Novel polymer system for molecular imprinting polymer against amino acid derivatives

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Molecular imprinting polymers (MIPs) against *N-Cbz-L*-Tyr were prepared utilizing different polymer systems and evaluated in HPLC mode. It was found that MIP utilizing cocktail functional monomers, acrylamide + 2-vinylpyridine showed better molecular recognition and better separation ability for the template molecule than those utilizing other functional monomers. MIP utilizing trimethylolpropane trimethacrylate as cross-linker showed higher load capacity and separation factor than those utilizing ethylene glycol dimethacrylate as cross-linker. Increasing the concentration of competing solvent, acetic acid weakened the ionic interaction and hydrogen bonding between the analyte and the functional monomers, 2-vinylpyridine and acrylamide, when the template enantiomer was separated by HPLC. Therefore increasing of the concentration of acetic acid leads to decreasing of capacity factor, separation factor and resolution.

Keywords Molecular imprinting polymer, chiral separation, chiral stationary phase

Molecular imprinting, a novel technology mimicking the "lock-to-key" phenomenon which happens between antigen and antibody when they interact in biological system, is performed by polymer with "predetermined" molecular recognition to the template molecule, and it has been found wide applications in areas, such as synthetic chemistry,[1] catalysis,[2,3] sensor design,[4,5] pharmaceutical assay[6,7] and chiral separation.[8-10] Generally, molecular imprinting is applied in two modes, *i.e.* covalent and non-covalent. Non-covalent molecular imprinting with its simplicity has made great progress in chiral separation with efforts of developing new functional monomer-cross-linker polymer systems.[11-16] Cocktail polymerization, in which two or more functional monomers are used simultaneously, has been tried to prepare molecular imprinting polymer (MIP) possessing high molecular recognition ability.[16] Recently, we introduced new cocktail functional monomers, acrylamide (AM) + 2-vinylpyridine (2-VP), for the imprinting of amino acid derivatives.[17,18] MIP utilizing these combined functional monomers showed higher molecular recognition ability and better chiral separation for the template enantiomer than those prepared from other functional monomers.

In this paper, recent progress on AM + 2-VP molecular imprinting system was reported. Series of MIPs against *N-Cbz*-Tyr utilizing 2-VP-ethylene glycol dimethacrylate (EDMA), AM + 2-VP-EDMA and AM + 2-VP-trimethylolpropane trimethacrylate (TRIM) polymer systems were prepared and evaluated in HPLC mode. The effects of functional monomer and cross-linkers on the selectivity of MIPs were discussed. Interactions between AM + 2-VP and the template molecule were investigated.

Experimental

Material

N-Cbz-D-Tyr and *N-Cbz-L*-Tyr were purchased from Sigma (St. Louis, MO, USA) and 2-VP from Acros (Pittsburgh, PA). EDMA and TRIM were obtained from Shanhu Chemical Plant (Shanghai, China). Azo-bis-isobutyronitrile (AIBN) and acrylamide (AM)

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were from Beijing Chemical Plant (Beijing, China). Inhibitors in the cross-linker and 2-VP were removed by activated carbon. All solvents were of HPLC or analytical grade.

Preparation of bulky MIP

MIPs against *N-Cbz-L*-Tyr were prepared in acetonitrile by photo polymerization according to reference.[17] 2-VP or 2-VP + AM were used as functional monomers and EDMA or TRIM as cross-linkers, respectively. In general, suitable amounts of template molecule, functional monomer, cross-linker and porogen solvent shown in Table 1 were mixed with free radical initiator azo-bisisobutyronitrile (AIBN) (1.0%, *W/V*, weight of AIBN to volume of monomers). The mixtures were degassed in a sonicating water bath, saturated with nitrogen for 15 min, and then irradiated by 366 nm light for 48 h at 0℃. The bulky polymers were ground, sieved and the fines were removed by repeated sedimentation in acetonitrile. Particles with diameters smaller than 35 μm were collected and evaluated in HPLC mode.

Table 1 Composition pre-polymerization mixture for *N-Cbz-L*-Tyr imprinted polymers

MIP	*N-Cbz-L*-Tyr (mmol)	Functional monomer (mmol)	Cross-linker (mmol)	Acetonitrile (mL)
P1	0.50	2-VP(4.00)	EDMA(20)	7.00
P2	1.25	AM(2.50) + 2-VP(2.50)	EDMA(25)	8.00
P3	1.00	AM(2.00) + 2-VP(2.00)	TRIM(4.00)	3.50

Chromatographic evaluation

The collected MIP particles were slurried in water/acetonitrile (50/50, *V/V*) and packed into 4 × 250 mm or 4 × 150 mm stainless steel HPLC columns at 20 MPa. The columns were eluted with acetonitrile/acetic acid (90/10, *V/V*) until a stable baseline was achieved. The LC-890A system from Beijing Xingda Technology Development Company comprised two LC-05C pumps and a LC-830 UV-VIS detector (Soma Optic LTD, Japan). The chromatograms were recorded and analyzed by a JS-3030 chromatography operation station (Johnsson Corporation, Dalian, China). The elution was performed at ambient temperature and monitored at 276 nm.

Acetone was used as void marker. Separation factors (α) were calculated according to the standard chromatographic theory.[19] The template peak was seriously asymmetric because of the nonlinear adsorption curve,[20] so the resolution was calculated as the resolution function (*f/g*) according to Ref. 21.

Results and discussion

Effect of functional monomer on the selectivity of MIPs

Molecular imprinting is a technology by which specific recognition sites can be produced by use of a template molecule in the polymerization procedure. The principle has been well documented and it was necessary to explore new polymer system including new functional monomer and cross-linker to improve the selectivity and load capacity.[22]

The most widely applied functional monomer in non-covalent molecular imprinting is methacrylic acid (MAA).[11,12] It interacts *via* hydrogen bonds with the amide, carbamate and carboxyl groups on amino acid derivatives. AM is another functional monomer evaluated,[13] and it was found that AM could form much stronger hydrogen bonds with the template than MAA. Basic functional monomers 4-VP[14,15] and 2-VP[16] were also introduced in non-covalent molecular imprinting. It was believed that pyridine formed strong ionic interaction with the carboxyl group in the template molecule. MIP prepared by cocktail polymerization with AM + 2-VP as combined functional monomers for the imprinting of amino acid derivatives showed higher molecular recognition ability and better chiral separation for the template enantiomer than those prepared from other functional monomers.[17,18]

Table 2 showed the chromatographic results of separation of *N-Cbz-DL*-Tyr on MIPs prepared from 2-VP and AM + 2-VP as functional monomers. The molar ratio

of template to 2-VP or AM + 2-VP employed in the preparation of MIPs was based on Ref. 16 and our optimization result,[18] because the molar ratio of template molecule to functional monomer has great effect on the molecular recognition property of the resultant MIPs.[14]

Chiral separation of the template enantiomer, *N-Cbz-DL*-Tyr, was achieved on MIP (P1) made of 2-VP as functional monomer (Table 2). We believed that the basic functional monomer 2-VP, as 4-VP,[14,15] interacts with the amide and carboxyl group on the template molecule *via* hydrogen bond and strong ionic interaction. Chiral separation of *N-Cbz-DL*-Tyr on MIP (P2) against *N-Cbz-L*-Tyr utilizing AM + 2-VP as combined functional monomers was shown in Fig. 1. It was obvious that the separation factor of *N-Cbz-DL*-Tyr on MIP (P2) was higher than the corresponding values obtained on MIPs made of AM, 2-VP or MAA + 2-VP as functional monomers (Tables 2, 3).

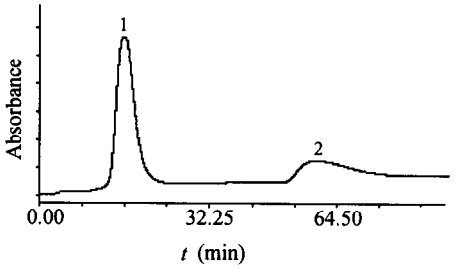

Fig. 1 Chiral separation of *N-Cbz-DL*-Tyr on the *N-Cbz-L*-Tyr imprinted MIP (P2). Column: 250 × 4 mm; mobile phase: acetonitrile/acetic acid (99.0/1.0, *V/V*); flow-rate, 0.5 mL/min; detection: 276 nm; 1. *N-Cbz-D*-Tyr, 2. *N-Cbz-L*-Tyr.

Table 2 Chiral separation of *N-Cbz-DL*-Tyr on the *N-Cbz-L*-Tyr imprinted chiral stationary phases utilizing different polymer systems

MIP	Sample amount (μg)	Capacity factor		Separation factor (α)	Resolution (f/g)	Flow-rate (mL/min)
		k_D'	k_L'			
P1	20	2.95	8.26	2.89	1.0	0.5
P2	20	3.86	13.67	3.54	1.0	0.5
P3	40	12.54	28.26	2.25	1.0	1.0
	100	12.00	21.32	1.78	0.9	0.5
	211	11.08	17.05	1.54	0.6	0.5

Column: P1 and P2, 250 × 4 mm, P3, 150 × 4 mm; mobile phase: acetonitrile/acetic acid (99.0/1.0, *V/V*). Detection: 276 nm.

Table 3 Separation factor and resolution obtained on other *N-Cbz-L*-Tyr imprinted MIPs

Functional monomer	Capacity factor		Separation factor (α)	Resolution (f/g)	Ref.
	k_D'	k_L'			
MAA + 2-VP[a]	1.78	3.01	1.69	0.68	23
AM[b]	0.29	0.63	2.19	0.28	24

[a] *N-Cbz-L*-Tyr:MAA:2-VP:EDMA = 1:4:4:40; [b] *N-Cbz-L*-Tyr:AM:EDMA = 1:4:20; [c] Capacity factors were not shown in the original Ref. 23 and 24.

This phenomenon was probably due to a combination of following points: First, interaction between functional monomers themselves would compete with that between functional monomers and the template molecule. Both AM and MAA could interact with 2-VP, but the interaction between AM and 2-VP is hydrogen bond while that between MAA and 2-VP is ionic. Ionic interaction is stronger than hydrogen bond, so the tendency for AM to interact with 2-VP is weaker than that for MAA to interact with 2-VP. It means that, comparing with the traditional cocktail functional monomers MAA + 2-VP, AM + 2-VP would interact more efficiently with the template molecule; Second, AM and 2-VP can form stronger hydrogen bonds and ionic bonds with the amide group and carboxyl group on the template molecule. This cooperative interaction would lead to a more stable complex between the template molecule and AM + 2-VP than that between the template molecule and other functional monomer. Therefore binding sites possessing better recognition of the template molecule would be formed in the MIP with AM + 2-VP as combined functional monomers.

Effect of cross-linker to chiral separation of MIPs

Other than functional monomer, cross-linker is another important factor that affects the chiral separation property of the MIPs. EDMA has been extensively used for the preparation of non-covalent molecular imprinting and more than 50 mol% in total composition of monomer should be used.[25] Cross-linker containing three or four vinyl groups was introduced into the imprinting of amino acid derivatives.[26,27] MIPs prepared from both pentaerythritol triacrylate (PETRA) and TRIM were superior to those from EDMA in load capacity, selectivity and resolution.

MIP against *N-Cbz-L*-Tyr utilizing AM + 2-VP as combined functional monomers and TRIM as cross-linker was prepared. The molar ratio of *N-Cbz-L*-Tyr to AM + 2-VP and TRIM were employed according to our optimization result[18] and Ref. 27. It indicated that TRIM based MIP (P3) had higher separation ability and load capacity than EDMA based MIP (P2), comparing the values obtained on MIP utilizing EDMA and TRIM as cross-linker. Baseline separation of 40 μg *N-Cbz-DL*-Tyr with separation factor of 2.25 was achieved on 150 × 4 mm column packed with P3 (Fig. 2). Even by increasing injection amount to 211 μg, *N-Cbz-DL*-Tyr was also resolved with separation factor of 1.54 and resolution of 0.57.

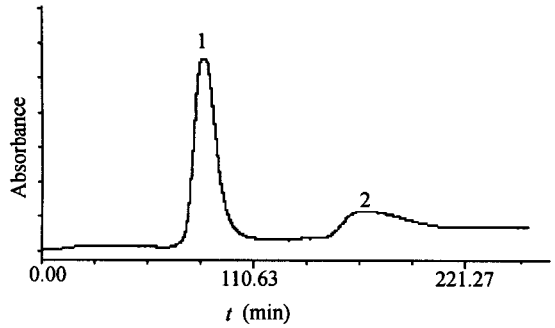

Fig. 2 Chiral separation of *N-Cbz-DL*-Tyr on the *N-Cbz-L*-Tyr imprinted MIP (P3). Column: 150 × 4 mm; mobile phase: acetonitrile/acetic acid (99.0/1.0, *V/V*); flow-rate: 0.5 mL/min; detection: 276 nm; 1. *N-Cbz-D*-Tyr, 2. *N-Cbz-L*-Tyr.

Following factors may give rise to higher separation ability and load capacity of TRIM based MIP.[26,27] Firstly, the molar ratio of the template molecule to the functional monomers was maintained constant (*N-Cbz-L*-Tyr :AM:2-VP = 1:2:2) for both EDMA based MIP and TRIM based MIP, but the molar ratio of functional monomer to EDMA (AM:2-VP:EDMA = 1:1:10) was lower than that to TRIM (AM:2-VP:TRIM = 1:1:2), therefore more template molecule was imprinted in unit weight of TRIM based MIP than in EDMA based MIP. This could result in more recognition cavities in unit weight of TRIM based MIP than in unit weight of EDMA based MIP, so the capacity factor and load capacity obtained on TRIM based MIP were increased comparing with those on EDMA based MIP. Secondly, EDMA contains two vinyl groups whereas TRIM contains three vinyl groups, so the cross-linking intensity of TRIM based MIP was higher than EDMA based MIP. This leads to better-defined recognition cavities possessing higher selectivity in TRIM based MIP than those cavities in EDMA based MIP.[27]

Interaction between AM + 2-VP *and the template molecule*

In non-covalent molecular imprinting the template molecule interacts with the functional monomer *via* hydrogen bond,[11,12] ionic interaction,[14-16] hydrophobic interaction.[28] For MIP made of AM + 2-VP as functional monomers, it has been found that there were hydrophobic and ionic interactions between the analyte and AM + 2-VP when water/acetonitrile were used as mobile phase.[17] In this paper, we investigated the hydrogen bond and ionic interactions between the template enantiomer and AM + 2-VP by changing the composition of the mobile phase.

Acetic acid could form hydrogen bond and ionic interaction with AM and 2-VP in the imprinting cavities, respectively, so it acts as a competing solvent in the mobile phase. With increasing the concentration of acetic acid, AM located at interior cavities would form hydrogen bond with acetic acid, so the hydrogen bond between AM and the amide group on *N-Cbz-DL*-Tyr would be weakened. On the other hand, 2-VP at interior cavities would be changed to pyridinium cation by acetic acid in the eluent and the acetate anion would interact with the pyridinium cation by ionic interaction. So the ionic interaction between the carboxylate anion of *N-Cbz-DL*-Tyr and the pyridinium cation would be weakened too. Therefore increasing of the concentration of acetic acid leads to decreasing of capacity factor, separation factor and resolution (Figs. 3 and 4).

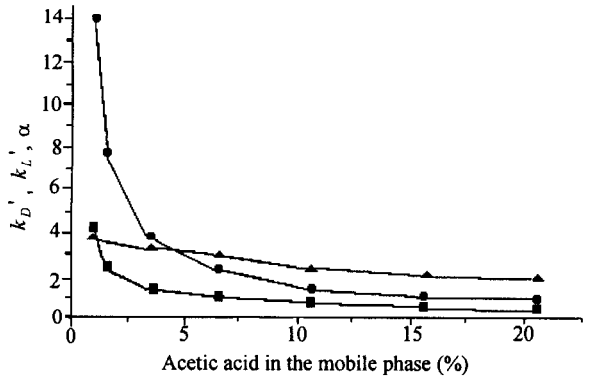

Fig. 3 Relationship between k', α and the concentration of acetic acid in the mobile phase on column P2. k_D' (■): capacity factor of *N-Cbz-D*-Tyr; k_L' (●): capacity factor of *N-Cbz-L*-Tyr; α (▲): chiral separation factor of *N-Cbz-DL*-Tyr.

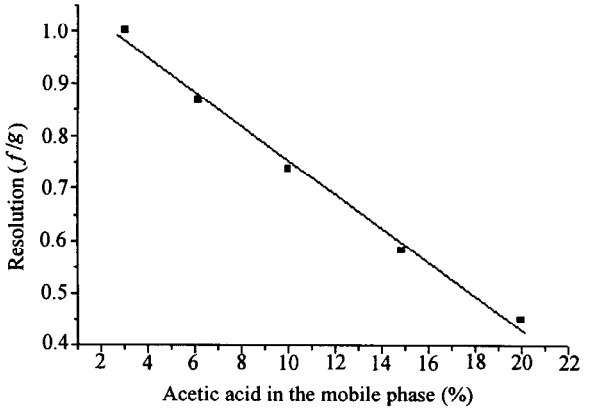

Fig. 4 Relationship between the resolution and the concentration of acetic acid in the mobile phase on column P2.

In this paper, MIPs against *N-Cbz-L*-Tyr were prepared utilizing different polymer systems and evaluated in HPLC mode. It was found that MIP utilizing cocktail functional monomers, AM + 2-VP showed better molecular recognition and better separation ability for the template molecule. TRIM based MIP showed higher load capacity and separation factor than EDMA based MIP. Ionic interaction and hydrogen bond between the analyte and 2-VP and AM were weakened by increasing the competing solvent acetic acid, therefore increasing of the concentration of acetic acid leads to decreasing of capacity factor, separation factor and resolution.

References

1. Tahmassebi, D.; Sasaki, T., *J. Org. Chem.*, **59**, 679 (1994).
2. Beach, H.V.; Shea, K.J., *J. Am. Chem. Soc.*, **116**, 379(1994).
3. Davis, M. E.; Katz, A.; Ahamad, W.R., *Chem. Mater.*, **82**, 1820(1996).
4. Kriz, D.; Ramström, O.; Mosbach, K., *Anal. Chem.*, **67**, 2142(1995).
5. Kriz, D.; Ramström, O.; Mosbach, K., *Anal. Chem.*, **69**, 345A(1997).
6. Vlatakis, G.; Andersson, L.I.; Muller, R.; Mosbach, K., *Nature*, **361**, 645(1993).
7. Schweit, L.; Andersson, L. I.; Nilsson, S., *Anal. Chem.*, **69**, 1179(1997).
8. Kempe, M.; Mosbach, K., *J. Chromatogr. A*, **649**, 3 (1995).
9. Wulff, G., *Angew. Chem., Int. Ed. Engl.*, **34**, 1812 (1995).
10. Meng, Z.-H.; Zhou, L.-M; Wang, Q.-H.; Zhu, D.-H., *Chin. J. Anal. Chem.* (in Chinese), **25**, 349 (1997).
11. Nicholls, I.A.; Ramström, O.; Mosbach, K., *J. Chromatogr. A*, **691**, 349(1995).
12. Andersson, L.I.; Mosbach, K., *J. Chromatogr.*, **516**, 313(1990).
13. Yu, C.; Mosbach, K., *J. Org. Chem.*, **62**, 4057 (1997).
14. Kempe, M.; Fischer, L.; Mosbach, K., *J. Mol. Recogn.*, **6**, 25(1993).
15. Kempe, M.; Mosbach, K., *J. Chromatogr. A*, **664**, 276(1994).
16. Ramström, O.; Andersson, L. I.; Mosbach, K., *J. Org. Chem.*, **58**, 7562(1993).
17. Meng, Z.-H.; Wang, J.-F.; Zhou, L.-M; Wang, Q.-H.; Zhu, D.-H., *Anal. Sci.*, **15**, 141(1999).
18. Wang, J.-F.; Meng, Z.-H.; Zhou, L.-M; Wang, Q.-H.; Zhu, D.-H., *Acta Chim. Sin.* (in Chinese), **57**, 1147(1999).
19. Poole, C.F.; Schuette, S.A., *Contemporary Practice of Chromatography*, Elsevier, Amsterdam: Holland, 1984.
20. Sellergren, B.; Shea, K.J., *J. Chromatogr. A*, **690**, 29(1995).
21. Mayer, V.R., *Chromatographia*, **24**, 639(1987).
22. Mayes, A.G.; Mosbach, K., *Trends Anal. Chem.*, **16**, 321(1997).
23. Meng, Z.-H.; Wang, J.-F.; Zhou L.-M.; Wang, Q.-H.; Zhu, D.-H., *Biomed. Chromatogr.*, **13**, 389 (1999).
24. Meng, Z.-H.; Wang, J.-F.; Zhou, L.-M; Wang, Q.-H.; Zhu, D.-H., *Chin. Chem. Lett.*, **10**, 69(1999).
25. Sellergren, B., *Makromol. Chem.*, **190**, 2703(1989).
26. Kempe, M.; Mosbach, K., *Tetrahedron Lett.*, **36**, 3565 (1995).
27. Kempe, M., *Anal. Chem.*, **68**, 1948(1996).
28. Yu, C.; Ramstrom, O.; Mosbach, K., *Anal. Lett.*, **30**, 2123(1997).

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