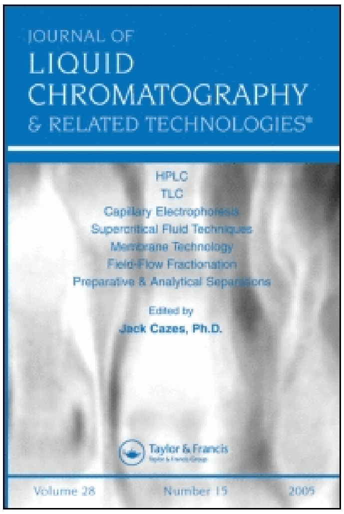

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

PREPARATION AND ENANTIOSEPARATION OF COPOLYMER CHIRAL STATIONARY PHASES WITH (1R,2R)-(+)-1,2-DIPHENYLETHYLENEDIAMINE AS THE CHIRAL BUILDING BLOCK

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To cite this Article Zhang, Jun-Yi , Chen, Jun , Yin, Chuan-Qi , Li, Shi-Rong and Bai, Zheng-Wu(2010) 'PREPARATION AND ENANTIOSEPARATION OF COPOLYMER CHIRAL STATIONARY PHASES WITH (1R,2R)-(+)-1,2-DIPHENYLETHYLENEDIAMINE AS THE CHIRAL BUILDING BLOCK', Journal of Liquid Chromatography & Related Technologies, 33: 1, 46 — 60

To link to this Article: DOI: 10.1080/10826070903430076

URL: http://dx.doi.org/10.1080/10826070903430076

Journal of Liquid Chromatography & Related Technologies, 33:46–60, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903430076

PREPARATION AND ENANTIOSEPARATION OF COPOLYMER CHIRAL STATIONARY PHASES WITH (1R,2R)-(+)-1,2-DIPHENYLETHYLENEDIAMINE AS THE CHIRAL BUILDING BLOCK

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□ *(1R,2R)-(+)-1,2-Diphenylethylenediamine was copolymerized with terephthaloyl chloride and toluene 2,4-diisocyanate at different fed ratios to give two polymeric chiral selectors. The selectors were immobilized onto 3-aminopropyl silica gel to afford corresponding chiral stationary phases. The enantioseparation evaluation reveals the shorter-chain polymeric selector possesses better enantioseparation ability. In normal phase mode, tert-butanol is advantageous for enantioseparation when it is used as one of the mobile phase compositions. The influence of other aspects including selector structure, sample loading and temperature on the enantioseparation ability of these chiral stationary phases was also studied.*

Keywords chiral stationary phase, copolymer, enantioseparation, high-performance liquid chromatography

INTRODUCTION

Polymer-type chiral stationary phases (CSPs) have attracted much more attention since 1980 s. Initially, natural polymers, such as cellulose, amylose and chitosan were usually applied to prepare CSPs, because of their ready availability.[1–5] Chiral columns, whose stationary phases were prepared from cellulose, have been commercialized for two decades. The corresponding CSPs, however, are only applicable for limited mobile phases because of their swelling and solubility.[6] Thus, cellulose derivatives were

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covalently immobilized on modified silica gel in a few works to overcome this drawback. In this case, another side-effect comes out that the chiral recognition ability of the CSPs decreases.[6] On the other hand, the effort was made to prepare synthetic polymer-type CSPs. Allenmark and coworkers developed network-polymeric CSPs that showed excellent enantioseparation ability,[7] and afterwards, this kind of CSP was commercialized. Recently the chiral selectors of polymer-type CSPs were mainly prepared by free radical polymerization, and were immobilized by radical reaction.[8–11] But these CSPs did not show satisfactory enantioseparation ability, although some of these polymers were appropriately designed, synthesized and well characterized. Saigo and coworker prepared a CSP by coating a chiral polymer on functionalized macroporous silica gel.[12] The chiral polymer was synthesized by the copolymerization of (1S,2S) (-)-1,2-diphenylethylenediamine (DPEDA) with terephthaloyl chloride (TPC).

In our previous work, a method to prepare polymer-type CSPs was developed.[13] The selectors of various molecular weights were synthesized by the copolymerization of 1,4-phenylene diisocyanate (PDI) with diisopropyl tartrate. To further investigate the impact of CSPs structures on enantioselectivity in chiral separation, in the follow-up works, the selectors were prepared by the copolymerization of (1 R,2 R)-(+)-1,2- DPEDA with 1,4-PDI and TPC;[14] (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol (ADPE) with 1,4-PDI; (1*S*,2*R*)-(+)-2-ADPE with 1,4-PDI and TPC.[15] A few trends between the structure and enantioseparation ability of the selectors were discussed. Continuing our previous work, to study the influence of polymer structure on enantioseparation ability, in this work, two polymers of different molecular weights were synthesized by the copolymerization of (1 R,2 R)-(+)-1,2-(DPEDA), TPC and toluene 2,4-diisocyanate (TDI). The related enantioseparation properties of these two CSPs were studied.

EXPERIMENTAL

Materials

(1 R,2 R)-(+)-1,2-DPEDA was purchased from Chengdu Likai Chiral Tech. Co., Ltd. (China). TPC was from Jiangxi Nanchang Pesticide Plant (China), and was purified by recrystallization from toluene. 3-Aminopropyltriethoxysilane (APTES) was obtained from Novel Organic Silicon Materials Co., Ltd. of Wuhan University (China) and redistilled before use. Silica gel (Lichrosorb Si 100) was obtained from Merck (Germany) with a particle size of 5 μm, a pore size of 100Å and a surface area of 300 m^2/g. N,N-Dimethylformamide (DMF) and triethylamine (TEA, used for CSP preparation) were dried over phosphorous pentoxide and redistilled. Toluene was refluxed with sodium and redistilled. All other

chemicals used for the synthesis of CSPs were of analytical grade and used as received.

Instrumentation

IR spectra were recorded on a Nicolet FT-IR instrument (USA) with KBr pellets. Nuclear magnetic resonance (NMR) spectra were performed on a Varian INOVA 500 spectrometer (USA), operating at 25°C. The solvent used was deuterated DMSO. Elemental analysis was performed on an Elemental VarioEL III CHNOS apparatus (Germany). The number-average molecular weights of chiral polymers were determined by gel permeation chromatography (GPC) on an Agilent 1100 chromatograph (USA) equipped with an Agilent G1362A RID detector and an Agilent PLgel (5 μm 10E5Å) column (300 mm × 7.5 mm). The stainless steel HPLC empty columns (250 mm × 4.6 mm) were purchased from Hypsil (UK). The CSPs were packed into the empty columns with an Alltech model 1666 slurry packer (USA). The enantioseparation was run on a Waters chromatograph (USA) equipped with a Waters 996 photodiode array detector, a Waters 600E Quat Pump, a Waters Millenium 32 system controller, a Waters 717 plus autosampler.

Synthesis of Chiral Polymers

A solution of (1 R,2 R)-(+)-1,2-DPEDA (3.75 g, 17.66 mmol) in 15 mL DMF was added dropwise into the mixture of TDI (1.55 g, 8.92 mmol), TPC (2.51 g, 12.35 mmol), and TEA (10 mL) in 20 mL DMF. The resulting solution was protected with nitrogen atmosphere, and stirred for 1 h at room temperature. Then the mixture was warmed to 80°C and stirred for an additional 12 h. After cooled down to room temperature, the mixture was filtered. The filtrate was dropped in 200 mL vigorously stirred toluene, during which solid precipitated. The solid was dissolved in DMF and precipitated by adding toluene again to yield polymer **1**. The structure of polymer **1** conformed to the absorbance occurred in FT-IR spectrum. The corresponding wave numbers of absorbance are shown as follows: 3634–3108 cm^{-1} (N–H, C=C–H); 2940 cm^{-1}, 2869 cm^{-1} (C–H); 2361 cm^{-1}, 2330 cm^{-1} (–NCO); 1660 cm^{-1}, 1491 cm^{-1}, 1442 cm^{-1} (–CONH–). The chemical shifts of its ^{1}H NMR are presented as follows: 2.49 (s, $-CH_3$); 4.90–5.34 (m, –CH–); 5.65 (m, –NHCONH–); 6.97–7.39 (m, aromatic H); 7.70–7.98 (aromatic H); 9.10–9.29 (–CONH–). Its specific rotation was measured as + 41.2° (c 0.52, DMF). The number-average molecular weight was determined as 5430 g/mol.

Polymer **2** was synthesized in the same manner with (1 R,2 R)-(+)-1,2-DPEDA (3.89 g, 18.34 mmol), TPI (1.38 g, 7.93 mmol), and TPC (2.49 g, 12.27 mmol). Its structure was confirmed by FT-IR spectrum. The wave numbers of absorbance are as follows: 3537–3140 cm^{-1} (N–H, C=C–H); 2970 cm^{-1}, 2932 cm^{-1} (C–H); 2526 cm^{-1}, 2490 cm^{-1} (–NCO); 1657 cm^{-1}, 1628 cm^{-1}, 1546 cm^{-1}, 1479 cm^{-1} (–CONH–). Its chemical shifts of ^{1}H NMR spectrum are as follows: 2.50 (s, $-CH_3$); 4.88–5.36 (m, –CH–); 5.64 (m, –NHCONH–); 6.77–7.47 (m, aromatic H); 7.67–8.06 (aromatic H); 9.08–9.34 (–CONH–). The specific rotation was measured as + 51.5° (c 0.51, DMF). The number-average molecular weight was determined as 9060 g/mol.

Synthesis of CSPs

Silica gel (19.66 g) was mixed with APTES (15 mL) and toluene (50 mL), and the resulting mixture was heated to reflux for 12 h. After the completion of reaction, the solid was filtered and washed thoroughly with tetrahydrogenfuran in a soxhlet apparatus. 3-Aminopropyl silica gel (21.61 g) was obtained after the removal of the solvent. The successful amination of silica gel was evident from FT-IR. The wave numbers of absorbance are as follows: 3422 cm^{-1}, 1652 cm^{-1} (N–H), 1115 cm^{-1} (Si—O). By elemental analysis, the contents of carbon, hydrogen, and nitrogen of 3-aminopropyl silica gel were found to be 5.55%, 1.74%, and 2.08%, respectively.

Polymer **1**, prepared above without further drying, was dissolved in 25 mL DMF, to which 3.98 g 3-aminopropyl silica gel and 10 mL TEA were added. The resulting mixture was gently stirred and kept at 85°C for 12 h. After cooled down to ambient temperature, the mixture was centrifugalized to remove the solution. The solid was washed with DMF and centrifugalized again till the centrifugate was almost colorless. The solid was further washed with acetone for 12 h in a soxhlet apparatus. After drying to constant weight, CSP **1** of pale yellow solid was given. CSP **1** was characterized by FT-IR. The wave numbers of the absorbance are as follows: 2935 cm^{-1} (C—H), 1667 cm^{-1}, 1535 cm^{-1} (—NH—CO—), 1097 cm^{-1} (Si—O). By element analysis, the contents of carbon, hydrogen, and nitrogen of CSP **1** were found to be 12.88%, 1.96%, and 2.83%, respectively.

CSP **2** was prepared in the same manner with 3.83 g 3-aminopropyl silica gel. It was characterized by FT-IR, and the wave numbers of absorbance are as follows: 2931 cm^{-1} (C–H), 1667 cm^{-1}, 1526 cm^{-1} (–NH–CO–), 1106 cm^{-1} (Si–O). The contents of carbon, hydrogen, and nitrogen were found to be 16.62%, 2.02%, and 3.11%, respectively.

Column Packing and the Determination of Column Efficiency

CSPs **1** and **2** were packed into empty columns through a slurry method, using chloroform as the solvent to form slurries, and hexane as the packing solvent.

The column efficiency was determined using the solution (1 mg/mL) of biphenyl in hexane as the probe, a mixture of hexane/isopropanol (90/10, v/v) as the mobile phase. The injection volume of biphenyl solution was 15 µL.

Chromatographic Conditions and Parameters

The chiral analyte solutions were prepared with acetonitrile, and filtered by 0.45 µm membrane. The buffer solutions (0.1 mol/L) were prepared with TEA and acetic acid. All mobile phases were filtered and degassed before use. The flow rate was set at 1.0 mL/min. Column temperature was at 25°C, except when indicated.

RESULTS AND DISCUSSION

Characterization of CSPs

The synthesized chiral polymers are structurally random (Figure 1). The linkage between the polymers and 3-aminopropyl silica gel is a urea, or an amide. The number-average molecular weight of polymer **2** is approximately double comparing to that of polymer **1** that is consistent with the excessive molar percentage of fed monomers. The carbon contents of CSPs, which are 12.88% for CSP **1**, and 16.62% for CSP **2**, show more structural units of polymer **2** were immobilized on the aminated silica gel due to the longer chain of polymer **2**. There are two absorbance bands of amides at 1667 cm^{-1} and 1535 cm^{-1} in F-T IR spectrum for CSP **1**; and at 1667 cm^{-1} and 1526 cm^{-1} for CSP **2**, indicating the successful immobilization of chiral polymers was achieved.

Enantioseparation Evaluation

The column efficiencies related to CSP **1** and CSP **2** were determined as 25200 and 29100 plates per meter, respectively. The enantioseparation ability of CSP **1** and CSP **2** was evaluated with structurally diversified chiral compounds (Figure 2). The chromatographic data are tabulated in Table 1. Obviously, the enantioseparation ability of CSP **1** is better than that of CSP **2**. CSP **1** was prepared from the shorter-chain polymer; while CSP **2** was prepared from longer-chain polymer. The trend between enantioseparation ability and the length of polymers accords with the observation in previous works.[13,14] The most compounds, separated both by these two CSPs, are resolved better on CSP **1** than on CSP **2**. These compounds separated by CSP **1** with satisfactory resolutions are similar in structures that one imidazole and two chloro-substituted phenyls contain in each compound.

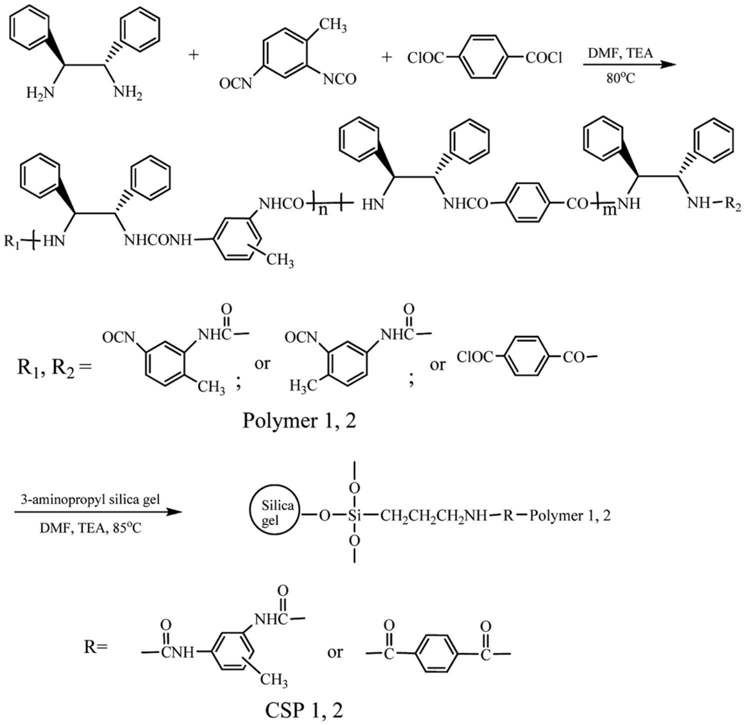

FIGURE 1 The synthetic scheme of CSPs.

Chloro-substituted phenyls are π-acidic in chemical nature. They strongly interact with the chiral selectors, which exhibit π-basicity resulting from many amide bonds connected to phenyls. Compounds 22, 24, and 25 were separated both by CSP **1** and CSP **2** because they are not π-base, and on the contrary, compounds 24 and 25 are strong π-acid. Compounds 23, 26, and 27, however, were not separated on these two CSPs because they are π-base.

In the same mobile phase conditions, it was found that the retention factors of compounds 8, 23, 26, and 27 are longer upon CSP **2** than upon CSP **1**. It may be related to longer molecular chain of polymer **2** that enwrapped these compounds relative to polymer **1**.

Effect of Alcohol-Contained Mobile Phases on Enantioseparation

Different from gas chromatography, in liquid chromatography, mobile phases are usually involved in the formation of diastereoisomers between selectors and chiral analytes. Consequently, mobile phase significantly impacts enantioseparation. The enantioseparation feature of CSP **1** in the mobile phases of various alcohols contained was investigated (Table 2).

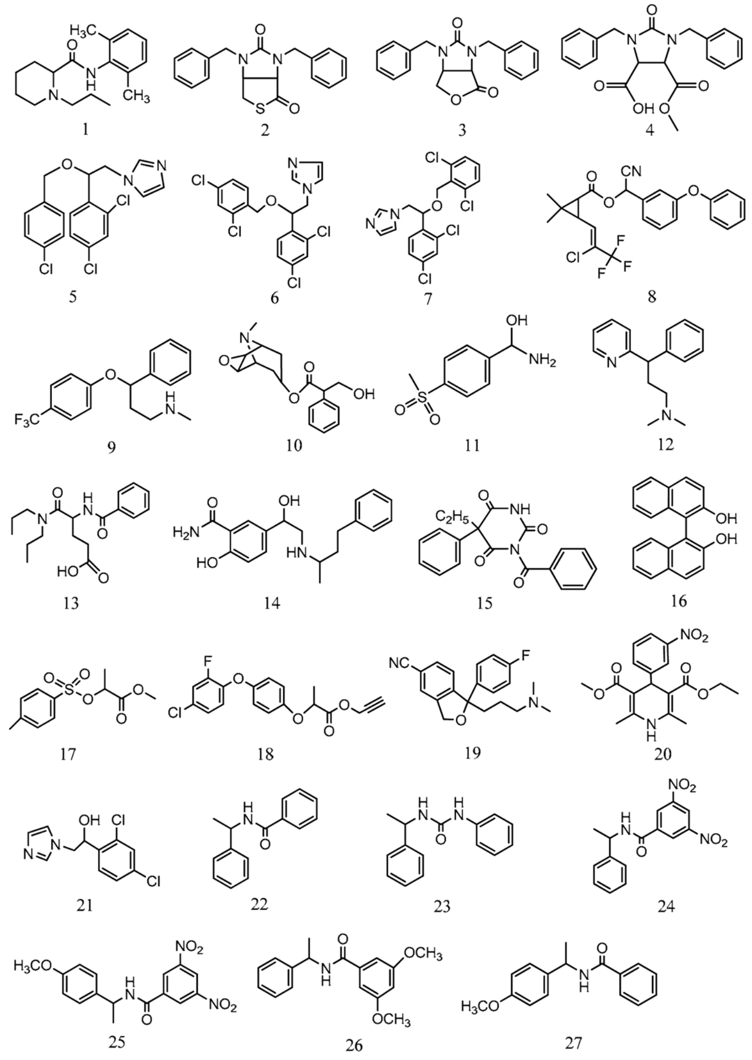

FIGURE 2 The structures of chiral compounds separated by CSP **1** and CSP **2**.

tert-Butanol is especially propitious for CSP **1** to demonstrate its enantioseparation ability. This tendency is possibly related to the polarity of these alcohols, which impacts the interaction between analytes and selectors by modulating the whole polarity of the mobile phases. Polarity of organic solvents depends on their dipole constant. Tert-Butanol's dipole constant is lowest among these used alcohols. On the other hand, the better enantioseparation in the presence of tert-butanol is possibly related to its molecular skeleton, which is suitable for the involvement of the formation of temporary diastereoisomers between analytes and chiral selectors.

TABLE 1 The Enantiomeric Separation of the Racemates on the CSP **1** and CSP **2**

	CSP **1**				CSP **2**			
S/N	k_1	α	R_s	Separation Condition	k_1	α	R_s	Separation Condition
1	12.29	1.27	1.23	methanol/water (40/60)	2.33	1.16	0.63	methanol/buffer[a] (70/30)
2	2.49	1.08	0.70	methanol/buffer[b] (60/40), 20°C	11.96	1.11	1.06	acetonitrile/water (40/60)
	2.69	1.89	4.78					
	5.09	1.19	1.10					
3	11.58	1.06	0.79	hexane/n-butanol (90/10)	6.94	1.12	0.67	hexane/n-propanol (90/10)
					7.79	2.21	5.66	
					17.79	1.12	0.89	
4	5.46	1.05	0.89	acetonitrile/buffer[c] (40/60)	1.29	1.03	0.34	acetonitrile/buffer[a] (70/30)
5	1.25	1.73	6.61	hexane/tert-butanol (80/20)	1.10	2.18	1.66	hexane/tert-butanol (70/30)
6	1.24	1.55	4.63	hexane/tert-butanol (80/20)	1.03	2.18	2.66	hexane/tert-butanol (70/30)
7	1.23	1.81	6.65	hexane/tert-butanol (80/20)	1.05	2.43	1.00	hexane/tert-butanol (70/30)
8	1.21	1.22	1.90	hexane/tert-butanol (98/2)	3.15	1.28	1.39	hexane/tert-butanol (98/2)
9	0.22	2.09	1.49	methanol/buffer[b] (60/40), 20°C	0.79	1.00	0	acetonitrile/buffer[a] (70/30)
10	0.11	1.48	0.69	methanol/buffer[b] (70/30)	0.46	1.00	0	acetonitrile/buffer[a] (70/30)
11	0.35	1.21	0.71	hexane/n-propanol (60/40)	0.64	2.01	0.75	methanol/buffer[b] (70/30)

(*Continued*)

TABLE 1 Continued

	CSP **1**				CSP **2**			
S/N	k_1	α	R_s	Separation Condition	k_1	α	R_s	Separation Condition
12	0.21	2.72	1.16	methanol/water (70/30)	0.31	1.87	1.32	methanol/buffer^b (70/30)
13	8.18	1.14	1.04	methanol/buffer^b (40/60)	1.11	1.00	0	hexane/ethanol (70/30)
14	0.24	2.14	0.66	hexane/ethanol (70/30)	0.55	1.00	0	hexane/iso-propanol (80/20)
15	12.22	1.15	0.80	methanol/buffer^b (50/50)	5.33	1.13	0.68	methanol/buffer^b (70/30)
16	5.65	1.07	1.15	hexane/tert-butanol (80/20)	6.00	1.17	0.91	methanol/water (70/30)
17	0.56	1.47	0.89	hexane/ethanol/iso-propa-propanol (60/20/20)	1.39	1.00	0	hexane/ethanol (70/30)
18	1.55	1.07	0.90	hexane/n-propanol (90/10)	0.60	1.00	0	acetonitrile/buffer^b (70/30)
19	0.21	2.12	1.56	methanol/buffer^b (60/40), 20°C	4.72	1.00	0	acetonitrile/water (40/60)
20	0.28	1.16	1.03	acetonitrile/buffer^b (70/30)	0.55	1.15	0.72	acetonitrile/buffer^a (70/30)
21	0.31	2.28	2.43	hexane/ethanol (50/50)	1.67	1.00	0	hexane/ethanol (70/30)
22	9.84	1.22	1.01	methanol/water (40/60)	1.88	1.06	0.42	methanol/water (70/30)
23	2.80	1.00	0	acetonitrile/water (40/60)	5.30	1.00	0	acetonitrile/water (40/60)
24	11.56	1.08	0.79	hexane/tert-butanol (80/20)	33.44	1.15	1.03	hexane/ethanol (95/5)
25	12.65	1.06	0.50	hexane/iso-propanol (80/20)	8.51	1.04	0.52	acetonitrile/water (40/60)
26	2.01	1.00	0	acetonitrile/water (40/60)	3.67	1.00	0	acetonitrile/water (40/60)
27	2.52	1.00	0	acetonitrile/water (40/60)	4.86	1.00	0	acetonitrile/water (40/60)

Retention factor (k_1): $(t_1\text{-}t_0)/t_0$, where t_1 is the retention time of the first-eluted enantiomer, and t_0 was determined by measuring the retention time of the solvent, which was used to prepared the samples; Separation factor (α): k_2/k_1, where k_2 was calculated from $(t_2\text{-}t_0)/t_0$, and t_2 refers to the retention time of the second-eluted enantiomer; Resolution (R_s): $2(t_2\text{-}t_1)/(w_1+w_2)$, in which w_1 and w_2 refer to, respectively, the bandwidth of the first-eluted enantiomer and the second-eluted enantiomer. pH value of the buffer solution: ^{a}8.67; ^{b}5.06; ^{c}4.00.

TABLE 2 The Enantiomeric Separation of Chiral Compounds on CSP **1** Under the Mobile Phases of Various Alcohols Contained

		Hexane/ethanol			Hexane/isopropanol			Hexane/n-propanol			Hexane/n-butanol			Hexane/tert-butanol		
S/N	v/v	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
6	70:30	0.53	1.56	1.72	0.83	1.62	0.70	0.59	1.41	0.86	0.60	1.29	0.71	0.74	1.86	4.03
	80:20	0.82	1.53	3.04	1.20	1.82	2.34	0.83	1.59	2.53	0.78	1.63	2.26	1.24	1.55	4.63
	90:10	1.64	1.56	1.85	3.98	1.31	1.02	2.01	1.43	1.42	1.74	1.33	0.92	3.80	1.37	2.72
8	70:30	0.41	1.05	0.32	0.46	1.09	0.48	0.38	1.07	0.39	0.36	1.08	0.45	0.40	1.12	0.62
	80:20	0.53	1.11	0.65	0.59	1.14	0.99	0.50	1.12	0.65	0.44	1.13	0.67	0.43	1.14	0.72
	90:10	0.78	1.15	0.88	0.83	1.18	1.11	0.69	1.16	0.79	0.59	1.16	0.78	0.71	1.17	1.27
	95:5	0.98	1.19	1.09	1.12	1.20	1.14	0.80	1.16	1.02	0.70	1.18	0.88	0.90	1.19	1.47
	98:2	1.32	1.24	1.46	1.31	1.24	1.50	0.99	1.16	1.29	0.85	1.15	1.35	1.21	1.22	1.90
16	70:30	4.04	1.04	0.41	8.21	1.04	0.31	5.15	1.05	0.44	5.20	1.05	0.48	4.52	1.08	0.85
	80:20	6.19	1.04	0.47	13.49	1.04	0.34	7.98	1.05	0.48	7.87	1.06	0.51	5.65	1.07	1.15
	90:10	12.84	1.04	0.50	Not available			16.42	1.06	0.51	15.20	1.06	0.60	22.24	1.07	0.60
24	70:30	2.36	1.04	0.47	6.09	1.06	0.43	3.05	1.05	0.42	3.59	1.05	0.44	7.80	1.07	0.57
	80:20	4.05	1.05	0.57	8.64	1.06	0.50	5.47	1.05	0.51	6.28	1.05	0.48	11.56	1.08	0.79
	90:10	10.36	1.05	0.61	Not available			16.70	1.06	0.61	17.59	1.06	0.62	Not available		

Influence of Chiral Compound Loading on Enantioseparation

To ensure a chiral compound is enantiomerically separated in bulk for industrial purpose, it is always desired that this compound is separated within a shorter retention time, and with a satisfactory resolution at a higher loading. The loading depends upon several factors, which include the chemical nature of chiral analyte, the property of CSPs, and separation condition, etc. Usually there is a conflict between a satisfactory resolution desired and a comparatively large amount of chiral compound loaded. Compound 8 was separated by CSPs **1** and **2** at different loadings (Figure 3). The resolutions descend as loadings increase. Comparing the variation trends of resolutions and loadings, the capacity of CSP **1** for chiral compound is better than that of CSP **2**.

Influence of Temperature on Enantioseparation

Many works reported that temperature affects enantioseparation. Grinberg and Armstrong and their coworkers investigated the influence of temperature on enantioseparation, and discovered enantioseparation trends within a definite temperature range.[16,17] In enantioseparation by

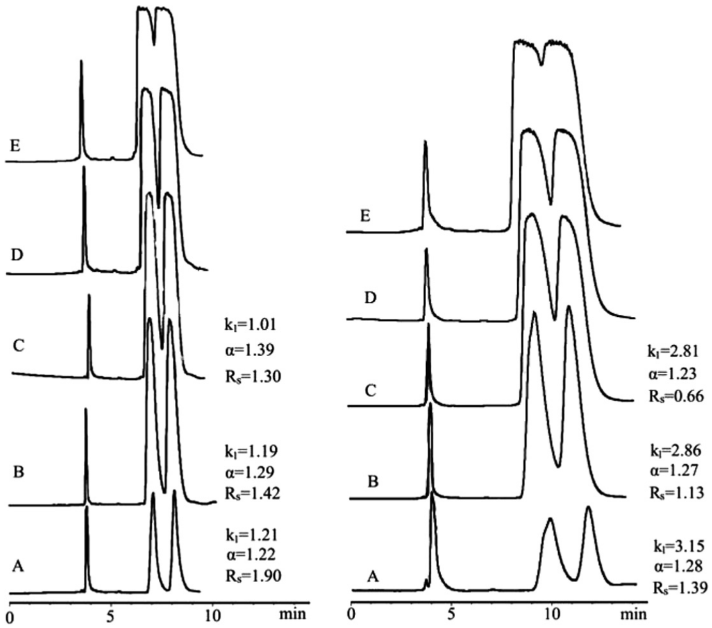

FIGURE 3 The Enantioseparation chromatograms of compound 8 on CSP **1** (the left) and CSP **2** (the right), respectively. Injection amount: (A): 15 μg; (B): 150 μg; (C): 300 μg; (D): 750 μg; (E): 1200 μg; Eluent: hexane/tert-butanol (98/2).

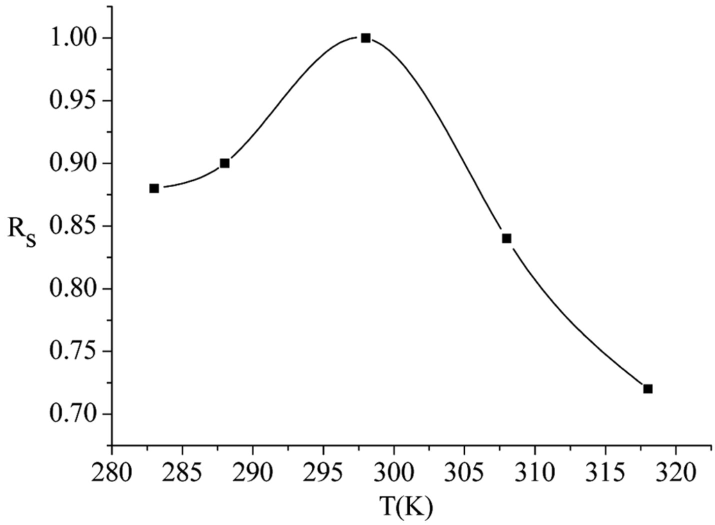

FIGURE 4 The Influence of temperature on the resolution of compound 13 separated by CSP **1**. Eluent: methanol/buffer (pH 5.06) (50/50).

HPLC, the van't Hoff equation is expressed by:

$$\ln k = -(\Delta H/RT) + (\Delta S/R) + \ln \Phi \tag{1}$$

Separation factor (α) is a measure of stereo selectivity during chiral recognition. According to $\alpha = k_2/k_1$, α can be expressed by:

$$\ln \alpha = -\Delta(\Delta G)/RT = -\Delta(\Delta H)/RT + \Delta(\Delta S)/R \tag{2}$$

In equations 1 and 2, R is gas constant; T is absolute temperature; Φ is the phase ratio. Figure 4 shows the resolutions at different temperature for compound 13 to be separated by CSP **1**. The resolution reaches a maximum value approximately at 298 K (25°C). The plot of lnk *vs* 1/T shows a linear correlation (Figure 5), suggesting that Φ is independent of temperature; And the plot of lnα *vs* 1/T exhibits the same feature (Figure 6). On the basis of slope and intercept in Figure 6, $\Delta(\Delta H)$ and $\Delta(\Delta S)$ were determined as $-0.89\,\mathrm{kJ \cdot mol^{-1}}$ and $-2.36\,\mathrm{J \cdot mol^{-1} \cdot K^{-1}}$. Because the values of $\Delta(\Delta H)$ and $\Delta(\Delta S)$ are negative, the enantioseparation of compound 13 is an enthalpy-derived process.

Influence of Selector Structure on Enantioseparation

In our previous work, two polymer-type CSPs were prepared, where 1,4-phenylene diisocyanate was applied instead of toluene 2,4-diisocyanate

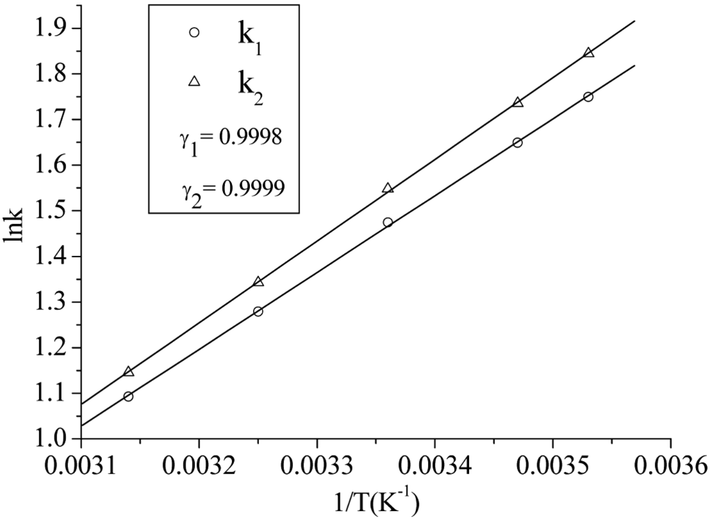

FIGURE 5 The linear correlation of lnk and 1/T concerning the separation of compound 13 on CSP **1**. Eluent: methanol/buffer (pH 5.06) (50/50).

in this work.[14] Two isocyanates are located at the para-position in 1,4-phenylene diisocyanate; and at the meta-position in toluene 2,4-diisocyanate. Therefore, the conformations of these two kinds of polymers are different. In addition, there is a methyl at the aromatic ring in toluene 2,4-diisocyanate. The stereo hindrance for the polymers to interact with analytes is also

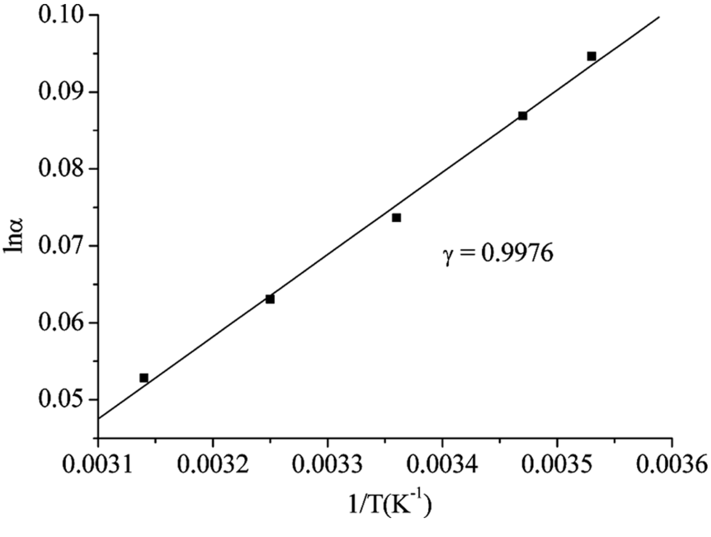

FIGURE 6 The linear correlation of ln α and 1/T concerning the separation of compound 13 on CSP **1**. Eluent: methanol/buffer (pH 5.06) (50/50).

different. However, the enantioseparation ability of the two CSPs in this work is just slightly improved. Therefore, the conformations of the polymers are not the leading effect for the enantioseparation of this type CSPs. Besides, the stereo hindrance caused by methyl is not large enough to result in prominent enantioselectivity difference for these CSPs to demonstrate improved enantioseparation ability.

CONCLUSION

The prepared CSPs show satisfactory enantioseparation ability. The CSP of shorter-chain polymer has better enantioseparation ability than the one of longer-chain polymer does. In normal phase condition, tert-butanol is advantageous for chiral recognition when it is employed as one of mobile phase compositions. In comparison with the previous work, the enantioseparation ability of the CSPs in this work is not essentially improved, although a different achiral monomer of toluene 2,4-diisocyanate is used, which produces a different polymer conformation and causes a little more stereo hindrance to chiral analytes.

ACKNOWLEDGMENT

Funding from National Natural Science Foundation of China (grant number: 20675061) is gratefully acknowledged.

REFERENCES

1. Blaschke, G. Chromatographic resolution of racemates. Angew. Chem. Int. Ed. Engl. **1980**, *19*, 13–24.
2. Okamoto, Y.; Yashima, E. Polysaccharide derivatives for chromatographic separation of enantiomers. Angew. Chem. Int. Ed. Engl. **1998**, *37*, 1020–1043.
3. Okamoto, Y.; Kaida, Y. Resolution by high-performance liquid chromatography using polysaccharide carbamates and benzoates as chiral stationary phases. J. chromatogr. A. **1994**, *666*, 403–419.
4. Franco, P.; Senso, A.; Minguillón, C.; Oliveros, L. 3,5-Dimethylphenylcarbamates of amylose, chitosan and cellulose bonded on silica gel-Comparison of their chiral recognition abilities as high-performance liquid chromatography chiral sationary phases. J. Chromatogr. A. **1998**, *796*, 265–272.
5. Senso, A.; Oliveros, L.; Minguillón, C. Chitosan derivatives as chiral selectors bonded on allyl silica gel: preparation, characterisation and study of the resulting high-performance liquid chromatography chiral stationary phases. J. Chromatogr. A. **1999**, *839*, 15–21.
6. Franco, P.; Senso, A.; Oliveros, L.; Minguillón, C. Covalently bonded polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography. J. Chrmatogr. A. **2001**, *906*, 155–170.
7. Allenmark, S.G.; Andersson, S.; Moller, P.; Sanchez, D. A new class of network-polymeric chiral stationary phases. Chirality. **1995**, *7*, 248–256.
8. Lee, Y.K.; Nakashima, Y.; Onimura, K.; Tsutsumi, H.; Oishi, T. Free-radical polymerization of (R)-(-)-1-(1-naphthyl)ethyl(2-methacryloylxyethyl)urea and chiral recognition. Macromolecules. **2003**, *36*, 4735–4742.

9. Lee, Y.K.; Yamashita, K.; Eto, M.; Onimura, K.; Tsutsumi, H.; Oishi, T. Synthesis of novel chiral poly (methacryate)s bearing urethane and cinchona alkaloid moieties in side chain and their chiral recognition abilities. Polymer. **2002**, *43*, 7539–7547.
10. Gasparrini, F.; Misti, D.; Villani, C. Chromatographic optical resolution on trans-1,2–diaminocyclohexane derivatives: theory and applications. Chirality. **1992**, *4*, 447–458.
11. Kurata, K.; Shimoyama, T.; Dobashi, A. Enantiomeric separation using temperature-responsive chiral polymers composed of valine diamide derivatives in aqueous liquid chromatography. J. Chromatogr. A. **2003**, *1012*, 47–56.
12. Saigo, K.; Chen, Y.; Kubota, N.; Tachibana, K.; Yonezawa, N.; Hasegawa, M. New chiral stationary phases for the high-performance liquid chromatographic resolution of enantiomers. Chem. Lett. **1986**, *4*, 515–518.
13. Huang, S.-H.; Bai, Z.-W.; Yin, C.-Q.; Li, S.-R. Covalently bonding chiral polyurethane on aminated silica gel: a new strategy to prepare chiral stationary phase for high-performance liquid chromatography. Chin. J. Polym. Sci. **2006**, *24*, 107–114.
14. Huang, S.-H.; Bai, Z.-W.; Yin, C.-Q.; Li, S.-R.; Pan, Z.-Q. Synthesis of polymer-type chiral stationary phases and their enantioseparation evaluation by high-performance liquid chromatography. Chirality. **2007**, *19*, 129–140.
15. Huang, S.-H.; Zhang, J.-Y.; Li, S.-R.; Yin, C.-Q.; Pan, Z.-Q.; Bai, Z.-W. The Preparation and Enantioseparation of Polymer-type Chiral Stationary Phases Derived from (1S,2R)-(+)-2-Amino-1,2–diphenylethanol. J. Liq. Chromatogr. Rel. Technol. **2008**, *31* (17), 2554–2574.
16. O'Brien, T.; Crocker, L.; Thompson, R.; Thompson, K.; Toma, P.H.; Conlon, D.A.; Feibush, B.; Moeder, C.; Bicker, G.; Grinberg, N. Mechanistic aspects of chiral discrimination on modified cellulose. Anal. Chem. **1997**, *69*, 1999–2007.
17. Péter, A.; Török, G.; Armstrong, D.W. High-performance liquid chromatographic separation of enantiomers of unusual amino acids on a teicoplanin chiral stationary phase. J. Chromatogr. A. **1998**, *793*, 283–296.