HPLC Enantioseparation with Cellulose Tris(3,5-dichlorophenylcarbamate) in Aqueous Methanol as a Mobile Phase

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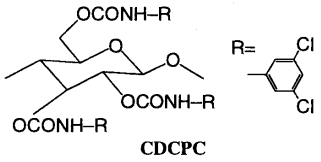
The appropriate design of mobile and stationary phase combinations allowed the use of cellulose tris(3,5-dichlorophenylcarbamate) (CDCPC) as the chiral stationary phase (CSP) in high-performance liquid chromatography (HPLC). Together with previous data obtained in *n*-hexane/2-propanol as a mobile phase the present study indicates very high chiral resolving ability of CDCPC.

The high chiral recognition ability of cellulose tris(3,5-dichlorophenylcarbamate) (CDCPC) has been known for a decade.[1] However, this material is very soluble in the common normal-phase HPLC eluents which are suitable for other polysaccharide-type CSPs.[1,2] Due to this reason, it was impossible to use intact CDCPC for HPLC enantioseparations. On the other hand, the corresponding dimethyl-substituted derivatives of cellulose and amylose are insoluble in *n*-hexane/2-propanol solution and belong to the most powerful CSPs for the HPLC enantioseparations.[1,2] Spectroscopic studies of CDCPC indicated that it possesses some unique properties from the viewpoint of the amount of free N-H and C=O groups and their acidity and basicity, respectively. In particular, the CDCPC contains higher amounts of free carbamate fragments compared with the corresponding dimethyl derivative of cellulose.[3e] These groups are the most likely interaction sites with chiral analytes. Therefore, several attempts were made in order to prepare some hybrid-type CSPs, containing both halogen and alkyl substituents.[3] The idea was that the former substituent will maintain a more universal chiral recognition ability and the latter one provides material stability by creating a higher number of intramolecular hydrogen bonds. The CSPs obtained in this way were stable in *n*-hexane/2-propanol solutions and exhibited quite interesting chiral recognition properties.[3] An alternative way for stabilizing the CSP is immobilization on a silica surface. This technique for polysaccharide derivatives was first applied by Okamoto et al. in 1987[4a] and recently used by several groups with various modifications.[4b-d] Both of above mentioned strategies resulted in CSPs with increased stability in organic solvents. However, these immobilized CSPs exhibited a somewhat lower chiral recognition ability. This may be the result of the loss of their regular higher-ordered structure. Therefore, the goal of this study was to use an intact CDCPC coated on the silica surface as a CSP.



CDCPC

The enantioseparation ability of polysaccharide-type CSPs in aqueous organic solvents is known.[1,2,5] The application of these CSPs in combination with aqueous-organic mobile phases are becoming increasingly important because this mode is better suited for biomedical samples. The most attractive advantage of separations using aqueous-organic mobile phases is a possibility of a direct sample injection without any pretreatment. Additional advantage is that achiral-chiral column-coupling is more powerful in the case of reversed-phase separations. In addition, recent studies indicated that polysaccharide-type CSPs may be successfully used even with pure alcohols as the mobile phases.[6] In some cases, even the separations not capable of being common normal- and reversed-phase eluents are possible in pure alcohols.[6e] For this reason, we re-examined the solubility of CDCPC in several alcohols and their aqueous mixtures. Although CDCPC is insoluble in pure methanol and *n*-hexane, it is very soluble in a binary (not even miscible) mixture of these two solvents in almost any ratio. Fortunately, the material is absolutely insoluble in aqueous methanol solutions for any ratio of the components. This opened the possibility to study CDCPC as a CSP for HPLC.

The packing material was prepared by dissolving the CDCPC in THF and coating it onto wide pore aminopropylsilanized silica (Daisogel-1000, 7 μm) as previously described.[1]

Results of a preliminary study presented below confirmed the previous expectations about the very high chiral recognition ability of CDCPC. At first, almost all racemates (Figure 1) previously resolved into enantiomers using this material in *n*-hexane/2-propanol (CDCPC is slightly soluble in this mobile phase and the column was destroyed after several runs)[1] were also resolved in aqueous methanol without any adjustment of the buffer and optimization of the separation conditions (Table 1). In addition, several chiral analytes not studied before (compounds **10**-**20** in Figure 1) were separated into enantiomers in the methanol-water mobile phase. In most cases, the selectivity of the enantioseparation was slightly lower in the aqueous methanol than in the *n*-hexane/2-propanol mobile phase. However, the plate numbers were rather high for the most resolved analytes in the aqueous methanol (Figure 2). Baseline enantioseparation of the several racemates with low capacity factors indicates that the material exhibits a quite high chiral recognition ability even in a polar medium. Under the unbuffered conditions, the neutral compounds were, as expected, better resolved than the charged ones. However, among the well resolved analytes, two contained a tertiary (compound **10**) and a primary amino (compound **20**) group. CDCPC seems to be an especially useful CSP for the separation of chiral binaphthyl and biphenyl derivatives (compounds **13**, **14**, **17**, **18**, and

Table 1. Enantioseparation of chiral compounds (**1**–**20**) of different structural groups using CDCPC as CSP[a]

	k_1'	k_2'	α	Rs		k_1'	k_2'	α	Rs
1[b]	3.00	3.75	1.25	2.7	**11**	1.00	1.12	1.12	1.0
2[b]	4.50	4.70	1.04	0.6	**12**	3.25	3.90	1.23	1.5
3[b]	4.90	4.90	1.00		**13**[b]	0.60	0.80	1.33	1.1
4[b]	2.00	2.25	1.12	1.3	**14**	1.63	2.00	1.23	1.2
5	3.00	3.00	1.00		**15**	3.75	3.75	1.00	
6	0.75	1.00	1.25	1.5	**16**	8.00	8.00	1.00	
7	3.30	3.45	1.05	0.7	**17**	2.50	3.00	1.20	1.5
8	5.80	5.80	1.00		**18**	3.00	4.50	1.50	2.2
9	1.00	1.00	1.00		**19**	3.50	3.50	1.00	
10	4.01	4.60	1.15	1.0	**20**	2.10	2.50	1.16	1.4

[a] For the definitions of k_1', k_2' (capacity factors), α (separation factor), and Rs (resolution factor), see ref. 1a. Column packing and separation conditions were as described in Fig. 2 if not indicated otherwise. [b] Eluent: MeOH/H_2O (70/30); flow rate: 1.0 mL/min.

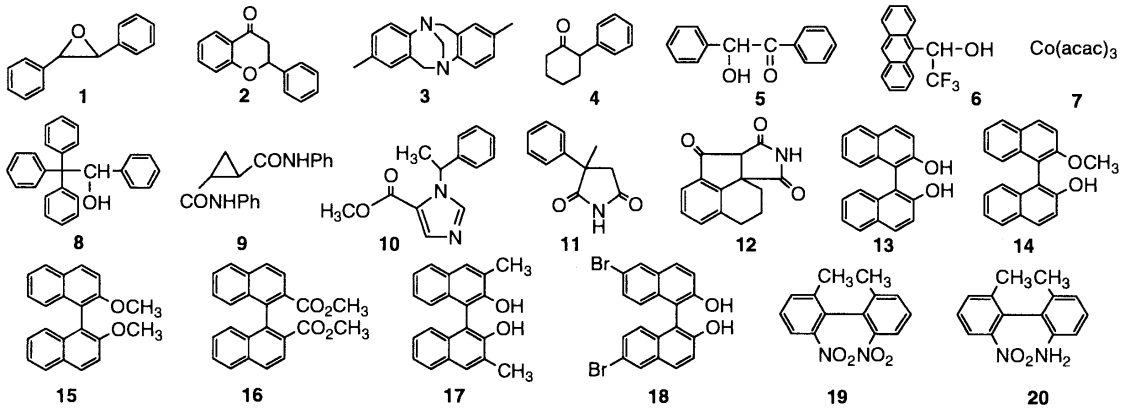


Figure 1. Structure of racemates.

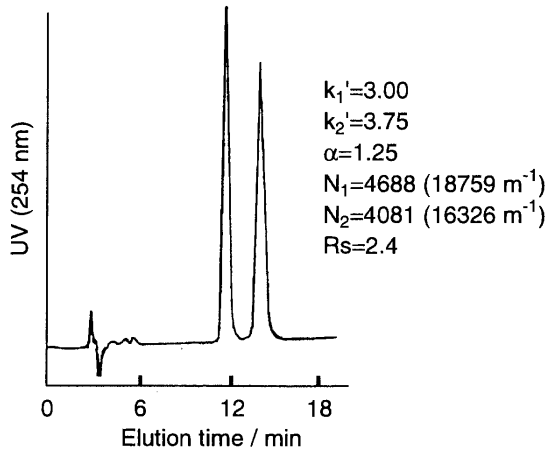


Figure 2. Enantioseparation of *trans*-stilbene oxide using CDCPC (coated 20% w/w on wide pore silica (aminopropylsilanized Daisogel-1000, 7 μm) as chiral stationary phase. Column: 4.6 x 250 mm; mobile phase: methanol/water 75:25 (v/v); flow-rate: 1 ml/min.

20) as well as for enantioseparation of succinimide derivatives (compounds **11** and **12**), an imidazole-containing animal-anesthetic (compound **10**) and antimicotic drugs, etc. Some interesting structure-enantioseparation dependencies were observed in the series of chiral biphenyl and binaphthyl derivatives.

Further studies on the screening of CDCPC under HPLC conditions and on its use in capillary electrochromatography (CEC) are in progress.

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References and Notes

1 a) Y. Okamoto, M. Kawashima, and K. Hatada, *J. Chromatogr.*, **363**, 173 (1986). b) Y. Okamoto and E. Yashima, *Angew. Chem., Int. Ed. Engl.* **37**, 1020 (1998).
2 E. Yashima, C. Yamamoto, and Y. Okamoto, *Synlett*, **1998**, 344.
3 a) B. Chankvetadze, E. Yashima, and Y. Okamoto, *Chem. Lett.*, **1993**, 745. b) B. Chankvetadze, E. Yashima, and Y. Okamoto, *J. Chromatogr. A*, **670**, 39 (1994). c) B. Chankvetadze, E. Yashima, and Y. Okamoto, *J. Chromatogr. A*, **694**, 101 (1995). d) E. Yashima, C. Yamamoto, and Y. Okamoto, *Polym. J.*, **27**, 856 (1995). e) B. Chankvetadze, L. Chankvetadze, S. Sidamonidze, E. Kasashima, E. Yashima, and Y. Okamoto, *J. Chromatogr. A*, **787**, 67 (1997).
4 a) Y. Okamoto, R. Aburatani, S. Miura, and K. Hatada, *J. Liq. Chromatogr.*, **10**, 1613 (1987). b) E. Yashima, H. Fukaya, and Y. Okamoto, *J. Chromatogr. A*, **677**, 11 (1994). c) C. Minguillon, P. Franco, L. Oliveros, and P. Lopez, *J. Chromatogr. A*, **728**, 407 (1996). d) N. Enomoto, S. Furukawa, Y. Ogasawara, H. Akano, Y. Kawamura, E. Yashima, and Y. Okamoto, *Anal. Chem.*, **68**, 2789 (1996).
5 a) K. Ikeda, T. Hamasaki, H. Kohno, T. Ogawa, T. Matsumoto, and J. Sakai, *Chem. Lett.*, **1989**, 108. b) A. Ishikawa and T. Shibata, *J. Liq. Chromatogr.*, **16**, 859 (1993).
6 a) J. Dingenen in: "A Practical Approach to Chiral Separations by Liquid Chromatography," ed by G. Subramanian, VCH, New York (1994), Chapt. 6, pp. 115-181. b) M. Girod, B. Chankvetadze, and G. Blaschke, *J. Chromatogr. A*, accepted. c) M. Meyring, B. Chankvetadze, and G. Blaschke, *J. Chromatogr. A*, accepted.