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Polymer- versus Silica-Based Separation Media: Elimination of Nonspecific Interactions in the Chiral Recognition Process through Functional Polymer Design

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A novel chiral stationary phase that contains a new chiral selector based on L-valine-3,5-dimethylanilide attached to monodisperse poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads has been prepared. The polymeric separation medium provides greatly enhanced enantioselectivities and reduced retention times when compared to the analogous silica-based chiral stationary phase in the separation of the enantiomers of 3,5-dinitrobenzamido derivatives of α-amino acids under normal-phase HPLC conditions. Separation factors (α) of up to 7 were achieved with the polymeric separation medium, which is also useful for the separation of large samples under overload conditions.

The separation of racemates to pure enantiomers is a difficult task. The "classical" methods of the separation of individual enantiomers such as crystallization[1] and distillation[2] date back more than 100 years. Chromatography and electrophoresis are other useful methods to achieve chiral separations.[3,4] However, while the separation of enantiomers using a solid support was first suggested almost a century ago,[5] it was not until 1960 that the first chromatographic enantioseparation was published. The number of chiral stationary phases (CSPs) for liquid chromatography that emerged during the last decade is tremendous, and more than 70 CSPs are now commercially available.[3,4,7]

Selectors in most commercial CSPs are attached to porous silica beads. Although the use of silica as a chromatographic support benefits from some obvious advantages, such as its commercial availability, resistance to swelling, and good efficiency, certain problems remain to be solved. For example, the presence of residual silanol groups on the surface of silica provides polar sites where nonspecific interactions with analyte enantiomers may occur. Such nonspecific interactions may decrease the chiral recognition.[4,8] End-capping the residual silanol groups after immobilization of chiral selectors to silica or coating of the silica with chirally modified polymers has led to CSPs of improved enantioselectivities.[13−18] However, these efforts only reduce but do not eliminate the effects of residual silanols.

In contrast, synthetic polymer beads have some characteristics, such as their stability over the entire range of pH and their wealth of surface chemistries, that make them well suited for the preparation of chiral separation media. In spite of these attractive properties, few chromatographic packings based on polymer beads are available for chiral separations.[3,4,15−17]

This article reports preliminary results obtained both with synthetic macroporous polymer beads and with silica, each provided with identical "brush" type chiral selector. Our general goal is to develop a *new platform*, different from the traditional silica, on which more efficient and better defined chiral stationary phases can be built.

EXPERIMENTAL SECTION

Materials. Spherical silica beads of Nucleosil (10 μm/300 Å) were purchased from Phenomenex (Torrance, CA). The glycidyl methacrylate and ethylene dimethacrylate monomers (Sartomer, Exton, PA) were distilled under vacuum prior to use. Azobis(isobutyronitrile) (AIBN) was obtained from Kodak (Rochester, NY), and cyclohexanol and dodecyl alcohol were from Aldrich (Milwaukee, WI). All other reagents and solvents used were of reagent and HPLC grades, respectively.

Chromatography. The chiral stationary phases were slurry packed at constant pressure of 15 MPa into 150 mm × 4.6 mm i.d. stainless steel HPLC columns using methanol as the dispersion liquid.

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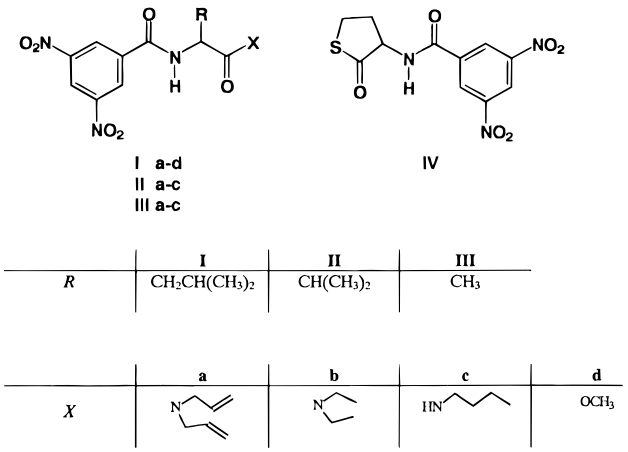

Figure 1. Structures of analytes used in this study.

A Waters HPLC system consisting of two 510 HPLC pumps, a 717 plus autosampler, and a 486 UV detector and Waters Millenium 2010 software were used throughout. 1,3,5-Tri-*tert*-butylbenzene (TTBB) was used to determine column void volume under normal phase HPLC conditions.

Racemic Analytes. *N*-(3,5-Dinitrobenzoyl)-α-amino acid methyl ester and alkyl amides **I**−**III** were prepared by methods similar to those previously reported.[18] Analyte **IV** was prepared by allowing homocysteine thiolactone to react with 3,5-dinitrobenzoyl chloride in the presence of triethylamine in dichloromethane. ^{1}H NMR and IR spectra are in agreement with the assigned structures shown in Figure 1.

Preparation of the Chiral Selector. ω-Undecylenyl 4-nitrophenylcarbonate (3). ω-Undecylenyl alcohol (**1**, 2.55 g) was added to a solution of 4-nitrophenyl chloroformate (**2**, 3.10 g) in 75 mL of anhydrous pyridine/tetrahydrofuran (1:2 by volume) at 0 °C with stirring. After the addition, the reaction mixture was allowed to warm up to room temperature and stirred overnight under nitrogen. The solution was diluted with ethyl acetate and washed with 1 mol/L HCl, water, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated under vacuum to afford 4.45 g of oily product **3** (89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (2H, d, J = 6.91 Hz), 7.43 (2H, d, J = 6.91 Hz), 5.83 (1H, m), 4.97 (2H), 4.29 (2H, d, J = 6.72 Hz), 2.05 (2H, m), 1.76 (2H, m), 1.58−1.23 (12H).

***N*-(*tert*-Butoxycarbonyl)-L-valine-3,5-dimethylanilide (6).** 2-Ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ, 8.54 g) was added to a solution of *N*-(*tert*-butoxycarbonyl)-L-valine (**4**, 6.52 g) in 45 mL of anhydrous dichloromethane with stirring, followed by addition of 4.18 g of freshly distilled 3,5-dimethylaniline (**5**). The resulting reaction mixture was stirred overnight and then washed successively with dilute aqueous HCl, water, and brine. The organic phase was dried over $MgSO_4$ and filtered. Evaporation of the filtrate in vacuo followed by crystallization from dichloromethane affords 9.04 g of the product, **6**, in 94% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (1H, br s), 7.12 (2H, s), 6.66 (1H, s), 5.52 (1H, d, J = 8.3 Hz), 4.16 (1H, m), 2.18 (6H, s), 2.12 (1H, m), 1.43 (9H, s), 1.11 (6H).

L-Valine-3,5-dimethylanilide (7). *N*-(*tert*-Butoxycarbonyl)-L-valine-3,5-dimethylanilide (**6**, 8.0 g) was placed in a 250 mL round-bottom flask containing 120 mL of 1:1 trifluroacetic acid/acetic acid solution, and the resulting mixture was stirred at ambient temperature for 6 h. The reaction mixture was concentrated in a rotary evaporator and poured into 25 mL of water. After neutralization with 1 mol/L KOH solution, the aqueous phase was extracted repeatedly with dichloromethane. The organic phases were combined, washed with water and brine, dried over $MgSO_4$, and filtered. Evaporation of the organic solvent gave 5.0 g of a pasty white solid in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.42 (1H, br s), 7.26 (2H, s), 6.73 (1H, s), 3.34 (1H), 2.45 (1H, m), 2.29 (6H, s), 1.68 (2H, br s), 1.02 (3H, d, J = 6.9 Hz), 0.85 (3H, d, J = 6.9 Hz).

ω-Undecylenyl L-valine-3,5-dimethylanilidocarbamate (8). L-Valine-3,5-dimethylanilide (**7**, 2.20 g) was added under stirring to a solution of ω-undecylenyl 4-nitrophenylcarbonate (**3**) and triethylamine (1.52 g) in 60 mL of dry tetrahydrofuran. The resulting reaction mixture was heated at reflux overnight, diluted with 100 mL of ethyl acetate, and washed with water and saturated sodium bicarbonate solution. The organic phase was dried over $MgSO_4$, filtered, and evaporated under vacuum to give a crude product, which was purified by flash chromatography on silica gel using ethyl acetate/hexane (1:3) to yield 3.51 g (84%) of the product as a pale-yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (1H, br s), 7.15 (2H, s), 6.72 (1H, s), 5.82 (1H, m), 5.53 (1H, d, J = 9.0 Hz), 5.03−4.90 (2H), 4.18 (1H, m), 4.07 (2H), 2.27 (6H, s), 2.14 (1H, m), 2.02 (2H, m), 1.60 (2H, m), 1.49−1.18 (12H), 1.02 (6H).

Silica-Based Chiral Stationary Phase (CSP 1). Dimethylchlorosilane (15 mL) and chloroplatinic acid (25 mg), which had been dissolved in 40 mL of tetrahydrofuran, were added to a 100 mL round-bottom flask containing 2.4 g of ω-undecylenyl L-valine-3,5-dimethylanilidocarbamate (**8**) in 15 mL of dry toluene. The reaction mixture was heated at 60 °C for 2 h with stirring under nitrogen, and the excess dimethylchlorosilane was removed with two small portions of dry toluene. The mixture was then treated with a solution of 10 mL of absolute ethanol, 10 mL of triethylamine, and 10 mL of diethyl ether. After the precipitated triethylamine hydrochloride was removed by filtration, the filtrate was concentrated and chromatographed on a silica gel column with 3:1 dichloromethane/hexane to afford 2.80 g of the chiral ethoxyorganosilane as a yellow oil in 89% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (1H, br s), 7.12 (2H, s), 6.70 (1H, s), 5.65 (1H, d, J = 8.7 Hz), 4.17 (1H, m), 4.10 (2H, quart, J = 7.1 Hz), 4.06 (2H), 2.25 (6H, s), 2.13 (1H, m), 1.61 (2H, m), 1.49−1.12 (19H), 1.05 (6H), 0.51 (2H), 0.08 (6H, s).

Dried silica beads (2.20 g) were added to a solution of the above chiral ethoxyorganosilane (2.75 g) in 15 mL of dry toluene. The resulting slurry was heated at reflux under nitrogen for 24 h. These chirally modified silica beads were washed thoroughly with dichloromethane and methanol. The content of chiral selector functionalities in silica beads is 0.20 mmol/g according to the result of elemental analysis (C, 6.5; H, 0.52; N, 0.50).

Uniformly Sized Macroporous Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) Beads. The monodisperse porous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) (GMA-EDMA) beads (**9**) were prepared according to a staged templated suspension polymerization process reported elsewhere.[19] The content of epoxide groups (1.53 mmol/g) was determined by volumetric titration.[20]

Reduced Beads, 10. To a slurry of 2.0 g of the GMA−EDMA beads **9** in 25 mL of dry tetrahydrofuran were added 10 mL of 1 mol/L $NaBH_3CN$ in tetrahydrofuran and a small amount of bromocresol green indicator. Boron trifluoride diethyl etherate

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was then introduced dropwise into the reaction mixture with gentle stirring until the color changed to yellow. The reaction was maintained at 50 °C for 5 h with occasional stirring while additional BF_3·OEt_2 was added periodically to maintain the acidity. Upon completion, the beads were washed successively with 1 mol/L NaOH, water, methanol, tetrahydrofuran, and ethyl ether and dried under vacuum. The content of epoxide groups remaining in the beads determined by titration was 0.28 mmol/g. This small amount of epoxides was deemed to be unreactive.

Activated Beads, 11. To 2.0 g of the above reduced beads, **10**, suspended in 30 mL of dry tetrahydrofuran were added 1.2 g of 4-nitrophenyl chloroformate and 0.4 g of triethylamine. After the addition, the reaction mixture was heated at 60 °C with stirring overnight. The modified beads were then washed repeatedly with tetrahydrofuran and ethyl ether and dried under vacuum. Nitrogen analysis indicates that the resulting beads contain 0.89 mmol/g of 4-nitrophenyl carbonate groups on their surface ($N = 1.25$).

Polymer-Based Chiral Stationary Phase (CSP 2). L-Valine-3,5-dimethylanilide (**7**, 1 g), and triethylamine (0.15 g) were added to 1.2 g of the polymer beads, **11**, suspended in 10 mL of dry tetrahydrofuran. The resulting slurry was heated at 60 °C with stirring overnight. The beads so modified with the chiral selector were then washed thoroughly with methanol and tetrahydrofuran. The selector content of the beads is 0.44 mmol/g based on elemental analysis ($N = 1.25$), assuming that all of the nitrogen originates from the chiral selector functionalities.

RESULTS AND DISCUSSION

The separation factor or enantioselectivity observed in the chromatographic separations of racemates is affected not only by the intrinsic capability of the immobilized chiral selector to differentiate between the two enantiomers of the analyte, but also by a number of other factors. These include, for example, the way by which the selector is tethered, the length of the tethering arm, the nature of the underlying support, and the presence of undesired and superfluous polar sites where nonspecific interactions between the CSP and the analyte enantiomers may occur. All of these factors have tremendous effects on the chiral recognition processes and, therefore, on the overall performance of the chiral separation medium.

Recently, we developed a staged templated suspension polymerization technique that leads to monodisperse glycidyl methacrylate polymer beads with excellent porous properties. These beads as well as others based on styrenic monomers have been used successfully as stationary phases for the chromatographic separations of both large and small molecules.[21−24] Our continuing search for new polymer-based separation media with improved performance led us to design a polymeric chiral stationary phase with a Pirkle-type π-basic chiral selector because this selector is known for its good enantioselectivity and its mechanism of chiral recognition is well understood.[25,26] It was expected that our ability to control the functionality, size distribution, and porous structure of polymer beads would afford media with greatly enhanced properties.

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Table 1. Characteristics of Macroporous Silica and Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate)

	silica, 300 Å	GMA−EDMA beads
particle size, μm	10	6
V_p, mL/g[a]	1.08	1.12
D_p, nm[b]	15	33
S_g, m^2/g[c]	81	84

[a] Pore volume determined by mercury intrusion porosimetry. [b] Median pore diameter determined from the pore volume. [c] Specific surface area, calculated from the BET isotherm of nitrogen.

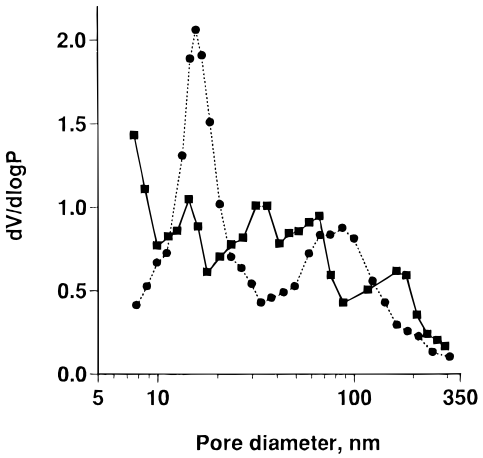

Figure 2. Differential pore size distribution curves for the silica beads (dotted line) and the monodisperse polymer beads (solid line) as determined by mercury intrusion porosimetry.

Characteristics of the Supports. Table 1 summarizes the results of BET nitrogen adsorption measurements and mercury intrusion porosimetry for both the silica and the poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads. Although the total pore volume and specific surface area are similar for the two supports, they differ in size and in median pore diameter. The latter value for the silica beads is actually only one-half the nominal value assigned by the manufacturer. Figure 2 shows the differential pore size distribution curves for the beads used in this study. This curve indicates a relatively broad pore size distribution.

The bead diameter of 6 μm was measured using scanning electron microscopy, a technique that also reveals the excellent monodispersity of the macroporous polymer beads (Figure 3).

Preparation of the Chiral Stationary Phases. Two chiral stationary phases, CSP 1 and CSP 2, were synthesized using an identical chiral selector derived from valine attached to both silica and modified poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads. A similar selector derived from proline has proven to be effective in the separation of π-acidic compounds.[16] The reaction path used for the preparation of two chiral stationary phases is shown in Figures 4 and 5. To facilitate the synthesis of polymer-based CSP 2, a carbamate functionality was used to tether the chiral selector to the polymer support. Because the carbamate group can be involved in the chiral recognition process, this functionality was also used in the tether of the silica-based chiral separation medium in order to compare stationary phases with similar chemistries. According to elemental analysis, the selector contents for CSP 1 and CSP 2 are 0.20 and 0.44 mmol/g, respectively. However, it should be noted that the density of the polymer beads is about one-half that of the silica beads used in

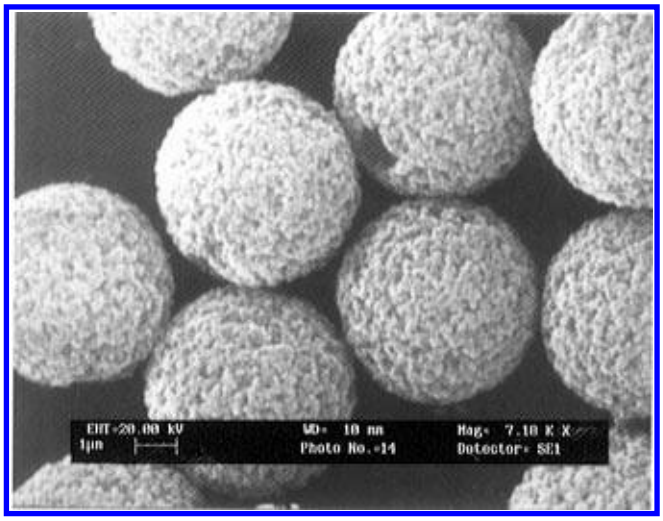

Figure 3. Scanning electron micrograph of the monodispersed macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads.

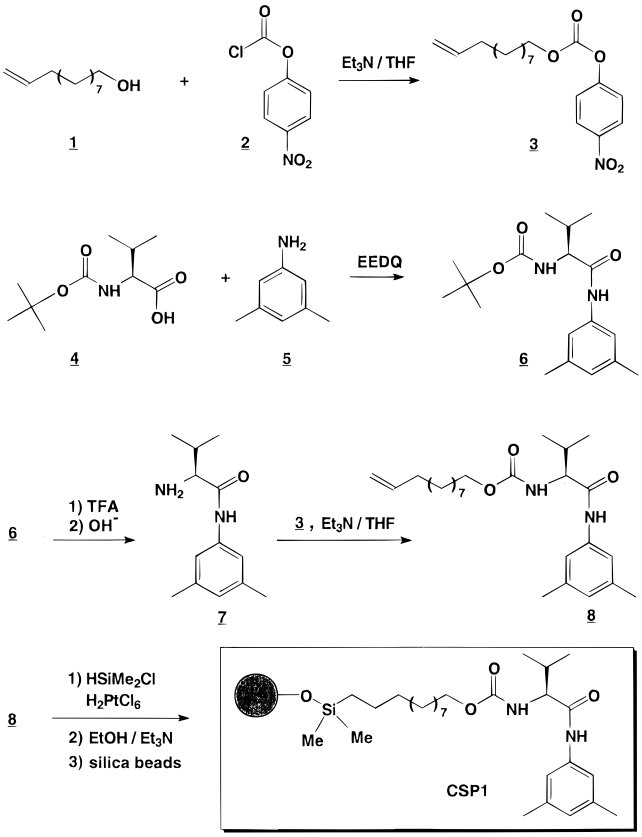

Figure 4. Synthetic strategy for the preparation of the silica-based chiral stationary phase (CSP 1).

this study; therefore, the number of selector functionalities per column is similar for both chiral stationary phases. This, in turn, diminishes the possible effect of the extent of functionalization on the separation properties of both CSPs and facilitates their direct comparison.

Obviously, it is impossible to prepare truly identical CSPs based on silica and on synthetic polymer beads because each type of starting material requires its own tethering chemistry. However, in both instances, the selector, the carbamate link to the tether, and the type of the tether are identical. Aside from the support itself, the only difference is the length of the tether (C_{11} for silica vs C_3 for polymer beads), a fact that would favor the silica CSP 1

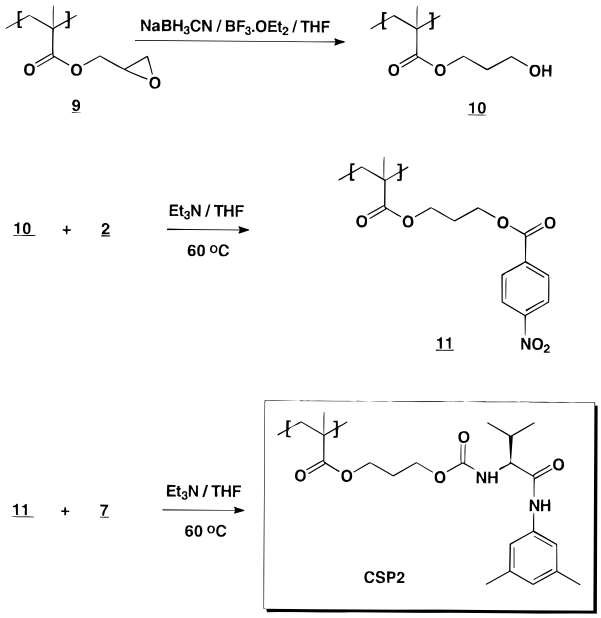

Figure 5. Synthetic strategy for the preparation of the polymer-based chiral stationary phase (CSP 2).

because it is known[27] that longer spacers often improve selectivity. To compare the effects of the matrices on the performance of both chiral separation media, no further chemical treatment such as capping of silanol functionalities was attempted.

Chromatographic Evaluation. (i) Separation of Enantiomers. The capabilities of both columns packed with chiral stationary phases CSP 1 and CSP 2 were evaluated in the model separations of 3,5-dinitrobenzamido derivatives of racemic α-amino acids. The best separations for both CSPs were achieved in a mobile phase consisting of 20% hexane and 80% dichloromethane. Figure 6 shows an example of typical separations obtained using the two chiral stationary phases. Table 2 summarizes all of the results of these chromatographic measurements carried out using identical chromatographic conditions that once again allow the assessment of the effect of the solid support. The synthetic polymer-supported CSP 2 phase exhibits substantially enhanced enantioselectivities and shorter retention times when compared to silica-based CSP 1 in almost all separations under normal-phase conditions. For example, separation factors (α) of 2.45 and 1.84 were observed on silica-based CSP 1 for the separation of enantiomers of the alanine derivatives **IIIa** and **IIIb**, respectively, while separation factors of 6.17 and 5.54 were obtained on polymeric CSP 2, representing a remarkable 2.5- and 3-fold increase in terms of the α value, respectively. Presumably, the lower level of enantioselectivity and longer retention times observed for CSP 1 are due to the presence of residual silanol groups on the silica surface. Using end-capping, Pirkle has provided clear evidence that these silanol groups increase retention times for both enantiomers of an analyte without differentiating between them, thus giving rise to diminished enantioselectivities.[8,12]

In contrast, these polar nonspecific interaction sites are absent on the surface of CSP 2, thus leading to a reduction in retention time and an enhancement in enantioselectivity. It can be assumed that these chromatographic results are essentially unaffected by the type of nonspecific interactions that are typical of phases with

(27) Bargmann-Leyder, N.; Truffet, J. C.; Tambute, A.; Caude, M. *J. Chromatogr. A* **1994**, *666*, 27−40.

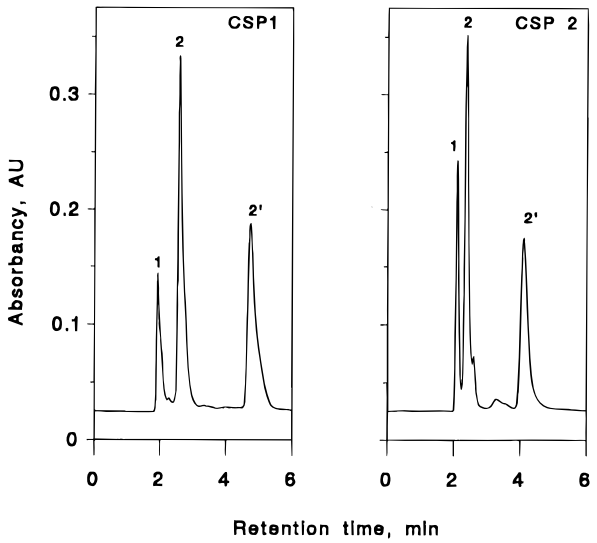

Figure 6. Separation of 3,5-dinitrobenzamidoleucine-*N,N*-diallylamide (**Ia**) enantiomers on chiral stationary phases CSP 1 and CSP 2. Conditions: column size, 150 mm × 4.6 mm i.d.; mobile phase, 20% hexane in dichloromethane; flow rate, 1 mL/min; injection, 7 μg; peaks, 1,3,5-tri-*tert*-butylbenzene (1), *R*-enantiomer (2), *S*-enantiomer (2′).

Table 2. Retention Factors *k′* and Separation Factors α Obtained for Enantioselective Separations of Racemic Compounds on Columns Packed with Chiral Stationary Phases CSP 1 and CSP 2[a]

	CSP 1				CSP 2			
analyte[b]	k_1'	k_2'	α	more retained	k_1'	k_2'	α	more retained
Ia	0.393	1.606	*4.09*	S	0.135	0.960	*7.11*	S
Ib	0.574	1.872	*3.26*	S	0.137	0.992	*7.25*	S
Ic	1.999	4.712	*2.36*	S	0.616	2.20	*3.57*	S
Id	0.213	0.606	*2.85*		0.135	0.413	*3.06*	
IIa	0.308	0.521	*1.59*	S	0.095	0.302	*3.06*	S
IIb	0.500	0.776	*1.55*	S	0.119	0.397	*3.34*	S
IIc	1.850	2.669	*1.44*	S	0.579	1.373	*2.37*	S
IIIa	0.425	1.042	*2.45*	S	0.103	0.635	*6.17*	S
IIIb	0.755	1.393	*1.84*	S	0.119	0.659	*5.54*	S
IIIc	3.191	5.476	*1.71*	S	0.794	1.74	*2.19*	S
IV	0.797	1.276	*1.60*		0.890	1.370	*1.54*	

[a] Chromatographic conditions: column, 150 × 4.6 mm i.d., mobile phase, 20% hexane in dichloromethane; flow rate, 1 mL/min; void marker 1,3,5-tri-*tert*-butylbenzene; UV detection at 254 nm. [b] For structures of the analytes, see Figure 1.

strongly acidic silanol groups. Therefore, another advantage of the polymeric platform is that the obtained separation factors approach more closely the intrinsic capability for enantioselectivity of the chiral selector itself based on the differences in the free energies ($\Delta\Delta G$ value) of the two diastereomeric complexes formed between the selector and the two enantiomers under the given conditions.

(ii) Column Capacity. Although analytical separations of racemic mixtures are invaluable for the characterization of mixtures and for process control, production scale chromatography is required for the racemic switch in the drug industry. The goal is to separate the largest possible amount of racemate in the smallest column. This makes overall column capacity a very important characteristic for chiral separations. While in analytical separations the amount injected is dictated by detector sensitivity,

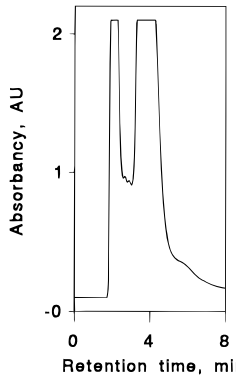

Figure 7. Enantioseparation of racemic 3,5-dinitrobenzamidoleucine-*N,N*-diallylamide (**Ia**) on chiral stationary phase CSP 2 under overload conditions. Conditions: column size, 150 mm × 4.6 mm i.d.; mobile phase, 20% hexane in dichloromethane; flow rate, 1 mL/min; injection, 0.5 mg.

throughput is much more important in preparative separations. The size of samples injected in these binary separations is always a trade-off between throughput and optical purity of both enantiomers. Therefore, high separation factors are advantageous because the peaks for the two enantiomers do not overlap very much, even if larger samples are injected. Figure 7 shows the separation of 0.5 mg of racemate **Ia** on a 150 mm × 4.6 mm column packed with CSP 2. This translates into a reasonably high capacity of 0.2 mg/mL of the stationary phase. Although this is no longer a baseline separation as found for analytical separations of microgram quantities, the resolution under overload conditions is still sufficient.

CONCLUSION

A new platform based on porous monodisperse poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads endowed with homogeneous surface chemistry is well suited for the engineering of a new generation of chiral separation media. We have demonstrated as an example that a chiral stationary phase prepared by attachment of a nearly identical chiral selector to this polymeric support showed greatly improved enantioselectivity over its silica-supported counterpart and afforded shorter retention times in the separation of model enantiomers.

Our results suggest that many inherent merits of synthetic polymer beads in general, and uniformly sized polymer beads in particular, deserve more attention in the development of novel and more efficient chiral stationary phases for chromatographic enantioseparations.

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