Poly(N^5-benzyl-L-glutamine)-Coated Silica Gels as Chiral Stationary Phase for Direct Resolution of Hydantoins

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SYNOPSIS

Poly(N^5-benzyl-L-glutamine) (PNLG), poly[N^5-(+)-1-phenylethyl-L-glutamine] [P(+)NLG], and poly[N^5-(±)-1-phenylethyl-L-glutamine) [P(±)NLG] were synthesized from poly[γ-(β-chloroethyl)-L-glutamate], which, in turn, was obtained by ester exchange of poly(γ-methyl-L-glutamate) with 2-chloroethanol. The PNLG was found to exist mainly in an α-helical conformation in the film state by using circular dichroic and infrared spectra. Moreover, the PNLG, P(+)NLG, and P(±)NLG have been coated on macroporous silica gels (10 μm) and packed as chiral stationary phases for direct chromatographic resolution of 10 hydantoins. The column bearing PNLG resolved satisfactorily four hydantoins (α = 1.34–1.73), and the enantioselectivity (α) increased with the bulkiness of 5-substituent in hydantoins. On the other hand, an increase in 2-propanol concentration of the eluent (2-propanol/hexane) resulted in a smooth decrease of the capacity factor. However, the selectivity (α) increased slightly, indicating that the α-helix of PNLG plays an important role in chiral resolution. From these results, it is concluded that the main chiral recognition force is the steric hindrance between the 5-substituents of hydantoin and the side chains of PNLG. The temperature effect on the resolution of the hydantoins by PNLG was also investigated. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

It is well known that surrounding us there exist many optically active (chiral) polymers. For example, starches and celluloses are the indispensable resources of life; enzymes and nucleic acids are the catalytic and hereditary substances, respectively, in biochemical processes. To mimic and elucidate the sophisticated functions played by natural polymers, many optically active polymers have been synthesized, and the relation between structures, properties, and functions as well as applications have been investigated.[1] The applications of optically active polymers are mainly as catalysts for asymmetric synthesis and as chiral stationary phases (CSP) for the direct optical resolution of enantiomers.[2,3] Certain optically active polymers can be absorbed on macroporous silica gels and used as effective packing materials for the direct optical resolution of enantiomers by high-performance liquid chromatography (HPLC).[3]

Since many biologically active compounds such as drugs and pharmaceuticals show different activity between their optical isomers, the procurement of optically active isomers and the determination of optical purity are becoming very important. This trend promoted the research and development of the preparation of chiral stationary phases (CSPs) with high recognition ability for enantiomers. CSPs can be silica gel-bonded low molecular weight compounds[4-8] or silica gel-absorbed optically active polymers.[9-14] Sometimes, optically active solid polymers can be used directly as packing materials, although their endurance to pressure is inferior to silica gel-absorbed ones.[15,16] For low molecular weight chiral stationary phases, the chiral recognition should involve a minimum of three simultaneous interactions with at least one of the enantiomers and at least one of these interactions is stereochemically dependent.[17] In polymeric CSPs, ordered conformation plays an important role in chiral recognition as well as in the simultaneous interactions

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between functional groups.[3] Some immobilized enzymes and polypeptides have been shown to be effective CSP for direct resolution of enantiomers.[18-21]

The objective of this work was to synthesize and characterize poly(N^5-benzyl-L-glutamine) (PNLG), poly(N^5-(+)-1-phenylethyl-L-glutamine) [P(+)NLG], and poly(N^5-(±)-1-phenylethyl-L-glutamine) [P(±)NLG] from poly(γ-methyl-L-glutamate). Chiral resolution of PNLG to 10 hydantoins, with the solvent and temperature effect, are presented and discussed.

EXPERIMENTAL

Chemicals

The *N*-carboxyanhydride (NCA) of γ-methyl-L-glutamate was synthesized from glutamic acid (Merck). It was recrystallized twice in ethyl acetate just before polymerization; the melting point was 96–98°C. Benzylamine, (+)-1-phenylethylamine, and (±)-1-phenylethylamine were from Merck and used as received. Triethylamine and 2-chloroethanol were purchased from Merck and Fluka, respectively, and used without further purification. The solvents used in the synthesis of NCA and polypeptides were purified and dried by the conventional method. Other chemicals were all pure reagents and used as received.

Measurements

Infrared spectra (IR) were recorded using an IR spectrophotometer, Model IR-810, from Japan Spectroscopic Co., at a resolution of 4 cm^{-1}. ^{1}H-NMR spectra were recorded using a Brucker 200 MHz spectrometer. Optical rotations were measured using a Jasco DIP-370 digital polarimeter at 27°C. The intrinsic viscosity of polymers was measured at 30°C using an Ostwald viscometer; the solvent was dichloroacetic acid (DCA).

The circular dichroic (CD) spectra of polymers were measured at room temperature on a Jasco J-720 spectropolarimeter. The thin films for CD measurement were prepared by casting their solutions (ca. 0.1 g/mL) on the outside surface of a quartz cell. The coated films were dried at ambient air and then *in vacuo* for 24 h at room temperature. Each CD measurement was repeated four times by rotating the sample cell by 60°, 120°, and 180° from the first position around the axis of the incident light beam to ascertain the absence of linear dichroism.

Direct chromatographic resolution of hydantoins was accomplished on a Hitachi L-6000 pump, a D-2000 chromatointegrator, and an L-4000 variable wavelength detector monitored at 254 nm. A Jasco digital polarimeter, Model DIP-370, equipped with a flow-through cell (50 × 3.0 mm [i.d.]) was employed for determining the polarity of separated enantiomers. The eluent was a mixture of hexane and 2-propanol (v/v = 9/1), and the flow rate was 0.5 mL/min at ambient temperature.

Synthesis of Poly(γ-methyl-L-glutamate) (PMLG)

As shown in Scheme 1, PMLG was prepared by ring-opening polymerization of NCA in 1,2-dichloroethane (DCE) using benzylamine or triethylamine as initiators. To a solution of NCA (*A*) in 30 mL DCE (1.15 g, 6.15 mmol) was added benzylamine (*I*) all at once ([*A*]/[*I*] = 600), and the mixture was allowed to react at 30°C for 96 h after purging with dry nitrogen. The gels that appeared were reprecipitated in methanol to obtained 0.50 g of PMLG. Yield 56.8%, [η] = 0.22 dL/g, [α] = −33.2° (c 0.5, DCE). IR (KBr) ν 3300 (amide A), 1630–1660 (amide I), 1520–1550 cm^{-1} (amide II). ^{1}H-NMR (CF_3COOD) δ 2.41 (s, 2H, β—C$\underline{H}_2$—); 2.62–2.69 (d, 2H, γ—C$\underline{H}_2$—), 3.87 (s, 3H, —O—C$\underline{H}_3$), 4.77–4.82 ppm (t, 1H, α—C$\underline{H}$—).

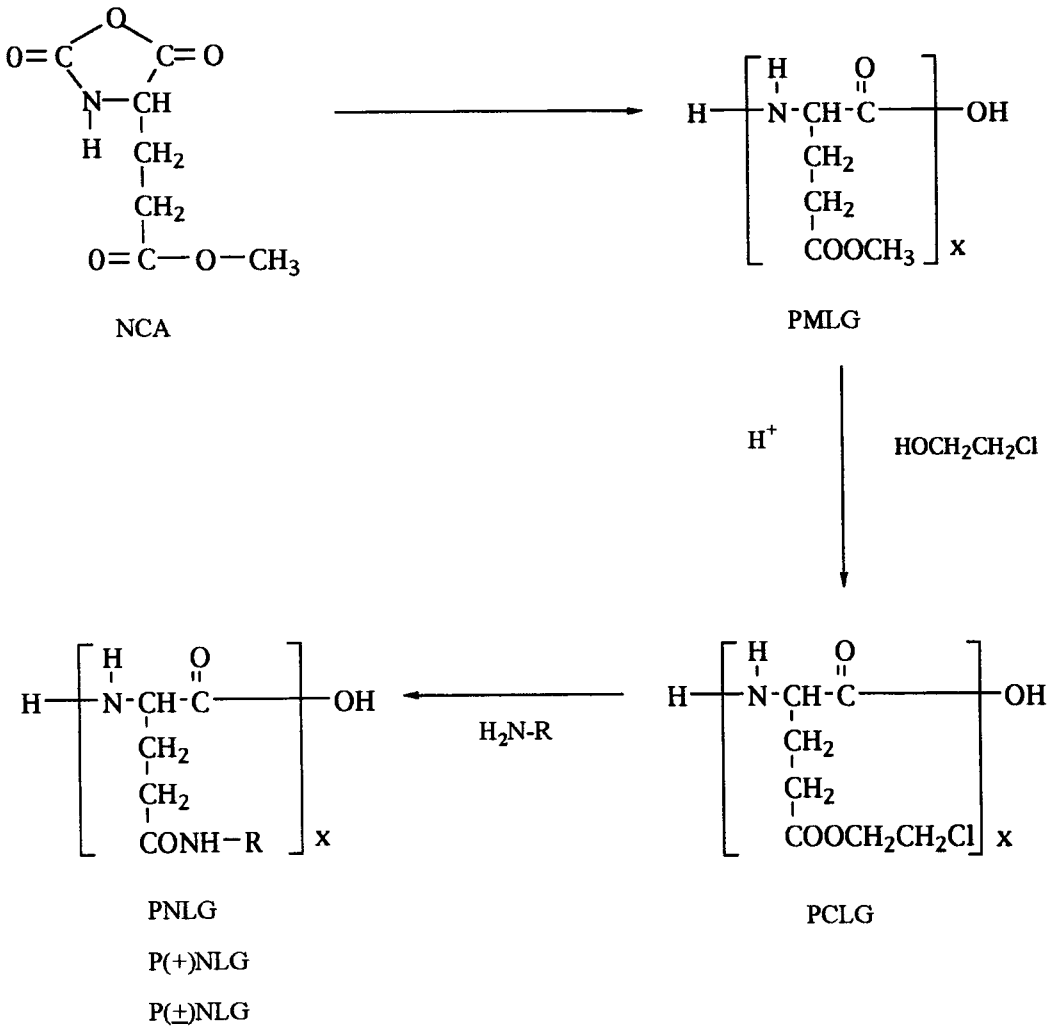
ANAL: Calcd for $(C_6H_9NO_3)_n$: C, 50.35%; H, 6.34%; N, 9.79%.
Found: C, 50.11%; H, 6.36%; N, 9.51%.

In case of initiation by tertiary amine, for example, to a solution of NCA in 110 mL DCE (4.13 g, 22.08 mmol) was added triethylamine (A/I = 600) quickly, and the mixture was allowed to react at 30°C for 96 h after purging with dry nitrogen. The gels were reprecipitated in methanol to obtain 3.15 g of PMLG. Yield 99.6%, [η] = 1.69 dL/g, [α] = −33.9° (c 0.5, DCE).

ANAL: Calcd for $(C_6H_9NO_3)_n$: C, 50.35%; H, 6.34%; N, 9.79%.
Found: C, 50.06%; H, 6.32%; N, 9.59%.

Synthesis of Poly[γ-(β-chloroethyl)-L-glutamate] (PCLG)[20]

For example, to a solution of PMLG ([η] = 0.22 dL/g]) in 20 mL DCE (1.10 g, 7.68 mmol) was added successively 10 mL of 2-chloroethanol and 2.90 g of *p*-toluenesulfonic acid (or H_2SO_4). Under vacuum (360 mmHg), the mixture was allowed to react at 60°C for 24 h. The PCLG was isolated by reprecipitating in large quantity of methanol. [η] = 0.20 dL/g, [α] = −22.2° (c 0.5, DCA). IR(KBr) ν 3300

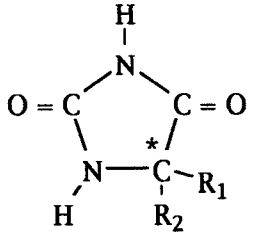


H_2N-R

PNLG: $H_2N-CH_2-C_6H_5$

P(+)NLG: (+)$H_2N-\overset{*}{C}H(CH_3)-C_6H_5$

P(±)NLG: (±)$H_2N-\overset{*}{C}H(CH_3)-C_6H_5$



Hydantoin

Scheme 1

(amide A), 1630–1650 (amide I), 1520–1550 cm^{-1} (amide II). ^{1}H-NMR(CF_3COOD) δ 2.12–2.36 (m, 2H, $\beta-C\underline{H}_2-$), 2.65 (s, 2H, $\gamma-C\underline{H}_2-$), 3.67–3.72 (d, 2H, $-C\underline{H}_2-Cl$), 3.76 (s, 3H, $-O-C\underline{H}_3$), 4.39–4.44 (t, 2H, $-O-C\underline{H}_2-$), 4.77 ppm (s, 1H, $\alpha-C\underline{H}-$). The chlorine content was found to be 14.8% by elemental analysis compared to 18.5% by calculation.

Nucleophilic Substitution of PCLG

The above-obtained PCLG with $[\eta]$ = 1.25 dL/g (0.8 g, 4.17 mmol) was directly dissolved in benzylamine, (+)- or (±)-1-phenylethylamine, respectively, and the mixtures were allowed to react at 60–80°C for 24 h. The products were isolated by reprecipitating in ethyl ether to obtain PNLG, P(+)NLG, and P(±)NLG, respectively.

Poly(N^5-benzyl-L-glutamine) (PNLG)

$[\eta]$ = 1.33 dL/g, $[\alpha]$ = −18.8° (c 0.5, DCA). IR(KBr) ν 3300 (amide A), 1630–1650 (amide I), 1520–1550 (amide II), 700 cm^{-1} (monosubstituted benzene). ^{1}H-NMR(CF_3COOD) δ 2.25–2.37 (d, 2H, $\beta-C\underline{H}_2-$), 2.74 (s, 2H, $\gamma-C\underline{H}_2-$), 4.53 (s, 2H, $-C\underline{H}_2-C_6H_5$), 4.76 (s, 1H, $\alpha-C\underline{H}-$), 7.23–7.33 ppm (t, 5H, $-C_6\underline{H}_5$). The content of chlorine was found to be lower than 0.1% from elemental analysis.

Poly[N^5-(+)-phenylethyl-L-glutamine] [P(+)NLG]

$[\eta]$ = 0.87 dL/g, $[\alpha]$ = −20.9° (c 0.5, DCA). IR(KBr) ν 3300 (amide A), 1630–1660 (amide I), 1530–1550 (amide II), 700 cm^{-1} (monosubstituted benzene). ^{1}H-NMR(CF_3COOD) δ 1.82–1.86 (d, 3H, $-CH-C\underline{H}_3$), 2.25 (s, 2H, $\beta-C\underline{H}_2-$), 2.69 (s, 2, $\gamma-C\underline{H}_2-$), 3.74–3.84 (m, 3H, $-O-C\underline{H}_3$), 4.68–4.71 (d, H, $-NH-C\underline{H}-CH_3$), 4.75 (s, 1H, $\alpha-C\underline{H}-$), 7.34–7.50 ppm (t, 5H, $-C_6\underline{H}_5$).

Poly[N^5-(±)-phenylethyl-L-glutamine] [P(±)NLG]

$[\eta]$ = 0.85 dL/g, $[\alpha]$ = −19.3° (c 0.5, DCA). The characteristic IR and ^{1}H-NMR absorptions are the same as those of P(+)NLG described above.

Synthesis of Hydantoins for Chromatographic Resolution

The racemic hydantoins were prepared from aldehydes or ketones as reported by Henze and Speer.[22] For example, into a 50% aqueous solution of ethanol (50 mL) was added 2.12 g of benzaldehyde (0.02 mol), 9.1 g of ammonium carbonate (0.08 mol), and 2.6 g of KCN (0.04 mol). The reaction mixture was concentrated to ca. 40 mL after reacting at 60°C for 2 h. The crystals appeared was isolated by filtration to obtain 2.23 g of hydantoin **H5**. The synthetic results of hydantoins **H1–H10** are summarized in Table I.

RESULTS AND DISCUSSION

Preparation of PNLG, P(+)NLG, and P(±)NLG

Ring-opening polymerization of the NCA in DCE resulted in poly(γ-methyl-L-glutamate) (PMLG) with intrinsic viscosities ($[\eta]$) at 0.22–1.69 dL/g depending on the initiator and $[A]/[I]$ ratio (Table II). Using benzylamine as initiator with $[A]/[I]$ = 600, the $[\eta]$ is 0.22 dL/g and the viscosity molecular weight ($\bar{M}_v$) is 1.45×10^4 as calculated by $[\eta] = 20.2 \times 10^{-5} \bar{M}_v^{0.73}$ of Suzuki et al.[23] On the other

Table I The Synthesis of Hydantoins H1–H10

Hydantoin	$-R_1$	$-R_2$	Yield (%)	mp (°C)
H1	$-H$	$-C_3H_7$	38.8	138–139
H2	$-H$	$-CH(CH_3)_2$	53.4	144–146
H3	$-H$	$-CH(C_2H_5)_2$	49.8	155–156
H4	$-H$	$-CH_2C_6H_5$	44.0	180–182
H5	$-H$	$-C_6H_5$	63.3	178–179
H6	$-H$	$-C_6H_4-CH_3$	67.2	180–182
H7	$-CH_3$	$-C_2H_5$	62.3	143–144
H8	$-CH_3$	$-C_3H_7$	46.2	124–125
H9	$-CH_3$	$-CH(CH_3)_2$	75.1	174–175
H10	$-CH_3$	$-C_6H_5$	73.7	194–195

Table II Synthesis of PMLG, PCLG, PNLG, P(+)NLG, and P(±)NLG

Polymer	Initiator	[A]/[I] Ratio[a]	[η] (dL/g)[b]	M_v(× 10^4)[c]	DP[d]	[α][e]
PMLG-1	ϕ-CH_2NH_2	600	0.22	1.45	101	−33.2°
PMLG-2	$N(Et)_3$	600	1.69	23.6	1650	−33.9°
PMLG-3	$N(Et)_3$	500	1.53	20.6	1440	−34.1°
PCLG-1[f]	—	—	0.20	1.27	71	−22.2°
PCLG-2[f]	—	—	1.31	16.7	925	−22.9°
PCLG-3[f]	—	—	1.25	15.6	855	−24.9°
PNLG[g]	—	—	1.33	17.0	780	−18.8°
P(+)NLG[g]	—	—	0.87	9.5	434	−20.9°
P(±)NLG[g]	—	—	0.85	9.2	416	+19.3°

[a] [A]/[I] ratio: the molar ratio of NCA and initiator.
[b] Measured in dichloroacetic acid at 27°C.
[c] Calculated by $[\eta] = 20.2 \times 10^{-5} M_v^{0.73}$ from Ref. 23.
[d] DP: degree of polymerization.
[e] Measured in 0.5 g/dL dichloroacetic acid at 27°C.
[f] The PCLG-1, PCLG-2, adn PCLG-3 were obtained from PMLG-1, PMLG-2, and PMLG-3, respectively.
[g] The starting polypeptide was PCLG-3 with [η] = 1.25 dL/g.

hand, initiation by triethylamine with [A]/[I] = 600 and 500 results in PMLGs with [η] = 1.69 and 1.53 dL/g, respectively. Clearly, triethylamine gives much higher molecular weight than does benzylamine. However, the degree of polymerization (DP) is not proportional to the [A]/[I] ratio. Therefore, the polymerization is not a living one irrespective of the initiator. Moreover, the specific rotations ([α]) of the isolated PMLGs are about −33.2 to −34.1° despite their molecular weight difference. The PCLGs, obtained by ester exchange of PMLG ([η] = 0.22–1.65 dL/g) with 2-chloroethanol, possess intrinsic viscosities ([η] = 0.20–1.31 dL/g) lower than those of the corresponding PMLGs, indicating that slight chain scission may have occurred during the reaction. The PNLG, P(+)NLG, and P(±)NLG were obtained by nucleophilic substitution of PCLG ([η] = 1.25 dL/g) with the corresponding amines (Scheme 1). The resulting PNLG shows higher viscosity ([η] = 1.33 dL/g) than that of starting PCLG, whereas for P(+)NLG and P(±)NLG, the viscosities ([η] = 0.85 − 0.87 dL/g) are much lower than that of PCLG. These results suggest that PCLG main chains could be readily cleavaged when phenylethylamine is used as both solvent and nucleophilic reagent.

Conformation in the Film State

Circular dichroic (CD) spectra have been proven to be an effective method for the study of ordered conformation of polypeptides.[24–26] Polypeptides forming as α-helical conformation possess one positive CD band at 190 nm and two negative bands at 208–210 nm and 222 nm. However, the amplitude and wavelength of the CD bands may shift slightly depending on solvent, side chain, and measuring state. To elucidate the contribution of ordered conformation to chiral separation, the CD spectra of PNLG, which resolved some hydantoins satisfactorily (see next section), were investigated. Figure 1 shows the CD spectra of PNLG in the film state. The negative CD bands appear at 228 and 207 nm, which can be attributed to components of the split π–π^* transition due to an exciton coupling of amide groups. The positive CD band appears at ca. 190 nm. From these results, it is concluded that PNLG exists mainly in α-helical conformation, although the helix may not

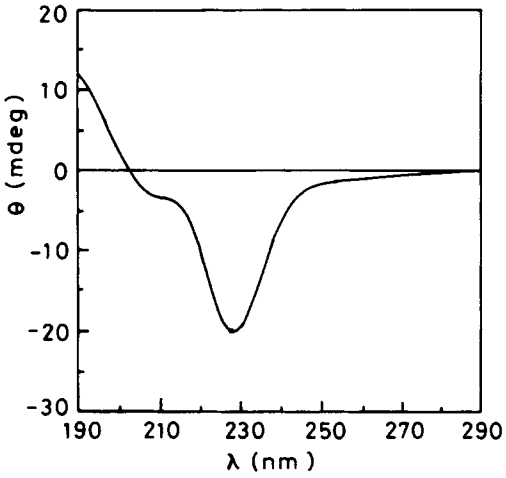


Figure 1 CD spectra of PNLG in the film state.

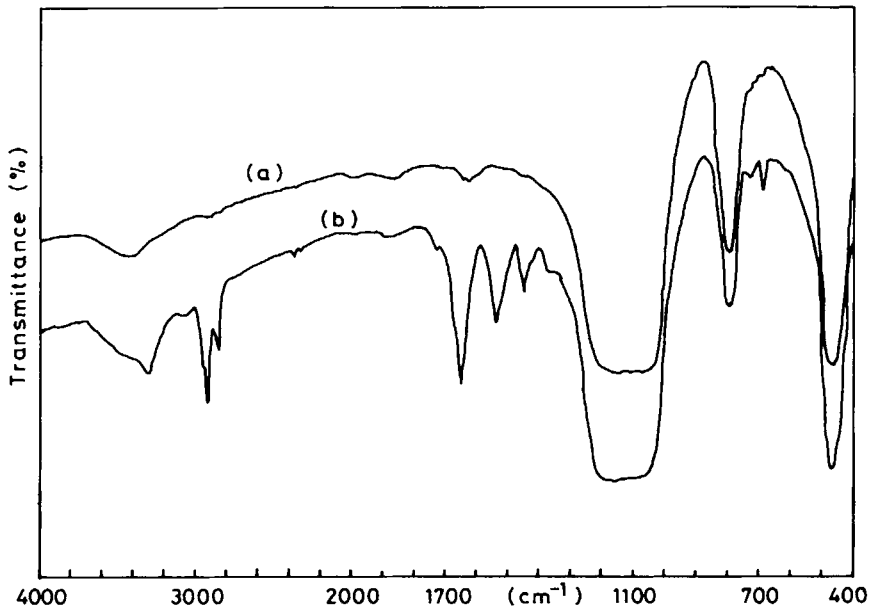


Figure 2 IR spectra of (a) silica gels and (b) PNLG-coated silica gels.

be a fully developed one. This can also be evidenced by its IR spectra, which shows characteristic absorptions of α-helix at ca. 1550 and 1650 cm^{-1}.

Chromatographic Resolution of Hydantoins H1–H10

Optically active polymers have been coated on macroporous silica gels and successfully packed as chiral stationary phases (CSPs) for direct resolution of racemates by high-performance liquid chromatography (HPLC).[3] Ordered structures such as α-helix and crystalline morphology have a synergistic effect to chiral separation of racemates.[11,12,27] The PNLG forms as α-helix and possesses many functional groups such as phenyl and amide chromophores. Expected is separation of hydantoins **H1–H10** (Scheme 1), which also possess many interactive functional groups.

Table III Chromatographic Resolution of Hydantoins H1–H10 by Columns Bearing PNLG and P(±)NLG[a]

	Substituent		PNLG[b]			P(±)NLG[b]	
Hydantoin	$-R_1$	$-R_2$	k_1'[c]	k_2'[c]	α[c]	k_1'	k_2'
H1	—H	$-C_3H_7$	1.41	—	1.00	0.38	—
H2	—H	$-CH(CH_3)_2$	1.13(+)[d]	1.50	1.34	0.35	—
H3	—H	$-CH(C_2H_5)_2$	0.62(+)	0.87	1.41	0.19	—
H4	—H	$-CH_2C_6H_5$	3.15	—	1.00	0.85	—
H5	—H	$-C_6H_5$	4.55	—	1.00	1.13	—
H6	—H	$-C_6H_4-CH_3$	3.20	—	1.00	0.68	—
H7	$-CH_3$	$-C_2H_5$	0.74	—	1.00	0.26	—
H8	$-CH_3$	$-C_3H_7$	0.58(+)	0.81	1.42	0.19	—
H9	$-CH_3$	$-CH(CH_3)_2$	0.56(+)	0.97	1.73	0.18	—
H10	$-CH_3$	$-C_6H_5$	1.62	—	1.00	0.56	—

[a] Eluent: hexane/2-propanol (v/v = 8/2); flow rate: 0.5 mL/min at 27°C.
[b] The coated PNLG and P(±)NLG are 16.6 and 14.5 wt %, respectively.
[c] The capacity factor $k_i' = (V_i - V_0)/V_0$, where V_i is the retention volume of the enantiomers and V_0 is the dead volume; the separation factor $\alpha = k_2'/k_1'$, where k_2' is the capacity factor of the more retained enantiomer.
[d] The first-eluted enantiomer is (*R*) – (+) – isomer.

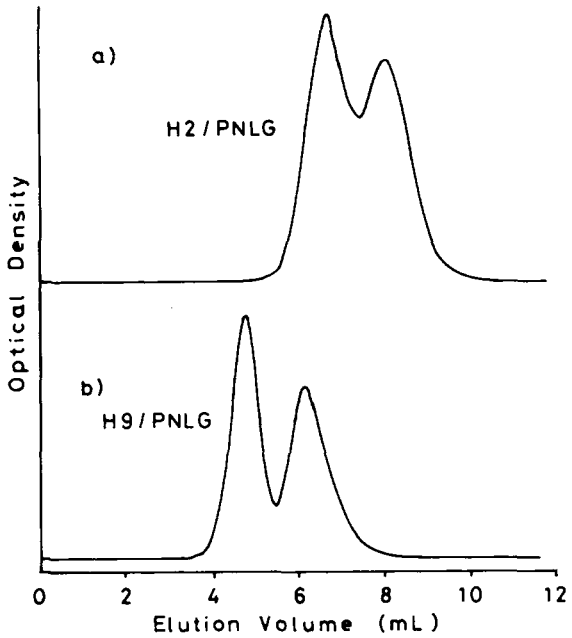


Figure 3 Resolution chromatograms of (a) (*R*, *S*)-5-isopropylhydantoin (**H2**) and (b) (*R*, *S*)-5-methyl-5-isopropylhydantoin (**H9**) by column bearing PNLG.

Macroporous silica gels, treated with dichlorodiphenylsilane, were coated with ca. 14–17 wt % of PNLG, P(+)NLG, and P(±)NLG, respectively, and packed as CSPs for direct HPLC resolution. For comparison, Figure 2 shows the IR spectra of silica gels and PNLG-coated (16.6 wt%) silica gels. Table III summarizes the chromatographic parameters (k'_1, k'_2, α) in the resolution of **H1–H10.** For columns bearing P(+)NLG and P(±)NLG, none of the hydantoins can be resolved and the capacity factors (k') are much smaller than those of PNLG. Therefore, in the following sections, we will discuss mainly the resolution results of the hydantoins by PNLG.

The column bearing PNLG resolves **H2, H3, H8,** and **H9** satisfactorily with enantioselectivity (α) at 1.34–1.73, and for each racemate, the first eluted enantiomer is the (*R*)-(+)-isomer. Moreover, the bulkier substituent in the 5-position (R_2) results in more efficient resolution. For example, the α value of **H3** (R_2 = 1-ethylpropyl) is 1.41 compared with 1.34 of **H2** (R_2 = isopropyl). In the case of disubstituted hydantoins (**H8** and **H9**), a similar tendency can also be observed. Consequently, steric hindrance should play an important role in the chiral recognition process. The resolution chromatograms of (*R,S*)-5-isopropylhydantoin (**H2**) and (*R,S*)-5-methyl-5-isopropylhydantoin (**H9**) are shown in Figure 3.

Table IV Solvent Effect for the Chromatographic Resolution of H2 and H9 by Column Bearing PNLG[a]

Eluent 2-Propanol/Hexane (v/v)	k'_1 (+)	k'_2 (−)	α
(*R*, *S*)-5-Isopropylhydantoin (**H2**)			
15 : 85	1.564	2.049	1.310
20 : 80	1.125	1.502	1.335
25 : 75	0.783	1.051	1.342
30 : 70	0.642	0.868	1.348
(*R*, *S*)-5-Methyl-5-isopropylhydantoin (**H9**)			
15 : 85	0.792	1.362	1.720
20 : 80	0.561	0.970	1.729
25 : 75	0.394	0.682	1.731
30 : 70	0.315	0.548	1.740

[a] The operation conditions are the same as those stated in Table III except that the compositions of the eluent are changed.

Solvent Effect in Chiral Separation

As described above, chiral recognition between a CSP and an enantiomer depends on three simul-

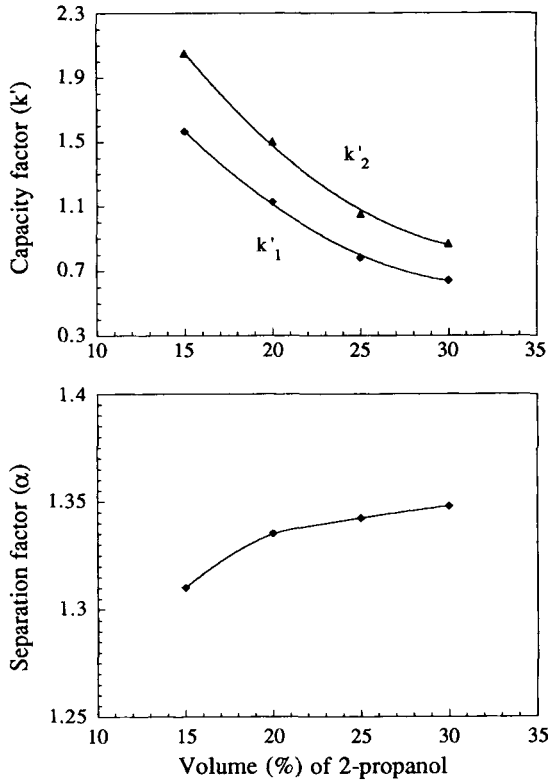


Figure 4 Influence of eluent composition on capacity factor (k') and selectivity (α) for the resolution of **H2**.

taneous interactions and/or ordered conformation. The former can be a $\pi-\pi$ interaction, a dipole–dipole interaction, hydrogen bonding, and steric hindrance, depending on the racemates and CSPs. The latter can be helical conformation or crystalline morphology of chiral polymers. The strength of these interactions determines the degree of chiral recognition, as seen by the order of elution (k') of the enantiomers, and the difference in their retention (α). Furthermore, the interactions are readily influenced by the chemical property of the eluent. For example, in our system, hexane is a nonpolar, nonselective solvent, serving only to adjust the strength of the mobile phases. However, 2-propanol interacts with the CSP through reciprocal hydrogen bonding at the amide groups.

To further elucidate the chiral recognition mechanism of PNLG to hydantoins, the effect of mobile phases was investigated by changing the ratio of 2-propanol/hexane. Table IV and Figures 4 and 5 show the variations of k'_1, k'_2, and α for (*R,S*)-5-isopropylhydantoin (**H2**) and (*R,S*)-5-methyl-5-isopropylhydantoin (**H9**), as the 2-propanol/hexane ratio changes from 15/85 to 30/70. Figure 6 shows the variation of resolution chromatograms of **H9** at different eluent compositions.

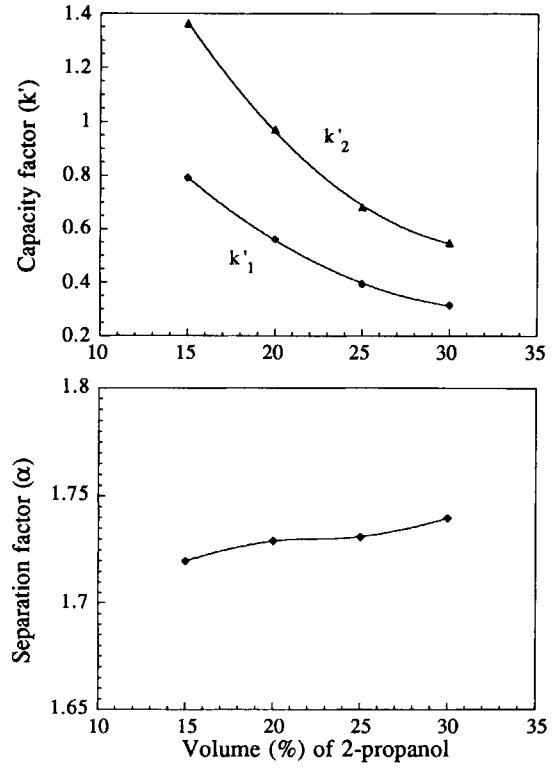


Figure 5 Influence of eluent composition on capacity factor (k') and selectivity (α) for the resolution of **H9**.

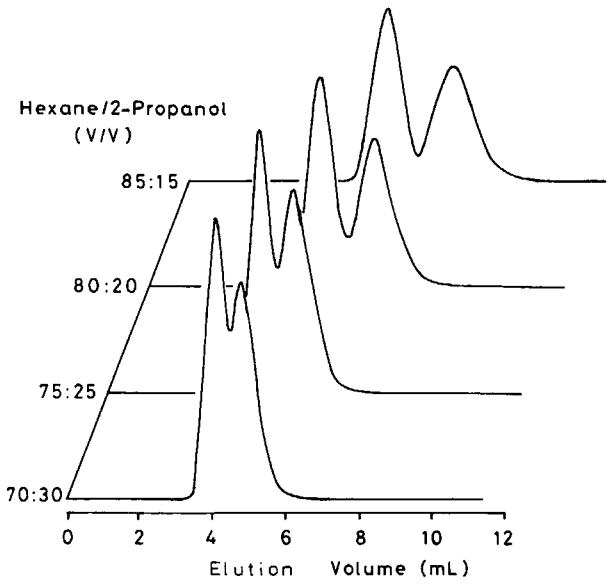


Figure 6 Variation of resolution chromatograms with eluent compositions for **H9**.

As can be observed, in each case, an increase in the 2-propanol concentration (increase in the polarity of the mobile phase) decreases the capacity factor k'_1 and k'_2 smoothly. This result indicates that hydrogen-bond association is the main retention force in the system. However, enantioselectivity (α) increases slightly with 2-propanol concentration for both **H2** and **H9**. The enhancement of enantioselectivity, at a high concentration of 2-propanol, should be attributed to the change of the ordered conformation of PNLG. It is reasonable to say that the side groups of PNLG are more extended and stabilized through the hydrogen-bonding interactions with 2-propanol. The extended side groups recognize hydantoins, through steric hindrance, much more effectively than do the irregular ones. However, a further increase in 2-propanol concentration (> 30%) may dissolve and elute out the coated PNLG. This is the general limitation of the coated polymeric chiral stationary phases.

Thermodynamics of Chiral Separation

The effect of the temperature on retention (k') and selectivity (α) are described by the van't Hoff equations[28]:

$$\ln k'_i = -\Delta H^0_i/RT + \Delta S^0_i/R + \ln \phi \quad (1)$$

$$\ln (k'_2/k'_1) = \ln \alpha = -\Delta\Delta H^0/RT + \Delta\Delta S^0/R \quad (2)$$

where ΔH^0 and ΔS^0 are, respectively, the enthalpy and entrophy changes associated with the enantio-

Table V Temperature Effect for the Chromatographic Resolution of H2 and H9 by Column Bearing PNLG[a]

Temperature (K)	k'_1	k'_2	α	ln k'_1	ln k'_2	ln α
(*R*, *S*)-5-Isopropylhydantoin (**H2**)						
284	1.55	—	1.00	0.44	—	0.00
289	1.36	1.83	1.35	0.30	0.60	0.30
294	1.24	1.64	1.32	0.22	0.50	0.28
299	1.11	1.43	1.29	0.10	0.36	0.25
304	1.02	1.28	1.25	0.021	0.24	0.22
309	0.95	1.15	1.22	−0.057	0.14	0.20
314	0.88	1.03	1.18	−0.13	0.03	0.16
				$\Delta H_1^{0\,b}$	$\Delta H_2^{0\,b}$	$\Delta\Delta H^{0\,c}$
				−4.07	−3.23	−2.94
				$\Delta S_1^{0\,b}$	$\Delta S_2^{0\,b}$	$\Delta\Delta S^{0\,c}$
				−12.89	−10.56	−2.67
(*R*, *S*)-5-Methyl-5-isopropylhydantoin (**H9**)						
284	0.75	—	1.00	−0.29	—	0
289	0.67	1.22	1.82	−0.40	0.20	0.60
294	0.62	1.10	1.79	−0.48	0.10	0.58
299	0.56	0.97	1.73	−0.58	−0.03	0.55
304	0.52	0.87	1.69	−0.66	−0.14	0.52
309	0.49	0.80	1.63	−0.72	−0.22	0.49
314	0.46	0.72	1.57	−0.78	−0.33	0.45
				ΔH_1^0	ΔH_2^0	$\Delta\Delta H^0$
				−3.85	−2.84	−1.09
				ΔS_1^0	ΔS_2^0	$\Delta\Delta S^0$
				−12.93	−10.62	−2.55

[a] The operation conditions are the same as those stated in Table III except that the temperatures are different.

[b] ΔH^0 (kcal/mol) and ΔS^0 (cal/K mol) are determined by the slope ($-\Delta H^0/R$) and intercept ($\Delta S^0/R$) of ln k' vs. $1/T$ plots as shown in Figures 9 and 10.

[c] $\Delta\Delta H^0$ (kcal/mol) and $\Delta\Delta S^0$ (cal/K mol) are determined by the slope ($-\Delta\Delta H^0/R$) and intercept ($\Delta\Delta S^0/R$) of ln α vs. $1/T$ plots as shown in Figures 9 and 10.

mer retention process, and ϕ, the phase ratio, which can be neglected; subscripts 1 and 2 refer to the enantiomeric forms of hydantoin. The above equations predict a linear inverse relationship between ln k' or ln α and temperature.

Chromatographic parameters obtained for **H2** and **H9** at different temperatures (284–314 K) are depicted in Table V and Figures 7 and 8. For both **H2** and **H9**, the k' and α decrease smoothly with increasing temperature, and the decrease rate of k'_2 is greater than that of k'_1. However, plots of ln k' or ln α vs. $1/T$ approach a linear relationship, as shown in Figures 9 and 10 for **H2** and **H9**, respectively. The values of ΔH^0 and ΔS^0 in Table V are determined from the slope and intercept of the straight lines in Figures 9 and 10 using eq. (1). The values of $\Delta\Delta H^0$ and $\Delta\Delta S^0$ are determined by eq. (2) using the same method. The $\Delta\Delta S^0$ of **H2** and **H9** are about −2.60 cal/K mol, whereas the $\Delta\Delta H^0$ of **H2** (−2.94 kcal/mol) is much greater than that of **H9** (−1.09 kcal/mol). Therefore, the resolution processes investigated are enthalpy-controlled. On the other hand, it is clear that decreasing temperature caused an enhancement of the chromatographic resolution. However, below 284 K, the peak broading occurs, which can be attributed to a slow mass transfer process at low temperature.

CONCLUSION

We have successfully synthesized poly[N^5-benzyl-L-glutamine) (PNLG), poly[N^5-(+)-1-phenylethyl-L-glutamine] [P(+)NLG], and poly[N^5-(±)-1-phenylethyl-L-glutamine) [P(±)NLG] from poly[γ-(β-chloroethyl)-L-glutamate)], which, in turn, was obtained by ester exchange of poly(γ-

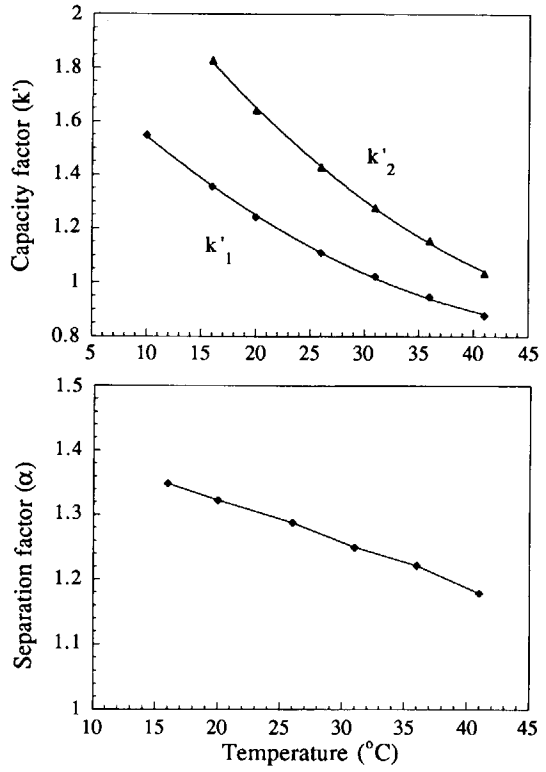


Figure 7 Effect of temperature on capacity factor (k') and selectivity (α) for the resolution of **H2**.

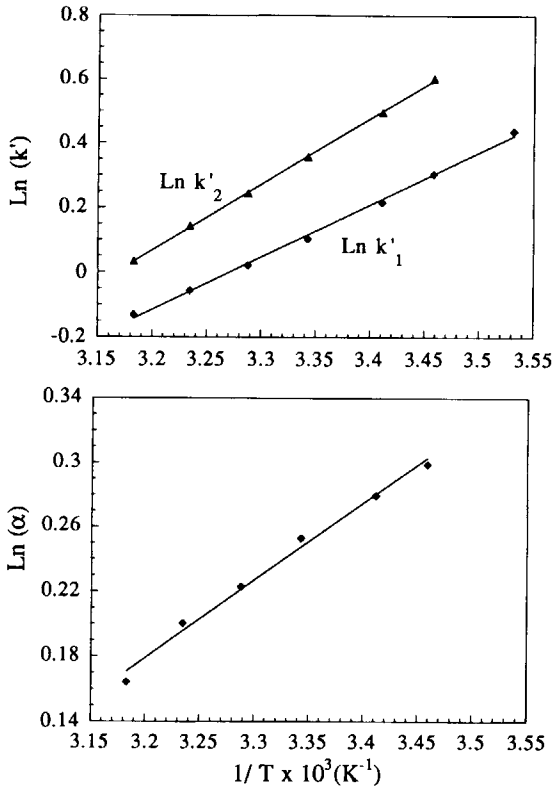


Figure 9 Plot of ln k' vs. $1/T$ and ln α vs. $1/T$ for the resolution of **H2**.

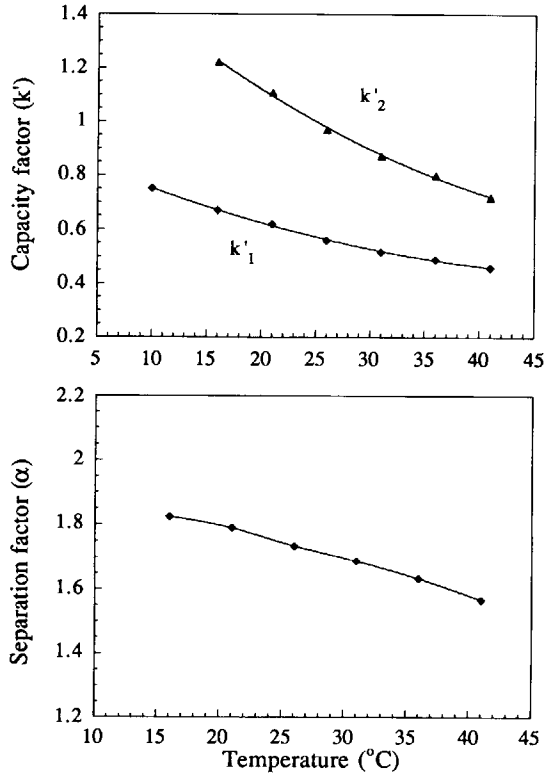


Figure 8 Effect of temperature on capacity factor (k') and selectivity (α) for the resolution of **H9**.

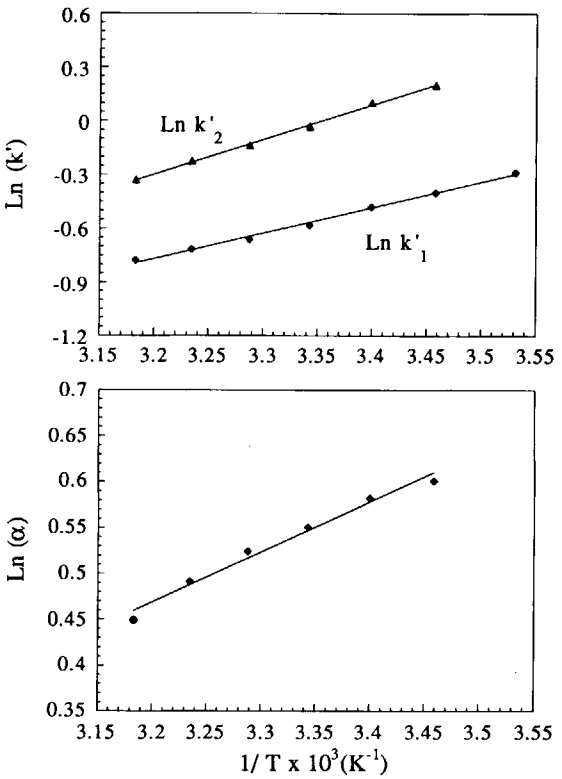


Figure 10 Plot of ln k' vs. $1/T$ and ln α vs. $1/T$ for the resolution of **H9**.

methyl-L-glutamate) with 2-chloroethanol. From CD and IR spectra, it is clear that the PNLG exists mainly as an α-helical conformation in the film state. As the chiral stationary phase, the PNLG showed efficient resolution to (*R,S*)-5-isopropylhydantoin, (*R,S*)-5-(1-ethylpropyl)hydantoin, (*R,S*)-5-methyl-5-isopropylhydantoin, and (*R,S*)-5-methyl-5-propylhydantoin. The enantioselectivity (α) were between 1.34 and 1.73 and increased with the bulkiness of 5-substituent in hydantoins. Increasing 2-propanol concentration in the eluent (2-propanol/hexane), the capacity factor (k') decreased smoothly, indicating that hydrogen bond association is the main retention force. However, the selectivity (α) increased slightly with 2-propanol concentration. From these results, it is concluded that the main chiral discrimination force is the steric hindrance between 5-substituents of hydantoin and side chains of the PNLG. The temperature effect on the resolution of two hydantoins by PNLG was also investigated to determine the thermodynamic parameters.

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