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Journal of Chromatography A, 950 (2002) 89–97

JOURNAL OF CHROMATOGRAPHY A

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Molecular recognition through the exact placement of functional groups on non-covalent molecularly imprinted polymers

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Received 26 September 2001; received in revised form 3 January 2002; accepted 10 January 2002

Abstract

To investigate the extent of the placement of functional groups in the non-covalent molecularly imprinted polymers contributing to selectivity, three kinds of molecularly imprinted methacrylic acid–ethylene glycol dimethacrylate copolymers were prepared at higher polymerization temperature (60 °C) using 2-L-phenylalanylamino-pyridine, 3-L-phenylalanylamino-pyridine, or 4-L-phenylalanylamino-pyridine as a template molecule, respectively. The enantiomeric recognition performance of these molecularly imprinted polymers (MIPs) in the high-performance liquid chromatography (HPLC) mode was investigated. The polymers exhibited efficient enantiomeric resolution of the racemic mixture of their printing molecules and could hardly resolve the enantiomers of other substrates that were structurally analogous to the imprinting molecules, but different from spatial interaction sites on the polymer with the methacrylic acid residues. Our results showed that the shape and spatial orientation of functionality of imprinted binding sites were critical for recognition and the recognition properties of MIPs were greatly influenced by the minor difference of N position on the pyridine ring and caused by the distance accuracy of functional groups in the binding sites. The implication of these findings with respect of the mechanism of recognition was discussed.

Keywords: Molecular recognition; Enantiomer separation; Chiral stationary phases, LC; Molecularly imprinted polymers

1. Introduction

The technique of molecular imprinting allows the formation of specific recognition sites in synthetic polymers via templates. Molecular recognition with imprinted polymers has been intensively studied in recent years [1,2]. In this way the specific binding sites can be introduced into polymers as the models of biological receptors and enzymes. In general, molecular imprinting processes depending upon the interactions which exist between the template guest and the host functional monomer(s) involve in: (a) covalent bonding; (b) non-covalent bonding. In our system, non-covalent interactions were used both in the preparation and in the subsequent evaluation of the imprinted polymers.

Imprinting technique was frequently used for the preparation of chiral cavities containing functional groups in the cross-linked polymers. The molecular recognition properties of molecularly imprinted polymers (MIPs) were determined by the ability of

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00058-4

resolution of enantiomers and discrimination between structurally related molecules, and influenced by the number of potential interaction sites [3]; the strength and position of the interactions [4–6]; rigidity of the template molecules and functional monomers [7]; and the shape of the cavity [8].

By the use of the molecular imprinting technique, highly enantio-selective and substrate-selective polymers were prepared by utilizing non-covalent interactions between the template molecule and methacrylic acid monomers at lower temperature [9]. Previous results showed that imprinted polymers could distinguish between enantiomers of the imprinting molecule and even discriminate between a wide range of enantiomers of structurally related molecules that have not been imprinted, but have the same interaction sites with functional monomers [10–13].

Wulff et al. [14–17] previously reported on the investigations of the selectivity. They located two functional groups on a more or less plane surface of silica through the formation of siloxane bonds using a similar imprinting procedure. However our interest was in the influence of the exact arrangement of functional groups on the recognition in a non-covalent molecularly imprinted polymer. In order to gain an understanding of the extent of the exact placement of functional groups contributing to the selectivity of the non-covalent polymers, we prepared three kinds of molecularly imprinted polymers using 2-L-PheNHPy, 3-L-PheNHPy, or 4-L-PheNHPy as a printing molecule, respectively (see Fig. 1). To avoid any shape selectivity, imprinting molecules selected have the same size and shape, but they are different from the relative position of the nitrogen atom on the pyridine ring. It is the difference that determined the spatial orientation of the functionality in imprinted binding sites in the cavity of the polymers. In the chromatographic mode the polymers could be used for baseline resolution of the original racemic target compound and could not separate the enantiomers of the molecules which were structurally analogous to printing molecules but different from spatial interaction sites with functionality in the polymers. We also investigated the effect of temperature on the retention factor of 3-L-form enantiomer in aqueous mobile phase. On the basis of observations we proposed the mechanism of recognition of the polymers.

2. Experimental

2.1. Materials

N-(*tert.*-butyloxycarbonyl)-L-phenylalanine was purchased from Advanced Chemtech (Louisville, KY, USA). *N*-(*tert.*-butyloxycarbonyl)-D,L-phenylalanine was supplied by Baosheng Company (Yangzhou, China, >98% ee). 2-aminopyridine, 3-aminopyridine and 4-aminopyridine were obtained from local suppliers. Methacrylic acid (MAA), chloroform, acetic acid, 2,2-azobisisobutyronitrile (AIBN) were obtained from Tianjin No.2 Chemical Reagent Factory and were purified by recrystallization or distillation prior to use. Ethylene glycol dimethacrylate (EGMA) was purified using an anion-exchange resin to remove inhibitor. Acetonitrile and methanol were of chromatographic grade. Other chemicals were analytical grade.

2.2. Equipment

The liquid chromatograph comprised a Waters 600 Pump quarternary gradient unit and a Waters 996 Photodiode Array Detector, connected to a Millennium 32 workstation. Manual injections were carried out using a 7725i injector with 10 μl sample loop. The solvents were degassed using In-Line Degasser (Waters, USA). Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on Varian Unity plus-400 (USA). UV spectra were performed on Shimadzu 2010-PC (Japan). Pore and surface area analysis were performed by N_2 adsorption on Autosorb-1-MP (Quantachrome Corporation, USA).

2.3. Synthesis of template molecules and their racemates

2.3.1. Synthesis of 2-L-PheNHPy and 2-D,L-PheNHPy

N-(*tert.*-butyloxycarbonyl)-L-phenylalanine (5.31 g, 20 mmol) or *N*-(*tert.*-butyloxycarbonyl)-D,L-phenylalanine (5.31 g, 20 mmol) was dissolved in dry $CHCl_3$ (30 ml) with stirring, then neutralized with triethylamine (2.8 ml) and cooled to −10 °C. To the mixture was added slowly ethyl chlorocarbonate (1.92 ml). Approximately 20 min later a precooled (−10 °C) solution of 2-aminopyridine (4.71 g, 50 mmol) in chloroform (20 ml) was added. The

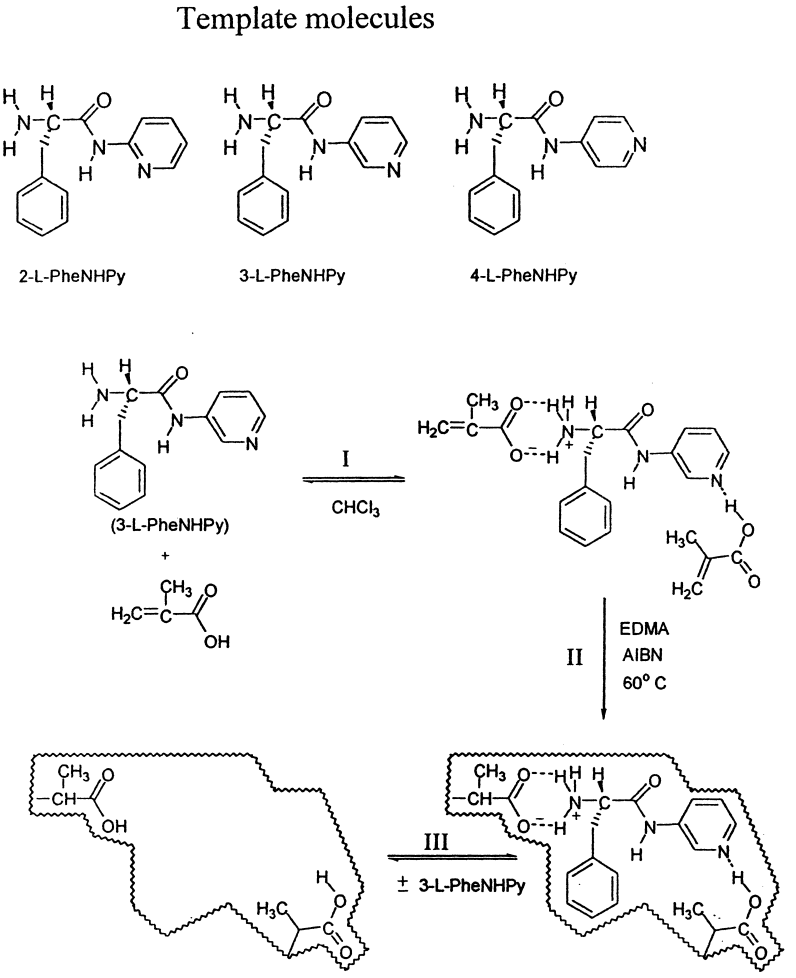

Fig. 1. Schematic representation of 3-L-PheNHPy imprinting procedure.

mixture was stirred for 5 h at −10 °C, and then overnight at room temperature. After evaporating the solvents, the residue was dissolved in ethyl acetate, washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated. The crude product was chromatographed on a silica column (60 g) using ethyl acetate–petroleum ether (1:8) as an eluent. 2-[[*N*-(*tert*.-butyloxycarbonyl)phenylalanyl]amino]-pyridine was obtained using the same eluent in a 1:2 ratio. The protecting group was removed using the method described in Section 2.3.4. The product of 2-L-PheNHPy was obtained: yield: 35%; mp: 112–113 °C; ^{1}H NMR ($CDCl_3$) δ 9.96 (1, s, NH), 7.04–8.31 (9, m, heterocyclic and aromatic), 3.37–3.77 (2, d, CH_2), 2.74–2.80 (1, m, CH), 1.50 (2, br, NH_2).

2-D,L-PheNHPy was obtained using the same procedure: yield: 37%; mp: 122–123 °C; ^{1}H NMR ($CDCl_3$) δ 9.97(1, s, NH), 7.04–8.31 (9, m, heterocyclic and aromatic), 3.37–3.77 (2, d, CH_2), 2.74–2.80 (1, m, CH), 1.50 (2, br, NH_2).

2.3.2. Synthesis of 3-L-PheNHPy and 3-D,L-PheNHPy

3-Aminopyridine (1.12 g, 13 mmol) and 1,3-dicyclohexylcarbodiimide (DCC) (2.59 g, 12.6 mmol) were dissolved in dry tetrahydrofuran (THF) (35 ml) and cooled in an ice bath. To the solution was added slowly *N*-(*tert*.-butyloxycarbonyl)-L-phenylalanine (3.18 g, 12 mmol) or *N*-(*tert*.-butyloxycarbonyl)-D,L-phenylalanine in THF (15 ml) within

20 min. The mixture was stirred for 2 h at 0 °C and overnight at room temperature. The urea was filtered out; the filtrate was concentrated. The crude product was recrystallized from ethanol–water (1:3). 3-[[*N*-(*tert*. - butyloxycarbonyl)phenylalanyl]amino] - pyri - dine was obtained. The protecting group was removed using the method described in Section 2.3.4. The product of 3-L-PheNHPy was obtained: 3-L-PheNHPy: yield: 78%; mp: 79–81 °C; ^{1}H NMR ($CDCl_3$) δ 9.61 (1, s, NH), 8.25–8.61 (4, m, heterocyclic), 7.25–7.37 (5, m, aromatic), 3.35–3.79 (2, d, CH_2), 2.79–2.85 (1, m, CH), 1.61 (2, br, NH_2).

3-D,L-PheNHPy was obtained using the same procedure: yield: 75%; mp: 88–90 °C; ^{1}H NMR ($CDCl_3$) δ 9.61 (1, s, NH), 8.25–8.61 (4, m, heterocyclic), 7.25–7.37 (5, m, aromatic), 3.36–3.79 (2, d, CH_2), 2.79–2.85 (1, m, CH), 1.55 (2, br, NH_2).

2.3.3. Synthesis of 4-L-PheNHPy and 4-D,L-PheNHPy

N-(*tert*.-butyloxycarbonyl)-L-phenylalanine (4.26 g, 16 mmol) or *N*-(*tert*.-butyloxycarbonyl)-D,L-phenylalanine (4.26 g, 16 mmol) was dissolved in dry acetonitrile (60 ml) and cooled in an ice bath. To the solution was added slowly DCC (1.65 g, 8 mmol) in acetonitrile (40 ml). The mixture was stirred for 1 h at 0 °C and an additional 2 h at room temperature. The cyclohexylurea was filtered out. To the filtrate were added 4-aminopyridine (0.74 g, 8 mmol) and pyridine (2 ml). The mixture was allowed to stand overnight and then the solvents were evaporated. The residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated. The crude product was chromatographed on a silica column (60 g) using ethyl acetate–methylene chloride (1:4), then the same solvents in a 2:3 ratio, finally ethyl acetate as eluents to obtain 4-[[*N*-(*tert*.-butyloxycarbonyl)-phenylalanyl]amino]-pyridine. The protecting group was removed using the method described in Section 2.3.4. 4-L-PheNHPy was obtained: yield 30%; mp: 93–94 °C; ^{1}H NMR ($CDCl_3$) δ 9.71 (1, s, NH), 7.33–8.52 (4, d, heterocyclic), 7.23–7.33 (5, m, aromatic), 3.34–3.77 (2, d, CH_2), 2.78–2.83 (1, m, CH), 1.58 (2, br, NH_2).

4-D,L-PheNHPy was obtained using the same procedure: yield: 32%; mp: 74–75 °C; ^{1}H NMR ($CDCl_3$) δ 9.73 (1, s, NH), 7.28–8.52 (4, d, heterocyclic), 7.23–7.28 (5, m, aromatic), 3.34–3.77 (2, d, CH_2), 2.77–2.83 (1, m, CH), 1.56 (2, br, NH_2).

2.3.4. Removal of the protecting group (general method)

To the corresponding *tert*.-butyloxycarbonyl (BOC) protecting derivative (450 mg) in dry methanol (5 ml) was added 15% HCl in methanol (4 ml). The mixture was stirred overnight at room temperature. The product was precipitated in dry ether for a few hours. The supernatant was decanted, and the crystal was washed several times with dry ether and finally purified by dissolving in dry methanol and precipitating with ether. The yield was quantitative. The sludge was dissolved in water and neutralized with aqueous solution of NaOH to pH 9–10. The product was extracted with ethyl acetate from the solution. The extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo to dryness.

2.4. Preparation of polymers

Polymers were prepared using MAA as functional monomer and EGDMA as a cross-linking agent, 2-L-PheNHPy, 3-L-PheNHPy or 4-L-PheNHPy as an imprint molecule. The general procedure of synthesis was as follows: In a 100-ml flask were added the template compound (0.69 g, 2.85 mmol), MAA (1.03 g, 12 mmol) and chloroform (20 ml). To the mixture were added EGDMA (11.89 g, 60 mmol) and initiator AIBN (20 mg). Then the mixture was transferred into a 50 ml glass ampoule and was degassed in a sonicating water bath. After nitrogen gas sparged into the solution for 5 min, the ampoule was sealed under vacuum. The mixture was shaken in a shaker bath at 60 °C for 24 h. Resulted bulk rigid polymer was ground and sieved. The particles from 38 μm to 25 μm were collected, placed into a template-separation apparatus and washed at 1.0 ml/min of the flow-rate with 10% acetic acid in methanol until the template could not be detected (λ_{max} = 264 nm) in the eluent. Then the particles were washed with methanol to remove residual acetic acid and dried to constant weight under vacuum at 60 °C.

2.5. Chromatographic evaluation

Particles were suspended in acetonitrile (30 ml), sonicated for 10 min and placed in a slurry reservoir with a single action reciprocating plunger pump (Alltech Associates, USA). The polymer was packed into 150 mm×4.6 mm I.D. stainless-steel columns using acetonitrile (200 ml) as the packing solvent. The polymer content in each column was approximately 0.8 g. Then the columns were washed on-line with a mixture of methanol and acetic acid (9:1, v/v) until a stable baseline was obtained. A solution of 100 nmol of each of the enantiomers of a given compound, prepared in acetonitrile, was injected for analysis in a total volume of 10 μl. The void volumes of the columns were determined by injection of acetone. The retention factors (k'), the separation factors (α) were calculated according to the standard chromatographic theory: $k'_{D} = (t_{D} - t_{0})/t_{0}$, $k'_{L} = (t_{L} - t_{0})/t_{0}$, $\alpha = k'_{L}/k'_{D}$, where t_{D} is the retention time of the D enantiomer, t_{L} is the retention time of the L enantiomer, and t_{0} is the retention time of the void marker [18]. The resolution factor (R_{s}) was obtained graphically as described elsewhere [19].

3. Results and discussion

3.1. Pore analysis of the polymers

In the N_2 adsorption a sample of polymer (25–38 μm in diameter) corresponding to ca. 20 m^2 (0.1~0.4 g) was degassed at 50 °C overnight under vacuum. The adsorption and desorption isotherms were then recorded using a 200-point pressure table and 15 s equilibration time. This gave a size distribution of pores between 17 and 3000 Å. The surface area was determined using the Brunauer–Emett–Teller (BET) model, the *t*-plot using the Harkins–Jura average thickness equation and the pore distribution using the Barrett–Joyner–Halenda (BJH) model. The results of this study were summarized in Table 1. Most of the cross-linked network polymers used for molecular imprinting have a wide distribution of pore sizes associated with various degrees of diffusional mass transfer limitations and a different degree of swelling. The sites associated with meso- and macropores (>20 Å) are expected to be easily accessible compared to those sites located in the smaller micropores (<20 Å) where the diffusion is slow.

Table 1
Surface area and pore analysis of the imprinted polymers

Polymer[a]	N_2 adsorption		
	Surf. area (BET)[b] (m^2/g)	Pore volume[c] (ml/g)	Pore diameter[d] (Å)
P2	145.0	0.227	62.76
P3	108.0	0.099	36.51
P4	76.4	0.074	24.61

[a] P2, P3 and P4 were prepared using 2-L-PheNHPy, 3-L-PheNHPy and 4-L-PheNHPy as the template molecule, respectively.
[b] Determined using the BET model on a seven-point linear plot.
[c] BJH cumulative adsorption pore volume of pores between 17 and 3000 Å.
[d] BJH desorption average pore diameter of pores between 17 and 3000 Å.

3.2. Chromatography

Polymers were evaluated in the HPLC mode using an isocratic scheme. Detection was at 254 nm. Elution conditions were optimized for every stationary phase. In order to compare the chromatographic data from all stationary phases, retention factors (k'), separation factors (α) were given. Racemic mixtures of all printing molecules were analyzed on all three chromatographic columns (see Tables 2 and 3).

Chromatographic data presented in Tables 2 and 3 showed that the molecularly imprinted polymers were able to recognize structurally subtle differences from the template molecules. In every group analyzed, the best separation was indicated to the printing molecule. Base-line separation was obtained for the enantiomers of template molecule in a short time. However, under these conditions other enantiomers of the substrates structurally analogous to the printing molecules could not be resolved or only had poor resolutions (see example shown in Fig. 2). Since the printing molecule had the same size and shape as its structural analogs, thus the difference of recognition property was likely caused alone by the exact placement of functional groups inside the cavity of the polymers.

Table 2
Chromatographic data for polymers imprinted with 2-L-PheNHPy, 3-L-PheNHPy, or 4-L-PheNHPy in organic mobile phase

MIP template	Substrate								
	2-D,L-PheNHPy			3-D,L-PheNHPy			4-D,L-PheNHPy		
	k'_D	k'_L	α	k'_D	k'_L	α	k'_D	k'_L	α
2-L-PheNHPy[a]	1.01	4.07	4.03	1.26	1.26	1.00	1.58	1.58	1.00
3-L-PheNHPy[b]	1.91	2.21	1.16*	1.53	5.43	3.55	1.57	1.57	1.00
4-L-PheNHPy[c]	0.99	2.71	2.74*	1.62	3.56	2.20*	0.79	5.38	6.81

Mobile phase: CH_3CN:CH_3COOH:H_2O (a=91:5:4, b=91:3:6, c=90.5:1.5:8). Temperature 23 °C; flow-rate 0.8 ml/min; loaded amounts: 100 nmol (24.1 μg).
*Poor resolution.

Table 3
Chromatographic data for polymers imprinted with 2-L-PheNHPy, 3-L-PheNHPy, or 4-L-PheNHPy in aqueous mobile phase

MIP template	Substrate								
	2-D,L-PheNHPy			3-D,L-PheNHPy			4-D,L-PheNHPy		
	k'_D	k'_L	α	k'_D	k'_L	α	k'_D	k'_L	α
2-L-PheNHPy[a]	0.82	3.14	3.83	0.06	0.06	1.00	0.10	0.10	1.00
3-L-PheNHPy[b]	1.15	1.80	1.57*	0.99	4.31	4.35	1.05	1.05	1.00
4-L-PheNHPy[c]	0.23	0.23	1.00	0.19	0.59	3.11*	0.60	5.37	8.95

Mobile phase: CH_3CN:0.05 *M* KP (a=6:4, pH=3.6; b, c=7:3, pH=4.7). Temperature 60 °C; flow-rate 0.8 ml/min; loaded amounts: 100 nmol (24.1 μg).
*Poor resolution.

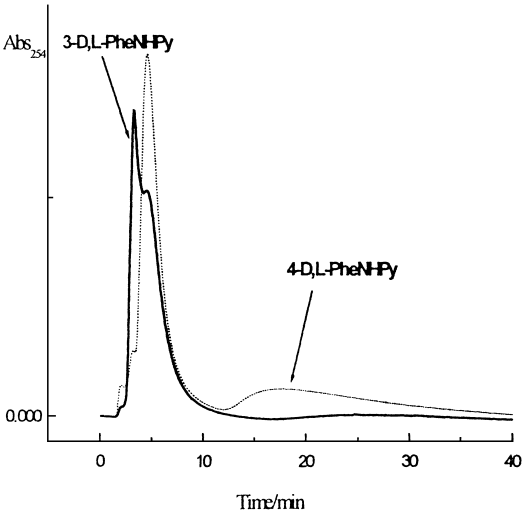

Fig. 2. Elution profiles of 3-D,L-PheNGPy and 4-D,L-PheNHPy applied on 4-L-PheNHPy-imprinted polymer. Mobile phase, MeCN—0.05 *M* KP (pH 4.7)(7:3, v/v); flow-rate, 0.8 ml/min; loaded amounts, 100 nmol.

3.3. The optimization of chromatographic conditions

In this study, the influence of mobile phase composition on the enantiomeric recognition properties of three imprinted polymers was investigated. We observed that the polymers gave better separation using acetonitrile-based mobile phase. In organic media, acetic acid could compete with the methacrylic acid residues in the polymers and weakened the interaction of substrates with the imprinted polymer. So the concentration of acetic acid in acetonitrile was an important factor for the separation. Due to the fact that the basicity of pyridyl group determined the interaction force with carboxylate group in the imprinted polymers, the mobile phase composition was optimized. For a polymer imprinted by 3-L-PheNHPy, 100 nmol of 3-D,L-PheNHPy was well resolved by eluent of 3% acetic acid and 4–8% water in acetonitrile (see Fig. 3).

Sellergren et al. [20] reported that improved chromatographic performance of polymers imprinted

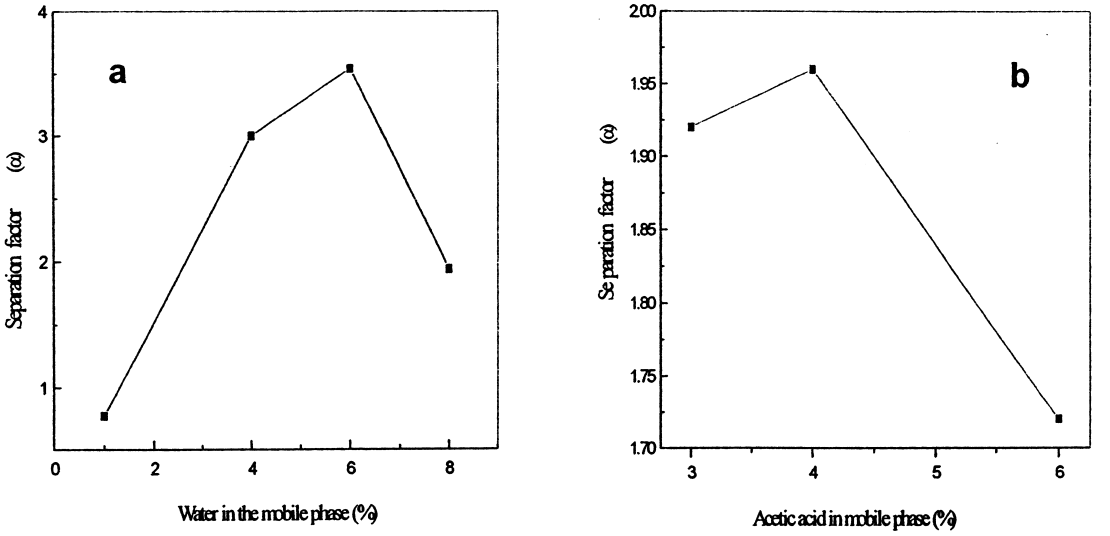

Fig. 3. The influence of the content of water (a) and acetic acid (b) in acetonitrile-based organic mobile phases on the separation factor (a) for the polymer imprinted by 3-L-PheNHPy. Conditions: Sample load, 100 nmol 3-D,L-PheNHPy; flow-rate, 0.8 ml/min.

with L-phenylalanine anilide was achieved by using aqueous buffer–organic solvent mixtures as a mobile phase. In our system, the better separation based on the acetonitrile–KH_2PO_4 (KP) mobile phase was observed. The chromatographic performance was similar to that in organic media (see Tables 2 and 3). This is different from those studies previously reported. Furthermore, the effect of pH value of the mobile phase on the enantiomeric separation was so sensitive that the slight change of pH value in the acetonitrile–KP-based mobile phase would lead to a bad resolution

In organic and aqueous mobile phase one common problem was that the more retarded peak was very broad, highly asymmetric, and tailing. This was probably caused by the fact that the molecularly imprinted polymers normally have unhomogeneous binding sites. The bad peak symmetry made it difficult to measure HPLC chromatogram parameters accurately. In order to distinguish the sample peak from the noise, a method of gradient elution was used for a carboxyl MIP with marginal improvement in peak symmetry [21]. For our system of 3-L-PheNHPy MIP in organic mobile phase, the use of the gradient elution greatly improved the peak symmetry and eliminated the tailing problem. The resolution was also improved with the increase in peak symmetry (see Fig. 4).

3.4. Proposal of recognition mechanism underlying the retention phenomenon

The interactions in the system of L-PheNHPh imprinted polymer was investigated in detail by Mosbach [22]. The recognition was suggested to be involved in the electrostatic and hydrogen bonding interactions between amino and amide groups of the substrate and carboxyl groups of the sites on the polymer. Results reported by Alexander [23] showed that no evidence provided the existence of weak hydrogen bond interactions between template molecule and binding monomer fragment except for electrostatic interaction. In our system of polymerization at higher temperature (60 °C) the weak hydrogen bond interactions were not found to be crucial.

In chromatographic mode the strength of interaction between the imprinted polymer and solute would vary greatly depending on the properties of used solvents. If the imprinted polymer specifically bound its template molecule through hydrogen bonds, this would be suppressed by increasing the concentration of a compound with higher hydrogen bonding capacity (e.g., water or methanol) in the mobile phase. We examined the chromatographic behavior of three molecularly imprinted polymers in the system of acetonitrile–potassium phosphate buffer (see Table 3). The results in Table 3 were similar

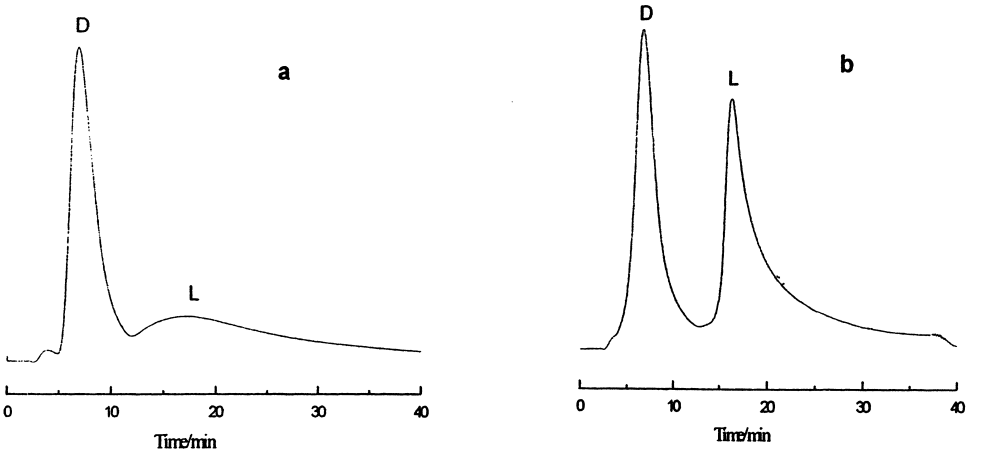

Fig. 4. Comparison of an enantiomeric separation using isocratic or gradient elution. MIP was made against 3-L-PheNHPy. (a) Mobile phase, CH_3CN:CH_3COOH:H_2O=91:3:6; flow-rate 0.8 ml/min: temperature 23 °C; loaded amounts: 100 nmol (24.1 μg) of 3-D,L-PheNHPy was injected in 10 μl of acetonitrile; k_D=1.53, k_L=5.43, α=3.55, R_s=1.91. (b) Gradient elution, solvent A: CH_3CN:CH_3COOH:H_2O= 86:8:6; solvent B: CH_3CN:CH_3COOH:H_2O=91:3:6; 0–7 min B, 7–8 min; temperature 23 °C; loaded amounts: 100 nmol (24.1μg) of 3-D,L-PheNHPy was injected in 10 μl of acetonitrile; k_D=1.44, k_L=4.98, α=3.46, R_s=3.50.

to that in Table 2. Since the increase of the content of water in the mobile phase did not cause the decrease of the selectivity of imprinted polymers, the electrostatic interaction seemed to play a primary role in the course of recognition.

To confirm the interaction of the recognition, the dependence of the retention factor k'_L of the 3-L-form enantiomer in aqueous mobile phase on temperature was investigated (see Fig. 5). The results showed that the change of k'_L value with temperature was very small, indicating that the effect of the temperature on the recognition was weak, which implied the hydrogen-bonding interaction of amide groups of the substrate with the carboxyl group in the polymer matrix was subordinate. Although it was very difficult to strictly describe the actual structure of these binding sites, several assumptions may be suggested. It seemed that the initial binding of the substrate to the polymers was the ionic bonding between the amine groups of the substrate and carboxylic acid residues in the cavity of the polymer. The second step in the recognition process was the formation of other interactions, such as hydrogen bonds or ionic interaction between nitrogen atom on the pyridine ring and carboxylic acid on the polymer, which was the most important account for discrimination of template molecule from structurally related molecules that had not been imprinted in our system. Moreover, by contrast we also noted the better resolution of D,L-PheNHPy on L-PheNHPy imprinted polymer prepared at 60 °C than that of D,L-PheNHPh in organic mobile phase [20]. So an additional interaction force may exist.

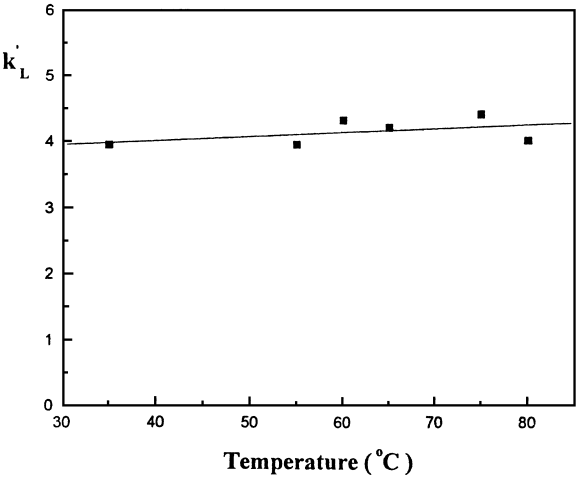

Fig. 5. Effect of temperature on the retention factor of the 3-L-form enantiomer for the 3-L-PheNHPy-imprinted polymer. Conditions: mobile phase, MeCN 0.05 *M* KP (pH 4.7)(7:3 v/v); flow-rate, 0.8 ml/min; loaded amounts: 100 nmol.

4. Conclusions

The molecular recognition properties of three imprinted polymers using three structurally similar molecules as template molecules were investigated. These non-covalent imprinted polymers had the ability of precise recognition of the original imprinting molecules and could distinguish the minor structural difference of substrates in the interaction sites. The possible recognition mechanism based on the chromatographic results suggested that the placement of functional groups at the recognition sites on non-covalent molecularly imprinted polymers was the predominating factor responsible for the resolution of enantiomers.

These observations may offer the new possibilities for the construction of highly selective adsorbents and enzyme-analog built catalysts.

Acknowledgements

We are grateful to State Key Fundamental R and D Projects (G 1999064707) and the Visiting Scholar Foundation of the Chinese Education Ministry for Key Laboratory for financial support of this work.

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