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Bonded cellulose-derived high-performance liquid chromatography chiral stationary phases
II. Influence of the porosity of the silica gel matrix on performance

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Abstract

Two cellulose derivatives bearing simultaneously 10-undecenoyl and 3,5-dimethylphenylaminocarbonyl groups in different ratios were chemically bonded on allylsilica gels of different porosity. The systematic study of the effect of the silica gel pore size on the bonding of the polysaccharide derivative and on the performance of the resulting chiral supports is presented.

Keywords: Chiral stationary phases, LC; Cellulose stationary phases; Enantiomer separation

1. Introduction

Owing to the chiral nature and ready availability of many natural polysaccharides, particularly cellulose and amylose, such compounds and their derivatives are among the most used as chiral selectors for optical resolution by HPLC [1].

Cellulose carbamates, like other derivatives, are easily prepared from cellulose by modification of the free hydroxyl groups in the glucose units. This type of derivative, classically obtained by reaction with isocyanates, can be coated on silica supports (frequently, macroporous γ-aminopropylsilica gel) and, at present, a number of them are available commercially [2,3]. A definite disadvantage with this type of chiral stationary phases (CSPs) is their incompatibility with many common organic solvents used in HPLC. The adsorbed polysaccharide derivatives are partially dissolved or swollen by solvents such as chloroform, tetrahydrofuran, toluene, ethyl acetate or acetone. Therefore, the choice of eluent is limited by this solubility. We have recently developed CSPs in which the cellulose derivatives are not adsorbed but chemically bonded to allylsilica gel or fixed on other matrices [4,5]. This property makes them resistant to usual solvents and very useful for the resolution of racemic compounds, slightly soluble in the solvents allowed on commercially available cellulose-derived CSP, even at preparative scale [6], in a wide range of HPLC solvents and conditions.

The fixation process is achieved either by heterogeneous coupling of double bonds on the allylsilica gel and on the cellulose derivative, or by reticulation of the 10-undecenoyl groups themselves. Both processes can affect the secondary structure of

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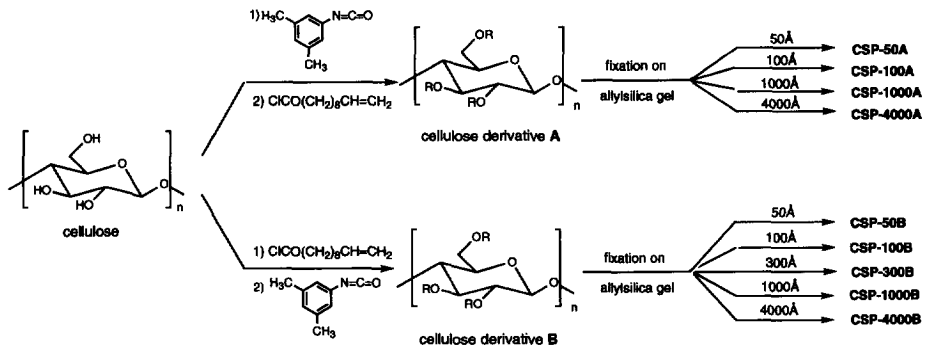

Fig. 1. Preparation of cellulose derivatives and chiral stationary phases.

the chiral selector, and consequently they affect the chiral discrimination ability of the resulting CSPs. Some variables remain to be optimized in this kind of CSPs for HPLC. In this report, the effects of porosity changes of the silica gel matrix either on the fixing of the polysaccharide derivative or on the chromatographic behaviour of the resulting CSPs are discussed.

2. Experimental

^{1}H-NMR spectra were measured using a Varian GEMINI-300 spectrometer. Elemental analyses were performed by the Serveis Científico-Tècnics de la Universitat de Barcelona (Spain). The CSPs were packed into stainless-steel tubes (150×4.6 mm I.D.) by the slurry method. The chromatographic experiments were performed on an HPLC system consisting of a Waters 600E pump, a Waters 717 auto sampler (Millipore, Milford, MA, USA) equipped with a Waters 996 photo-diode array detector and a Perkin-Elmer 241LC polarimetric detector (Perkin-Elmer, Uberlingen, Germany). The volume of sample injected was 3 μl. The void volume was determined using tri-*tert.*-butylbenzene.

2.1. Cellulose derivatives and chiral stationary phases

Cellulose derivatives A and B were prepared by the method indicated in Fig. 1 and previously described [4,5,7]. The ratios of the two kinds of substituent for each glucose unit on A and B were calculated from their elemental analyses (Table 1). The same values for the ratios of substituents on glucose units were obtained from ^{1}H-NMR data (pyridine-d_5, 300 MHz, 70°C).

The cellulose derivatives were fixed on allylsilica gel matrices. The various allylsilica gels were prepared from spherical 5 μm silica gels of different pore size (Nucleosil 50-5, 100-5, 300-5, 1000-5 and 4000-5, Macherey-Nagel) by reaction with allyltriethoxysilane followed by treatment with hexamethyldisilazane.

Table 1
Characterization of cellulose derivatives

Cellulose derivative	Elemental analyses of cellulose derivatives			Substitution of glucose units		
	%C	%H	%N	Undec.	Carbam.	Total[a]
A	66.4	7.18	5.06	0.65	2.09	2.74
B	65.8	6.79	5.97	0.31	2.44	2.75

[a] The maximum substitution degree of a glucose unity being 3 (number of OH).

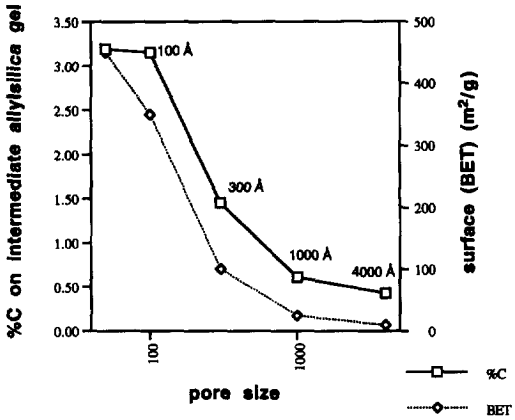

Fig. 2. Relationship between silica gel pore size (logarithmic scale) and the carbon content on intermediate allylsilica gels.

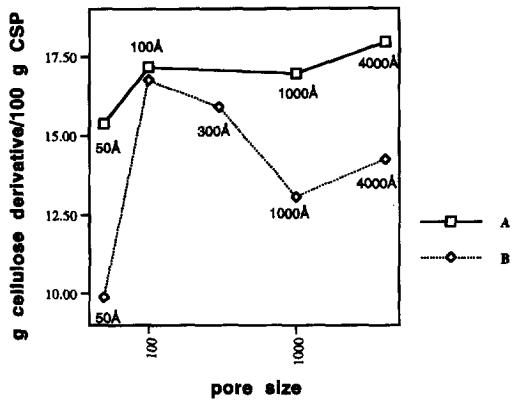

Fig. 3. Relationship between silica gel pore size (logarithmic scale) and the amount of cellulose derivative fixed on the CSP.

3. Results and discussion

Usually, macroporous γ-aminopropylsilica gel (pore diameter of 1000 or 4000 Å) was used as a matrix on which polysaccharide derivatives are coated [8]. In the present kind of bonded CSP, due to the interaction between the polysaccharide derivative and the matrix surface, an investigation of the effect of the silica gel pore size on the bonding and on the performance of the resulting supports was needed.

3.1. Influence of the pore size of the matrix on the bonding of cellulose derivative

As is well known, the greater the pore size, the smaller the number of allyl groups on the silica surface. In Fig. 2 the relationship between the percentage of carbon on allylsilica gel, directly related to the quantity of allyl groups, and the pore size is shown. Therefore, the number of fixation groups diminishes as pore size increases. As a

Table 2
Characterization of chiral stationary phases

CSP	Surface (BET)[a] (m^2/g)	Intermediate allylsilica gel		Elemental analyses of final chiral supports			g cellulose derivative/ 100 g phase[b]	HEPT[c] (cm)
		%C	%H	%C	%H	%N		
50A	450	3.19	1.27	12.30	2.00	0.78	15.38	$7.89 \cdot 10^{-3}$
100A	350	3.15	1.00	13.80	2.53	0.87	17.16	$7.11 \cdot 10^{-3}$
1000A	25	0.61	0.59	11.30	1.67	0.86	16.96	$6.04 \cdot 10^{-3}$
4000A	10	0.43	0.42	11.00	1.69	0.91	17.95	$8.09 \cdot 10^{-3}$
50B	450	3.19	1.27	7.81	1.43	0.59	9.88	$8.89 \cdot 10^{-3}$
100B	350	3.15	1.00	13.40	2.10	1.00	16.75	$8.90 \cdot 10^{-3}$
300B	100	1.45	0.73	10.70	1.41	0.95	15.91	$9.89 \cdot 10^{-3}$
1000B	25	0.61	0.59	8.01	1.12	0.78	13.06	[d]
4000B	10	0.43	0.42	8.84	0.95	0.85	14.24	[d]

[a]Data given by the supplier.
[b]Based on elemental analyses.
[c]Calculated using 1,3,5-tri-*tert.*-butylbenzene.
[d]These supports could not be packed in columns by the slurry method.

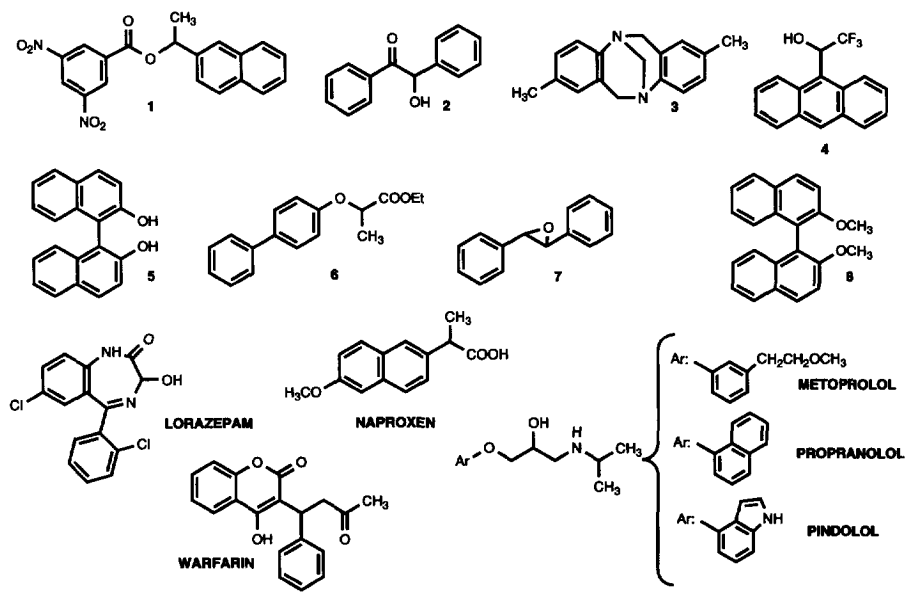

Fig. 4. Chemical structures of racemic test compounds.

consequence, the fixation of cellulose derivatives depends on porosity of the silica gel matrix.

Two different derivatives of cellulose were used as chiral selectors. The study was started by bonding cellulose derivative A on several spherical 5 μm allylsilica gel matrices of different pore size. A parallel study indicated that cellulose derivative B had the best molar ratio of substituents [7]. Therefore, a second series of CSPs based on this chiral selector was also included. The characterization of the chiral supports obtained is reported on Table 2.

In spite of the decrease in the number of fixation

Table 3
Chromatographic results of CSPs of the A series obtained using heptane–2-propanol as the mobile phase

Racemic compounds	CSP-50A			CSP-100A			CSP-1000A			CSP-4000A			Mobile phase[a]
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
1	5.87	1.13	1.15	5.17	1.07	-	6.91	1.09	-	8.79	1.11	-	(a) 90:10
2	4.08	1.19	1.78	3.56	1.15	-	4.05	1.18	-	4.64	1.19	1.03	(a) 98:2
3	4.74	1.14	1.02	2.71	1.15	-	1.03	1.44	1.06	1.62	1.56	1.27	(a) 98:2
4	1.59	1.58	3.57	1.01	1.49	1.29	1.21	1.61	1.50	1.60	1.65	1.93	(a) 90:10
5	12.4	1.13	0.95	11.0	1.00	-	13.9	1.00	-	15.9	1.18	-	(a) 98:2
6	0.96	1.32	2.20	0.80	1.28	1.01	0.79	1.34	1.01	0.95	1.38	1.29	(a) 98:2
7	0.69	1.41	1.85	0.58	1.00	-	0.64	1.17	-	0.79	1.29	0.97	(a) 98:2
Lorazepam	8.80	1.30	1.83	7.66	1.35	0.89	8.46	1.45	0.91	9.81	1.50	1.24	(a) 90:10
Warfarin	3.67	1.62	2.77	2.81	1.65	1.56	5.16	1.46	1.64	3.71	1.81	1.68	(a) 90:10
Metoprolol	6.33	1.16	-	3.35	1.08	-	1.06	1.08	-	1.07	1.32	-	(b) 90:10:0.1
Propranolol	4.64	1.27	1.31	2.41	1.33	-	1.14	1.59	1.14	1.29	1.84	1.78	(b) 90:10:0.1
Pindolol	5.21	2.16	5.23	3.83	1.89	1.66	2.17	3.01	2.32	2.38	4.05	3.68	(b) 80:20:0.1
Naproxen	4.46	1.18	1.69	4.15	1.16	0.67	4.38	1.19	0.81	4.91	1.23	1.15	(c) 98:2:0.5

k'_1=Capacity factor for the first eluted enantiomer; α=selectivity factor; R_s=resolution. Column: 15×0.46 cm I.D. UV detection, $\lambda_{230\ nm}$ (**1**, **3**, **5**, **8**, lorazepam, warfarin, metoprolol and naproxen), $\lambda_{254\ nm}$ (**2**, **4**, **6**, **7** and pindolol) and $\lambda_{280\ nm}$ (propranolol).

[a](a)=heptane–2-propanol, flow-rate: 1 ml/min; (b)=heptane–2-propanol–DEA, flow-rate: 1 ml/min; (c)=heptane–2-propanol–TFA, flow-rate: 0.5 ml/min.

Table 4
Chromatographic results of CSPs of the A series obtained using heptane–chloroform as the mobile phase

Racemic compounds	CSP-50A			CSP-100A			CSP-1000A			CSP-4000A			Mobile phase[a]
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
2	2.78	1.11	1.27	1.36	1.00	-	1.45	1.17	-	1.66	1.21	0.99	80:20
4	2.89	2.94	6.11	1.36	1.73	2.49	1.76	1.95	2.90	2.12	2.09	3.59	50:50
5	2.80	1.41	1.87	1.32	1.20	-	1.65	1.37	1.13	1.89	1.44	1.46	60:40
6	3.56	1.21	2.00	0.98	1.23	1.00	1.31	1.29	0.94	1.46	1.27	1.02	95:5
7	1.75	2.13	5.76	0.74	1.32	1.08	1.05	1.67	2.00	1.24	1.88	2.65	95:5
8	4.03	1.11	-	1.16	1.11	-	1.65	1.11	-	1.96	1.08	-	95:5
Lorazepam	16.4	1.13	1.04	9.35	1.22	1.14	5.31	1.43	1.30	5.51	1.51	1.94	40:60
Warfarin	2.97	1.74	1.27	0.89	2.33	1.41	0.94	2.30	1.23	1.09	2.34	1.30	50:50

k'_1=Capacity factor for the first eluted enantiomer; α=selectivity factor; R_s=resolution. Column: 15×0.46 cm I.D. UV detection, $\lambda_{240\ nm}$ (**7**, **8**, and lorazepam), $\lambda_{254\ nm}$ (**2**, **4** and **6**) and $\lambda_{280\ nm}$ (**5** and warfarin).
[a]Mobile phase=heptane–chloroform; flow-rate: 1 ml/min.

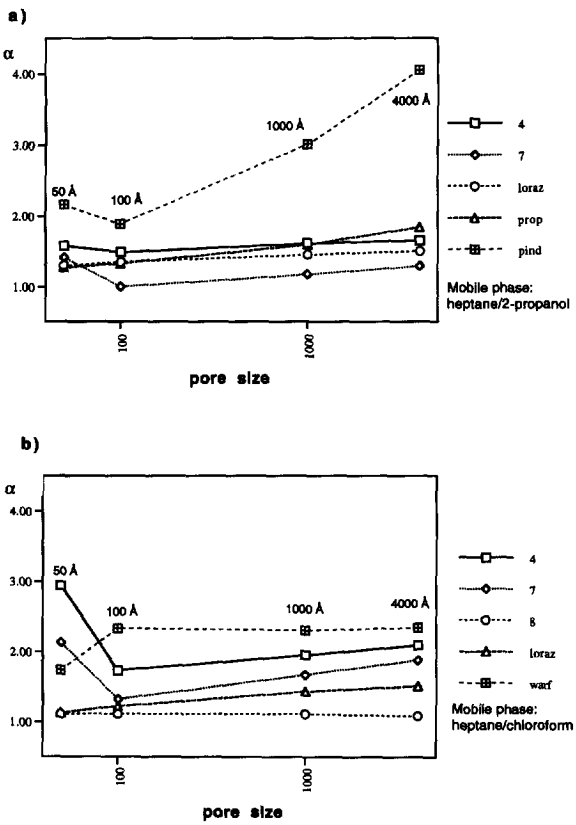

Fig. 5. Relationship between silica gel pore size (logarithmic scale) and α values in the A series. Mobile phase: (a) heptane–2-propanol, (b) heptane–chloroform.

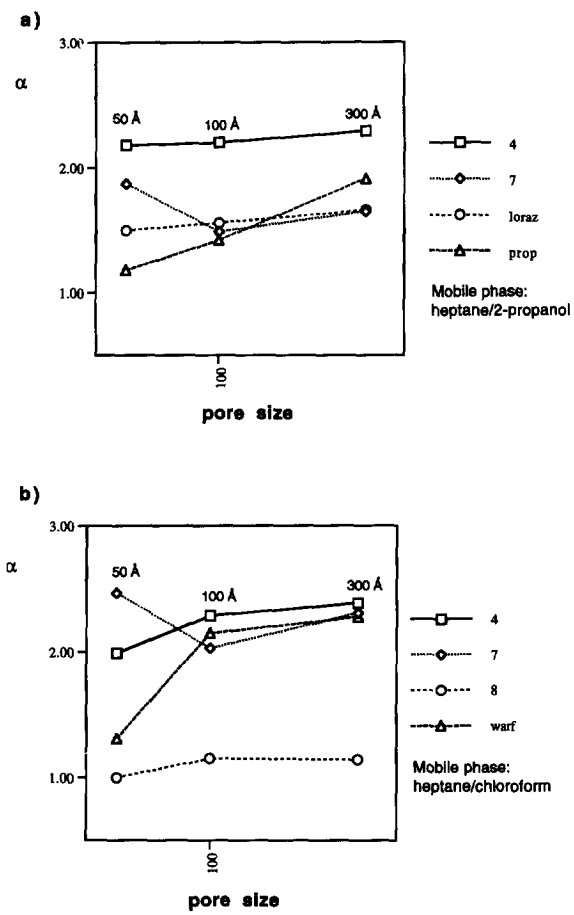

Fig. 6. Relationship between silica gel pore size (logarithmic scale) and α values in the B series. Mobile phase: (a) heptane–2-propanol, (b) heptane–chloroform.

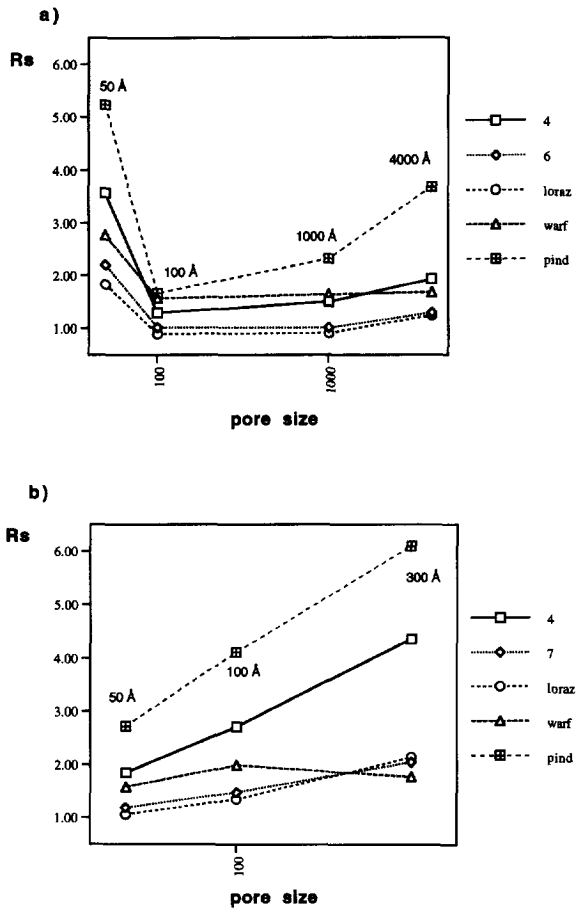

Fig. 7. Relationship between silica gel pore size (logarithmic scale) and resolution (mobile phase, heptane–2-propanol). (a) A series, (b) B series.

groups on the matrix, in the A series, with a higher number of 10-undecenoyl groups on the cellulose derivative, the percentage of bonded chiral selector was not reduced (Fig. 3), and the chromatographic supports obtained were properly packed. In fact, the decrease of allyl groups on the matrix was, in this case, balanced by the reticulation process between the double bonds of the more abundant 10-undecenoyl groups. However, in the B series the content of cellulose derivative diminished as the pore size increased, because in this series the reduced quantity of 10-undecenoyl groups did not ensure the fixation of the cellulose derivative through a reticulation process. This could also explain the problems encountered when attempting to pack CSP-1000B and CSP-4000B in columns. These CSPs could not be correctly packed by the slurry method using several solvents. Packing problems with related CSPs have been reported [9], but attributed in that case to the high amount of chiral selector coated on the matrix (above 15% w/w cellulose derivative loading). In both series, the slight increase of organic material percentage between 1000 and 4000 Å CSPs can be attributed to the accessibility of allyl groups. Thus, though the number of allyl groups is smaller in 4000 Å matrices, they could be more accessible than in allylsilica gel of 1000 Å.

In both series, a smaller percentage of cellulose derivatives on 50 Å CSPs is observed. It seems possible that a part of allyl groups could be in the cavities, where they might not be accessible to the cellulose derivative. Therefore, not all the allyl groups will be involved in the fixation of the cellulose derivative and it is only fixed on the matrix surface. As a consequence, the degree of fixation on them can be compared with that on 4000 Å phases. There, even if the quantity of allyl groups is lower, they are in cavities readily accessible to the polysaccharide. This effect is more noticeable in the B series, where CSP-50B has the least organic content. In CSP-50A, this phenomenon is offset by the fixation of polysaccharide derivative A by reticulation of 10-undecenoyl groups of cellulose derivative A.

3.2. Influence of the pore size of the matrix on performance of CSPs

In Tables 3–6, several chromatographic results obtained with the synthesized CSPs are presented for some racemic compounds (Fig. 4), using either heptane–2-propanol or heptane–chloroform mixtures as mobile phases. Generally, the selectivity factor increases with pore size, as is illustrated on Fig. 5 for the A series and on Fig. 6 for the B series. This effect can be attributed to the better accessibility of the chiral selector for racemic compounds as the pore size increases. Only CSP-50A constitutes an exception. In some cases, CSP-50A shows higher α values than CSP-100A or even than CSP-4000A. This effect is clearer on the resolution factor (Fig. 7). The improvement in enantioselectivity could be associated, again, with the accessibility of the chiral selector, placed on the particle's surface in 50 Å

Table 5
Chromatographic results of CSPs of the B series obtained using heptane–2-propanol as the mobile phase

Racemic compounds	CSP-50B			CSP-100B			CSP-300B			Mobile phase[a]
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
1	2.86	1.00	-	7.02	1.17	-	6.21	1.13	-	(a) 90:10
2	2.41	1.31	-	4.74	1.33	1.55	4.11	1.40	2.39	(a) 98:2
3	5.43	1.00	-	3.20	1.21	-	1.83	1.28	1.11	(a) 98:2
4	0.70	2.18	1.85	1.34	2.20	2.71	1.45	2.29	4.36	(a) 90:10
5	5.63	1.15	-	14.2	1.18	-	12.3	1.00	-	(a) 98:2
6	0.61	1.00	-	1.01	1.23	-	0.84	1.23	-	(a) 98:2
7	0.39	1.87	1.19	0.86	1.49	1.48	0.81	1.65	2.05	(a) 98:2
Lorazepam	5.09	1.50	1.06	8.74	1.56	1.35	8.24	1.66	2.14	(a) 90:10
Warfarin	1.90	1.96	1.58	3.33	2.17	1.99	3.35	2.31	1.77	(a) 90:10
Metoprolol	8.38	1.00	-	3.22	1.20	-	1.84	2.10	2.73	(b) 90:10:0.1
Propranolol	5.71	1.18	-	3.01	1.42	1.33	2.63	1.91	2.77	(b) 90:10:0.1
Pindolol	5.39	2.45	2.72	3.79	4.11	4.11	3.17	5.68	6.09	(b) 80:20:0.1
Naproxen	3.52	1.00	-	5.64	1.22	1.18	5.00	1.25	1.35	(c) 98:2:0.5

k'_1=Capacity factor for the first eluted enantiomer; α=selectivity factor; R_s=resolution. Column: 15×0.46 cm I.D. UV detection, $\lambda_{230\ nm}$ (**1**, **3**, **5**, **8**, lorazepam, warfarin, metoprolol and naproxen), $\lambda_{254\ nm}$ (**2**, **4**, **6**, **7** and pindolol) and $\lambda_{280\ nm}$ (propranolol).
[a](a)=heptane–2-propanol, flow-rate: 1 ml/min; (b)=heptane–2-propanol–DEA, flow-rate: 1 ml/min; (c)=heptane–2-propanol–TFA, flow-rate: 0.5 ml/min.

CSPs. In the case of CSP-50B, the lowest enantioselectivity in the series can be ascribed to the minor quantity of chiral selector on it. In CSP-100A and CSP-100B, although their content of chiral selector is higher than in the rest of the supports, the pore diameter could allow the polysaccharide derivative partially to enter the cavities. Therefore, not all the cellulose derivative is accessible to racemic compounds. Only the part on the matrix surface is able to act as a chiral selector. Further studies are needed to confirm the validity of this hypothesis.

Above 300 Å, the resulting CSPs in B series show a considerable amount of aggregated particles, which prevents the correct packing of those CSPs.

4. Conclusion

Usually, the performance of the chiral stationary phases increases with pore size when the pore diameter is large enough to allow the penetration of macromolecules. However, the choice of the matrix

Table 6
Chromatographic results of CSPs of the B series obtained using heptane–chloroform as the mobile phase

Racemic compounds	CSP-50B			CSP-100B			CSP-300B			Mobile phase[a]
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
2	2.12	1.13	-	2.20	1.15	0.85	1.90	1.40	2.35	80:20
4	1.74	1.99	2.20	2.39	2.29	3.75	2.73	2.39	5.20	50:50
5	1.79	1.18	-	1.94	1.32	1.07	2.11	1.39	1.80	60:40
6	2.27	1.00	-	1.73	1.17	-	2.49	1.17	-	95:5
7	1.04	2.47	2.36	1.41	2.03	3.88	2.11	2.31	4.81	95:5
8	2.44	1.00	-	1.80	1.15	-	2.41	1.14	-	95:5
Lorazepam	18.4[b]	1.00	-	13.8	1.18	-	>25			50:50
Warfarin	2.19	1.31	0.94	1.32	2.15	1.74	1.41	2.28	2.31	50:50

k'_1=Capacity factor for the first eluted enantiomer; α=selectivity factor; R_s=resolution. Column: 15×0.46 cm I.D. UV detection, $\lambda_{240\ nm}$ (**7**, **8**, and lorazepam), $\lambda_{254\ nm}$ (**2**, **4** and **6**) and $\lambda_{280\ nm}$ (**5** and warfarin).
[a]Mobile phase=heptane–chloroform; flow-rate: 1 ml/min.
[b] Heptane–chloroform (40:60).

will be limited by the quantity of fixing groups, both on cellulose derivative and on the silica gel surface.

A pore size of 50 Å can prevent the cellulose derivative bonding in some cases (CSP-50B). Nevertheless, depending on the cellulose derivative, reticulation between alkenoyl double bonds can offset the difficulties of fixation on these pore size matrices, and it is possible to obtain CSPs with satisfactory chromatographic behaviour (CSP-50A).

To date, several variables have already been optimized in this type of CSPs, resistant to common HPLC conditions, but other studies are in course. These will allow us to know more about this new kind of CSP for HPLC.

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