_3$ groups remained. Hence, the sol-gel processes could not react completely, forming an intact network structure which could not provide the recognition sites on the MIP. Based on the results of the above descriptions, the optimum rates of hydrolysis and condensation with the highest selectivity were obtained by the quantity of ammonium hydroxide at 40 μL.

Moreover, a nonimprinted polymer (NR10-40) was prepared in parallel and under optimum conditions. Comparison of the MIP (MR10-40) and NIP (NR10-40) showed that the α of MIP (283.9) was greater than the NIP (2.45). This result indicated that the MIP possessed remarkable recognition ability under optimum conditions.

CONCLUSIONS

The experimental results showed that MIP were successfully prepared in this research. The results indicated that adding a suitable amount of water could effectively increase selectivity of the MIP. The template removed by calcination at 600°C obtained greater efficiency and high selectivity. In addition, the rate of hydrolysis or condensation was significantly affected by adjusting the pH appropriately. Consequently, the most significant results were observed through a comparison of the imprinted MIP prepared under optimum conditions and the NIP, which showed that the selectivity of the molecular imprinted polymer was greater than the selectivity of the nonimprinted polymer.

References

1. Perez-Moral, N.; Mayes, A. G. Anal Chim Acta 2004, 504, 15.
2. Busi, E.; Basosi R.; Ponticelli, F.; Olivucci, M. J Mol Catal A: Chem 2004, 217, 31.
3. Yan, H. Y.; Row, K. H. Int J Mol Sci 2006, 7, 155.
4. Rajinder, S. G.; Manuel, M.; Gustavo, L. Micropor Mesopor Mater 2005, 85, 129.
5. Marta, E. D.; Rosana B. L. Microchim Acta 2005, 149, 19.
6. Haginaka J. J Chromatogr B 2008, 866, 3.
7. Tangbin, L.; Xin, T.; Songjun, L. Polym Plast Technol Eng 2007, 46, 613.
8. Kyung, S. L.; Dae, S. K.; Beom, S. K. Biotechnol Bioprocess Eng 2007, 12, 152.
9. Keith, F.; Edmond, M.; Fiona, R. Anal Chim Acta 2006, 566, 60.
10. Hongyuan, Y.; Longmei, J.; Kyung, H. R. J Liq Chromatogr Relat Technol 2005, 28, 3147.
11. Haupt, K.; Mosbach K. Chem Rev 2000, 100, 2495.
12. Sun, H. W.; Qiao, F. X.; Liu, G. Y. J Chromatogr A 2006, 1134, 194.
13. Yin, J.; Yang, G.; Chen, Y. J Chromatogr A 2005, 1090, 68.
14. Martin, P. D.; Jones, G. R.; Stringer, F.; Wilson, I. D. Analyst 2003, 128, 345.
15. Bŕuggemann, O.; Visnjevski, A.; Burch, R.; Patel, P. Anal Chim Acta 2004, 504, 81.
16. Yan, H.; Row, K. H. Biotechnol Bioprocess Eng 2006, 11, 357.
17. Cunliffe, D.; Kirby, A.; Alexander, C. Adv Drug Deliv Rev 2005, 57, 1836.
18. Wayne C.; Patrick D.; Peter M. Anal Chim Acta 2005, 542, 52.
19. Collinson, M. M. Anal Chem 1999, 29, 289.
20. Cauli, M. M. Behav Pharmacol 2005, 16, 63.
21. Barbara, B.; Elio, D.; Paolo, C. Electroanal 2007, 19, 385.
22. Hung, S. W.; Yen, L. T.; Jia, Y. W.; Hui, C. J Chromatogr B, 2006, 836, 57.
23. Jokinen, M.; Gyorvary, E.; Rosenholm, J. B. Colloid Surface A 1998, 141, 205.
24. Siouffi, A. M. J Chromatogr A 2003, 1000, 801.