ELSEVIER

Journal of Chromatography A, 728 (1996) 407–414

JOURNAL OF CHROMATOGRAPHY A

Bonded cellulose-derived high-performance liquid chromatography chiral stationary phases I. Influence of the degree of fixation on selectivity

Cristina Minguillón[a,*], Pilar Franco[a], Laureano Oliveros[b], Pilar López[b,1]

[a]*Laboratori de Química Farmacèutica, Facultat de Farmàcia, Universitat de Barcelona, Avda. Diagonal s/n, E-08028 Barcelona, Spain*

[b]*Laboratoire de Chimie Générale, Conservatoire National des Arts et Métiers, 292 Rue Saint-Martin, F-75141 Paris Cedex 03, France*

Abstract

Four mixed 10-undecenoyl–3,5-dimethylphenylaminocarbonyl derivatives of cellulose, with an increasing proportion of alkenoyl groups, were synthesized and chemically bonded on allylsilica gel. The influence of the degree of fixation of the cellulose derivative on the matrix for the four resulting chiral stationary phases on their selectivity is discussed.

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

1. Introduction

The chiral recognition ability of cellulose and amylose derivatives has been attracting scientific and industrial attention since 1967, when the use of cellulose acetate as a stationary phase for chromatography was first reported [1]. Nevertheless, their potential for chiral discrimination has been used extensively only in the last two decades. Okamoto and co-workers [2–4] have described a large number of chiral stationary phases (CSPs) for HPLC based on phenylcarbamates of cellulose and other polysaccharides coated on macroporous γ-aminopropylsilica gel. In spite of the ability in the resolution of a very large range of racemic compounds shown by these CSPs, their limitation is due to the solubility of many polysaccharide derivatives in a number of solvents.

Although chemical bonding of a polysaccharide derivative to a silica gel matrix is possible, only few attempts can be found in the literature [5,6], none of which, however, has led to a commercially available CSP. Recently, we have described an easy way to bond polysaccharide derivatives on chromatographic support materials, by polymerization of a mixed 10-undecenoyl–3,5-dimethylphenylaminocarbonyl derivative of cellulose on the matrix (silica gel or

* Corresponding author.

[1] Present address: Instituto de Ciencia de Materiales de Aragón, Facultad de Ciencias, Universidad de Zaragoza-CSIC, E-50009-Zaragoza, Spain.

0021-9673/96/$15.00
SSDI 0021-9673(95)01123-4

otherwise) [7,8]. The CSPs obtained can be utilized with any of the solvents normally used in liquid chromatography, in either normal- or reversed-phase conditions. Moreover, the resolution on a preparative scale of racemic compounds, slightly soluble in mobile phases used with classical cellulose-derived CSPs, is possible by the use of more polar solvents, which are nevertheless incompatible with the commercially available polysaccharide-derived CSPs [9].

It is known that the helical secondary structure of the polysaccharide derivative used as a chiral selector is very important for the enantioselectivity [10,11]. The fixation process used to obtain the new CSPs, and also the nature of its matrix or the solvent used as the mobile phase in the separation, among other factors, can affect this structure and therefore the chiral recognition ability of the resulting CSPs. In this work, the influence of the molar ratio of substituents (10-undecenoyl and 3,5-dimethylphenylaminocarbonyl) on the mixed cellulose derivative used as a chiral selector was studied to obtain the best chiral recognition abilities for the resulting supports. Four cellulose derivatives were synthesized and subsequently covalently bonded to allylsilica gel. The characteristics and performances of the resulting CSPs are discussed.

2. Experimental

^{1}H NMR spectra were measured using a Varian Gemini-300 spectrometer. Elemental analyses were performed in a CE Instruments Model EA 1108 apparatus using standard conditions 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 Model 600E pump, a Waters Model 717 autosampler (Millipore, Milford, MA, USA) and equipped with a Waters Model 996 photodiode-array detector and a Perkin-Elmer (Überlingen, Germany) Model 241LC polarimetric detector. The volume of sample injected was 3 μl. The void volume was determined using tri-*tert.*-butylbenzene.

2.1. Preparation of cellulose derivatives

The chiral selectors and CSPs were prepared as indicated in Fig. 1 [7,8]. Cellulose derivatives were synthesized by the successive reaction of cellulose [Avicel (Merck) suspended in pyridine at about 100°C] with 10-undecenoyl chloride and 3,5-dimethylphenyl isocyanate. Molar ratios of reagents and reaction times are given in Table 1. The resulting products were isolated as the insoluble fraction in methanol. They were redissolved in chloroform, reprecipitated in methanol and thoroughly washed with methanol and hot ethanol, in order to remove the strongly insoluble N,N′-bis(3,5-dimethylphenyl)urea, obtained as a by-product. All derivatives were characterized by their ^{1}H NMR spectra and elemental analysis.

Cellulose derivative **A** (^{1}H NMR, pyridine-d_5, 300 MHz, 70°C), δ: 1.0–1.8 (m, $C^{3'}H_2$–$C^{8'}H_2$); 2.0–2.3 (m, $C^{2'}H_2$ and $C^{9'}H_2$); 2.05 and 2.11 (s + s, 2-ArCH_3 and 3-ArCH_3); 2.32 (s, 6-

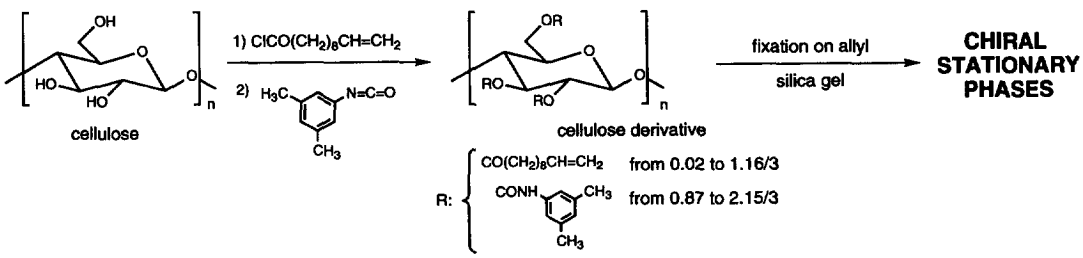

Fig. 1. Preparation of chiral stationary phases.

Table 1
Characterization of cellulose derivatives

10-Undecenoyl chloride		3,5-Dimethylphenyl isocyanate		Cellulose derivative	Analyses of cellulose derivatives			Substitution of glucose units[c]		
								Undec.	Carbam.	Total[d]
M[a]	t[b] (h)	M[a]	t[b] (h)		C (%)	H (%)	N (%)			
0.4	1	3.6	15	**A**	63.7	6.37	6.26	0.02	2.15	2.17
0.4	2	3.6	15	**B**	65.8	6.79	5.97	0.31	2.44	2.75
1.2	2	2.4	15	**C**	64.6	7.05	4.57	0.50	1.54	2.04
2.2	15	1.2	8	**D**	66.1	8.19	2.52	1.16	0.87	2.03

[a] M = mol of reagent/mol glucose units.
[b] Time of reaction in pyridine solution at reflux temperature.
[c] Calculated from the elemental analysis.
[d] The maximum degree of substitution of a glucose unit being 3 (number of OH).

$ArCH_3$); 3.45 (m, C^5H); 3.75 (m, C^4H); 4.65 (m, C^1H and C^6H_2); 5.25 (m, $C^{11'}H_2$=, C^2H); 5.52 (m, C^3H); 5.95 (m, $C^{10'}H$=); 6.40 (s, 6-ArHb); 6.61 and 6.71 (s + s, 2-ArHb and 3-ArHb); 7.30 (s, 6-ArHa); 7.34 (s, 2-ArHa and 3-ArHa); 9.24 and 9.33 (ba, 2-NH and 3-NH); 10.27 (ba, 6-NH).

The ^1H NMR spectra (Fig. 2) show the increase in alkenoyl residues from cellulose derivative **A** to **D** and the reduction of carbamate moieties. In the latter, the signals corresponding to the aromatic substitution on the 6-position of the glucose units disappear.

After the verification of the absence of N,N'-bis(3,5-dimethylphenyl)urea (non-appearance of the singlet absorption at δ 2.26, assigned to the methyl groups in that compound, in the ^1H NMR spectrum), the cellulose derivatives were characterized by elemental analysis. In Table 1 the calculated compositions of the cellulose derivatives obtained are shown.

2.2. Preparation of chiral stationary phases

The cellulose derivatives were fixed on allylsilica gel (Nucleosil 100-5; Macherey–Nagel) in a radical polymerization reaction by means of the C–C double bonds on the 10-undecenoyl groups [7]. The cellulose derivative was first coated on allylsilica gel (20%, w/w). After the evaporation of the solvent, the solid material was allowed to react for 2 h at 100°C in the presence of a 2% (w/w) of AIBN (α,α'-azobisisobutyronitrile). The CSPs thus obtained were suspended in chloroform and heated at reflux for 2 h, then the suspension was filtered off and the solid washed with chloroform and acetone. The resulting CSPs were characterized by elemental analysis (Table 2).

3. Results and discussion

The fixation process takes place either by reaction of 10-undecenoyl groups on the cellulose with allyl groups on the silica gel (heterogeneous coupling of double bonds) or by reticulation of 10-undecenoyl groups themselves. Both processes can affect the helical secondary structure of the chiral selector. In this study, the amount of allyl groups on the silica is the same for all CSPs and therefore, if there are enough 10-undecenoyl groups on the cellulose derivative, the degree of heterogeneous coupling will also be the same. On the other hand, a stronger effect on the secondary structure of the cellulose derivative could be expected if the amount of 10-undecenoyl groups was too high, owing to an increase in the reticulation process. Moreover, an increase in the number of 10-undecenoyl groups on the cellulose results in a decrease in the number of arylcarbamate units, which will

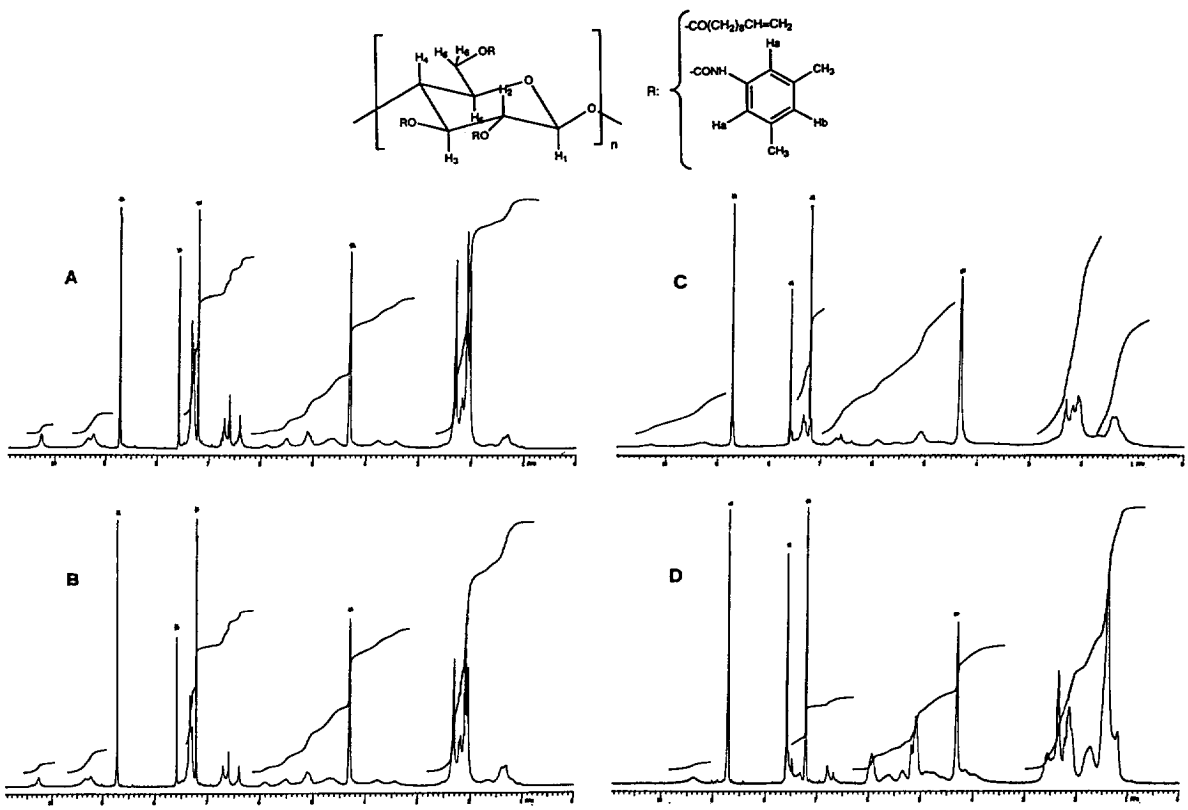

Fig. 2. ^{1}H NMR spectra (300 MHz) of cellulose derivatives in pyridine-d_5 at 70°C. Asterisks indicate signals corresponding to the solvent.

modify the chiral recognition ability of the resulting cellulose derivative. The total fixation process results in insolubilization of the cellulose derivatives after the reaction.

The percentages of cellulose derivative fixed on the matrix, based on the elemental analyses of the resulting CSPs, are given in Table 2. All the cellulose derivatives were satisfactorily bonded to allylsilica gel. Only CSPA shows a lower degree of fixation of cellulose derivative, which

Table 2
Characterization of CSPs

CSP	Cellulose derivative	HETP (cm)[a]	Analyses			g of cellulose derivative per 100 g phase[b]
			C (%)	H (%)	N (%)	
CSPA	**A**	$9.37 \cdot 10^{-3}$	10.10	1.98	0.79	12.63
CSPB	**B**	$8.90 \cdot 10^{-3}$	13.40	2.10	1.00	16.75
CSPC	**C**	$8.33 \cdot 10^{-3}$	13.16	1.96	0.82	17.93
CSPD	**D**	$8.16 \cdot 10^{-3}$	12.19	2.00	0.43	17.03

[a] Calculated using 1,3,5-tri-*tert.*-butylbenzene.
[b] Based on elemental analyses.

Table 3
Chromatographic results obtained using heptane–2-propanol as mobile phase

Racemic compound	CSPA			CSPB			CSPC			CSPD			Mobile phase[a]
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
1	3.28	1.00	–	7.02	1.17	–	5.40	1.00	–	2.52	1.00	–	(a) 90:10
2	3.38	1.28	–	7.79	1.22	–	7.51	1.00	–	4.25	1.00	–	(a) 98:2
3	2.18	1.37	–	4.74	1.33	1.55	3.96	1.16	–	2.30	1.00	–	(a) 98:2
4	2.22	1.00	–	3.20	1.21	–	3.29	1.10	–	3.10	1.00	–	(a) 98:2
5	0.75	2.22	1.55	1.34	2.20	2.71	1.03	1.70	1.35	0.85	1.23	0.96	(a) 90:10
6	5.77	1.00	–	14.2	1.18	–	9.13	1.30	–	4.99	1.19	1.06	(a) 98:2
7	0.53	1.00	–	1.01	1.23	–	0.83	1.19	–	0.56	1.27	1.10	(a) 98:2
8	0.42	1.69	–	0.86	1.49	1.48	0.64	1.00	–	0.38	1.00	–	(a) 98:2
Lorazepam	4.06	1.43	–	8.74	1.56	1.35	8.67	1.85	1.29	6.58	1.32	1.31	(a) 90:10
Warfarin	1.71	2.17	1.53	3.33	2.17	1.99	2.79	1.73	1.25	2.11	1.63	2.85	(a) 90:10
Metoprolol	2.23	1.47	1.17	3.22	1.20	–	5.35	1.00	–	5.62	1.00	–	(b) 90:10:0.1
Propranolol	2.29	1.51	1.10	3.01	1.42	1.33	4.15	1.00	–	3.99	1.00	–	(b) 90:10:0.1
Pindolol	2.45	4.61	2.63	3.79	4.11	4.11	3.98	1.56	1.32	4.28	1.00	–	(b) 80:20:0.1
Naproxen	3.37	1.13	–	5.64	1.22	1.18	4.32	1.20	0.76	2.82	1.09	–	(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 at 230 nm (**1**, **2**, **4**, **6**, lorazepam, warfarin, metoprolol and naproxen), 254 nm (**3**, **5**, **7**, **8** and pindolol) and 280 nm (propranolol).

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

corresponds to a lower amount of reacting groups on **A**.

In Tables 3 and 4, the chromatographic results obtained with these CSPs are presented for some of the racemic compounds tested (Fig. 3). In Fig. 4 several chromatograms are shown. The chiral

Table 4
Chromatographic results obtained using heptane–chloroform as mobile phase

Racemic compound	CSPA			CSPB			CSPC			CSPD			Heptane–chloroform
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
2	3.21	1.20	–	5.59	1.25	1.19	5.24	1.05	–	3.94	1.00	–	90:10
3	1.12	1.15	–	2.20	1.15	0.85	1.71	1.05	–	1.51	1.00	–	80:20
5	1.30	2.42	1.95	2.39	2.29	3.75	1.92	1.86	2.53	1.48	1.36	1.62	50:50
6	1.89	1.40	–	3.97	1.31	1.39	3.54	1.10	–	3.00	1.00	–	70:30
7	1.09	1.00	–	1.73	1.17	–	1.24	1.19	–	0.86	1.20	0.92	95:5
8	0.78	2.37	1.77	1.41	2.03	3.88	0.96	1.47	1.48	0.50	1.00	–	95:5
Lorazepam	9.30	1.00	–	13.8	1.18	–	18.9	1.13	–	17.6	1.04	–	50:50
Oxazepam	8.25	1.38	1.08	14.1	1.24	1.05	18.9	1.00	–	16.6	1.00	–	50:50
Warfarin	0.71	2.40	1.59	1.32	2.15	1.74	1.84	1.70	1.11	1.82	1.66	1.16	50:50

k'_1 = Capacity factor for the first-eluted enantiomer; α = selectivity factor; R_s = resolution. Column, 15 × 0.46 cm I.D.; flow-rate, 1 ml/min. UV detection at 240 nm (**2**, **8**, lorazepam and oxazepam), 254 nm (**3**, **5** and **7**) and 280 nm (**6** and warfarin).

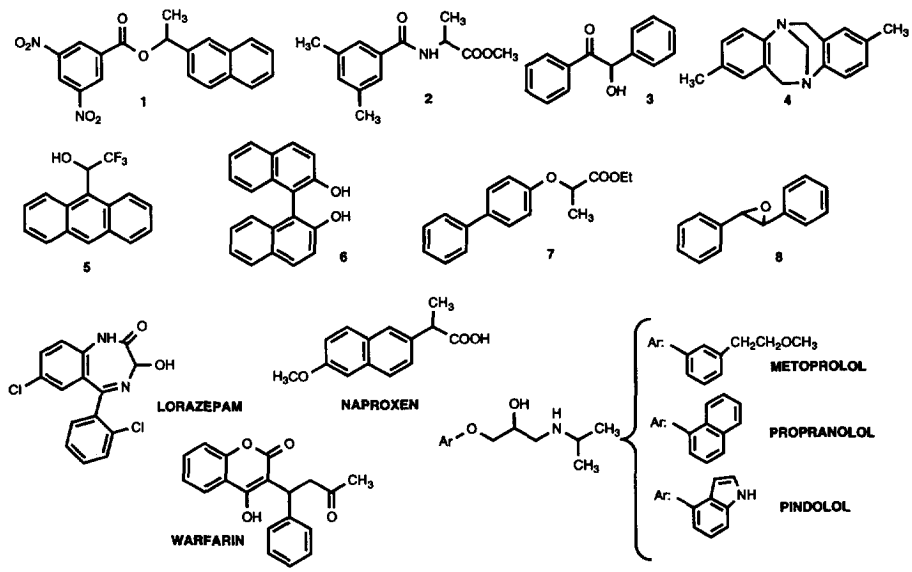

Fig. 3. Structures of racemic test compounds.

recognition ability of CSPC and CSPD for most of the racemic compounds tested is lower than that of CSPA and CSPB, using either heptane–2-propanol or heptane–chloroform as mobile phase. CSPA shows higher α values than CSPB in the resolution of the racemic compounds that it is able to resolve. However, no noticeable chiral discrimination was observed with certain analytes that are resolved on other CSPs in the study. This decrease in the enantioselectivity and the short retention time shown by CSPA for most of the analytes can be attributed to the smaller amount of cellulose derivative fixed on this support.

The chromatographic data show that CSPB presents overall the best chiral recognition ability, using either heptane–2-propanol or heptane–chloroform as mobile phase. This CSP is able to resolve the widest range of the racemic compounds tested, although not always with the highest selectivity factor. The more satisfactory chiral recognition behaviour of CSPB can be attributed, to a first approximation, to the higher number of carbamate residues or to the total degree of substitution of glucose units. Nevertheless, in the graphical comparisons between selectivity factors for several racemic compounds and the quantity of 10-undecenoyl groups (Fig. 5a), phenylcarbamate groups (Fig. 5b) and the total degree of substitution of glucose units (Fig. 5c), only an inverse relationship between α and the quantity of alkenoyl groups, on the cellulose derivative used as chiral selector, can be established. CSPB, with less free hydroxyl groups, shows selectivity values similar to those of CSPA (Fig. 5a). On the other hand, the selectivity values of CSPB, with more phenylcarbamate groups than CSPA, are similar to or even lower than those shown by the latter. The important decrease in the resolving ability of CSPC and CSPD, relating to CSPA and CSPB, could be attributed to the higher reticulation degree existing in the former. This is related to the results obtained in a previous study [8]. There, for the same cellulose derivative, when the fixation process takes place exclusively by means of a reticulation, owing to the lack of reactivity of the matrix, the resulting CSPs show a lower chiral recognition ability than the CSP in which allylsilica gel is the matrix. In this CSP, part of

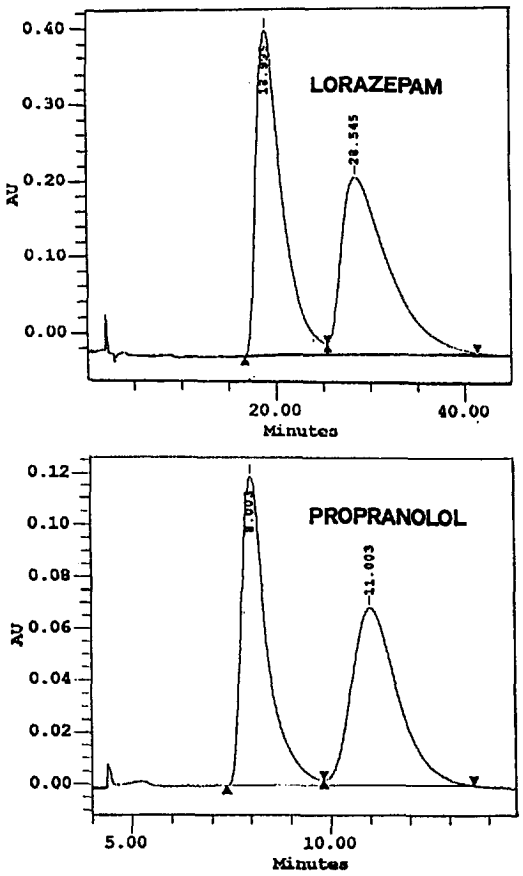

Fig. 4. Optical resolution of lorazepam [heptane–2-PrOH (90:10), $\alpha = 1.56$, $R_s = 1.35$, $\lambda = 230$ nm] and propranolol [heptane–chloroform–DEA (50:50:0.1), $\alpha = 1.49$, $R_s = 1.86$, $\lambda = 280$ nm] on CSPB; flow-rate, 1 ml/min.

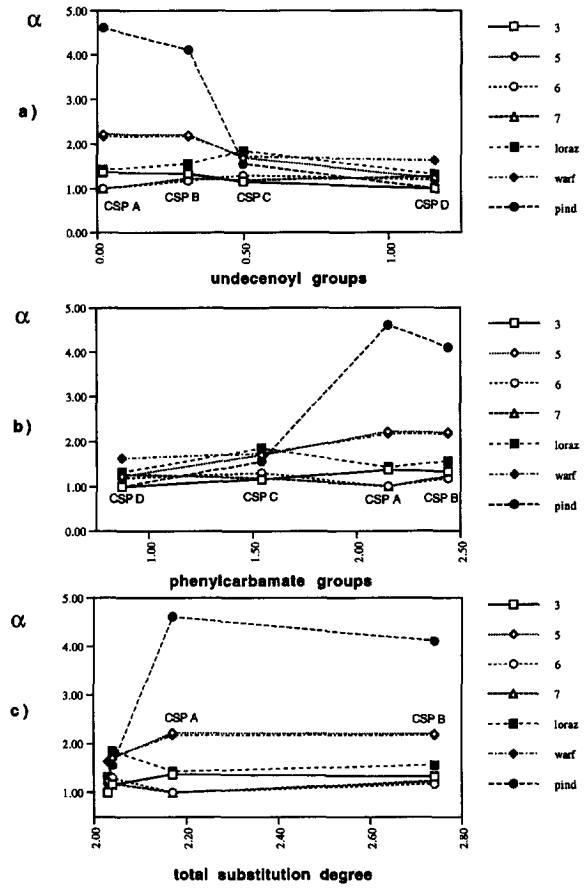

Fig. 5. Relationship between α (selectivity factor) and (a) the amount of 10-undecenoyl groups per glucose unit, (b) the amount of phenylcarbamate groups per glucose unit and (c) the total degree of substitution of glucose units in cellulose derivatives. Mobile phase: heptane–2-propanol.

the 10-undecenoyl groups are involved in a heterogeneous reaction with the allyl groups on the matrix and the reticulation degree is lower.

Acknowledgements

Financial support from the Comisión Interministerial de Ciencia y Tecnologia and from the Generalitat de Catalunya (Project No. QFN94-4605) of Spain is acknowledged. Pilar Franco gratefully thanks the Comissió Interdepartamental de Recerca i Innovació Tecnològica (Generalitat de Catalunya) for a doctoral fellowship. Pilar López gratefully thanks the Consejo Superior de Investigaciones Científicas for a Post-doctoral fellowship.

References

[1] A. Lüttringhaus, V. Hess and H.J. Rosenbaum, Z. Naturforsch., Teil B, 22 (1967) 1296.
[2] Y. Okamoto, M. Kawashima and K. Hatada, J. Am. Chem. Soc., 106 (1984) 5357.
[3] Y. Okamoto, M. Kawashima and K. Hatada, J. Chromatogr., 363 (1986) 173.

[4] Y. Okamoto and Y. Kaida, J. Chromatogr. A, 666 (1994) 403.
[5] Y. Okamoto, R. Aburatani, S. Miura and K. Hatada, J. Liq. Chromatogr., 10 (1987) 1913.
[6] E. Yashima, H. Fukaya and Y. Okamoto, J. Chromatogr. A, 677 (1994) 11.
[7] L. Oliveros, C. Minguillón and P. López, Fr. Pat., 2 714 671.
[8] L. Oliveros, P. López, C. Minguillón and P. Franco, J. Liq. Chromatogr., 18 (1995) 1521.
[9] L. Oliveros, C. Minguillón, B. Serkiz, F. Meunier, J.P. Volland and A. Cordi, J. Chromatogr. A, 729 (1996) 29.
[10] G. Hesse and R. Hagel, Chromatographia, 6 (1973) 277.
[11] A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, Chromatographia, 19 (1984) 280.