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Journal of Chromatography A, 791 (1997) 37–44

JOURNAL OF CHROMATOGRAPHY A

Bonded cellulose-derived high-performance liquid chromatography chiral stationary phases III.[1] Effect of the reticulation of the cellulose derivative on performance[2]

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Received 12 May 1997; received in revised form 5 August 1997; accepted 5 August 1997

Abstract

The influence of the reticulation of a mixed 10-undecenoyl/3,5-dimethylphenylaminocarbonyl derivative of cellulose on the enantioselectivity of several high-performance liquid chromatography (HPLC) chiral stationary phases (CSPs) was investigated. Three cellulose-derived chiral columns were obtained by reticulation of the same cellulose derivative on three end-capped silica gels of different pore size. The performance of the obtained supports was studied and compared with that of three bonded cellulose-derived CSPs prepared by fixation of the same cellulose derivative on allylsilica gel. © 1997 Elsevier Science B.V.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Cellulose stationary phases; Lorazepam; Warfarin; Naproxen; β-Blockers

1. Introduction

Chiral stationary phases (CSPs) based on cellulose and amylose derivatives, together with the chemically bonded protein phases, are at present the CSPs for high-performance liquid chromatography (HPLC) that show the broadest application domain in the resolution of enantiomeric mixtures [1].

Polysaccharide-derived CSPs are usually prepared by coating the polysaccharide derivative onto macroporous γ-aminopropylsilica gel [2,3]. Some attempts to bond the polysaccharide derivative on the matrix have been described in the literature [4–6]. However, the use of a mixed 10-undecenoyl/3,5-dimethylphenylaminocarbonyl cellulose derivative allows the fixation of the chiral selector on the matrix [7–9] in a very easy way. As already stated by Okamoto and coworkers [4,5], the chiral recognition ability of polysaccharide-derived CSPs is greatly affected by immobilization. Those CSPs whose chiral selector is fixed on the matrix usually show a lower resolving

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[1]For Part I, see Ref. [11].

[2]Presented at the 8th Jornadas de Análisis Instrumental, 22–25 October 1996, Barcelona, Spain.

0021-9673/97/$17.00
PII S0021-9673(97)00836-4

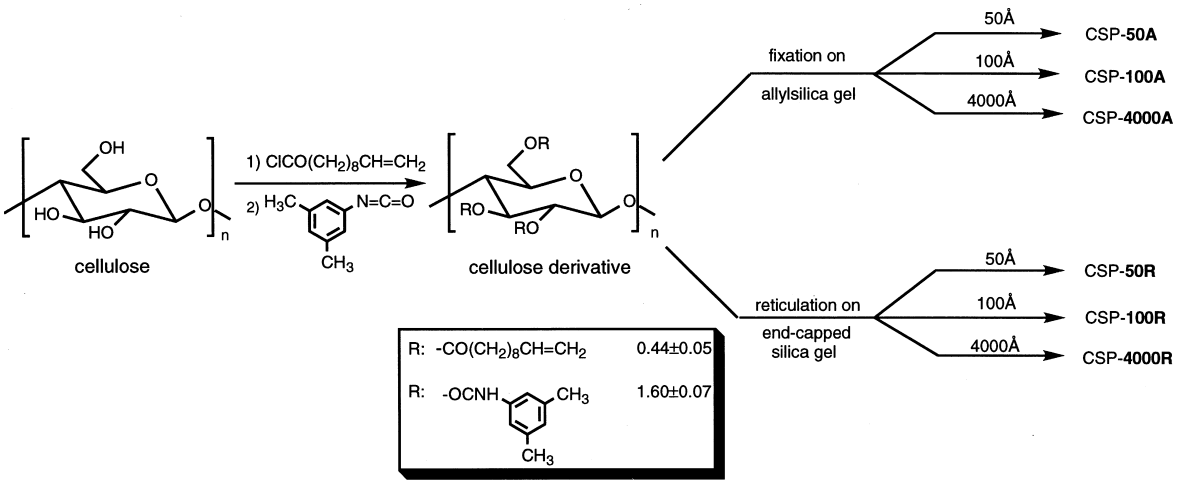

Fig. 1. Preparation of chiral stationary phases.

ability than the coated CSPs [4,5]. In the present case, the fixation takes place either by heterogenous 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 occur simultaneously, and may affect the chiral discrimination ability of the resulting CSPs. However, reticulation is the only possible fixation mechanism when double bonds are not available on the matrix surface [7,10]. In this case, the polymerization of the mixed cellulose derivative leads to a kind of net on the matrix surface, which results in the insolubilization of the polysaccharide derivative.

Some chromatographic results obtained in previous studies [7,10] suggested that the accessibility of the cellulose derivative to the racemic compounds is very important in the chiral discrimination. This accessibility is affected not only by the pore size of the matrix, but also by the fixation mechanism of the polysaccharide derivative. Moreover, the fixation process may also result in a modification of the secondary structure of the polysaccharide derivative. In order to establish the effect of the reticulation process on the discrimination ability of the resulting supports, three CSPs were prepared by reticulation of a mixed cellulose derivative on end-capped silica gels of different pore size (50, 100 and 4000 Å). The chromatographic results were compared with those of three other columns prepared from the same cellulose derivative, but fixed on allylsilica gel. The characteristics and performances of both series of CSPs are compared.

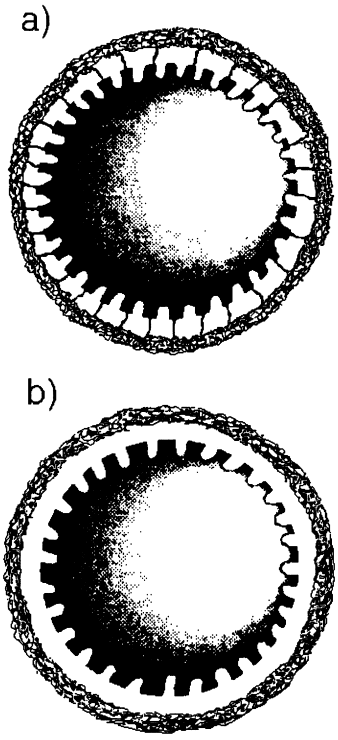

Fig. 2. Schematic representation of porous spherical particles of silica gel of the CSPs: (a) A series; (b) R series.

Table 1
Characterization of chiral stationary phases

CSP	Pore volume[a] (ml/g)	Surface (BET)[a] (m^2/g)	Intermediate silica gel[b]		Elemental analyses of final chiral supports			g Cellulose derivative per 100 g phase[c]	HETP[d] (cm)
			% C	% H	% C	% H	% N		
50A	0.8	450	3.09	1.11	12.66	1.97	0.89	18.68	$7.58\cdot10^{-3}$
100A	1.0	350	4.20	1.19	15.58	2.30	0.94	19.73	$7.11\cdot10^{-3}$
4000A	0.7	10	0.47	0.32	10.80	1.16	0.89	18.68	$7.76\cdot10^{-3}$
50R	0.8	450	2.42	1.05	11.56	1.73	0.73	15.32	$5.47\cdot10^{-3}$
100R	1.0	350	3.63	1.21	12.65	2.14	0.71	14.90	$6.70\cdot10^{-3}$
4000R	0.7	10	0.45	0.31	11.01	1.23	0.79	16.58	$5.40\cdot10^{-3}$

[a] Data given by the supplier (Macherey–Nagel).
[b] For the A series is allylsilica gel and for the R series end-capped silica gel.
[c] Based on elemental analyses (% N).
[d] Calculated using 1,3,5-tri-*tert*.-butylbenzene.

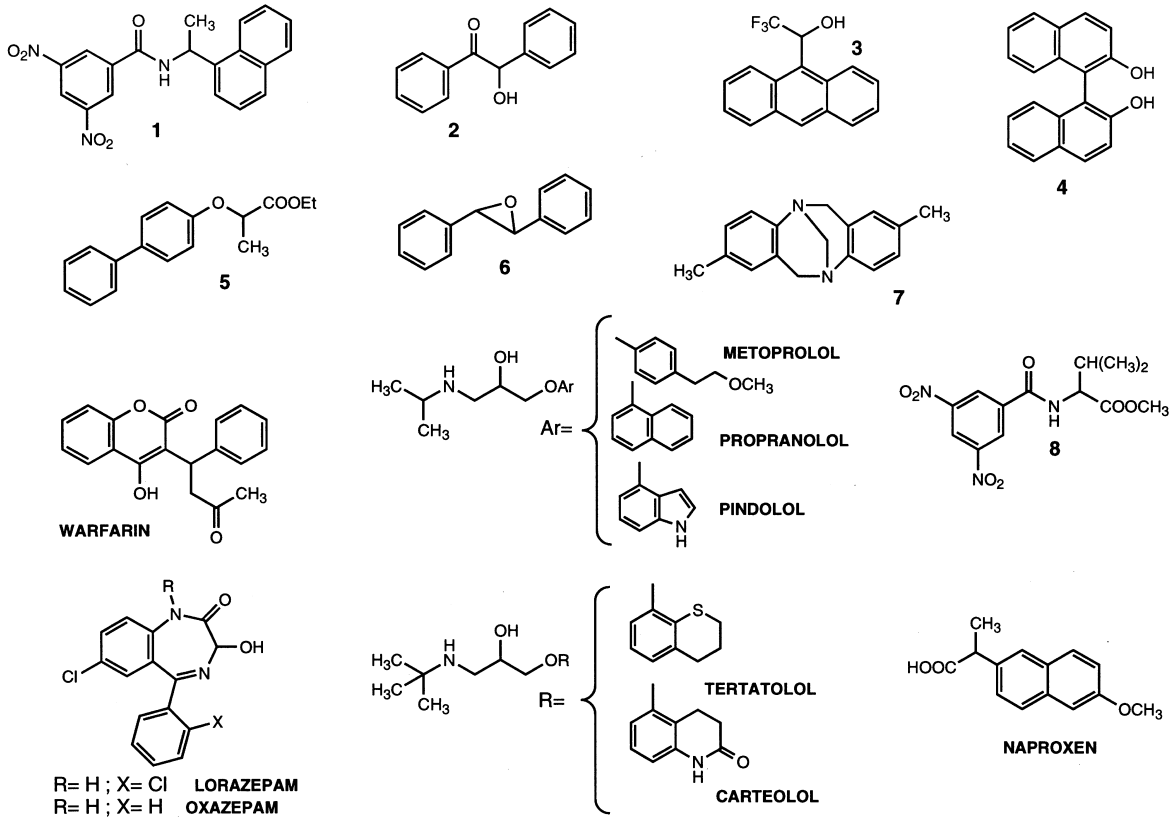

Fig. 3. Chemical structures of racemic test compounds.

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

Racemic compounds	CSP-50R			CSP-100R			CSP-4000R			CSP-50A			CSP-100A			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	k_1'	α	R_s	k_1'	α	R_s	
1	6.61	1.00	–	4.25	1.19	–	6.00	1.00	–	4.96	1.26	0.94	6.12	1.28	1.88	6.08	1.29	0.78	(a) 80:20
2	5.10	1.23	0.91	1.37[b]	1.17	–	4.03	1.21	0.71	3.41	1.19	1.14	2.03[b]	1.22	1.70	3.52[b]	1.23	1.27	(a) 98:2
3	1.73	1.90	1.76	0.58[c]	1.59	1.16	1.21	1.89	1.38	1.40	1.66	2.40	0.87[c]	1.73	2.97	1.54	1.80	2.59	(a) 90:10
4	3.62	1.21	–	2.22	1.00	–	2.73	1.28	–	2.99	1.19	0.72	2.96	1.16	1.21	3.21	1.21	0.61	(a) 90:10
5	1.05	1.19	0.49	0.44[b]	1.24	–	0.82	1.22	–	0.80	1.21	0.79	0.65[b]	1.25	1.20	0.79	1.24	0.88	(a) 98:2
6	0.91	1.23	–	0.46[b]	1.00	–	0.82	1.00	–	0.70	1.09	–	0.64[b]	1.27	1.30	0.83	1.08	–	(a) 98:2
7	2.48	1.07	–	1.31	1.00	–	0.60	1.39	–	1.69	1.11	–	1.48	1.10	–	0.66	1.40	1.08	(a) 90:10
Lorazepam	13.25	1.64	0.96	6.72	1.34	0.79	9.13	2.03	1.09	9.40	1.57	1.54	9.84	1.64	3.48	8.87	1.80	1.86	(a) 90:10
Oxazepam	5.32	1.00	–	2.70	1.17	–	3.78	1.00	–	9.60[b]	1.00	–	9.90[b]	1.27	1.76	9.34[b]	1.00	–	(a) 80:20
Warfarin	26.09	1.07	–	14.76	1.37	–	3.92	1.66	0.72	13.09	1.15	–	4.85	1.81	3.03	5.31	1.49	0.86	(a) 90:10
Metoprolol	13.67	1.00	–	3.38	1.11	–	1.26	1.00	–	6.30	1.00	–	5.56	1.08	–	1.24	1.35	0.78	(b) 90:10:0.1
Propranolol	10.14	1.00	–	3.34	1.12	–	1.43	1.30	–	5.16	1.11	0.62	5.63	1.16	–	1.79	1.53	1.61	(b) 90:10:0.1
Pindolol	10.21	1.62	1.76	4.45	2.14	1.77	2.42	3.13	1.56	5.31	2.00	2.91	5.51	2.84	5.34	2.84	3.98	4.52	(b) 80:20:0.1
Tertatolo	9.60	1.06	–	3.58	1.32	1.03	1.22	1.95	1.16	5.31	1.19	1.09	5.69	1.31	0.79	1.82	1.92	2.17	(b) 90:10:0.1
Carteolol	9.22	1.06	–	9.61	1.10	–	5.13	1.00	–	12.82	1.07	–	17.64	1.16	–	5.22	1.28	0.67	(b) 90:10:0.1
Naproxen	6.23	1.18	–	5.28	1.12	–	4.63	1.22	–	3.56	1.19	1.33	4.47	1.16	1.31	4.56	1.19	1.07	(c) 98:2:0.5

k_1', capacity factor for the first eluted enantiomer; α, selectivity factor; R_s, resolution. Column: 150×4.6 mm I.D.

UV detection, $\lambda_{230\ nm}$ (4, 6 and metoprolol), $\lambda_{254\ nm}$ (1, 2, 3, 5, 7, lorazepam, oxazepam, warfarin, pindolol, tertatolol, carteolol and naproxen) 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.

[b] Heptane–2-propanol (90:10).

[c] Heptane–2-propanol (80:20).

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 a HPLC system consisting of a Waters 600E pump, a Waters 717 autosampler (Millipore, Milford, MA, USA) equipped with a Waters 996 photodiode 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. Preparation of the cellulose derivative

The cellulose derivative was prepared by the method shown in Fig. 1 as previously described [7,11]. The degree of substitution of this cellulose derivative with both substituents was calculated from the elemental analysis (% C, 64.38; % H, 6.97; % N, 4.76). The calculated ratio of substituents on glucose units thus obtained agrees with the data from ^{1}H-NMR (pyridine-d_5, 300 MHz, 70°C).

2.2. Preparation of the chiral stationary phases

Various end-capped silica gels were prepared from spherical 5 μm silica gels of different pore size (Nucleosil 50-5, 100-5 and 4000-5, Macherey–Nagel, Düren, Germany) by reaction with hexamethyldisilazane in toluene. The cellulose derivative previously prepared was reticulated on these end-capped silica gels yielding the R series.

Various allylsilica gels were prepared as previously described [8] from the same spherical 5 μm silica gels (Nucleosil 50-5, 100-5 and 4000-5, Macherey–Nagel) by reaction with allyltriethoxysilane followed by treatment with hexamethyldisilazane. The same cellulose derivative was fixed on the allylsilica gel matrices [11], resulting in the three CSPs that

Table 3
Chromatographic results of CSPs of the R series obtained using heptane–chloroform as mobile phase

Racemic compounds	CSP-50R			CSP-100R			CSP-4000R			Mobile phase[a]
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
1	6.31	1.17	–	3.88	1.27	–	4.26	1.26	–	(a) 50:50
2	5.81	1.16	1.14	3.76	1.22	0.97	3.74	1.33	1.33	(a) 90:10
3	4.69	1.62	2.80	1.77	1.99	2.55	2.25	2.26	3.11	(a) 50:50
4	16.48	1.00	–	3.68	1.04	–	4.82	1.24	0.74	(a) 75:25
5	1.72	1.10	–	0.72	1.17	–	0.73	1.20	–	(a) 90:10
6	1.26	1.38	1.57	0.67	1.43	0.87	0.88	1.40	1.28	(a) 90:10
7	4.62	1.00	–	1.79	1.00	–	0.18	1.25	–	(a) 50:50
8	3.92	1.14	–	2.65	1.13	–	2.36	1.26	–	(a) 50:50
Lorazepam	8.00	1.00	–	3.74	1.06	–	3.36	1.40	0.68	(a) 25:75
Oxazepam	7.09	1.00	–	10.99	1.22	0.74	13.20	1.19	–	(a) 50:50
Warfarin	16.69	1.00	–	0.91	1.44	1.24	0.34	2.54	1.57	(a) 25:75
Metoprolol	6.82	1.00	–	2.51	1.00	–	0.51	1.39	0.70	(b) 50:50:0.5
Propranolol	13.65	1.00	–	3.09	1.12	–	1.22	1.44	0.98	(b) 50:50:0.5
Pindolol	32.34	1.26	1.76	4.83	2.18	2.33	2.20	3.34	2.78	(b) 25:75:0.5
Tertatolol	12.3	1.00	–	1.64	1.15	–	0.47	1.68	1.03	(b) 50:50:0.5
Naproxen	8.47	1.00	–	4.68	1.00	–	3.41	1.14	–	(c) 50:50:0.5

k'_1, capacity factor for the first eluted enantiomer; α, selectivity factor; R_s, resolution. Column: 150×4.6 mm I.D.
UV detection, $\lambda_{240\ nm}$ (6), $\lambda_{254\ nm}$ (1, 2, 3, 4, 5, 7, 8, lorazepam, oxazepam, warfarin, tertatolol and naproxen) and $\lambda_{270\ nm}$ (metoprolol, propranolol and pindolol).
[a] (a)=Heptane–chloroform, flow-rate: 1 ml/min; (b)=heptane–chloroform–DEA, flow-rate: 1 ml/min; (c)=heptane–chloroform–TFA, flow-rate: 0.5 ml/min.

constitute the A series. All resulting CSPs were characterized by elemental analysis (Table 1).

3. Results and discussion

The insolubilization of the mixed cellulose derivative is achieved by means of its alkenoyl chains. On the one hand, in the R series only the reticulation of the cellulose derivative is possible, due to the absence of reactive groups on the surface of the end-capped silica gel (Fig. 2a). On the other hand, in the A series the fixation takes place both by coupling of the double bonds on allylsilica gel and by reticulation of the 10-undecenoyl groups themselves (Fig. 2b). However, as the cellulose derivative is the same in both series, the relative extent of this latter process is lower than in the R series. The chromatographic results obtained for some of the racemic compounds tested (Fig. 3) are shown in Tables 2 and 3.

3.1. Influence of the reticulation process of the cellulose derivative on performance

When CSPs of the same pore size are compared (Table 2), α values are normally higher in CSPs of the A series. This result seems to be connected with the higher degree of crosslinking of the chiral selector in the R series. It is likely that the reticulation process had a stronger effect on the secondary structure of the chiral selector than the heterogeneous fixation on allylsilica gel. This could explain the differences in selectivity observed when a mixed cellulose derivative was fixed on several chromatographic matrices with different characteristics [7]. Even in those cases where the selectivity factors were higher in the R series than in the A series (i.e., for 2, 3 and lorazepam in CSP-50R/A), resolution values were always better in the series where the chiral selector was fixed on allylsilica gel (Fig. 4).

3.2. Influence of the pore size of the matrix on performance of CSPs in the R series

A previous study [10] has shown that, in general, selectivity factors increase with pore size. This is also the case in the R series, where the chiral selector

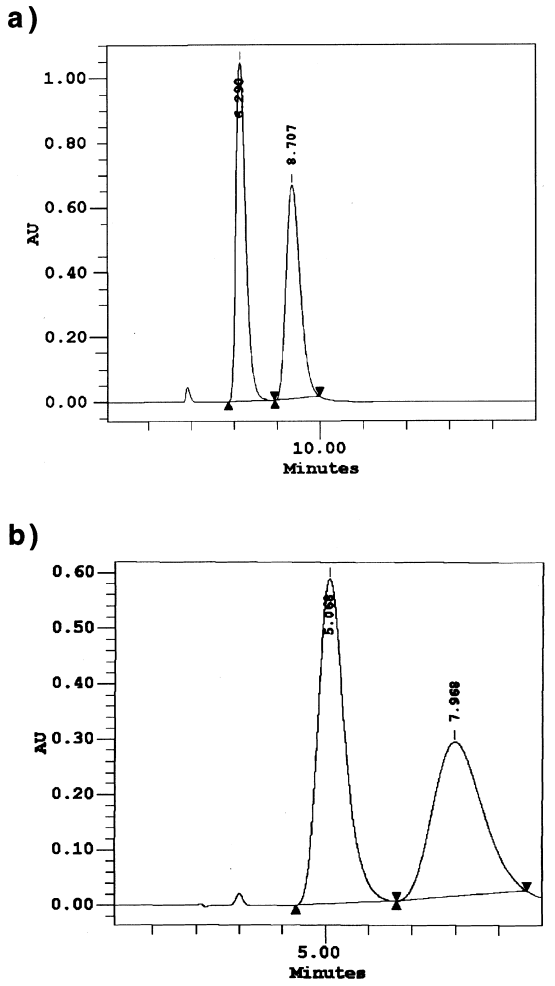

Fig. 4. Resolution of compound 3 in heptane–2-propanol (90:10) and flow-rate: 1 ml/min: (a) column CSP-50A, α: 1.66 and R_s: 2.40; (b) column CSP-50R, α: 1.90 and R_s: 1.76.

was reticulated on the matrix. In most cases, CSP-4000R showed the highest selectivity factors in the R series, as illustrated on Fig. 5 and the resolution factor (R_s) was also higher in this CSP. This fact was more evident when chloroform was used as mobile phase modifier (Fig. 5b).

Capacity factors were always higher for CSP-50R than for the other members of the same series, though longer retention times did not lead to any noticeable chiral discrimination. This phenomenon may be attributable to the distribution of the cellulose derivative on the matrix. In a silica gel particle of 50 Å pore size, the cellulose derivative is pre-

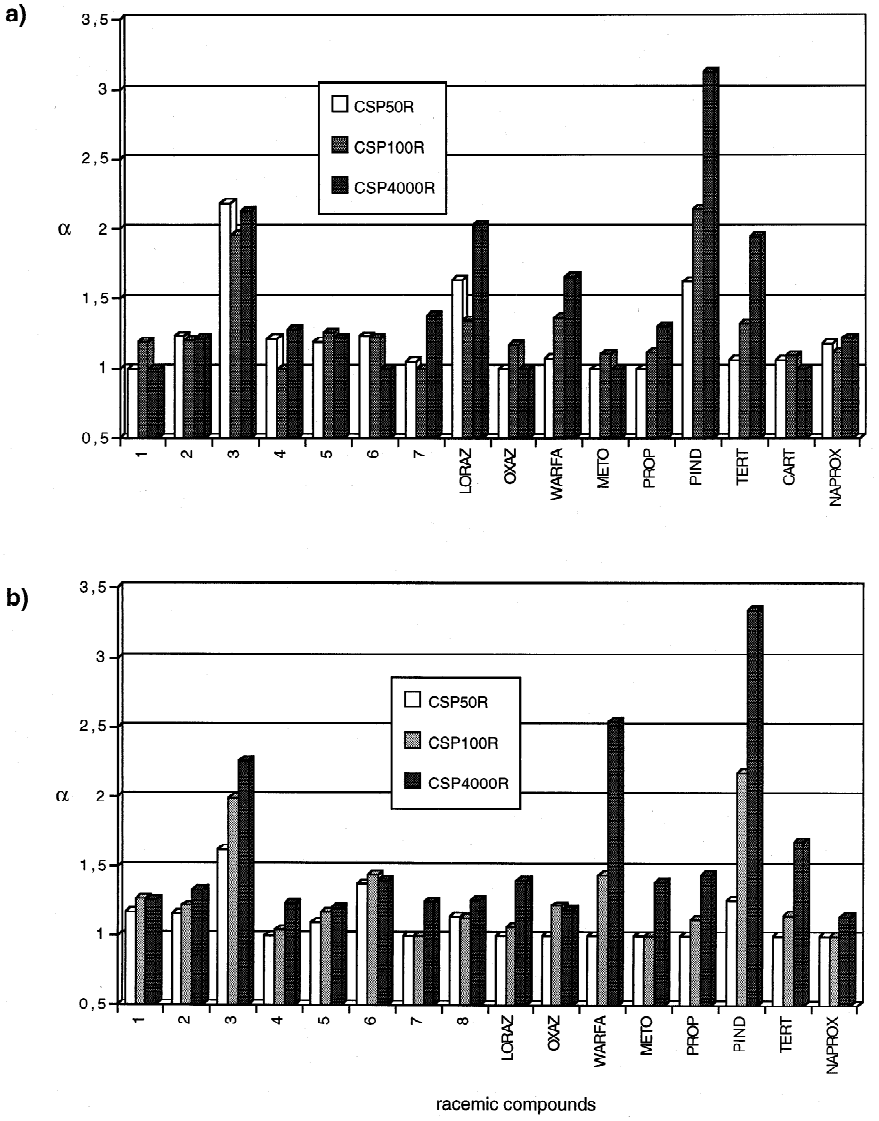

Fig. 5. Comparison of selectivity factors in the R series using as mobile phase: (a) heptane–2-propanol; (b) heptane–chloroform. Comparisons for the different CSPs are made under the same chromatographic conditions for every racemic compound.

vented from entering the pores, due to their small size, even more reduced by the end-capping treatment. It is already known that the end-capping process, like other surface modifications, leads to a decrease in the mean pore size [12,13]. Thus, the polysaccharide derivative must be spatially arranged by reticulation in a thick layer on the surface of the matrix particle (Fig. 6). The number of accessible chiral recognition sites in CSP-50R and CSP-100R

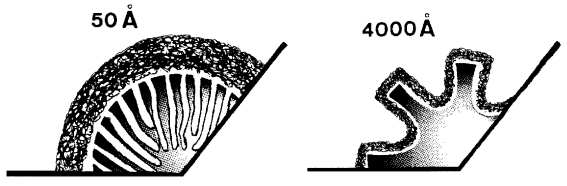

Fig. 6. Schematic representation of the different distribution of the cellulose derivative on the surface of a particle of silica gel depending on the pore size of the matrix.

should be lower than in CSP-4000R. This could result in a relatively slow mass transfer and hence in high retention times. Although the amount of cellulose derivative per gram of support is of the same order of magnitude for all three CSPs of the R series, CSP-4000R can accommodate a higher amount of accessible cellulose derivative on its surface [14]. This could be the reason for the reduction in the chiral recognition ability shown by CSP-50R for most of the racemic compounds tested. In a 4000 Å pore size particle, the cellulose derivative is spread all over the surface of the silica, including pores. Therefore, the accessibility of the cellulose derivative to the analyte is higher than in CSPs with small pores and the chiral recognition might be better than in the latter.

4. Conclusions

Usually, when reticulation of the cellulose derivative is the only possible mechanism for its fixation on the matrix surface, the chiral recognition ability is lower than in those CSPs where the fixation is partially achieved by chemical bonding of the cellulose derivative on the matrix. The spatial arrangement of the cellulose derivative and its accessibility, also influenced by the pore size of the silica gel, are largely responsible for the decrease of chiral recognition.

Acknowledgements

Financial support from Comisión Interministerial de Ciencia y Tecnologia and from the Generalitat de Catalunya (Project No. QFN94-4605) of Spain is acknowledged. The authors thank the NATO International Scientific Exchange Programme for a Collaborative Research Grant (No. CGR 950983). P.F. gratefully thanks the Comissió Interdepartamental de Recerca i Innovació Tecnològica (Generalitat de Catalunya) for a doctoral fellowship.

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