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Immobilized halogenophenylcarbamate derivatives of cellulose as novel stationary phases for enantioselective drug analysis

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Dedicated to Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

Three different halogeno-phenylcarbamate derivatives of cellulose have been prepared and coated on silica gel. The coated materials have been immobilized and their chiral recognition ability as chiral stationary phase (CSP) has been evaluated with a set of reference racemates, including several drugs such as lormetazepam, glutethimide, and warfarin, using various mobile phase mixtures. The novel phases were found to exhibit unique enantioselective properties compared with more established polysaccharide-based CSPs. A good resolution of all racemates could be successfully achieved on at least one of the immobilized CSPs. Moreover, it has been pointed out that selectivity may considerably vary with the composition of the mobile phase.

Keywords: Chiral stationary phase; Enantiomer; Cellulose derivatives; Polysaccharides; Mobile phase

1. Introduction

Among all chiral stationary phases (CSPs) which have been so far investigated, the cellulose and amylose-based phases have proved to be remarkably versatile and extremely powerful as well on an analytical [1–4] as on a preparative scale [6–8]. It is well established that the enantioselectivity of these phases strongly varies with the type of derivatising group attached to the glucose moieties of the polysaccharide [1–5]. This characteristic has been extensively exploited to modulate the chiral recognition ability of the polysaccharide-based CSPs. However, a number of polysaccharide derivatives are more or less soluble in organic solvents, even in the presence of only small amount of polar modifiers in apolar solvent such as hexane or heptane which are usually applied as the mobile phase with this kind of CSPs. This feature prevents their utilization as sorbents for chromatographic resolution. This is particularly marked for most of the halogenophenylcarbamate derivatives of cellulose. Okamoto and his group

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already earlier recognized the high chiral recognition power of these derivatives but they pointed to their high solubility in alkane–alcohol mixtures [9–14], thus considerably limiting their utilization. However, applying the photochemical technique that we elaborated for the immobilization of polysaccharide-based phases [7,15–17], we were now able to prepare a series of stable CSPs derived from halogenophenylphenylcarbamate derivatives of cellulose which could be used for the chromatographic resolution of racemic compounds.

2. Experimental

2.1. Preparation of the chiral stationary phases

Tris(3,4-dichlorophenylcarbamate) of cellulose I. Ten gram of dried cellulose are suspended in 57.5 g of 3,4-dichlorophenylisocyanate (Fluka, Buchs, Switzerland) in a round-bottomed flask. About 120 ml pyridine are added to this suspension followed by 0.5 ml of dibutyltin dilaurate. The suspension is heated with stirring for 24 h at 130 °C (bath temperature). The solution is cooled down to 50 °C and 800 ml of methanol are added to precipitate the polymeric cellulose derivative. The precipitate is filtrated and dissolved in 300 ml of methylene chloride, and 1 l of methanol is added to this solution. The cellulose derivative is precipitated by addition of 100 ml of water. After stirring for 3 h at room temperature, the suspension is filtrated and again dissolved in a mixture consisting of 120 ml of methylene chloride and 300 ml of methanol. Fifty milliliter of water are added to this solution which is filtrated. The solid residue is dried under vacuum. Yield: 19.8 g (44.2%). Elemental analysis: Calculated: C 44.66; H 2.64; N 5.79; Cl 29.29; O 17.63. Found: C 43.99; H 3.05; N 5.70; Cl 28.03; O 19.31.

Tris(3,5-dichloropenylcarbamate) of cellulose II. Dried cellulose (8.1 g) are suspended in 95 ml pyridine containing 0.2 ml dibutyltin dilaurate. 3,5-Dichlorophenylisocyanate (41.4 g; Aldrich, Buchs, Switzerland) are slowly added at room temperature to this suspension. After complete addition, the suspension is stirred for 40 h at 130 °C (bath temperature). The obtained solution is cooled down to 50 °C and diluted with 100 ml of methanol. Methanol (500 ml) are added to this solution to precipitate the polymeric cellulose derivative. The precipitate is filtrated and washed with 200 ml of methanol. The solid cake is dissolved in a mixture consisting of 70 ml of methanol and in 170 ml of methylene chloride. Methanol (800 ml) are added to this solution and the obtained precipitate is filtrated. The wet cake is again dissolved in 130 ml of methylene chloride and 700 ml of methanol are added. The last operation is repeated a second time. The solid residue is dried under vacuum at 60 °C. Yield: 31.78 g (87%). Elemental analysis: Calculated: C 44.66; H 2.64; N 5.79; Cl 29.29; O 17.63. Found: C 44.18; H 3.29; N 5.40; Cl 27.36; O 19.88.

Tris(3-trifluoromethyl-5-chloropenyl)carbamate of cellulose III. Ten gram of dried cellulose are suspended in 150 ml of pyridine containing 0.4 ml dibutyltin dilaurate. (3-trifluoromethyl-5-chlorophenyl)isocyanate (61.6 g; Fluka, Buchs, Switzerland) are slowly added at room temperature to this suspension. After complete addition, the suspension is stirred for 23 h at 130 °C (bath temperature). The obtained solution is cooled down to room temperature and diluted with 500 ml of methanol. Water (100 ml) are added to this solution to precipitate the polymeric cellulose derivative. The precipitate is filtrated and dissolved in 200 ml of methylene chloride and 100 ml of acetone. 1 l of methanol and 100 ml of water are added to this solution. The obtained suspension is filtrated and again dissolved in a mixture consisting of 40 ml of acetone and 100 ml of methylene chloride. After dilution of this solution with 500 ml of methanol, 100 ml of water are added to precipitate the cellulose derivative. After filtration of the suspension, the solid residue is dried under vacuum. Yield: 38 g (74.5%). Elemental analysis: Calculated: C 43.58; H 2.32; F 20.67; Cl 12.86; N 5.08; O 15.48. Found: C 43.66; H 2.44; F 20.80; Cl 12.40; N 4.94; O 15.76.

Coating of the cellulose derivative on silica gel has been performed similarly for the three derivatives. Typically, 1 g of the cellulose derivative was dissolved in a mixture consisting of 2 ml of methanol and 20 ml of tetrahydrofurane. The

obtained solution is divided into three equal portions. The first portion is mixed with 3.0 g of silica gel (Nucleosil Si 4000, 7 μm, from Macherey-Nagel), preliminarily modified with 3-aminopropyl-triethoxysilane [1]. The suspension is evaporated under vacuum in a rotavapor. The same procedure is repeated in the same manner with the two other portions.

Immobilization of the silica-coated materials was achieved similarly for the three cellulose derivatives. About 4 g of coated I-III are suspended in a mixture consisting of 240 ml of methanol and 240 ml of water in a 750-ml glass flask. The suspension is stirred with a mechanic stirrer and irradiated using a high pressure immersing mercury lamp surrounded by a Pyrex cooling jacket. After 16 h, the suspension is filtrated off and treated without stirring with a mixture ethanol/chloroform/tetrahydrofurane 20/50/100 (in volume) for 16 h. The suspension is filtrated, washed with 100 ml of tetrahydrofurane. The solid residue is suspended again in 30 ml of tetrahydrofurane. During moderate magnetic stirring, 300 ml of hexane were added dropwise to the above suspension (1.6 ml/min). After complete addition, the suspension is filtrated and dried at 120 °C for 2 h.

Based on the elemental analysis (carbon content), the following amounts (weight) of immobilized cellulose derivative were found for the three CSPs: CSP I: 23.7%; CSP II: 20.2%; CSP III: 24.1%.

2.2. Chromatography

Packing of the columns (stainless-steel column, 250 × 4 mm I.D., Macherey-Nagel) with the CSP materials obtained as above was performed using the slurry method in ethanol under constant pressure (150 bar).

The high-performance liquid chromatography (HPLC) systems used in this study consisted of (1) a Shimadzu SPD-6AV pump, a variable wavelength Shimadzu LC-6A UV–visible detector in series with a Perkin–Elmer (Model 241) polarimeter and a Reodyne injector fitted with a 20 μl sample loop; the apparatus was connected to a PC and the data were managed with the GINA-NT (Bional AG, Dietikon, Switzerland) chromatographic software; (2) a Shimadzu LC-10AD pump, a variable wavelength Shimadzu SPD-10A UV–visible detector in series with a Jasco OR-990 chiral detector and a Reodyne injector fitted with a 20 μl sample loop; the apparatus was connected to a PC loaded with the Shimadzu chromatographic software (version 1.62).

The mobile phase consisted of different mixtures as indicated in the Tables 1 and 2. Chromatographic runs were performed at ambient temperature at a flow rate of 0.7 ml/min. Tri-*tert*-butylbenzene peak was taken as a reference to determine the dead time of the columns.

3. Results and discussion

3.1. Preparation of the chiral stationary phases

In this study, three different halogenophenylcarbamate derivatives of cellulose were investigated. The structures of the used cellulose derivatives, 3,4- and 3,5-dichlorophenylcarbamate, and 3-trifluoromethyl-4-chlorophenylcarbamate are shown on Fig. 1.

They were obtained by reaction of cellulose with the corresponding halogenophenylisocyanates. High degree of substitution have been obtained (see experimental part). The halogenophenylcarbamate derivatives of cellulose were coated on macroporous silica gel (4000 Å, 7 μm) used as a carrier. Coating is achieved by mixing silica gel with a solution of the cellulose derivative in tetrahydrofurane/methanol, followed by evaporation of the organic solvent, according to the technique developed by Okamoto and his group [1]. However, all these polysaccharide derivatives are soluble in alcohol–hydrocarbon mixtures. Therefore, their practical application as CSPs is greatly limited with these traditional mobile phases [8–14]. Recently it has been shown that pure alcohols such as methanol, ethanol or 2-propanol can be used as mobile phases with the 3,5-dichlorophenylcarbamate derivative of cellulose [14,18], but the utilization of these solvents is excluded with the other halogenophenylcarbamate derivatives of cellulose reported in this work.

Table 1
Chromatographic results obtained on CSPs I–III for the racemates 1–15; HPLC column 4 mm i.d ×250 mm; flow rate 0.7 ml/min; mobile phase; A, hexane/2-propanol 90/10; B, heptane/chloroform 50/50

Racemate	CSP I				CSP II				CSP III			
	Mobile phase A		Mobile phase B		Mobile phase A		Mobile phase B		Mobile phase A		Mobile phase B	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	6.18	1.51	9.99	1.32	3.23	1.75	12.30	1.60	2.99	1.53	12.36	1.39
2	8.92	1.06	7.42	1.91	4.35	1.35	8.76	1.47	5.58	1.18	10.79	1.21
3	9.88	1.16	38.67	1.26	7.39	1.19	24.81	1.29	4.90	1.20	45.30	1.32
4	0.89	1.79	0.80	1.34	0.74	1.52	1.21	1.00	0.45	1.41	0.79	1.00
5	5.12	1.26	2.42	1.10	4.14	1.20	3.27	1.12	3.13	1.20	1.86	1.11
6	5.38	1.18	11.29	1.34	4.24	1.00	9.42	1.46	2.08	1.41	8.75	1.84
7	*	*	34.33	1.22	*	*	30.22	1.13	*	*	28.45	1.19
8	Not eluted		Not eluted		Not eluted		Not eluted		Not eluted		Not eluted	
9	Not eluted		Not eluted		Not eluted		Not eluted		Not eluted		Not eluted	
10	4.90	1.05	8.54	1.26	3.45	1.00	8.52	1.34	2.19	1.00	11.65	1.10
11	25.37	1.00	36.43	1.26	17.15	1.46	31.86	1.20	19.71	1.00	32.96	1.34
12	*	*	31.24	1.57	*	*	27.82	2.02	*	*	29.12	1.88
13	0.88	1.36	5.15	1.31	0.63	1.56	4.87	1.57	0.39	1.23	4.19	1.38
14	0.24	1.67	1.35	2.06	0.21	1.57	1.40	2.36	0.15	1.00	1.46	1.84
15	2.11	1.25	4.62	1.15	1.69	1.22	5.02	1.15	1.08	1.22	4.61	1.13

*, Compound insoluble in the mobile phase.

Both the 3,4-dichloromethylphenyl carbamate and 3-trifluoromethyl-4-chlorophenylcarbamate derivatives of cellulose are readily soluble in methanol and ethanol. The latter derivative had not been examined as CSP so far. To solve the problem linked to the high solubility of these materials, we applied the strategy that we recently developed to immobilize polysaccharide derivatives [7,15–17]. The general scheme for immobilizing the cellulose-based CSPs is shown on Fig. 1. The silica-coated materials I–III are suspended in a mixture of water/methanol in a glass flask. The suspension is irradiated for 16 h using an immersing UV-mercury lamp according to a process recently developed in our laboratory [15]. The exact mechanism of immobilization is not yet elucidated [17], but it presumably occurs through cross-linking between the polymeric chains. After irradiation, the materials are filtered off and the non-immobilized part is removed by extraction

Table 2
Chromatographic results obtained on CSPs I–III for the racemates 1–15; HPLC column 4 mm i.d × 250 mm; mobile phase, C, heptane/chloroform/ethanol 50/50/1 (volume); D, heptane/chloroform/ethanol 50/50/2 (volume); flow rate 0.7 ml/min

Racemate	CSP I				CSP II				CSP III			
	Mobile phase C		Mobile phase D		Mobile phase C		Mobile phase D		Mobile phase C		Mobile phase D	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	1.01	1.41	0.81	1.37	1.13	1.61	0.67	1.52	1.00	1.73	0.47	1.51
2	1.19	1.14	0.98	1.08	1.39	1.00	1.39	1.00	1.35	1.14	0.62	1.18
3	4.50	1.18	3.17	1.17	7.84	1.23	2.76	1.21	5.93	1.32	1.66	1.29
4	0.31	1.46	0.20	1.54	0.21	1.41	0.24	1.32	0.57	1.00	0.15	1.00
5	0.69	1.25	0.63	1.15	0.61	1.18	0.59	1.16	0.53	1.00	0.45	1.00
6	2.19	1.53	1.68	1.49	2.05	1.59	1.37	1.50	1.86	2.16	0.86	2.00
7	4.39	1.41	3.39	1.41	2.47	1.34	1.88	1.35	3.95	1.46	1.53	1.47
8	13.96	1.58	10.64	1.60	16.48	1.63	7.38	1.68	12.58	1.78	5.09	1.67
9	11.97	2.68	8.07	2.55	22.59	1.95	6.02	1.88	12.15	2.30	3.58	2.15
10	1.57	1.18	0.89	1.15	1.19	1.21	0.78	1.10	1.11	1.00	0.46	1.00
11	7.66	1.12	3.97	1.08	4.21	1.10	3.05	1.14	7.64	1.15	3.16	1.00
12	3.71	1.89	2.97	1.88	3.88	2.49	2.30	2.47	2.59	2.42	1.73	2.39
13	1.25	1.31	0.94	1.27	1.16	1.71	0.67	1.55	1.16	1.32	0.46	1.24
14	0.37	1.63	0.28	1.57	0.32	1.86	0.25	1.55	0.41	1.51	0.14	1.59
15	1.51	1.18	1.27	1.17	1.46	1.21	1.10	1.20	1.33	1.22	0.73	1.19

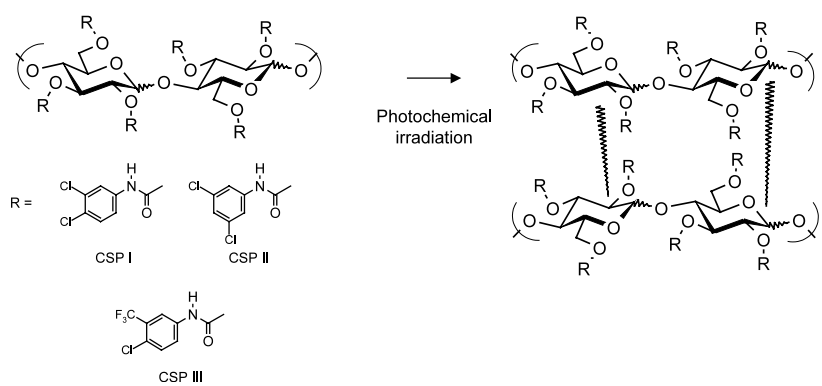

Fig. 1. Immobilization process and structures of the CSPs I–III.

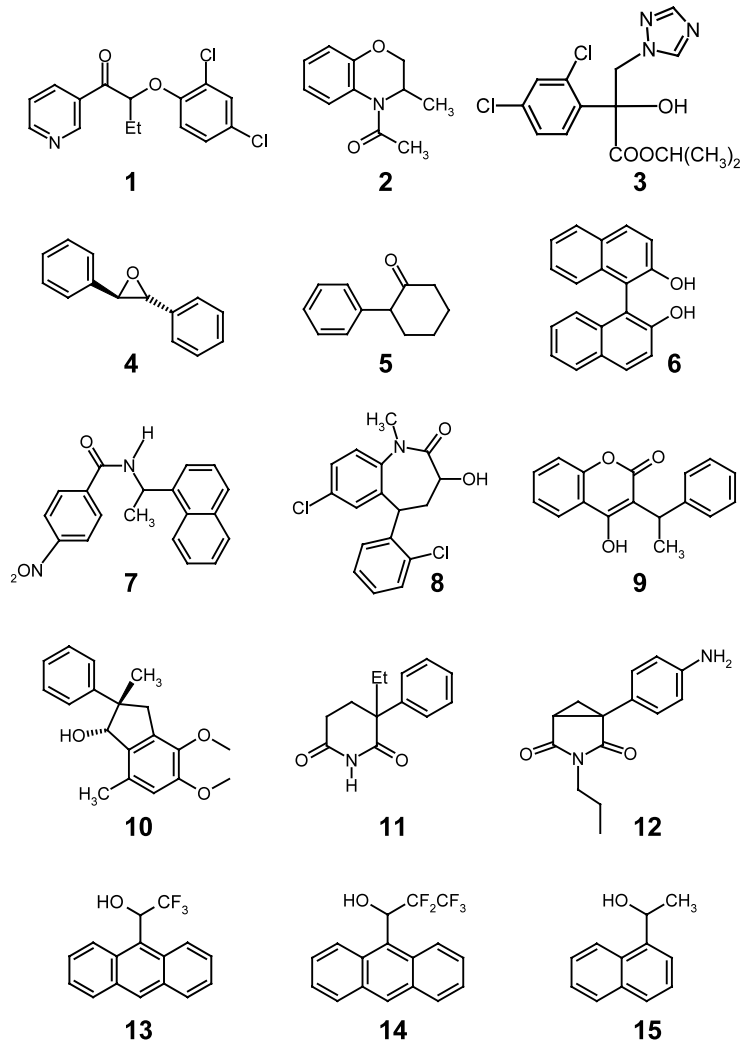

Fig. 2. Structures of the racemates.

with a mixture of tetrahydrofurane/chloroform/ethanol. Packing of the stationary phase has been performed using the slurry technique.

3.2. Chromatographic properties of the CSPs

Table 1 shows the enantioselectivity (α) and the capacity factor k'_1 obtained for various test racemates (Fig. 2) on the cellulose-based CSPs I–III after immobilization, using hexane/2-propanol (90/10 volume) or heptane/chloroform (50/50 volume) as the mobile phase. Thanks to the immobilization, the CSPs can be used with these mobile phases, which are known to dissolve the corresponding non-immobilized phases.

For all compounds, a good separation of the enantiomers could be achieved on at least one of the investigated immobilized CSPs and mobile phase conditions, with an enantioselectivity ranging between 1.22 and 2.36, except for lormetazepam **9** and warfarin **10** which are not eluted. Selected chromatogrammes illustrating these separations are shown on Fig. 3. CSPs I and II show the highest success rate but for compound

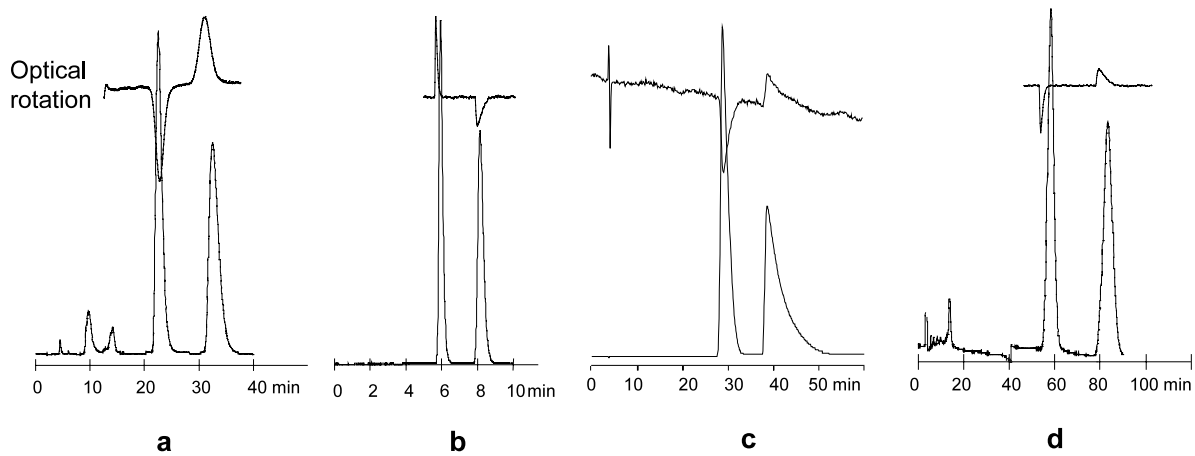

Fig. 3. Chromatogrammes (upper line, optical rotation) of the enantioselective separation of (a) compound **1** (CSP I, mobile phase A); (b) compound **4** (CSP I, mobile phase A); (c) compound **2** (CSP I, mobile phase B); (d) compound **11** (CSP II, mobile phase A).

6, CSP III exhibits the best enantioselectivity ($\alpha = 1.84$) using the mixture chloroform/heptane as the mobile phase.

The crucial influence of the mobile phase on retention and selectivity is clearly demonstrated in Table 1. For some racemates (**1**, **4**, **5**, **11**, **15**), mobile phase A (hexane/2-propanol 90/10) gives the best results, while for another series of racemates (**2**, **3**, **6**, **7**, **10**, **14**) mobile phase B (hexane/chloroform 50/50) provides the best enantioselectivity. However, with mobile phase B, excessively long retention times (large k'_1 values in Table 1) are mostly observed, making the separations not very useful on a practical point of view. But, we found that addition of small amounts of ethanol in the mobile phase composition can considerably reduce retention time. An example is shown on Fig. 4 for the separation of the enantiomers of the diuretic compound **10** [19] on CSP II. A strong tailing and long retention times are observed when using heptane/chloroform (50/50 volume) as the mobile phase. Addition of 1 part of ethanol to this mobile phase causes a dramatic reduction of the retention time and suppression of the tailing effect.

The large decrease of retention time systematically observed when small amounts of ethanol are added to the mobile phase mixture heptane/chloroform is clearly evidenced when comparing the data summarized in Table 2 with those reported in Table 1 under the mobile phase conditions B. Decrease of k'_1 by a factor 3–12 is generally observed when one part of ethanol is added. This decrease of k'_1 is mostly associated either with a reduction or an improvement of enantioselectivity which is shifting in the same way for the three CSPs, indicating that similar interaction mechanisms govern the chiral recognition process with

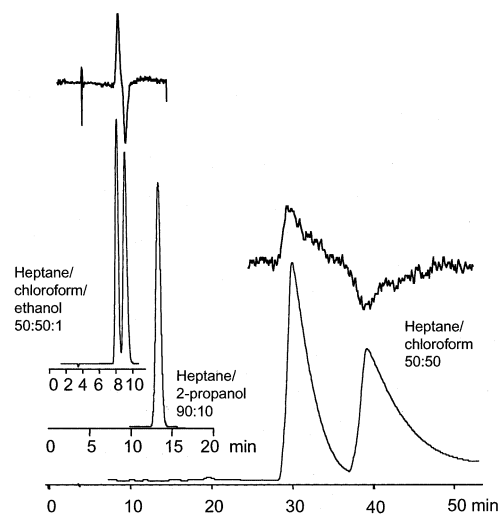

Fig. 4. Influence of the mobile phase composition on selectivity and retention of racemate **10** on CSP II.

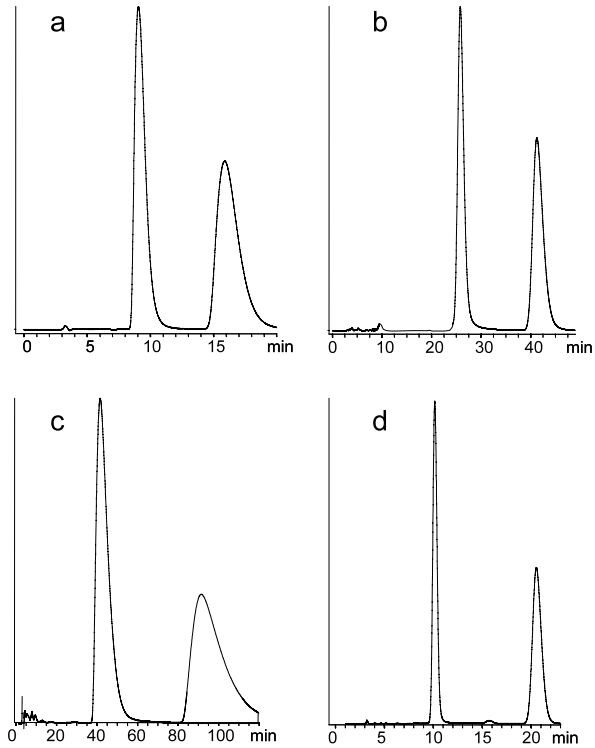

Fig. 5. Chromatographic separation of the enantiomers of (a) binaphthol **6** (CSP III, mobile phase C), (b) lormetazepam **8** (CSP II, mobile phase D), (c) warfarin **9** (CSP III, mobile phase C), and (d) aromatase inhibitor **12** on (CSP II, mobile phase D).

all CSPs. However, the largest improvements of selectivity are observed with CSP III which provides a good or even the best separation of the enantiomers of compounds **1**, **3**, **7**, **15**, of the chiral ligand binaphthol **6** (Fig. 5a), and of the drugs lormetazepam **8** (Fig. 5b), warfarin **9** (Fig. 5c), and the aromatase inhibitor **12** [20].

By increasing the amount of ethanol in the mobile phase to two parts (Table 2, mobile phase D), the retention times could generally be further reduced (factor 2–3) without affecting the enantioselectivity too much, but allowing fast separations to be achieved. Increase of separation rate by more than 25 times have been reached (compounds **1** and **10**) when changing from mobile phase B to mobile phase D.

Although only three different mobile phase combinations are reported in this work, it is likely that the application of further variations may still improve the separation. The results emphasize that the effect of the mobile phase on the chiral recognition ability of the polysaccharide-based CSPs has by no means be fully exploited. Even if no general rule could be established from the collected data, these results points to the importance of screening a broad variety of mobile phases in order to successfully achieve the separation of the enantiomers of a defined racemate on a particular CSP.

4. Conclusion

Several polyhalogeno–phenylcarbamoyl derivatives of cellulose have been immobilized on macroporous silica and successfully applied as CSPs for the separation of enantiomers. The chromatographic results demonstrate the effectiveness of the immobilization process, making possible the use of CSPs, which would not have been applicable in the non-immobilized form. The novel approach considerably extends the possibility to modulate the chiral recognition properties of the polysaccharide-based phases.

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