

Preparation of inorganic molecularly imprinted polymers with higher adsorption and selectivity by sol–gel method

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Received 30 August 2005; accepted 14 March 2006

Available online 18 April 2006

Abstract

Preparation of inorganic molecularly imprinted polymers (IMIPs) with higher adsorption and selectivity has been developed on caffeine as model compound by sol–gel processes. In our study, by introducing pore-forming agent, lactic acid, into sol–gel process, the porosity of IMIPs was enhanced and the performance of IMIPs was thus improved. And, by introducing base catalyst in the sol–gel process, large pore volume was obtainable, and the caffeine adsorption of IMIPs was increased. Competition adsorption experiments between caffeine (CAF) and structure analogous molecule, theophylline (TH), were determined by HPLC analysis. It was found that adding pore-forming agent method produced better caffeine adsorption (ca. 20 $\mu\text{mol/g}$) than by adding base catalyst. But adding base catalyst method was found to yield better selectivity (ca. 4) (Selectivity (α) is the ratio of CAF bound to TH bound.) In addition, caffeine adsorption of IMIPs with template removed by calcination is two times that by extraction without sacrificing the selectivity of IMIPs.

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Keywords: IMIPs; Inorganic molecularly imprinted polymers; Sol–gel; Template

1. Introduction

Molecular recognition is the basic concepts in nature. Pauling [1] was the first person to discuss the lock and key mechanism to explain molecular recognition. This conception has been translated into molecularly imprinted polymers. Polymeric materials with specific sites are producible through the use of various templates [2,3]. In the molecular imprinting process, functional monomers are either covalently [2] or non-covalently [4] interacted with a template, which is fixed into the polymeric matrix by polymerization. After removal of the template, the functional groups of the polymeric matrix can then rebind the same template or its analogue. Molecularly imprinted polymers have been successfully applied in many fields, such as analytical applications (stationary phase packing in HPLC column), separation (selectively adsorption of template) [5], also as artificial antibodies [6], recognition unit of sensor [7], and catalysis operations [8]. Because most investigations of imprinted polymers were

produced with pure organic polymers, there are very had been limited reports on inorganic molecularly imprinted polymers (IMIPs).

Sol–gels are mainly inorganic-based. The inorganic matrix is formed by sequential hydrolysis and condensation of metal alkoxides in aqueous acid or base with a mutual cosolvent. Sol–gels possess have several physical properties, as they exhibit porous, rigid, good optical and mechanical properties and chemically inert. Poljakow [9] first removed water from a silica gel in an atmosphere of benzene, xylene or toluene, respectively. In 1949 Dickey [10] observed that template, methyl orange, during the preparation of silica gel. It showed that, after extraction, the adsorption of methyl orange is more than a non-imprinted gel. Pinel et al. [11] used sol–gel chemistry to generate imprinted gel with (–)-menthol as template. They discovered that the adsorption showed no difference between imprinted and non-imprinted gel. Maier and co-workers [12] used sol–gel process to prepare new catalyst materials. In this research, they found that the amorphous microporous oxide remembered the kinetic diameter of the alcohol. In other words, it can be said to remember the shape of imprinted molecules. His investigation provided another door

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for molecular imprinting to enter into inorganic oxides. Bohnen [13] used micro-porous silica gel to adsorb selectively the imprinted molecule (–)–bornel. Katz and Davis [14] used aromatic rings with one, two or three 3-aminopropyltriethoxysilane side groups as template during the sol–gel polymerization process. When the aromatic cores was removed, the shape-selective catalytic material was produced. Marx and Liron [15] used acrylic and hybrid organic–inorganic sol–gels polymeric systems to prepare propranolol-imprinted polymers. Leung et al. [16] used 2,4-dichlorophenoxyacetic acid as template and 3-[*N,N*-bis(9-anthrylmethyl)amino]propyltriethoxysilane as function monomer. The result discovered selective respond to 2,4-dichlorophenoxyacetic acid. Finally, McLoughlin and co-workers [17] used a 3-monomer sol–gel system to imprint the template of lisinopril dihydrate. The material showed that the imprinted gel demonstrated enhanced selectivity over the non-imprinted gel.

As mentioned above, some studies have been made on IMIPs, but the above results did no touch on the influence of varying parameters of sol–gel process on the product, such as the ratio of water to silane, value of pH, and the amount of pore-forming agent. The purpose here is to explore a little further into IMIPs. IMIPs were synthesized on model compound, caffeine by adding pore-forming agent and base catalyst sol–gel processes, respectively. Influences of the above conditions on the structure of IMIPs are discussed. In addition, the template can be removed by extraction and calcination. Therefore, the influence on caffeine adsorption of IMIPs prepared by these two removing template methods is also reported.

2. Experimental

2.1. Materials

All materials were used as received from the manufacturer without pre-purification. Tetraethoxysilane (TEOS), ethanol (EtOH), and hydrochloric acid (HCl), were purchased from Merck. Ammonium hydroxide was purchased from TEDIA. Caffeine (the template) and Theophylline (structure analogous molecule) were purchased from Lancaster and SIGMA, respectively. Lactic acid was purchased from J.T.Baker.

2.2. Inorganic molecularly imprinted material synthesis

2.2.1. Adding pore-forming agent method

The pH of deionized water was adjusted using hydrochloric acid to 1.2. TEOS (20.8 g) and acidic water (7.2 g) was well mixed before added in alcohol (14.4 g) as co-solvent. The so-formed mixture was stirred in 60 °C water bath for 3 h to form the precursor. Template molecule caffeine was dissolved completely with or without lactic acid and the solution was stirred at room temperature. The precursor was then added into the solution. And allowed for reaction in 60 °C oven in closed system for 1 day, then followed by reaction in open system for 2 days. The monoliths obtained were ground and sieved to obtain particle of size between 38 and 53 μm. The template molecule was removed by calcination at 600 °C or extraction using methanol.

2.2.2. Adding base catalyst method

Caffeine was added into a mixture of TEOS and acidic water as prepared above and stirred for 3 h at room temperature. Ammonium solution is then added to adjust the pH. It was placed in the 60 °C oven for 1 day for closed system reaction. The monoliths were ground and sieved to obtain a particle size between 38 and 53 μm by sieving. The template molecule was removed via calcination at 600 °C.

2.3. Competition adsorption experiment

Selectivity of IMIP was obtained by competition adsorption experiment. Caffeine and structure analogous molecule (theophylline) were added into water to prepare the standard solution (100 ppm). Two hundred milligrams IMIP was added into 10 ml of the standard solution and stirred at room temperature for 30 min. Adequate solution was taken and injected in the HPLC and the concentration of caffeine and theophylline were recorded. It is found to be different from the standard solution. HPLC analysis was performed by Waters 510 HPLC pump equipped with a SFD UV/vis Detector S32109 (273 nm). Samples were analyzed on a 150 mm × 4.6 mm 5 μ 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).

2.4. Nitrogen adsorption–desorption isotherms

Porosity measurements were performed using a Micromeritics ASAP 2010 analyzer. All samples were degassed at 200 °C for 24 h before measurement. BET surface area and pore parameters were measured using 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.

3. Results and discussion

There is no argument on the point that a lot of parameters exist in preparation of IMIPs by sol–gel process. How to form porosity in IMIPs with self-assembly between an imprinted molecule and inorganic matrix is important? In general, in acidic sol–gel process a lot of hydroxyl groups were produced after hydrolysis of silicon alkoxide. These hydroxyl groups promoted the interaction between imprinted molecule and inorganic matrix. But in acidic catalyst, entangled linear chains were formed in sols and thus resulting dense network structure. Hereby porous network structure was achieved by using pore-forming agent or base catalyst while adsorption and selectivity of IMIPs were improved. In addition, another advantage of sol–gel process is that it can be carried out under mild condition [18]. During the preparation of IMIPs, the template, caffeine, was incorporated into inorganic matrix and the imprinted cavities and specific binding sites of hydroxyl group in a predetermined orientation were formed, after removing the caffeine. Most of us would accept that there are two methods to remove the imprinted molecule via calcination and extraction. Because inorganic matrix was produced via hydrolysis and condensation of metal alkoxides and

Table 1
Preparation conditions and characteristics of IMIPs prepared with varied amount of caffeine via sol–gel process

Run no.	Caffeine (g)	LAC (g)	Appearance
P-1	0		White
P-2	0.17		Black
P-3	0.34	0	Black
P-4	0.69		Black

the inorganic matrix was able to remain the same even under high temperature, not only extraction can be employed to remove imprinted molecule, but calcination can also be used. Therefore, the characterizations of IMIPs prepared by using extraction and calcination to remove template are also discussed.

3.1. IMIP prepared via adding pore-forming agent

The precursor is formed by mixing TEOS, H₂O and EtOH in ratio of TEOS:H₂O:EtOH = 1:4:4, and allow reaction at 60 °C for 3 h. The hydrolysis reaction replaces ethoxy groups of TEOS with hydroxyl groups. The hydrogen bonding could be formed between above hydroxyl groups and caffeine. The caffeine is fixed by non-covalent bond in the rigid inorganic matrix via condensation reaction in further sol–gel process. Preparation conditions and characteristics of IMIPs with varied amount of caffeine are shown in Table 1. Differences in appearance of IMIP was obtained after calcination at 600 °C. The appearance of P-1, which excluded caffeine as the template was white. However, the appearance of P-2, P-3 and P-4 that contained caffeine as the template were black. It is considered that caffeine cannot be completely removed in inorganic matrix by calcination. Hydrolysis of alkoxide is enhanced in acidic catalyst, forming entangled linear chains sols and thus producing dense structure in IMIPs. Zheng et al. [19] found that the porosity of inorganic matrix could be enhanced by introducing hydroxyacetic acid derivatives. Hence, lactic acid was used to improve the porosity of IMIPs. Preparation conditions and results of IMIPs prepared by introducing pore-forming agent, lactic acid, are tabulated in Table 2. It is observed that all samples of IMIPs exhibit white appearance, after calcination. By using lactic acid as pore-forming agent, not only the porosity of IMIPs is improved but the adsorption of caffeine is also promoted. The results also confirmed Zheng's investigation. In addition, we used P-5 to investigate the caffeine adsorption of IMIPs with different adsorption

Table 2
Preparation conditions and characteristics of IMIPs prepared with varied amount of caffeine in the present of lactic acid as pore-forming agent

Run no.	Caffeine (g)	LAC (g)	Appearance	CAF (μmol/g)	TH (μmol/g)	α
P-5	0			11.8	5.3	2.23
P-6	0.08			17.6	7.1	2.48
P-7	0.17			18.9	9.2	2.05
P-8	0.34	8.32	White	17.9	8.1	2.21
P-9	0.69			19.0	8.5	2.24
P-10	1.04			18.2	8.3	2.19

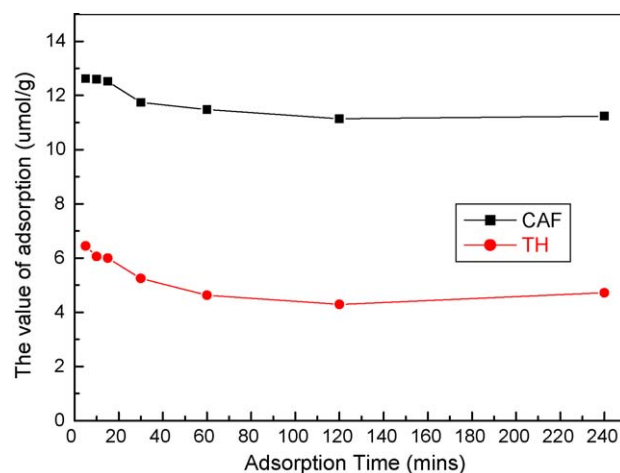


Fig. 1. The relationship between the value of adsorption and adsorption time of P-5 (200 mg P-5 was added into 10 ml standard solution).

times (Fig. 1). It showed that the values of caffeine and theophylline adsorption were kept constant when the adsorption time was greater than 30 min. Hence, the adsorption time was kept at 30 min for the binding experiments. But, the higher value of adsorption was obtained in the beginning. It is considered that the different kinetics of water and caffeine (or theophylline) uptake in the pore structure, therefore, in the first period the consumption of caffeine (or theophylline) from the solution by adsorption would be partially compensated by an increase in solution concentration due to preferential water uptake. In order to investigate the effect of introducing lactic acid in the system, quantity of lactic acid added is studied as tabulated in Table 3. When small amount of lactic acid is added in the system, appearance of the IMIP changed from black to yellow, after calcination. On the other hand, the appearance of IMIPs changed from yellow to white as the added quantity is increased in the system. In addition, caffeine adsorption of IMIPs is directly proportional to the amount of lactic acid added in the system. It is concluded that effect of introducing lactic acid as pore-forming agent can be achieved. On the other hand, as more lactic acid is added into the system as shown in P-16 that sudden decrease caffeine adsorption. It is likely that loose silica network is produced when large quantities of lactic acid is added. The silica network results in decrease of caffeine adsorption of P-16.

The template in IMIPs can be removed by extraction or calcination. In order to compare the difference of these methods, two

Table 3
Preparation conditions and characteristics of IMIPs prepared with varied amount of lactic acid as pore-forming agent

Run no.	Caffeine (g)	LAC (g)	Appearance	CAF (μmol/g)	TH (μmol/g)	α
P-11		0	Black	–	–	–
P-12		0.3	Yellow	–	–	–
P-13		2.58		8.1	3.6	2.25
P-14	0.34	5.42		13.6	5.6	2.43
P-8		8.32	White	17.9	8.1	2.21
P-15		16.6		20.6	10.1	2.04
P-16		25		15.5	6.4	2.42

Table 4
Characteristics of IMIPs prepared by different removing the template molecule method

Run no.	Method	CAF ($\mu\text{mol/g}$)	TH ($\mu\text{mol/g}$)	α	Micropore ^a	
					Surface area (m^2/g)	Volume (cm^3/g)
P-17	Extraction	8.3	4.0	2.08	47.60	0.018
P-9	Calcination	19.0	8.5	2.24	59.56	0.024

^a The micropore surface area and micropore volume was obtained by *t*-plot analysis.

methods of removing template were carried out on P-9 and P-17, respectively. The results are shown in Table 4. It indicates that caffeine adsorption of IMIPs prepared by calcination is more than that by extraction, without sacrificing the selectivity. In addition, the micropore surface area of P-9 was slight higher than P-17. We may say that the removal of template using calcination was much more efficiently and it resulted in higher caffeine adsorption. Hence, the more micropore fraction was obtained and the better template removal efficiency was observed. Similar results had been observed for polymeric imprinted materials [20]. The adsorption isotherms and pore size distributions of IMIPs prepared by different removing template methods is presented in Fig. 2. The results indicated that the characterization of the IMIP prepared by extraction (P-17) and calcination (P-

9) was almost the same (Fig. 2a and b). But, the BET surface area of IMIP using calcination ($333 \text{ m}^2/\text{g}$) was also slight higher than extraction ($325 \text{ m}^2/\text{g}$). On the other hands, pore size distribution of the IMIPs prepared by calcination is almost the same as that by extraction. It is considered that the inorganic matrix of IMIPs prepared by calcination was the same as that by extraction. In other words, the inorganic matrix prepared by calcination was not destroyed and influenced under high calcination temperature. In addition, the morphology of IMIPs prepared by different removing template methods was determined by scanning electron microscopy (SEM) (Fig. 3). It showed that IMIPs prepared by extraction (P-17) and calcination (P-9) produced smooth and rough surface of IMIP, respectively. The smooth surface of IMIP indicates that methanol cannot diffuse into the inner of IMIP to extract the template. Therefore, the inner template remained in inorganic matrix of IMIP. The rough surface, the similar value of selectivity and the appearance of IMIP indicate the template is completely removed by calcination without destroying the structure of IMIP. In addition, when P-17 had been immersed in aqueous solution for another 2 weeks, the concentration of caffeine in the solution is zero (data not shown). It indicated that the dense silica network of P-17 is not swollen so that the caffeine in P-17 would not diffuse into the aqueous solution.

3.2. IMIP prepared via adding base catalyst

In general, dense structure of IMIPs is obtained when it was prepared in acidic condition. It was found in the preceding section that porosity and caffeine adsorption of IMIPs is enhanced by addition lactic acid as pore-forming agent. Further, the selectivity of IMIP is around two in all preparation conditions with adding pore-forming agent. There is another method for preparing porous IMIPs. That is to adjust the pH value during sol-gel process. The template, caffeine, was dissolved in acidic water and then tetraethoxysilane was added to the above solution. After hydrolysis reaction, the non-covalent bonding (hydrogen bonding) was formed between caffeine and the hydroxyl groups. The condensation reaction was facilitated after adding a base catalyst and the shape of caffeine was captured in the gel. It is considered that adding base catalyst method easily forms loose network structure of IMIPs. Preparation conditions and characteristics of IMIPs with varying volume of water via adding base catalyst method are shown in Table 5. The appearance of IMIPs are white and more than $10 \mu\text{mol/g}$ caffeine adsorption of IMIPs are obtained when the $\text{H}_2\text{O}/\text{TEOS}$ (molar ratio) is greater than two. But in I-1, when $\text{H}_2\text{O}/\text{TEOS}$ is equal to two (molar ratio), the phase separation phenomena occur, that is to say, the

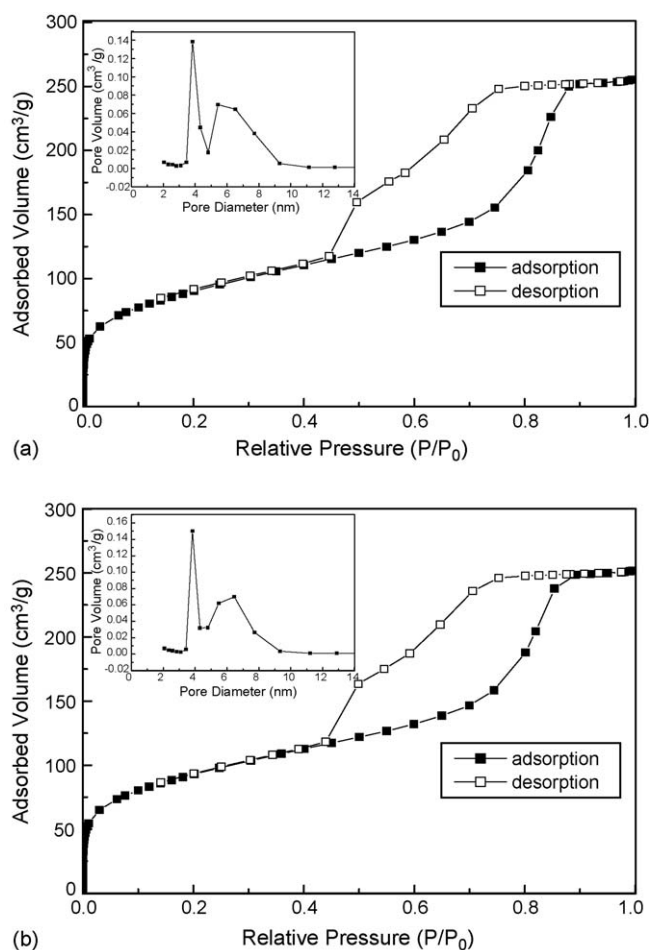


Fig. 2. N_2 adsorption-desorption isotherms and pore size distribution of the IMIPs prepared by different removing the template molecule method (a) extraction (P-17) and (b) calcination (P-9).

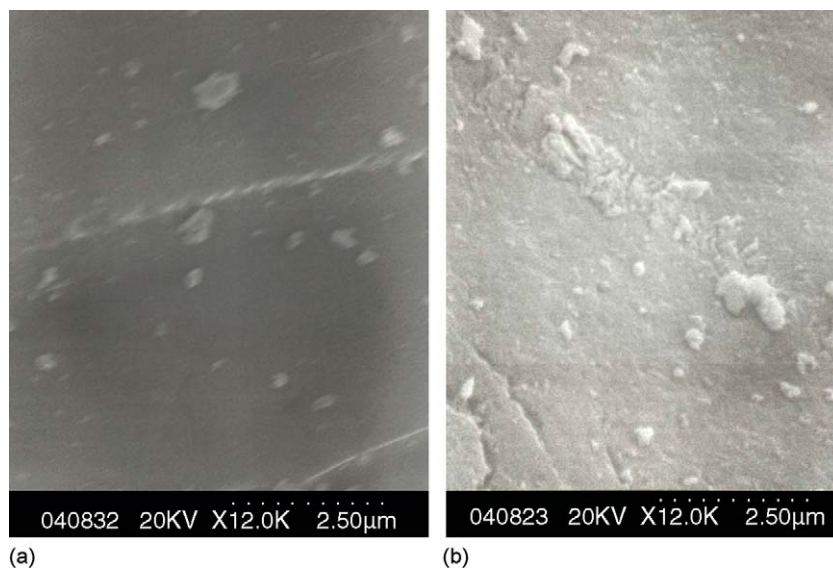


Fig. 3. SEM photographs of the IMIPs prepared by different removing the template molecule method (a) extraction (P-17) and (b) calcination (P-9).

Table 5

Preparation conditions and characteristics of IMIPs prepared by adding varied amount of water via adding base catalyst method

Run no.	H ₂ O/TEOS (molar ratio)	CAF (µmol/g)	TH (µmol/g)	α
I-1	2	–	–	–
I-2	3	10.9	2.9	3.76
I-3	4	15.6	5.7	2.74
I-4	5	14.9	3.6	4.14

The pH 6 is fixed of the final system.

mixture cannot be mono-phase. It is considered that because of the less of water, tetraethoxysilane is insufficient for hydrolysis. This phenomenon is disappeared by increasing the amount of water. One may notice that higher selectivity of more than four, I-4, which H₂O/TEOS is equal to five, is achievable. It is obvious that the selectivity of IMIPs prepared by adding base catalyst is higher as compared with that prepared by adding pore-forming agent. Hence, the H₂O/TEOS was fixed to five, by introducing varied amount of base catalyst to change the pH value of the final system to investigate the effect of pH value in the system. The results are shown in Table 6. The white appearance of IMIPs is obtained on all samples after calcination. The selectivity of IMIPs rise to four and above, especially

Table 6

Preparation conditions and characteristics of IMIPs prepared by different value of pH via adding base catalyst method

Run no.	pH	CAF (µmol/g)	TH (µmol/g)	α
I-5	5.5	14.3	3.9	3.67
I-6	6	14.9	3.6	4.14
I-7	7	11.9	2.9	4.10
I-8	7.5	13.1	3.9	3.36

The molar ratio, H₂O to TEOS, is fixed (H₂O/TEOS = 5).

when pH value are 6 and 7. The more large value of selectivity of IMIP is an indicative of its high selectivity for caffeine over the structure analogous molecule, theophylline. Therefore, it is simple to form a network with porous structure and higher selectivity of IMIPs under these conditions. In addition, caffeine adsorption and selectivity of IMIPs preparing via adding pore-forming agent and base catalyst method are much higher than those of MIPs prepared by organic copolymer, P(AN-co-4-Py), reported earlier (caffeine adsorption = 0.32 (µmol/g), α = 1.4) [21]. Under adding base catalyst method, the selectivity of IMIPs is higher than adding pore-forming agent method, while the caffeine adsorption is lower (Fig. 4). It is considered that the porosity of IMIPs prepared via adding pore-forming agent method is improved by using lactic acid, and the higher caffeine adsorption is obtained. In addition, the gelation times

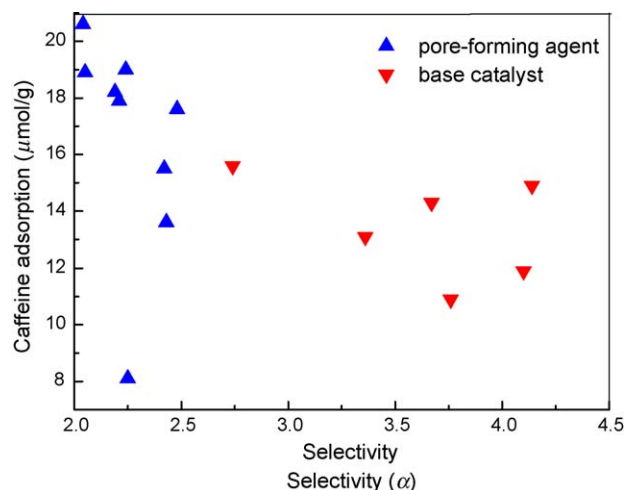


Fig. 4. Relationship between the caffeine adsorption and selectivity of IMIPs by different methods (blue triangle: adding pore-forming agent method; red triangle: adding base catalyst method).

decrease and the shape of caffeine is captured in the gel by adding base catalyst. The direction of better selectivity is obtained. In addition to this, the caffeine adsorption larger than theophylline adsorption not only under adding pore-forming agent method but also adding base catalyst method. We may say that the interactions of caffeine (or theophylline) with hydroxyl group of inorganic matrix were influenced by the different acidity of caffeine ($pK_a = 3.6$) and theophylline ($pK_a = 5.2$). It is considered that caffeine was in a weakly protonated state, while the theophylline would be essentially neutral. Hence, the caffeine adsorption is larger than theophylline adsorption for all samples.

4. Conclusion

Hence, higher caffeine adsorption and selectivity of IMIPs were successfully synthesized by adding pore-forming agent and base catalyst, respectively. In the sol–gel process, we have demonstrated that improved porosity of IMIPs with higher caffeine adsorption by using pore-forming agent, lactic acid. On the other hand, higher selectivity of IMIPs was obtained by introducing base catalyst into sol–gel process. The caffeine adsorption of IMIPs by calcination to remove template is two times that by extraction. All these IMIPs can be used as the stationary phase of column chromatography.

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