Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 804 (2004) 53–59
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Influence of polymerization temperature on the molecular recognition of imprinted polymers

Yan Lu, Chenxi Li*, Xudong Wang, Pingchuan Sun, Xianghua Xing

State Key Laboratory of Functional Polymer Materials for Adsorption and Separation, Institute of Polymer Chemistry, Nankai University, Tianjin 300071, PR China

Abstract

This paper aimed at investigating the influence of polymerization temperature on the molecular recognition of molecularly imprinted polymers (MIPs) based on multiple non-covalent interactions. 3-L-Phenylalanylaminopyridine (3-L-PheNHPy) imprinted polymers were prepared using azobisnitriles as either thermal initiators or photoinitiators at various temperatures of 10, 40 and 60 °C, respectively. These polymers were subsequently evaluated in the high-performance liquid chromatographic (HPLC) mode for enantioselectivity. An unexpected result shows that polymer prepared at 40 °C has the highest enantioselectivity, but not the polymer prepared at lower temperature of 10 °C. Further, the effect of elution temperature and sample load on the selectivity of polymers was investigated in detail. In order to get a better understanding of the "exception", the influence of polymerization temperature on the polymerization extent and polymer morphology was studied by FT-IR spectrum test, cross-polarization magic angle spinning (CP-MAS) ^{13}C NMR spectra experiment and pore analysis. Based on these results we attribute this "exception" to that there is a tradeoff between the extent of polymerization and stabilization of the template–functional monomer complexes. And an optimal polymerization temperature can be found for each combination of template and monomer.

Keywords: Polymerization temperature; Molecular recognition; Molecular imprinting polymers

1. Introduction

Molecular imprinting is a useful technique for the preparation of functional materials with molecular recognition properties. Recently, molecularly imprinted materials have been used in variety of applications, such as chromatographic stationary phase, immunoassay-type analyses and sensor technologies [1–3]. Molecularly imprinted polymers (MIPs) have become an increasingly active field of study for the construction of new materials capable of molecular recognition [4].

In principle, MIPs are synthesized by cross-linking complexes of template molecules and functional monomers. After removing the template molecules from the polymers, binding sites are formed by functional monomer derived residues complementary for the template molecules. According to the principle, the stability of monomer–template complexes present in the solution prior to polymerization as well as the polymerization reaction itself undoubtedly play a dominant role in determining the recognition performance of the polymers. An understanding of the physical rules governing the quality and quantity of MIPs recognition sites will contribute to design polymerization systems for producing polymers with good recognition property. It is surprising that only relatively limited efforts have been devoted to the analysis of the role of the polymerization temperature on template–functional monomer complexes and polymerization reaction.

It is well known that the position of the equilibrium between free template–monomer(s) and their corresponding complexes is a product of both temperature and pressure [5]. Recently the Sellergren group showed that high pressure (1000 bar) polymerization could be used to enhance the selectivity of the resultant imprinted polymers [6]. In present work, our interest was focused on the influence of polymerization temperature on the recognition of MIPs. Usually lower temperature will stabilize the template–functional monomers complexes. Previously, much research found that lower temperature of polymerization is favorable to the preparation of MIPs based on electrostatic interaction [7–9], due to the greater strength of electrostatic interactions at lower temperatures. It must be noted that polymerization

* Corresponding author. Tel.: +86-222-350-6382; fax: +86-222-350-2749.
E-mail address: nkulicx@yahoo.com.cn (C. Li).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.10.013

temperature also affects the polymerization process (e.g. reaction completeness and reaction rate) and polymer structure (e.g. pore structure and swelling properties), which in turn influence the quality and quantity of MIPs recognition sites. Thus, it may be that there is a tradeoff between the polymerization extent and stabilization of the template–functional monomer complexes. And an optimal condition of polymerization temperature may be found for each combination of template and monomer.

In this paper, we synthesize three polymers at different polymerization temperature of 10, 40 and 60 °C, respectively using 3-L-PheNHPy as template molecule. The molecular recognition properties of polymers are characterized in high-performance liquid chromatographic (HPLC) mode and the influence of sample loads on the selectivity of the polymers is studied in detail. The results show that the polymer prepared at 40 °C has both the highest enantioselectivity and the largest sample load capacity. Further the properties of polymers themselves are also analyzed to examine the effect polymerization temperature on the polymerization extent and polymer morphology. Based on these results we attribute the highest enantioselectivity of the polymer prepared at 40 °C to that the specific temperature condition ensures both the monomer–template complex stability and the high polymerization extent.

2. Experimental

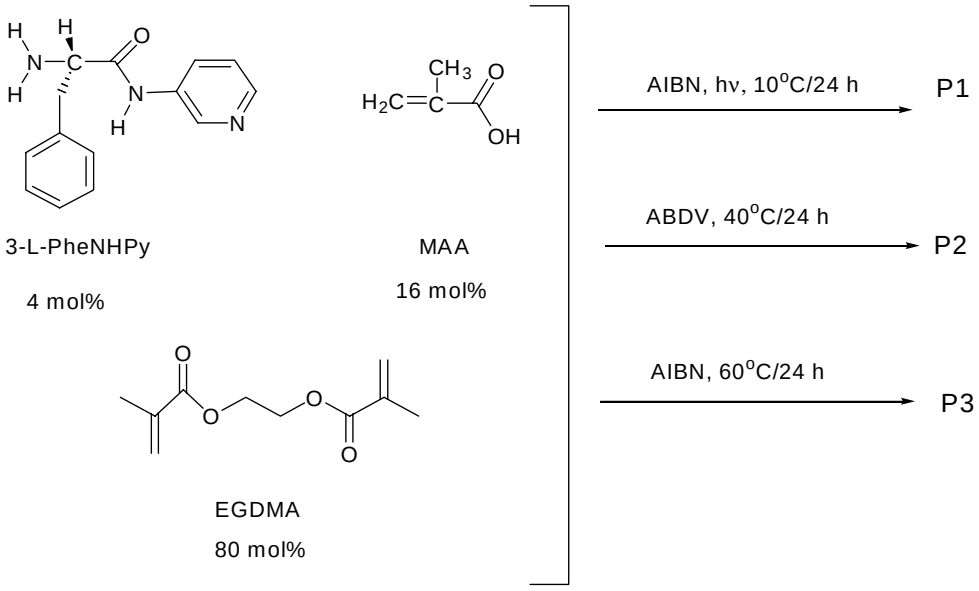
2.1. Materials and instruments

3-L-Phenylalanylaminopyridine (3-L-PheNHPy) and 3-D,L-phenylalanylaminopyridine (3-D,L-PheNHPy), were prepared by condensation of L- or D,L-PheOH with aminopyridine [10,11]. Methacrylic acid (MAA), 2,2-azobisisobutyronitrile (AIBN) and azobisdivaleronitrile (ABDV) were obtained from Tianjin No. 2 Chemical Reagent Factory and were purified by distillation or recrystallization prior to use. Ethylene glycol dimethacrylate (EGDMA) was purified by anion-exchange resin to remove inhibitor. Acetonitrile and methanol are of chromatographic grade. Other chemicals are analytical grade and obtained from commercial sources.

All chromatographic evaluation was done using a Waters 600 pump and a Waters 996 Photodiode Array Detector, connected to a Millennium 32 workstation. Manual injections were carried out using a 7725i injector with 25 μl sample loop. The solvents were degassed using In-Line Degasser (Waters, USA). A LAUDA constant temperature bath oscillator (Germany) was used. The FT-IR spectrum was recorded on a Bio-RADFTS 6000 spectrometer. The cross-polarization magic angle spinning (CP-MAS) ^{13}C NMR spectra were performed on Varian Unity plus-400 MHz instrument (USA). Pore and surface area analysis was performed on Autosorb-1-MP (Quantachrome Corporation, USA).

2.2. Preparation of polymers

Three polymers (P1, P2 and P3) were prepared as shown in Scheme 1. A typical preparation process for block molecularly imprinted polymer was described using P1 as an example. To 0.7 g (2.9 mmol) of template (3-L-PheNHPy), and 1.0 g (12 mmol) of the functional monomer (MAA) in 15 ml of chloroform was added 11.9 g (60 mmol) cross-linker (EGDMA). The 20 mg (0.1 mmol) 2,2-azobisisobutyronitrile was used as an initiator. The mixture was transferred into a 50 ml glass ampoule, degassed in a sonicating bath, and purged with nitrogen for 5 min. Then



Scheme 1. Schematic illustration of the preparation of 3-L-PheNHPy-imprinted polymers. Abbreviations used: AIBN: 2,2′-azobis(2-methylpropionitrile); ABDV: 2,2′-azobis(2,4-dimethylvaleronitrile).

the ampoule was sealed under vacuum. The ampoule was placed at ca. 10 cm distance from a standard laboratory UV light source (365 nm) in a waterbath thermostatted at 10 °C and turned at regular intervals for a symmetric exposure. After 24 h the resultant bulk rigid polymer was ground in a mortar and sieved to collect the 25–38 μm fraction (the gravimetric yield of polymerization is about 93%). The resulting particles were placed into a template-separation apparatus made by us and washed at 1.0 ml/min of the flow rate with 10% acetic acid methanol solution until the template could no longer be detected (λ_{max} = 260 nm) in the elutant (the extraction results in about 67% recovery of the template). Then the particles were washed with methanol to remove residual acetic acid and dried to constant weight under vacuum at 60 °C. IR (KBr) of P1: 3454, 2992, 2959, 1730, 1639, 1567, 1549, 1478, 1454, 1398, 1260, 1159, 959, 879, 816, 756, and 515 cm^{-1}. CP-MAS ^{13}C NMR of P1: δ 177, 167, 137, 62 (br), 58 (br), 46, 24 (br), 18 ppm. The level of unsaturation was estimated by a comparison of the integrals at 177 and 167 ppm corresponding to non-conjugated and conjugated carbonyl carbons as described elsewhere [12].

2.3. Spectroscopic analysis

Single-contact ^{13}C CP-MAS NMR spectra were obtained using a Varian Unity plus-400 MHz spectrometer equipped with an auxiliary high power amplifier and a solid-state probe with magic angle spinning capability. The untemplated samples were packed in a Kel F rotor, which was spun at ca. 6 kHz. The spectra were obtained in a 16 kHz window using 4 K time-domain data points. For each spectrum 4000 scans were accumulated.

2.4. Pore analysis

Pore and surface area analysis were performed by N_2 adsorption on Autosorb-1-MP. In the N_2 adsorption a sample of polymer (25–38 μm) corresponding to ca. 20 m^2 (0.2–0.4 g) was degassed at 170 °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 pore size distribution of pores between 30 and 2000 Å. The surface area was determined using the BET model, the *t*-plot using Harkin–Jura average thickness equation and the pore distribution using the BJH model [13].

2.5. Swelling analysis

Swelling experiments were performed as described previously [14]. The 300 mg of the untemplated polymer particles with the mesh size 25–38 μm was packed in 1 ml solid-phase extraction cartridges. Cartridges were filled with 1 ml of chloroform. After 6 h equilibration at 20 °C, the excess of solvent was removed from the polymer by applying reduced pressure for 1 min, and the weight of the swollen polymer was measured. The swelling ratio (S_r) of the polymers was calculated from the following equation:

$$S_r = \frac{m_s - m_0}{m_0}$$

where m_s is the mass of the swollen polymer and m_0 the mass of dry polymer.

2.6. Chromatographic evaluation

A 1.5 g amount of polymer was subsequently suspended in 30 ml acetonitrile. The suspensions were sonicated for 10 min and placed in a slurry reservoir with a single action reciprocating plunger pump (Alltech Associates, USA). The particles (25–38 μm) were packed into 150 mm × 4.6 mm i.d. stainless-steel column with 200 ml acetonitrile as the packing solvent. The column contained approximately 0.8 g (dry mass) of polymer after packing. The columns were then washed on-line with methanol–acetic acid (9:1, v/v) until a stable baseline was obtained. The flow rate was 0.8 ml/min, the solution of substrate in acetonitrile was injected for analysis. The mobile phase was a 70:30 (v/v) mixture of pure acetonitrile and an aqueous buffer (0.05 M) of potassium dihydrogen phosphate. Subsequent evaluations were carried out at various temperatures using an HPLC system comprising a thermostatted column oven (waters, USA). All analyses were performed in triplicate. The void volumes of the columns were determined by injection of acetone. Retention factors (k'), separation factors (α) were calculated according to standard chromatographic theory as $k'_D = (t_D - t_0)/t_0$, $k'_L = (t_L - t_0)/t_0$, $\alpha = k'_L/k'_D$, where t_D is the retention time of the D-enantiomer, t_L the retention time of the L-enantiomer, and to is the retention time of the void marker.

3. Results and discussion

3.1. Spectroscopic analysis

In order to study the effect of the polymerization temperature on the polymerization extent, FT-IR spectroscopic analysis and CP-MAS ^{13}C NMR test were performed. The content of remaining carbon–carbon double bonds in MIPs is an important indication of polymerization extent. FT-IR spectra of the untemplated polymers (P1, P2 and P3) were given in Fig. 1. The resonance of interest from the vinyl group is indicated. The result may allow an estimate of the extent of unreacted double bonds. A well-resolved band at 1639 cm^{-1} is attributed to C=C stretch and the broad band at 3300–3700 cm^{-1} to the carboxyl OH stretch. The FT-IR spectra of all polymers were indistinguishable indicating that they all contained approximately the same amount of unreacted double bonds. In order to determine the amount of the remaining double bonds in the polymers, CP-MAS ^{13}C NMR test was performed, referring to the pioneer work [12]. In principle, in our system the carbonyl bond in an

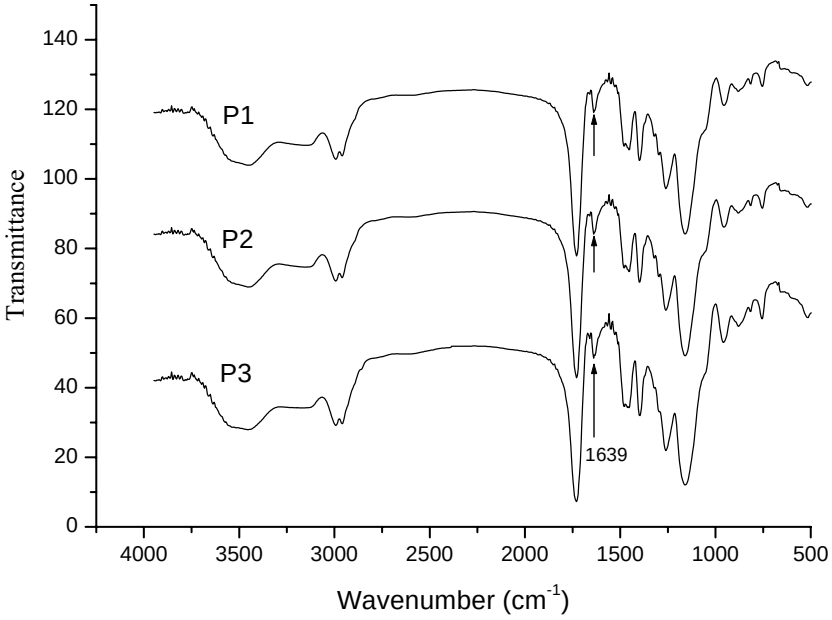


Fig. 1. FT-IR spectra of polymers of P1, P2 and P3. The arrow at 1639 cm^{-1} indicates the band arising from the stretch of carbon–carbon double bond.

Table 1
Effect of polymerization temperatures on the amount of unreacted methacrylic groups

Polymer	Polymerization temperature (°C)	Fraction of unreacted units (mol%)
P1	10	12.9
P2	40	9.6
P3	60	4.1

unreacted acrylate group is conjugated with a double bond, which should shift ^{13}C carbonyl resonance about 10 ppm upfield compared to the reacted units. So based on CP-MAS ^{13}C NMR experiments of P1, P2 and P3 a level of unsaturation of ca. 12.9, 9.6 and 4.1, respectively, was found (Table 1). From the Table 1 it was found that the extent of polymerization increased with increasing polymerization temperature. The number of unreacted double bonds is related to the heterogeneity of the cross-link density, which in turn will affect the stiffness of the chains linking the agglomerates during phase separation from the solution. Further it will influence the integrity and the number of the high-selectivity binding sites. So, this may explain the lowest enantioselectivity of P1 even in lower sample load (Fig. 3).

3.2. Pore and swelling analysis

Often unreacted double bonds remains in the polymer will leading to the change of pore volume, surface areas, and swelling properties. In present work, pore and swelling analysis of P1, P2, and P3 were also performed. The results were presented in Table 2. It is clear from Table 2 that polymerization temperature has a significant effect on the morphology of the polymers. Swelling ratio increased with decreasing temperature of polymerization, which was consistent with the results of CP-MAS NMR. Larger pore volume and surface area were obtained with increasing

Table 2
Comparison of molecularly imprinted polymers prepared under different conditions

Polymer[a]	k'_L	α	Swelling ratio chloroform	N_2 adsorption		
				Surface area (BET)[b] (m^2/g)	Total pore volume[c] (cm^3/g)	Average pore diameter[d] (nm)
P1	10.520	4.54	2.61 ± 0.02	3.515	0.006811	7.8
P2	8.748	5.80	2.52 ± 0.03	47.62	0.08545	7.2
P3	5.467	4.891	1.82 ± 0.02	169.6	0.2891	6.8

Note: The print molecule was 3-L-phenylalanylaminopyridine (3-L-PheNHPy). Particles of 25–38 μm were prepared from the resulting polymers as described. Chromatographic analyses were performed in the HPLC mode (150 mm × 4.6 mm, i.d. columns) with isocratic elution (0.8 ml/min) at 40 °C. Samples consisted of 50 nmol of 3-D,L-phenylalanylaminopyridine. Eluent, MeCN, 0.05 M KP (pH 4.7) (7:3, v/v).

[a] P1, P2, and P3 were prepared at 10, 40, and 60 °C, 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.

polymerization temperature, indicating the formation of more defined sites and/or more sites being available for binding. In theory, the polymerization temperature can affect the polymer morphology in different ways due to complexity of the process of phase separation. The free radical initiator decomposes, generating free radicals and forming cross-linked nuclei or domains, which soon become insoluble and precipitate in the reaction medium forming globules. On one hand, the higher polymerization temperatures lead to the formation of a larger number of free radicals and a larger number of growing nuclei and globules. The formation of a larger number of globules at higher temperature is compensated by their small size. The polymer composed of smaller globules will have a larger number of smaller pores and larger surface area [14]. On the other hand, temperature also affects the phase separation of the polymers from the solution through the solvation of forming nuclei. Normally an increase in temperature improves the nuclei solubility. Thus, at higher temperature the precipitating nuclei will have a higher molecular weight. As a result, both the nuclei and the voids between them would be larger [14,15]. Obviously, an increased surface area and pores volume for the polymers P2 and P3 serves as an indication that the first process plays a more important role in determining their morphology. Although P3 has more binding sites, its sample load capacity is very low. Thus, there must be another factor affecting its enantioselectivity. In present work, we studied the enantioselectivity of the three polymers in detail.

3.3. Polymer selectivity

In order to study the effect of polymerization temperature on the recognition of the MIPs, several 3-L-PheNHPy imprinted polymers were prepared at various polymerization temperatures using azobisnitriles as initiators as shown in Scheme 1. The recognition properties of these polymers were estimated by examining their enantioselectivity in HPLC mode. In present work, the aqueous buffer—organic solvent mobile phase was used to improve the excessive peak broadening referring to pioneer work [16]. The results were partly presented in Table 2. Usually it was considered that the chemical interactions between template molecule and the functional monomers would be stronger at lower temperature, and therefore printing efficiency would be increased. That is, such polymers would exhibit higher substrate and/or enantioselectivity. However, from Table 2 it is interesting to note that P2 shows the highest enantioselectivity (significantly α values, $\alpha = 5.80$), while P1 exhibits the lowest selectivity.

In a similar investigation Sellergren [7] compared materials polymerized at 40 and 60 °C (subsequently annealing both 90 °C and then 120 °C) and found that higher retention factors and better resolution were observed at lower temperature and the separation factors were almost unchanged. Sellergren and Shea [17] also compared materials obtained by either thermal polymerization at 60 °C or photopolymerization at 15 °C and found the latter to be superior stationary phases for the resolution of racemic phenylalanine anilide.

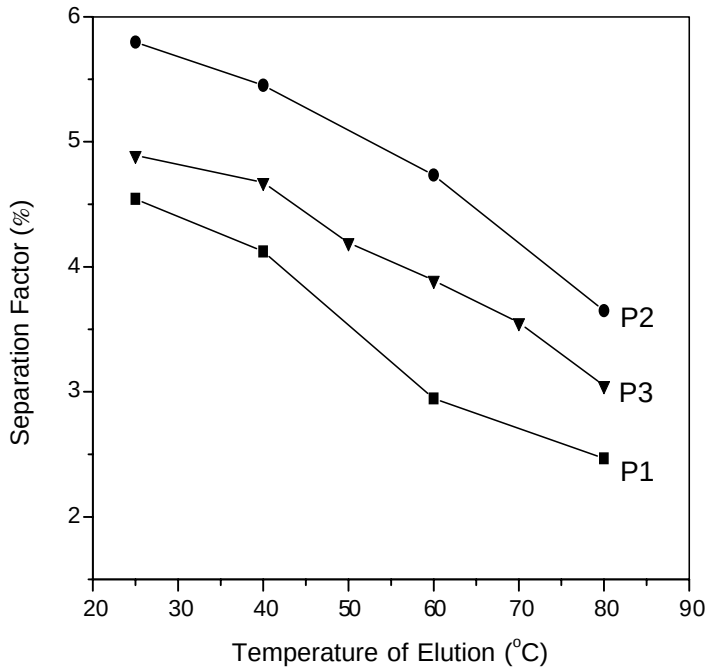


Fig. 2. Effect of temperature of polymerization and elution on separation factor (α). Polymers were prepared as described using thermal initiation of AIBN at 60 °C (▼) or ABDV at 40 °C (●), and photoinitiation of AIBN at 10 °C (■). Chromatographic conditions: mobile phase, MeCN, 0.05 M KP (pH 4.7) (7:3, v/v); flow rate, 0.8 ml/min; sample load, 50 nmol.

In fact, the performance of the materials mentioned above was shown after high-temperature treatment of the initially formed polymer. In general, the heat treatment accompanied the morphology of polymer (e.g. swelling properties and pore structure) would influence selectivity of polymer. Thus, these results could not account directly for the effect of the polymerization temperature on the selectivity of the MIPs. It is possible that the simultaneous influence of polymerization temperature on polymerization reaction itself as well as on the stability of the template–monomer complexes results in different results observed in our system of multi-point non-covalent interaction.

3.4. Temperature of elution versus selectivity

In order to confirm further the results observed in Table 2, the influence of temperature of elution on the selectivity of the polymers was studied in detail. The results were presented in Fig. 2. From Fig. 2, it was clearly seen that there was a large dependence of separation factor on the temperature of elution. The selectivity factors of three imprinted polymers decreased with increasing elution temperature, due to higher temperature of elution decreasing the interactions between the imprint molecule and the polymers while affecting slightly the interactions between non-imprint molecule and the polymers. Over all elution temperatures investigated, imprinted polymer P2 always showed highest separation factors.

3.5. Sample load versus retention and selectivity

As we know polymerization temperature affects both the polymerization reaction itself and the extent and nature of the template–functional monomers complexes present in the solution prior to polymerization. Decreasing the polymerization temperature could promote the formation of template assemblies. By stabilizing the template–monomer assemblies, it is possible to achieve a large number of imprinted sites [18]. That is, larger sample load capacity of imprinted polymer stationary phase would be obtained. Therefore, in order to understand the effect of polymerization temperature on the stability of template–functional monomer complexes, it was necessary to evaluate the influence of sample load on

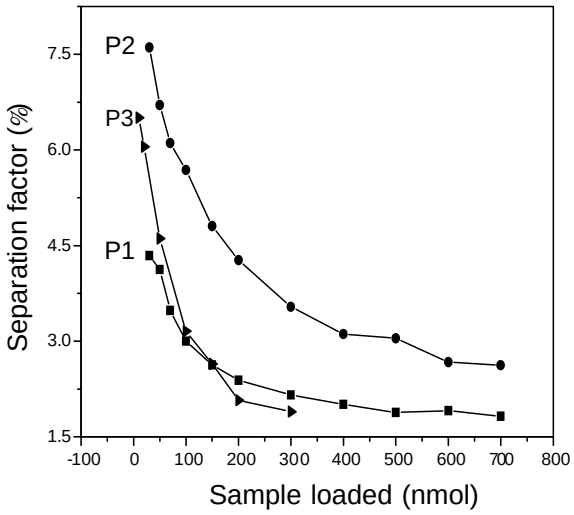


Fig. 3. Separation factors (α) as functions of load amounts on polymers. Polymers were prepared as described using thermal initiation of AIBN at 60 °C (▼) or ABDV at 40 °C (●), and photoinitiation of AIBN at 10 °C (■). Mobile phase, MeCN, 0.05 M KP (pH 4.7) (7:3, v/v); flow rate, 0.8 ml/min; temperature of elution, 40 °C.

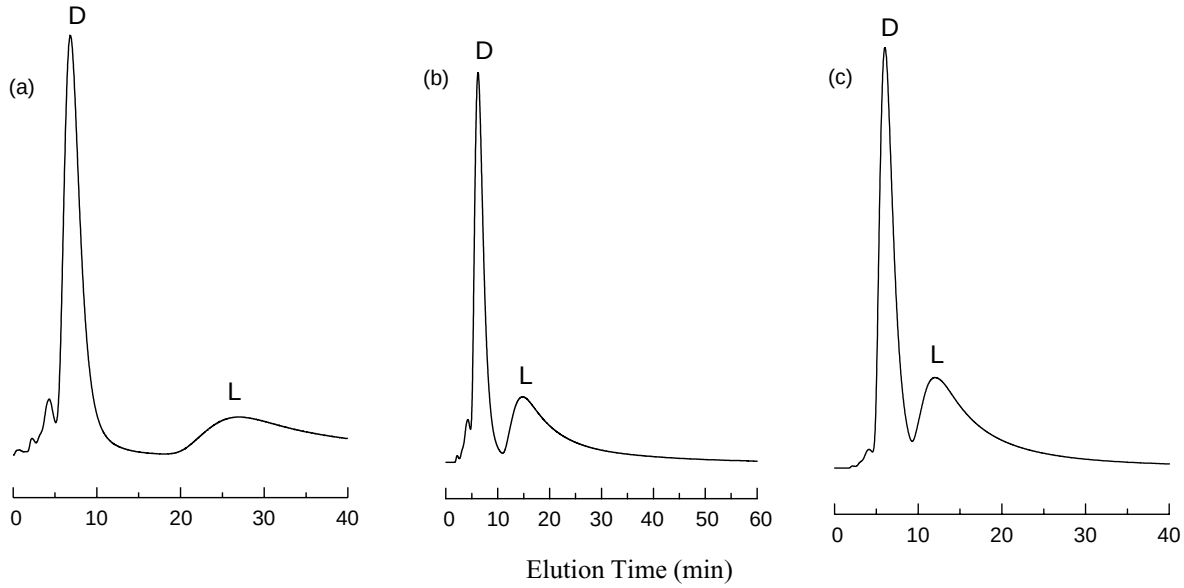


Fig. 4. Elution profiles of different amounts of 3-D,L-PheNHPy applied on P2 prepared at polymerization temperature of 40 °C. Mobile phase, MeCN, 0.05 M KP (pH 4.7) (7:3, v/v); flow-rate, 0.8 ml/min; temperature of elution, 25 °C. The amounts of 3-D,L-PheNHPy injected were (a) 50 nmol; (b) 200 nmol; and (c) 500 nmol.

the selectivity of the imprinted polymers. The results were partly presented in Figs. 3 and 4.

Since high-affinity binding sites are present in limiting numbers in MIPs [19], a decrease in retention and selectivity would be expected to result from higher sample loads. Fig. 3 gives the plots of separation factor versus sample loads. Retention factor k' remains essentially constant for D-form enantiomer over all loading range used. On the other hand, retention factor k' of L-form enantiomer deeply decreases with increasing the sample loads. These results show that there may be two distinct types of binding sites on the polymers. The limited number of imprinted sites that binding the imprint enantiomer and the non-selective sites that occupy the bulk of the polymer. For all polymers, the separation factor α decreases deeply for sample loads from ~10 to 70 μg. It is found polymer P2 always exhibits highest selectivity over all sample loads used while the sample load capacity of polymer P3 is very low.

It is believed from earlier reported that in main single-point interaction system sample load capacities of imprinted polymers that polymers prepared by thermal initiation [20,21] are superior to those prepared by photoinitiation [8] allowing in the former case sample loads of ca. 2.4 mg/g (column temperature: 80 °C) and in the latter case 0.5 mg/g polymer, respectively, with resolved peak maxima. However, in our system mainly containing two-point interaction more sample load capacity was observed on polymer P1 (ca. 0.7 μmol) than that on polymer P3 (ca. 0.3 μmol). In theory, the template–functional monomer complexes are the most stable at 10 °C, resulting in the highest sample load capacity, but the fact is that the sample load capacity (number of available binding sites) of polymer P2 is highest although in comparison with the amount of template added to the monomer mixture (theoretical maximum number of sites) this number is very low.

4. Conclusion

The present results indicate that the polymerization temperature plays a crucial role in the performance of the synthesized materials. The polymer's affinity and specificity were significantly improved by optimizing the polymerization temperature. Polymerization temperature has a complex effect on the monomer–template complex and polymerization reaction itself. Lower polymerization temperature is advantageous to the stability of the template–functional monomer assemblies in the pre-polymerization mixture. However, higher polymerization temperature is favorable for completeness of polymerization reaction, which in turn improves the quality and quantity of MIPs recognition sites and increases the enantioselectivity of polymers. This study indicates the possibility of modulating the polymer's properties by optimizing the temperature regime. And optimal condition of temperature should be found for each combination of template and monomer.

Acknowledgements

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

References

[1] G. Wulff, Angew. Chem. Int. Ed. Engl. 34 (1995) 1812.
[2] S. Vidyasankar, F.H. Arnold, Curr. Opin. Biotechnol. 6 (1995) 218.
[3] R.J. Ansell, O. Ramstrom, K. Mosbach, Clin. Chem. 42 (1996) 1506.
[4] (a) K. Mosbach, O. Ramstrom, Biotechnology 14 (1996) 163;
(b) K.J. Shea, Trends Polym. Sci. 2 (1994) 166;
(c) T. Takeuchi, J. Matsui, Acta Polym. 47 (1996) 471.
[5] I.A. Nicholls, K. Adbo, H.S. Andersson, P.O. Andersson, J. Ankarloo, J. Hedin-Dahlstrom, P. Jokela, J.G. Karlsson, L. Olofsson, J. Rosengren, S. Shorvi, J. Svenson, S. Wikman, Anal. Chim. Acta 435 (2001) 9.
[6] B. Sellergren, C. Dauwe, T. Schneider, Macromolecules 30 (1997) 2454.
[7] B. Sellergren, Macromol. Chem. 190 (1989) 2703.
[8] D.J. O'Shannessy, B. Ekberg, K. Mosbach, Anal. Biochem. 177 (1989) 144.
[9] H.S. Andersson, J.G. Karlsson, S.A. Piletsky, A.-C. Koch-Schmidt, K. Mosbach, I.A. Nicholls, J. Chromatogr. 848 (1999) 39.
[10] J. Altman, M. Gorecki, M. Wilchek, J.R. Votano, A. Rich, J. Med. Chem. 27 (1984) 596.
[11] Y. Lu, C.X. Li, X.H. Liu, W.Q. Huang, J. Chromatogr. 950 (2002) 89.
[12] T. Hjertberg, T. Hargitai, P. Reinholdsson, Macromolecules 23 (1990) 3080.
[13] S.J. Gregg, K.S.W. Sing, Adsorption, Surface Area and Porosity, second ed., Academic Press, London, 1982.
[14] S.A. Piletsky, E.V. Piletska, K. Karim, K.W. Freebairn, C.H. Legge, A.P.F. Turner, Macromolecules 35 (2002) 7499.
[15] C. Viklund, F. Svec, J.M. Frechet, J. Chem. Mater. 8 (1996) 744.
[16] B. Sellergren, K.J. Shea, J. Chromatogr. 690 (1995) 29.
[17] B. Sellergren, K.J. Shea, J. Chromatogr. 635 (1993) 31.
[18] B. Sellergren, J. Chromatogr. 906 (2001) 227.
[19] G. Vlatakis, L.I. Andersson, R. Muller, K. Mosbach, Nature 361 (1993) 645.
[20] B. Sellergren, Chirality 1 (1989) 63.
[21] B. Sellergren, M. Lepisto, K. Mosbach, J. Am. Chem. Soc. 110 (1988) 5853.