Comparison of Molecular Adsorption Ability of the Molecularly Imprinted Polymers Prepared by Ethylene Glycol Dimethacrylate and Trimethylolpropane Trimethacrylate as Cross Linkers

Zikant Saenkasa,[1] Chaiyavat Chaiyasut,[1] Roongnapa Srichana,[2] Sirivipa Piyamongkol[1]

[1]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90110, Thailand*

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ABSTRACT: The target of this study was to synthesize the molecularly imprinted polymers (MIPs) of L-phenylalanine as the solid phases for characterization of molecular adsorption by molecularly imprinted solid phase extraction (MISPE). These MIPs, in microscale, were synthesized using thermal (40°C)-compared with thermal (65°C)-initiated polymerization process. Itaconic acid was chosen as the functional monomers, and either ethylene glycol dimethacrylate or trimethylolpropane trimethacrylate (TRIM) was used as the cross linker and was compared together. The influences of several parameters on the properties of the MIPs were investigated, especially physical robustness from the percentage yields and molecular adsorption from the percentage recovery by MISPE. The best yields were obtained from polymers made using TRIM and thermal (65°C)-initiated polymerization. However, there were no significant differences in molecular adsorption. It was concluded that these parameters can be considered to synthesize MIPs for chiral separation in advance steps such as other related chromatographic techniques. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 2325–2330, 2007

Key words: molecularly imprinted polymers; molecularly imprinted solid phase extraction; molecular adsorption

INTRODUCTION

Most chiral compounds currently used are the mixtures of stereoisomers or racemates. The separations of these racemates are necessary, especially in industrial production, qualitative, and quantitative analyses. The molecularly imprinted polymers (MIPs) are most widely used in chiral separation because it can be predicted for selectivity and reproducibility. Molecularly imprinted solid phase extraction (MISPE) provides a method for the microscale preparation of solid phase for determination of molecular adsorption in which this method can be considered in screening test of molecular adsorption ability prior to the synthesis of MIPs for chiral separation in advance steps such as other related chromatographic techniques.

The synthesis of MIPs is elucidated in Figure 1. The functional monomer is employed for their function to interact by noncovalent interactions with the print molecule. The polymerization reaction fixes the print molecule in a bulky solid or highly crosslinked polymer in which the interactions between the print molecule and the monomers are preserved. This process gives rise to imprint possessing steric hindrance and chemical complementary to the print molecule. Subsequent elution of the print molecule leaves recognition sizes and shapes with affinity for the original print molecule.

There are many publications mentioning the preparation of MIPs, which refer to the preparation as chiral stationary phases (CSPs) for objective studies, such as the work of Haginaka et al.[1] Their experiment was the preparation of MIPs as CSPs for (S)-naproxen. The purpose of study was to compare molecular recognition ability of the MIPs prepared by thermal and redox polymerization techniques. Zhang et al.[2] studied about noncovalent bonding. Their experiment indicated influence of intramolecular H-bonding of templates on the molecular recognition of MIPs. This study was undertaken to understand the molecular recognition mechanism of MIPs for the prediction of the selectivity of MIPs.

As for MISPE, Lai and Wu[3] have developed MISPE method of selective detection for the rapid screening of cephalexin in human plasma and serum. Also, the work of Vaidya et al.[4] involved a process for the preparation of MIPs useful for separation of enzymes. The selectivity was evaluated by contacting the imprinted polymer with aqueous so-

Correspondence to: C. Chaiyasut (Chaiyavat@yahoo.com).

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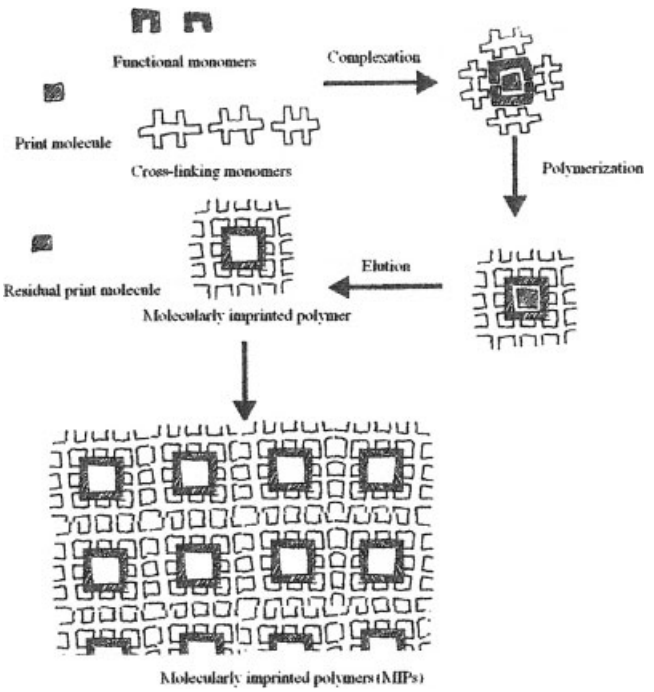

Figure 1 Scheme of polymerization process.

lution containing imprinted enzyme or a mixture of imprinted enzyme and other enzymes and isolating the enzyme-adsorbed polymer.

The works of Suedee et al.[5–7] discuss an evaluation method of MIPs by TLC. These works involved chiral separation of various adrenergic and antimalarial drugs by TLC. This method based on MIPs as CSPs was applied. Their method showed a rapid, sensitive, and reliable method for quality control of optically active compounds. Their MIPs were able to resolve diastereomeric pairs of their own print molecule and other stereoisomers structurally related to the print molecule.

Regarding the preparation of MIPs as CSPs in modern chromatographic column, Tan and Remcho[8] did chiral separation employing MIPs as CSPs in column. Enantiomeric separations of D- and L-dansyl phenylalanine were achieved in both OT-LC and OT-CEC modes with good selectivity and efficiencies.

For the chiral selective evaluation by CE techniques, there are interesting works by L. Schweitz et al.[9,10] Their review was about MIPs-based CSPs for CE. The CE techniques provided a high degree of separation efficiency and short separation times. The most successful approach utilized capillary columns filled with a monolithic, superporous imprinted polymer obtained by an *in situ* photo-initiated polymerization process for chiral separation of local anesthetics.

Later, not only the MIPs were synthesized as the CSPs for modern chromatographic columns, but also some parameters of these columns were improved for efficiency resolution; for example, the works of Chaiyasut et al.[11–13] indicated the modifying nature of the stationary phase because the electrovoltage was applied for the separation of the neutral solutes. They have been developed to direct measurement of the electroosmotic flow (EOF) velocity in relation between the EOF velocity and pH.

The intention of this study was to synthesize the MIPs of L-phenylalanine (L-phe) as the solid phases for determination of molecular adsorption by MISPE. These MIPs, in microscale, were synthesized using thermal (40°C)-compared with thermal (65°C)-initiated polymerization process. Itaconic acid (ITA) was chosen as functional monomers, and either ethylene glycol dimethacrylate (EGDMA) or trimethylolpropane trimethacrylate (TRIM) was used as the cross linker and was compared together. In the case of print molecule, L-phe was used as the molecular models. This model possesses an amino group and a carboxyl group on a chiral carbon together with hydrophobic part (Fig. 2).

Generally, molar ratio of composition on polymerization of MIPs from several articles is as follows: print molecule (mmol)/functional monomer (mmol)/cross linker (mmol)/solvent (mL) = 1 : 4–6 : 20–30 : 4–10. For this experiment, the molar ratio of composition on polymerization of MIPs was 1 : 2–8 : 20–40 : 16. The influences of several parameters on the properties of the MIPs were investigated, especially physical robustness from the percentage yields and molecular adsorption from the percentage recovery by MISPE.

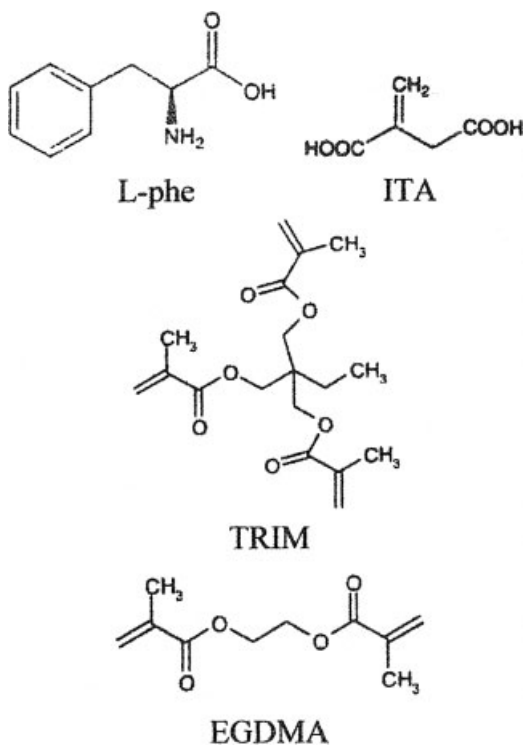

Figure 2 Structure of main substances.

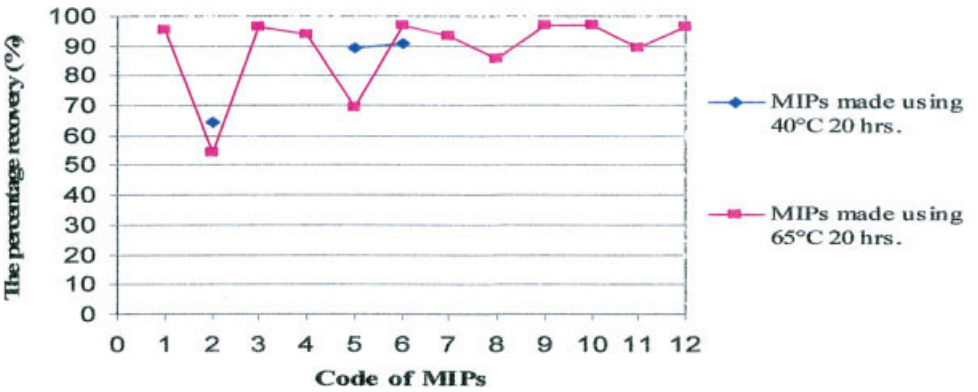

Figure 3 The percentage recovery from comparison between MIPs made at 40°C for 20 h and 65°C for 20 h. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

These effects, including the type and amount of cross linkers, together with effects of polymerization conditions on microscale preparation, were studied.

EXPERIMENTAL

Standard substances and other related compounds

L-Phenylalanine (L-phe), ethyleneglycol dimethacrylate (EGDMA), itaconic acid (ITA), and trimethylolpropane trimethacrylate (TRIM) were purchased from Aldrich (USA). 2,2[prime]-Azobisisobutyronitrile (AIBN) was supplied from Janssen Chimica (Geel, Belgium). Acetonitrile (ACN) and other solvents were of analytical grade. All chemicals were used without further purification.

Preparation of molecularly imprinted polymers

A prepolymerization mixture containing 0.25 mmol of L-phe (0.0413 g/0.25 mL in dil. HCl), ITA (1.0 or 2.0 mmol), TRIM or EGDMA (5.0, 7.5, or 10.0 mmol), and 0.1 mmol of AIBN dissolved in ACN later, composed as described in Table I, was prepared. A glass tube containing the prepolymerization mixture was ultrasonicated for 5 min and then purged with nitrogen gas for 5 min. The glass tube ends were tightly sealed. To perform the polymerization, the glass tube was placed in a hot air oven with protection from light at 40°C for 20 h or placed in a hot air oven with protection from light at 65°C for 20 h. It was then stored at room temperature until elution step.

A control polymer was also synthesized under the same conditions as described earlier, but without the addition of L-phe.

Elution of the print molecule

The polymers were removed from the glass tubes and ground using a mortar and pestle. After drying, the polymers were sieved by the same sieves (mesh size, 100 μm). To remove print molecule and residue, the polymers were kept in 10% acetic acid in methanol for 48 h with intermittent shaking, followed by washing several times with water–acetonitrile (50%, v/v) and filtered. The absence of the print molecule in the final rinse, as determined by UV spectropho-

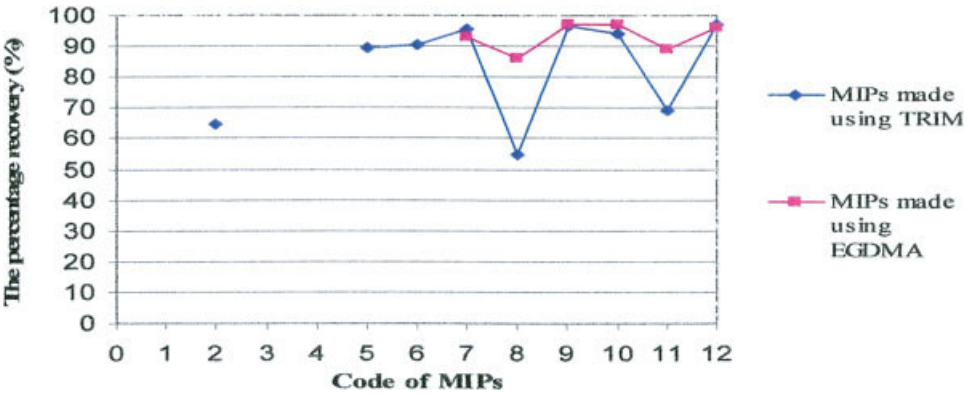

Figure 4 The percentage recovery from comparison between MIPs made using TRIM and EGDMA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Schematic Representation of Composition and Conditional Syntheses

Print molecule (0.25 mmol)	Functional monomer	Cross linkers	Initiator (0.1 mmol)	Solvent (qs. to 6 ml)	Conc. of functional monomer (mmol)	Conc. of cross linker (mmol)	Reaction temp.
L-Phe	ITA	TRIM	AIBN	ACN	1.0	5.0	40°C
		EGDMA			2.0	7.5	65°C
						10.0	

Calculation of the percentage yield:
Weight of reactant (g) was weight of prepolymerization mixture.
The first weight of product (g) was weight of MIPs before elution (dry state).
The first yield (%) = [the first weight of product (g) × 100]/weight of reactant (g).
The last weight of product (g) was weight of MIPs after elution (dry state).
The last yield (%) = [the last weight of product (g) × 100]/weight of reactant (g).

tometry, confirmed the removal of the print molecule from polymers. MIPs were finally dried under ambient temperature.

Characterization of the molecular adsorption by MISPE

Characterization of the molecular adsorption by MISPE was done by a screening test to indicate the molecular recognition of sizes and shapes, not chiral recognition, in term of adsorption value. The meaning of this term showed that the print molecule in solution form was adsorbed on surface area of the polymer. The more the polymer adsorbed, the higher the adsorption value was. The adsorption value of MIPs was compared with the adsorption value of control polymers (Blanks) and was shown in terms of the percentage recovery.

Procedure of MISPE

A reservoir was packed with 0.2 g of the dried polymer and 10 mL of 0.0025*M* of L-phe solution was added into the reservoir. The solvent for this solution was 2% 1*M* HCl in methanol because 1*M* HCl was the solvent of L-phe, and methanol was used as wetting agent for polymers. The mixture was shaken and then allowed to stand for 8 h, before filtered by using filtration paper. The concentration of L-phe in the filtrate was detected by UV–visible spectrophotometry. The percentage recovery was determined as shown in Figures 3 and 4.

Evaluation of MISPE

The adsorption value of MIPs can be calculated from a calibration graph ($y = mx \pm c$). This graph was achieved by standardization from a serial concentration of L-phe at maximum wavelength. The calibration equation was $y_1 = 129.5x_1 - 0.0045$. Where, x_1 was the concentration of filtrate of print molecule solution compared with 0.2 g of MIP (mol/L) and y_1 was the UV absorbance of filtrate of print molecule solution compared with 0.2 g of MIP.

Statistical assessments

Statistical assessments were done by SPSS. In the case of comparison of multiparameters, *P* value from analysis of variance (ANOVA) was calculated. Since Ho was hypothesis, a *P* value from ANOVA ≤ 0.05, $\mu_1 \neq \mu_2$ (reject Ho), meant the multiparameters gave significant difference (95% of confidence). Conversely, when the *P* value from ANOVA [mt] 0.05, $\mu_1 = \mu_2$ (accept Ho), multiparameters gave no significant difference.

In the case of comparison of two parameters, *P* value from *F*-test (two-sample for variances) was calculated. If *P* value from *F*-test (two-sample for variances) was more than 0.05, *P* value from *t*-test (two-sample assuming equal variances) would be calculated to find out the significance. Conversely, if *P* value from *F*-test (two-sample for variances) was less than 0.05, *P* value from *t*-test(two-sample assuming unequal variances) would be calculated to find out the significance. Since Ho was hypothesis, a *P* value from *t*-test ≤ 0.05, $\mu_1 \neq \mu_2$ (reject Ho), would indicate that the parameter of MIPs gave significant difference (95% of confidence). Conversely, if *P* value from *t*-test [mt] 0.05, $\mu_1 = \mu_2$ (accept Ho), the parameter of MIPs gave no significant difference. The meaning of parameters in column of statistical conclusions was the best composition of MIPs that gave significant difference.

RESULTS AND DISCUSSION

The best yields were obtained from polymers made using TRIM and thermal (65°C)-initiated polymerization as shown in Table II. They indicated that TRIM gave more reaction than EGDMA because of more

TABLE II
Percentage Yields of MIPs of L-Phe from Syntheses

Molar ratio of MIPs (L-phe)	The first yield (%)	Sample, *n*	The last yield (%)	Sample, *n*
Cross inkers				
TRIM (overall)	45.82 ± 25.26	12	19.19 ± 11.14	12
EGDMA (overall)	16.54 ± 17.82	12	11.05 ± 12.65	12
TRIM (mmol)				
5.0	35.16 ± 35.67	4	10.76 ± 11.82	4
7.5	62.89 ± 14.79	4	26.03 ± 11.29	4
10.0	39.40 ± 15.93	4	20.79 ± 5.26	4
EGDMA (mmol)				
5.0	17.06 ± 19.83	4	8.92 ± 10.31	4
7.5	12.88 ± 14.88	4	8.28 ± 9.56	4
10.0	19.68 ± 22.77	4	15.95 ± 18.47	4
Functional monomers				
ITA (overall)	31.18 ± 26.09	24	15.12 ± 12.38	24
ITA (mmol)				
1.0	33.56 ± 30.52	12	15.39 ± 13.72	12
2.0	28.79 ± 21.91	12	14.85 ± 11.49	12
Conditional syntheses				
40°C, 20 h (overall)	23.02 ± 29.57	12	7.28 ± 10.09	12
65°C, 20 h (overall)	39.34 ± 20.09	12	22.96 ± 9.18	12

Values are mean ± SD.

methacrylate chains to react strongly through covalent bonding with ITA and these conditional syntheses gave more complete reactions.

However, there were no significant differences in molecular adsorption from the percentage recovery by MISPE. Table III showed that polymers gave no significant difference in noncovalent bonding with L-phe and molecular fitting.

It has been shown that MIPs can also be synthesized for chiral separation using TRIM or EGDMA, including thermal (40°C)- or thermal (65°C)-initiated polymerization. However, MIPs can be synthesized for yield using TRIM or EGDMA and thermal (65°C)-initiated polymerization. It was concluded that these parameters can be considered to synthesize MIPs for chiral separation in advance steps. However, the prospects for future development of MIPs include using optimum molar ratio, optimum composition, and optimum conditional synthesis are needed.

TABLE III
Percentage Recovery of MIPs of L-Phe from MISPE (MIPs 0.2 g)

Molar ratio of MIPs (L-phe)	The percentage recovery (%)	Sample, *n*
Cross linkers		
TRIM (overall)	83.55 ± 16.19	9
EGDMA (overall)	93.14 ± 4.71	6
TRIM (mmol)		
5.0	71.59 ± 21.43	3
7.5	95.23 ± 1.72	2
10.0	86.68 ± 12.02	4
EGDMA (mmol)		
5.0	89.54 ± 5.18	2
7.5	97.18 ± 0.01	2
10.0	92.68 ± 5.01	2
Functional monomers		
ITA (overall)	87.39 ± 13.46	15
ITA (mmol)		
1.0	90.07 ± 9.68	7
2.0	85.03 ± 16.39	8
Conditional syntheses		
40°C, 20 h (overall)	81.60 ± 14.77	3
65°C, 20 h (overall)	88.83 ± 13.40	12

Values are mean ± SD.

CONCLUSIONS

MISPE remains a method for providing simple, rapid, reliable, and inexpensive analysis. MISPE may provide a potentially simple method for adsorbing chiral compounds. This is a useful method for estimating the molecular adsorption ability prior to the synthesis of MIPs for chiral separation in advance steps such as other related chromatographic techniques. However, the synthesis of MIPs is a chemical reaction and demands the understanding of chiral recognition theory and polymer. Moreover, the synthesis of MIPs is made more difficult by the fact that there are several parameters for consideration, such as the crosslinking ratio, conditional synthesis, type of component, and nature of print molecule. These parameters can influentially affect the chemical, physical, and chiral recognition properties of the MIPs.

In the future, these properties can be conveniently improved and characterized, increasing the efficacy of MIPs for research and development in the field of syntheses and analyses.

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