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Solvent versatility of bonded cellulose-derived chiral stationary phases for high-performance liquid chromatography and its consequences in column loadability

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Abstract

The chromatographic behaviour of a 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose bonded on allylsilica gel is tested using four organic mobile-phase modifiers (2-propanol, chloroform, tetrahydrofuran and ethyl acetate). The advantages of the broad choice of solvents offered by this kind of chiral stationary phases and their resistance are discussed. The loadability of the column and its dependence both on the racemate to be resolved and on the solvent used as mobile phase are also discussed. © 1998 Elsevier Science B.V.

Keywords: Chiral stationary phases, LC; Mobile phase composition; Benzodiazepines; Warfarin; β-Blockers; Naproxen

1. Introduction

Cellulose and amylose-derived chiral stationary phases (CSPs) have been widely used for analytical and preparative chromatographic separation of a broad variety of racemic compounds [1]. At present, many of these CSPs are commercially available, either as coated materials onto silica gel for chromatography [2,3], or as pure polymeric beads, especially for preparative purposes [4,5]. Nevertheless, the productive scale-up of chiral separations with polysaccharide-derived CSPs is usually limited by the reduced choice of compatible solvents. Solvents, such as chloroform or tetrahydrofuran, in which the polysaccharide derivatives used as chiral selectands are dissolved or swollen, cannot be used at high percentages in the mobile phase. Therefore, the choice of eluents is limited to alcohols, alcohols/water, some ethers and hydrocarbons. However, a large number of drugs and their chemical precursors are slightly soluble in these systems. This is not a limitation in analytical chromatography since the concentration of the sample is very low. However, a mobile phase in which the sample had a good solubility, and which does not affect the stability of either the CSP or the analyte, should be preferable in preparative applications. Moreover, the loading capacity of the CSP should be as high as possible.

The development of 10-undecenoate/arylcarbamates or benzoates of polysaccharides fixed on different chromatographic matrices by a direct radicalary reaction [6–9] has led to CSPs with

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remarkable resolving ability that are more resistant than coated CSPs. In previous studies [6–11], they were tested using heptane–2-propanol or heptane–chloroform mixtures and in reversed-phase conditions [6]. However, the broad choice of solvents offered by chemically bonded polysaccharide-derived CSPs should be investigated due to their potential applicability.

In this study, the discrimination ability of a 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose bonded on allylsilica gel is studied using 2-propanol, chloroform, tetrahydrofuran and ethyl acetate as mobile-phase modifiers. Under these conditions, the column loadability for several racemic compounds is also investigated. Moreover, some experiments are undertaken to test the resistance of the unpacked material to solvents such as pyridine and toluene.

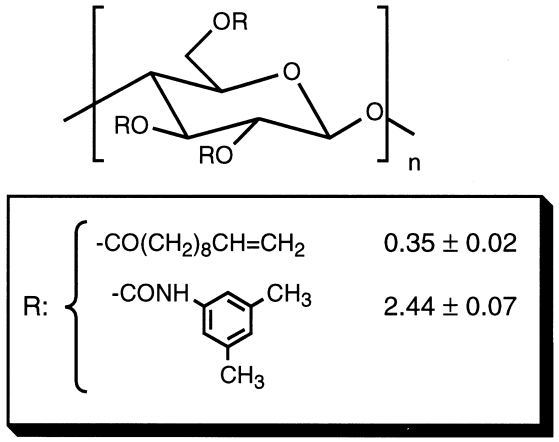

Fig. 1. Mixed cellulose derivative.

2. Experimental

Elemental analyses were performed in a CE Instruments apparatus (Model EA 1108, Carlo Erba, Milan, Italy) using standard conditions by the Serveis Científico-Tècnics de la Universitat de Barcelona (Spain). The CSP was packed into stainless steel tubes (150×4.6 mm I.D.) by the slurry method. The height equivalent to a theoretical plate (HETP) of CSPA was 8.90×10^{-3} cm for 1,3,5-tri-*tert*.-butylbenzene using heptane 100% (flow-rate, 1 ml/min). The chromatographic experiments were performed on an HPLC system consisting of a Waters 600E pump, a Waters 717 autosampler (Millipore, Milford, MA, USA) and 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, except when indicated. The void volume was determined using tri-*tert*.-butylbenzene.

2.1. Preparation of the cellulose derivative and the chiral stationary phases

The chiral selector was prepared as previously described [7]. The ratios of the two substituents for each glucose unit were calculated from elemental analysis (Fig. 1, derivative B in Ref. [7]: %C, 65.80; %H, 6.79; %N, 5.97).

The mixed cellulose derivative was fixed on allylsilica gel, previously prepared from spherical silica gel (Nucleosil 100-5, Macherey-Nagel, Düren, Germany) by successive reaction with allyltriethoxysilane and hexamethyldisilazane [8]. The resulting material was exhaustively washed in chloroform and acetone. The elemental composition of the resulting CSP was: %C, 13.40; %H, 2.10; %N, 1.00. A portion of this material was packed into a stainless steel chromatographic column (CSPA). A second portion of the obtained material was additionally washed in pyridine and acetone leading to CSPB. Finally, a third aliquot was treated with pyridine, toluene and acetone to yield CSPC.

3. Results and discussion

3.1. Chromatographic resolution on the bonded cellulose-derived CSPA

Polysaccharide-derived CSPs are considered as chiral supports with a high loadability. This characteristic, together with their wide application domain, makes them especially suitable for preparative scale enantioseparations. However, the solubility of the chiral selector in coated CSPs limits the choice of solvents. Very recently, tetrahydrofuran, chloroform, ethyl acetate and *N*,*N*-dimethylacetamide at low percentages have been used in chemically bonded cellulose and amylose-derived CSPs [12–14].

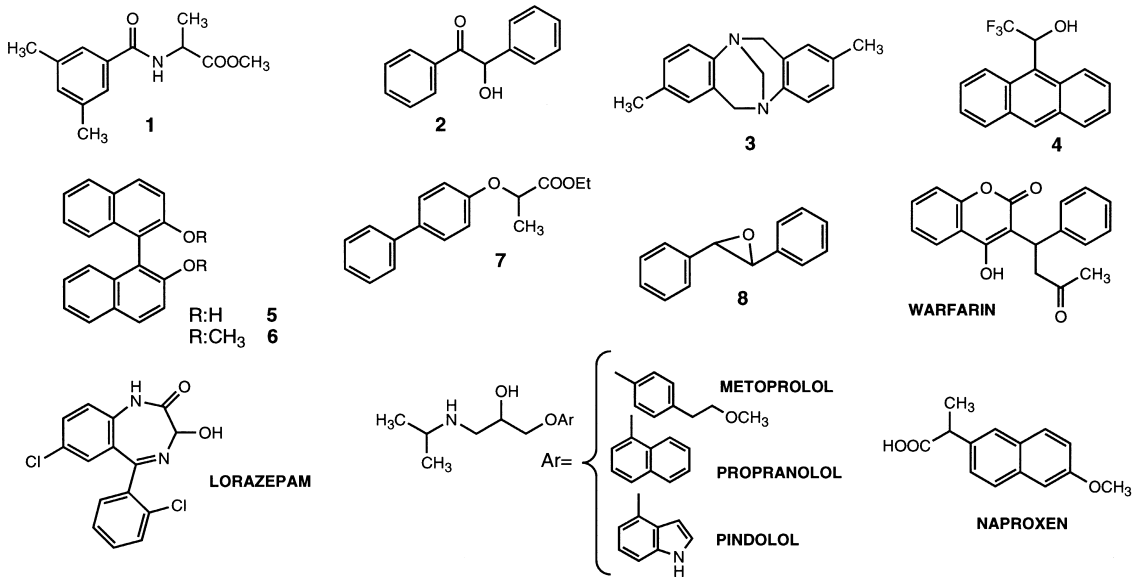

Fig. 2. Chemical structures of racemic test compounds.

The bonded cellulose-derived column studied here allowed the chromatographic separation of racemic compounds in different conditions. The chromatographic results using four mobile-phase modifiers (2-propanol, chloroform, tetrahydrofuran and ethyl acetate) for the racemic compounds tested (Fig. 2) are presented in Table 1. In order to assess the performance and stability of the column, it was reconditioned in the original conditions (heptane–2-propanol) after the use of each of the modifiers tested. Racemic compound **4** was used as standard compound to control selectivity and resolution. The same column was used to carry out all the tests and no loss of performance or efficiency was observed after its exhaustive use with each solvent. Acetone was also tested as a mobile-phase modifier [15]. However, in this case, the coincidence of the UV absorbance of this solvent with the maxima UV absorptions for most of the racemic compounds tested prevented proper detection of peaks. No restriction on the percentage of the polar solvents was applicable, because neither the potential solubility of the cellulose derivative nor the pressure in the HPLC system were limiting factors.

The racemic compounds were chosen on the basis of their resolution using heptane–2-propanol as a mobile phase. The results showed that the mixed cellulose derivative resolved a number of the racemic compounds tested in any of the solvents used as mobile-phase modifiers. Using heptane–2-propanol and heptane–chloroform a higher number of the racemic compounds tested were resolved than when using heptane–tetrahydrofuran or heptane–ethyl acetate mixtures. Tetrahydrofuran was the least efficient of the solvents tested. No inversion in the elution order of the enantiomers seemed to occur when the mobile phase composition was changed[1]. Furthermore, the highest separation factors were obtained with 2-propanol and chloroform for most of the analytes tested.

3.2. Resistance to solvents of the bonded cellulose-derived CSPs

Bonded polysaccharide-derived chiral supports are usually washed in chloroform after the fixation process and before packing [7]. Due to the solubility of the mixed 10-undecenoate–3,5-dimethylphenylcarbamate of cellulose in this solvent, this process allows the removal of the polysaccharide derivative that is not fixed onto the chromatographic matrix.

[1]It has to be taken into account that the sign of the optical rotation can be modified by the composition of the mobile phase, resulting in a non-detectable inversion when using polarimetric detection.

Table 1
Chromatographic results obtained on CSPA using different mobile-phase modifiers

Racemic compounds	Heptane–2-propanol				Heptane–chloroform				Heptane–tetrahydrofuran				Heptane–ethyl acetate			
	k'_1	α	R_s	UV[m]	k'_1	α	R_s	UV[n]	k'_1	α	R_s	UV[o]	k'_1	α	R_s	UV[p]
1	7.79	1.22	0.89	98:2[a]	5.59	1.25	1.19	90:10[d]	3.35	1.23	1.14	90:10[g]	7.20	1.19	1.00	90:10[j]
2	4.74 (+)	1.33	1.55	98:2[a]	2.20	1.15	0.85	80:20[d]	2.73 (+)	1.31	1.59	90:10[g]	3.98	1.24	1.19	90:10[j]
3	3.20 (+)	1.21	0.81	98:2[a]	2.36	1.00	—	80:20[d]	1.96 (+)	1.16	—	90:10[g]	6.01	1.00	—	90:10[j]
4	1.34 (*R*)	2.20	2.71	90:10[a]	2.39 (*R*)	2.29	3.75	50:50[d]	1.79 (*R*)	2.02	3.75	90:10[g]	0.91 (*R*)	2.04	3.20	80:20[j]
5	2.98	1.25	0.74	90:10[a]	3.97	1.30	1.39	70:30[d]	4.11	1.11	—	90:10[g]	14.4 (+)	1.11	—	90:10[j]
6	1.28	1.11	—	98:2[a]	1.80	1.15	-	95:5[d]	1.06	1.00	—	90:10[g]	1.30	1.00	—	90:10[j]
7	1.01 (+)	1.23	0.78	98:2[a]	1.73	1.17	0.73	95:5[d]	0.61	1.00	—	90:10[g]	1.71	1.13	—	98:2[j]
8	0.86 (−)	1.49	1.48	98:2[a]	1.41 (−)	2.03	3.88	95:5[d]	0.61	1.00	—	90:10[g]	1.21 (−)	1.82	2.32	98:2[j]
Lorazepam	8.74 (−)	1.56	1.35	90:10[a]	13.8	1.18	0.72	50:50[d]	4.69	1.11	—	75:25[g]	>18	—	—	80:20[j]
Warfarin	3.33	2.17	1.99	90:10[a]	1.32	2.15	1.74	50:50[d]	1.44	1.74	2.46	75:25[g]	7.08	1.58	2.11	80:20[j]
Metoprolol	3.22	1.19	0.66	90:10:0.1[b]	2.47	1.12	-	50:50:0.5[e]	1.46	1.00	—	75:25:0.5[h]	10.3	1.08	—	80:20:0.5[k]
Propranolol	3.01	1.42	1.33	90:10:0.1[b]	3.27	1.49	1.85	50:50:0.5[e]	1.44	1.29	0.77	75:25:0.5[h]	6.86	1.08	—	60:40:0.5[k]
Pindolol	3.79	4.11	4.11	80:20:0.1[b]	>14			50:50:0.5[e]	2.68	1.24	1.19	50:50:0.5[h]	10.8	1.42	1.79	60:40:0.5[k]
Naproxen	5.64 (−)	1.22	1.18	98:2:0.5[c]	3.45	1.07	—	60:40:0.5[f]	3.41	1.00	—	90:10:0.5[i]	9.19	1.04	—	90:10:0.5[l]

k'_1, capacity factor for the first eluted enantiomer; α, selectivity factor; R_s, resolution. Column: 15×0.46 cm. The configuration or the sign of the first-eluted enantiomer is shown in parentheses.

[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; [d]heptane–chloroform; flow-rate, 1 ml/min; [e]heptane–chloroform–DEA; flow-rate, 1 ml/min; [f]heptane–chloroform–TFA; flow-rate, 0.5 ml/min; [g]heptane–THF; flow-rate, 1 ml/min; [h]heptane–THF–DEA; flow-rate, 1 ml/min; [i]heptane–THF–TFA; flow-rate, 0.5 ml/min; [j]heptane–AcOEt; flow-rate, 1 ml/min; [k]heptane–AcOEt–DEA; flow-rate, 1 ml/min; [l]heptane–AcOEt–TFA; flow-rate, 0.5 ml/min.

[m]UV detection: $\lambda_{230\ nm}$ (**1**, **3**, **5**, **6**, lorazepam, warfarin, metoprolol and naproxen); $\lambda_{254\ nm}$ (**2**, **4**, **7**, **8** and pindolol); and $\lambda_{280\ nm}$ (propranolol). [n]UV detection: $\lambda_{240\ nm}$ (**1**, **6** and lorazepam); $\lambda_{254\ nm}$ (**2**, **3**, **4**, **7** and **8**); and $\lambda_{280\ nm}$ (**5**, warfarin, metoprolol, propranolol, pindolol and naproxen). [o]UV detection: $\lambda_{230\ nm}$ (**1**, **3**, **5**, **6**, lorazepam and warfarin); $\lambda_{254\ nm}$ (**2**, **4**, **7**, **8** and naproxen); $\lambda_{270\ nm}$ (metoprolol); and $\lambda_{280\ nm}$ (propranolol and pindolol). [p]UV detection: $\lambda_{254\ nm}$ (**4**, **8**, pindolol and naproxen); $\lambda_{270\ nm}$ (**7**, warfarin and metoprolol); and $\lambda_{280\ nm}$ (**1**, **2**, **3**, **5**, **6** and propranolol).

Therefore, the resistance of the resulting material to this solvent is ensured.

Nevertheless, in the present study, more exhaustive washing of the chiral support before packing were carried out to investigate the resistance of the CSP to solvents such as pure pyridine (CSPB) or toluene (CSPC). The comparison of the elemental analysis after the various treatments showed that the organic content in the washed material slightly diminished from CSPA to CSPB, but no change was observed from CSPB to CSPC.

Relating to the comparison of the chromatographic behaviour of these three columns, results were in good agreement with the elemental analysis. Thus, selectivity values were slightly better in CSPA and equivalent for CSPB and CSPC (Fig. 3a). However, the reduction of the organic content in CSPB and CSPC, compared with CSPA, resulted in an improvement of the resolution, due to the reduction in the width of the chromatographic peaks (Fig. 3b). Retention times were also longer for CSPA.

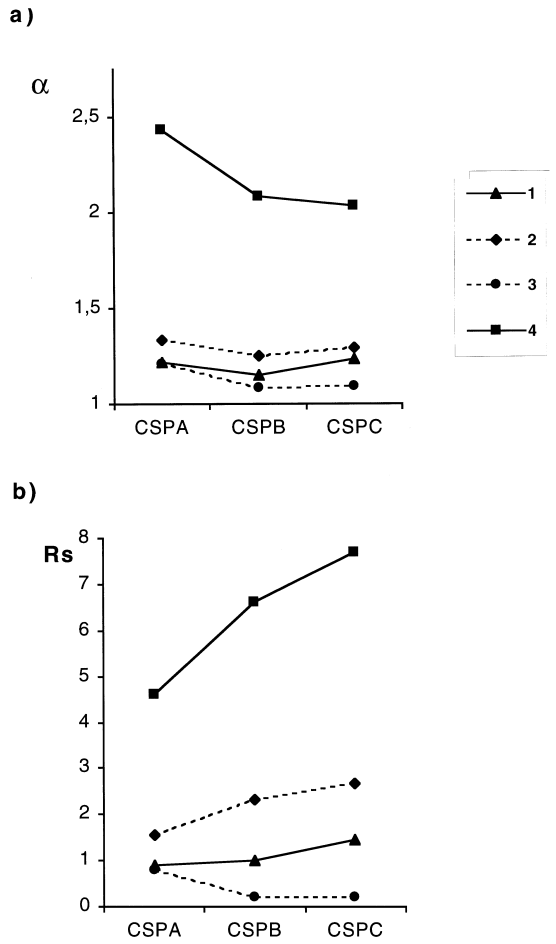

Fig. 3. Evolution of α (a) and R_s (b) from CSPA to CSPC using heptane–2-propanol mixtures.

3.3. Considerations about the relative cost of separations on CSPA

When a given separation is to be scaled-up, the highest selectivity factors are not always necessary. Other parameters, such as composition of the mobile phase or time of analysis, might have direct consequences on the loading capacity of the column and on the cost of the separation. Therefore, they should also be taken into account. Thus, racemic compound **1** is resolved with similar selectivity factors ($\alpha \approx 1.2$) using the four mobile-phase systems. However, the shortest retention time was observed when using heptane–tetrahydrofuran. This is also the case for **2**. This racemate showed nearly identical α values in 2-propanol and tetrahydrofuran mixtures, the latter solvent being the one which least retained this compound. In these cases, the use of tetrahydrofuran led to shorter times of analysis and less solvent consumption.

The relative injection cost of the analytical sepa-

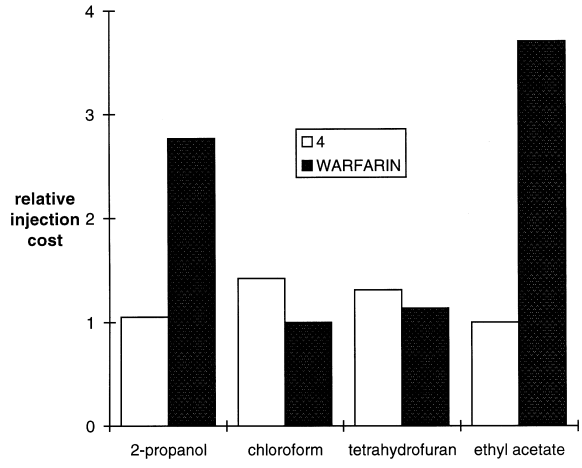

Fig. 4. Comparison of the relative injection cost (analytical separation) for the resolution of racemic **4** and warfarin using the four different mobile-phase modifiers.

ration, as a function of the volume and the composition of the mobile phase used for an individual analysis is also an important issue. Thus, if **4** and warfarin are considered, heptane–ethyl acetate led to the cheapest separation for compound **4**, whereas chloroform and tetrahydrofuran reduced the cost of the resolution of warfarin (Fig. 4). The four chromatographic resolutions for warfarin are presented in Fig. 5.

The solubility of the racemic compound to be separated in the mobile phase should also be considered for preparative separations. The solubility of a number of drugs in hydrocarbon–alcohol mixtures is low. However, in the case under study, the use of heptane–chloroform or heptane–tetrahydrofuran mixtures, in the separation of warfarin, not only led to the shortest times of analysis, but also allowed the optical resolution with considerable amounts of chloroform (50%) and tetrahydrofuran (25%) in the mobile phase. These high percentages of polar mobile phase modifier improve the sample solubility and reduce the relative cost of the separation.

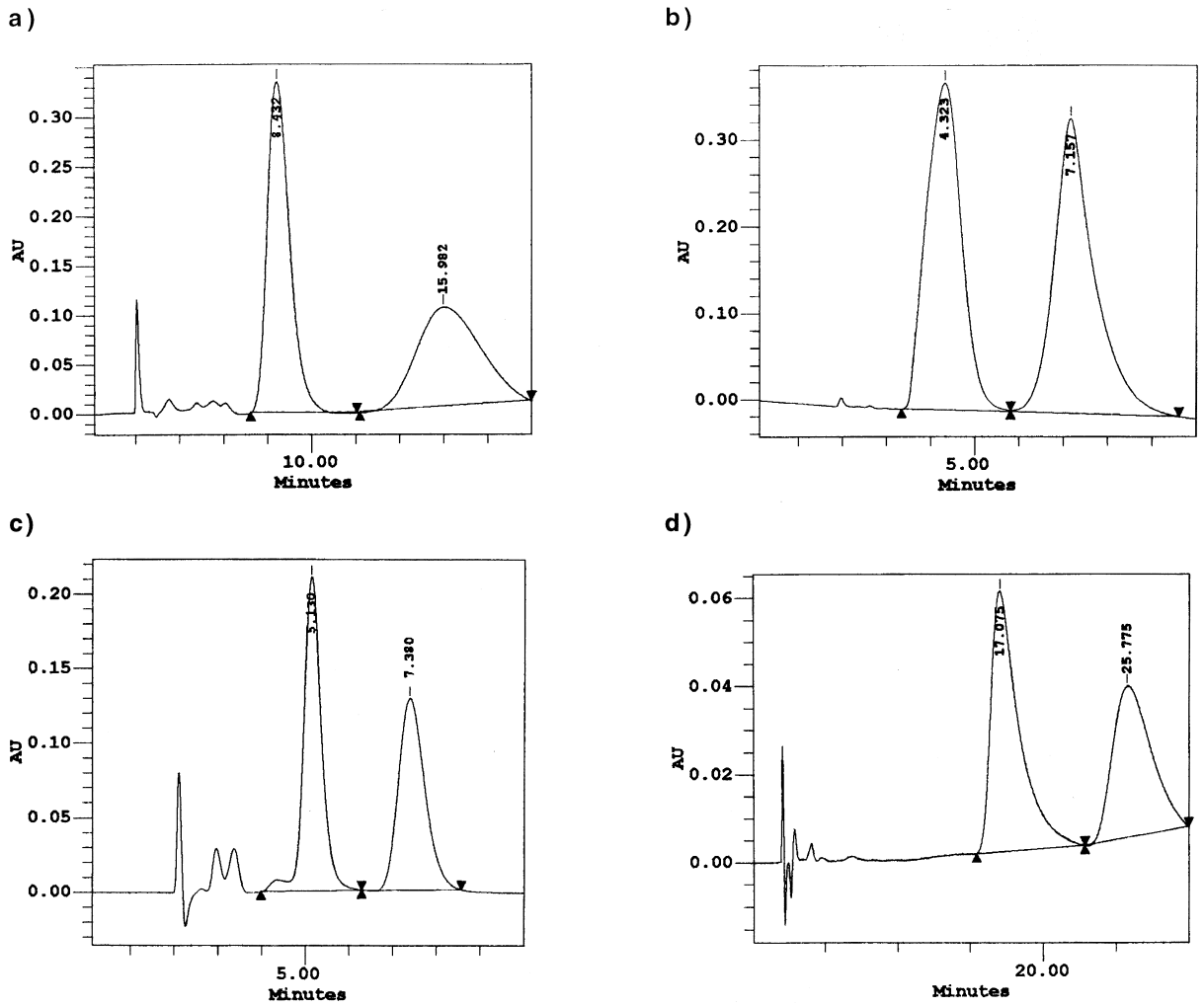

Fig. 5. Optical resolution of warfarin: (a) heptane–2-propanol (90:10), $\lambda_{230\ nm}$; (b) heptane–chloroform (50:50), $\lambda_{280\ nm}$; (c) heptane–tetrahydrofuran (75:25), $\lambda_{230\ nm}$; (d) heptane–ethyl acetate (80:20), $\lambda_{270\ nm}$.

Table 2
Loadability for compound **4** on CSPA

	Mobile-phase modifier			
	2-Propanol (10%)	Chloroform (50%)	Tetrahydrofuran (10%)	Ethyl acetate (20%)
α	2.20	2.29	2.02	2.04
Mass of injected solute (mg)	0.35	6.00	3.44	3.80
Injected solute (mmol)	1.3	21.7	12.5	13.8
Injected volume[a] (μl)	140	800	32	38
Time of analysis (min)	10	15	12	10
Productivity (mg/day)	50	576	413	547

Flow-rate, 1 ml/min. UV detection: $\lambda_{280\ nm}$ (to avoid the UV saturation) and polarimetric detection: $\lambda_{546\ nm}$.
[a]The sample was dissolved in the pure organic modifier.

3.4. Loadability of the bonded cellulose-derived CSPA

Since the bonded 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose used in this study allowed the use of different solvent mixtures, its loading capacity was investigated for several racemic compounds using the considered mobile-phase modifiers.

Racemate **4** was injected in quantities varying from 2.5 to 6.75 mg on the analytical CSPA column (150×4.6 mm I.D.) using the four different mobile-phase modifiers. Although this compound was resolved in the four cases with a values of the same order (2.02–2.29), the amount of sample injected for 'touching-band' separation (R_s=1) [16,17] was different depending on the mobile phase used (Table 2). Thus, when using heptane–chloroform (50:50) as a mobile phase, 6.00 mg could be resolved in a single run. In contrast, by using heptane–2-propanol (90:10) only the resolution of 0.35 mg was achieved. The amount of sample (**4**) that can be separated per day in this column is presented in Table 2. Considering the loading capacity of the column in every solvent, the comparison of the relative separation cost of 1 mg of racemate (Fig. 6) shows that chloroform and ethyl acetate are the organic modifiers that lead to the cheapest resolution. In Fig. 7, the chromatogram of the saturation conditions in heptane–ethyl acetate is presented.

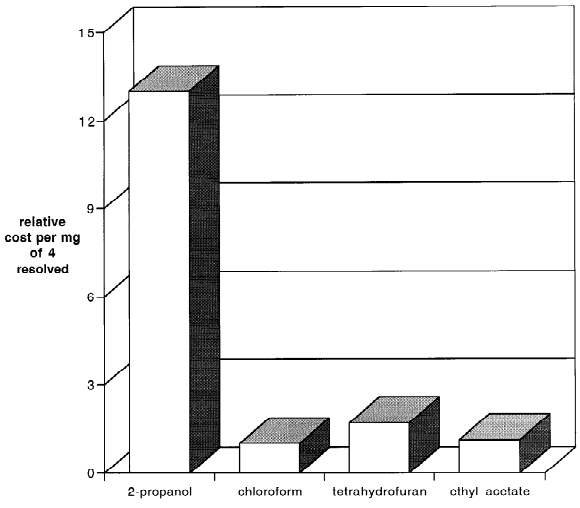

Fig. 6. Comparison of the relative solvent cost for the resolution of 1 mg of racemate **4**, using the four different mobile-phase modifiers, on the basis of the loadability of the column and the solvent consumption.

The loadability of the column was also studied for racemic compounds **2** and **8** in heptane–2-propanol (98:2). The amounts of sample resolved in a single run were 0.30 mg (1.4 mmol) and 0.22 mg (1.1 mmol), respectively. In heptane–chloroform, 0.30 mg (1.5 mmol) of racemate **8** were separated. The results show that the loading capacity of the column depends on the racemic compound to be resolved as well as on the eluent used for the separation.

4. Conclusions

Mixed derivatives of cellulose, such as 10-undecenoate/3,5-dimethylphenylcarbamate, exhibited a high resistance to solvents when bonded on

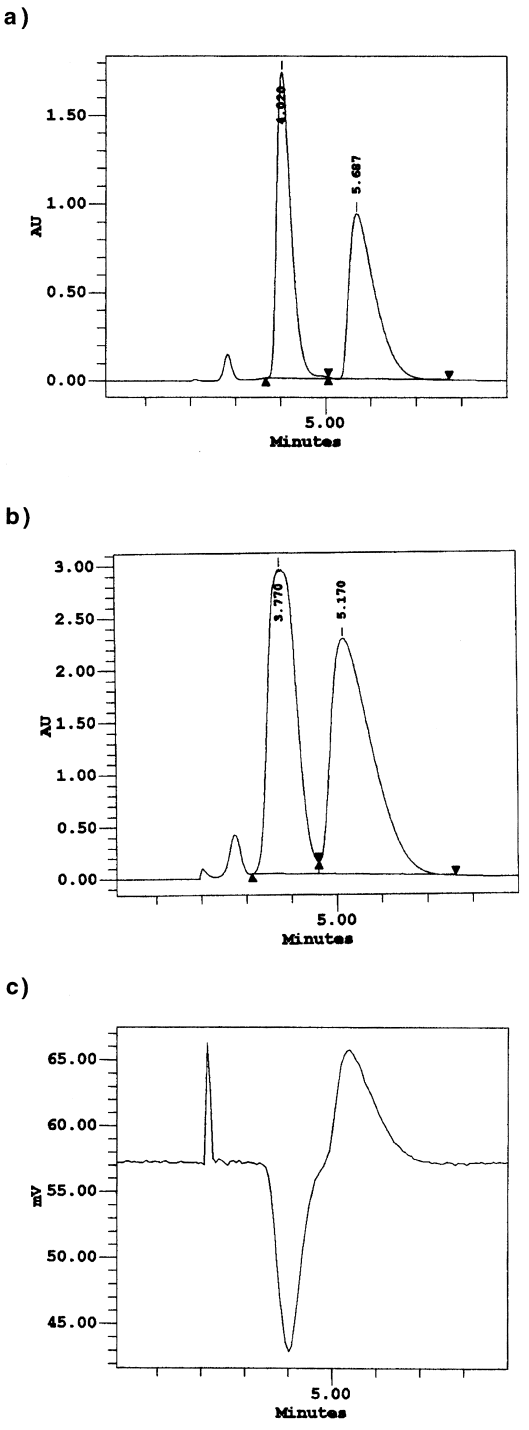

Fig. 7. Chromatogram of racemic compound **4** in heptane–ethyl acetate (80:20): (a) 1 mg/10 μl; (b) 3.8 mg/38 μl; UV detection $\lambda_{280\ nm}$ and polarimetric detection at 546 nm (c).

allylsilica gel, and a remarkable chiral discrimination ability in a number of them. This solvent versatility is particularly convenient for preparative purposes. The choice of the mobile phase composition to be used in the separation will be determined in every case as a function of the racemic compound to be resolved, its solubility in the eluent and the time of analysis. The loadability of the support will be also dependent on the racemic compound tested and the mobile phase used. The wider choice of solvents to be used with these bonded CSPs broadens the applicability of chiral polysaccharide-derived supports for HPLC.

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