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(*R*)- and (*S*)-Naphthylethylcarbamate-substituted β-cyclodextrin bonded stationary phases for the reversed-phase liquid chromatographic separation of enantiomers

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ABSTRACT

(*R*)- and (*S*)-naphthylethylcarbamate-β-cyclodextrin bonded phases were originally developed for the normal-phase separation of enantiomers. Although their selectivity resembled that of some of the earlier substituted cellulosic phases, the functionalized cyclodextrin stationary phases were much more stable as they were bonded to the silica support and not adsorbed. Because of their stability, the naphthylethylcarbamate-β-cyclodextrin stationary phase was utilized in reversed-phase separations. It was found that a completely different set of enantiomers was resolved by this column in the reversed-phase mode. This included racemic pesticides such as Dyfonate, Ruelene, Ancymidol and Coumachlor; as well as a variety of pharmacologically active compounds such as Tropicamide, Indapamide, Althiazide, Tolperisone, a sulfonamide from Merck Sharp & Dohme that has been resolved only by indirect methods, and over twenty others. It appears that the naphthylethylcarmabate-β-cyclodextrin bonded phase is a highly effective multimodal chiral stationary phase. The concept of a multimodal chiral stationary phase is discussed. The effect of pH and the configuration of the naphthylcarbamate group is considered as well.

INTRODUCTION

Recently a series of functionalized, cyclodextrin (CD) bonded, stationary phases was introduced for the normal-phase liquid chromatographic (LC) separation of enantiomers [1]. These chiral stationary phases (CSPs) seemed to resemble more closely the functionalized cellulosic stationary phases (produced in Japan) than the original native CD bonded-phase packings [2–4]. They were able to resolve a variety of racemates using either hexane–isopropanol mobile phases or (for more strongly retained polar analytes) mobile phases of 100% alcohol or acetonitrile. Inclusion complexation did not appear to play an important role in these separations. However, unlike the cellulosic stationary phases that are adsorbed onto large pore silica gel, the functionalized CD phases are covalently bonded. This makes them more stable and allows them to be used in a greater variety of mobile phase conditions.

The naphhylethylcarbamate (NEC)-functionalized β-CD stationary phase seemed to be the most widely applicable of all the various derivatives (Fig. 1). Also, the NEC moiety contains a stereogenic center and can be produced in *R*, *S* or racemic forms [1]. In fact the (*R*)- and (*S*)-NEC-β-CDs often had different enantioselectivities

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in normal-phase LC. The number of NEC substituents on the CD affected both the selectivity and efficiency [1,5]. In general, enantioselectivity (*i.e.*, α values) tended to increase with increasing degree of substitution. However, very high degrees of substitution tended to produce stationary phases of poor efficiency (presumably due to mass transfer problems). As a compromise, a degree of substitution of five NEC groups per CD molecule was used. This product seemed to give good selectivity and efficiency for the largest number of racemates [1].

Originally it was thought that the bulky substituents at the mouth of the CD cavity (Fig. 1) would interfere with inclusion complexation, which is very important in the reversed-phase mode. Early, studies with the original native CD bonded phases seemed to indicate that inclusion complexation was necessary for chiral recognition in the reversed-phase mode [4]. Thus, it is no surprise that the derivatized CD columns (operated in the normal-phase mode) and native CD columns (operated in the reversed-phase mode) separate different types of racemates. Thus far no reversed-phase separations have been attempted on the NEC-functionalized β-CD supports. In this work we evaluate the use of these columns in the reversed-phase mode. Particular attention is paid to their enantioselectivity and whether it is similar or different from other cyclodextrin bonded stationary phases in any mode.

EXPERIMENTAL

Materials and methods

Cyclobond I stationary phase was obtained from Advanced Separation Technologies (Whippany, NJ, U.S.A.). (*R*)- and (*S*)-1-(naphthyl)ethyl isocyanate were obtained from Aldrich (Milwaukee, WI, U.S.A.). Pyridine was used as the solvent for the isocyanate-derivatized phases. The mixture was refluxed until all the water was removed (as an azeotrope into a Dean-Stark trap). The derivatizing agent was added (neat) and the mixture was refluxed for about 4 h. The isocyanate-derivatized β-CD bonded phases were collected on a fritted glass filter and washed with approximately

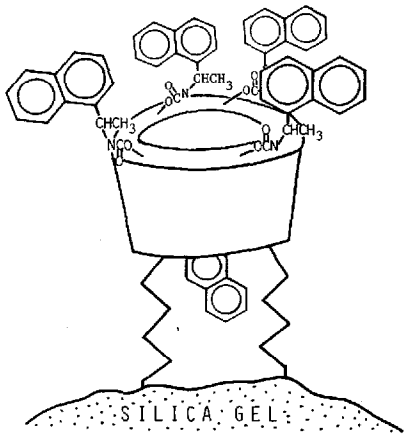


Fig. 1. A simplified model of the NEC-functionalized β-CD bonded stationary phase. In this model the NEC degree of substitution is five and there are two linkage chains from the CD to the silica gel support.

100 ml of pyridine followed by 200 ml of methanol and then air-dried.

The bonded sorbents were submitted for carbon analysis. The surface concentration was calculated according to the equation below:

$$(\mu\text{mol/m}^2) = \frac{\%\text{C} \cdot 10^6}{S[1200N_c - \%\text{C}(M - 1)]}$$

where N_c is the number of carbons in the ligand, M is the molecular weight of the ligand, and S is the surface area of the substrate, which, according to the manufacturer, is 170 m^2/g. For β-CD ($N_c = 42$, $M = 1135$), the coverage was calculated to be 0.20 $\mu mol/m^2$. To determine the degree of substitution on the β-CD of the derivatized β-CD phases, the % C from the CD + linkage chain was subtracted from the total % C and $M - 1$ was substituted by M in the denominator. The degrees of substitution for each of the phases was calculated. The sorbents were all packed into 250 × 4.6 mm I.D. stainless steel columns.

The structures of all resolved racemates are given in Table I. All compounds were obtained from Sigma or Aldrich except for Terodiline (N-*tert.*-butyl-1-1-methyl-3,3-diphenylpropylamine) which was the generous gift of Dr. Marit Olsson (Kabi Pharma, Stockholm, Sweden). Compounds **29** and **30** were the generous gifts of Dr. D. Rothley and Professor Oelschlager (University of Frankfurt). Ancymidol, Ruelene and Dyfonate were obtained from Chem. Service (West Chester, PA, U.S.A.). The 5,6-dihydro-4-[(2-methylpropyl)amino]4H-thieno{2,3-β}thiopyran-2-sulfonamide-7,7-dioxide was obtained from Merck Sharp & Dohme (West Point, PA, U.S.A.).

The chromatographic experiments were done on a Shimadzu LC-6A Chromatograph interfaced with a C-R3A Chromatopac Data System. Detection was accomplished using a variable wavelength detector.

RESULTS AND DISCUSSION

Although the NEC-functionalized β-CD was not expected to be an effective CSP in the reversed-phase mode, the opposite turned out to be true (Fig. 2). Table I lists 30 racemates that were resolved on the (*R*)- or (*S*)-NEC-β-CD bonded phases. It is apparent from this data that the configuration of the NEC group plays an important role in the enantiomeric separation process. Twenty-one of these compounds were resolved best (or exclusively) on the (*S*)-NEC-β-CD column, five resolved best (or exclusively) on the (*R*)-NEC-β-CD, and four of the racemates seemed to resolve equally well on either column. Also the data indicate that pH plays a major role in both the retention and chiral recognition of most of these analytes. This is to be expected for ionizable compounds when using hydro–organic mobile phases. Often pH was shown to be a critical parameter for other reversed-phase CSP-based separations, including the original, native β-CD bonded phases and protein-based stationary phases [6–8]. Optimization of pH improved the resolution of essentially all of the racemates in this study (Table I). Over one third of the compounds could be resolved at one pH but not another (Table I).

The most interesting feature of these reversed-phase separations involves the types of compounds resolved on the (*R*)- and (*S*)-NEC-β-CD columns. In no case were the compounds in Table I effectively resolved by the same columns in the

TABLE I

SEPARATION DATA AND STRUCTURES OF RACEMIC ANALYTES SEPARATED IN THE REVERSED-PHASE MODE ON THE (*R*)- AND (*S*)-NEC-*β*-CD STATIONARY PHASES

k' = Capacity factor of the first eluted enantiomer; α = separation factor; buffer = 1% triethylammonium acetate.

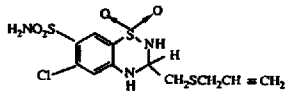
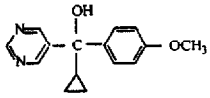
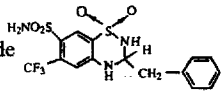
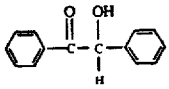
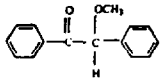
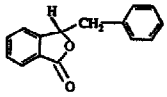
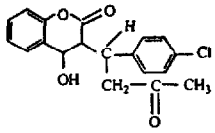
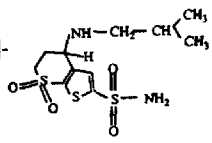
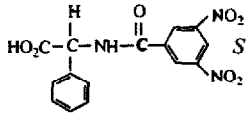
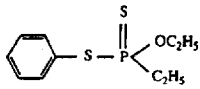
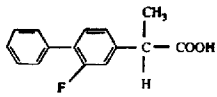
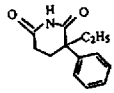
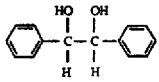
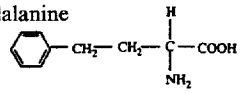
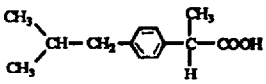
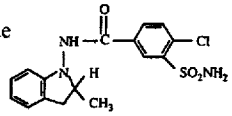
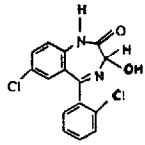
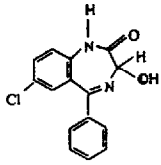
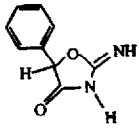
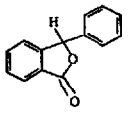
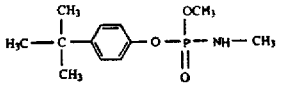
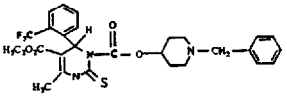
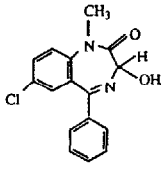
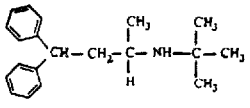
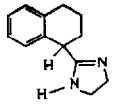
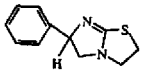
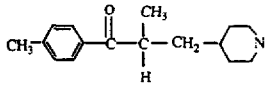
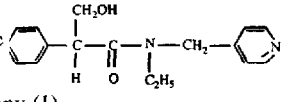
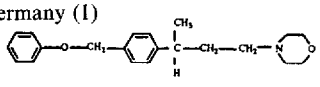
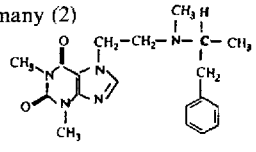
Compound	Structure	Configuration of the NEC group	Mobile phase acetonitrile–buffer	pH of buffer	k'	α
1 Althiazide		*R*	20:80	4.5	8.9	1.03
		S	20:80	7.1	6.8	1.02
		S	20:80	4.5	3.0	1.03
2 Ancymidol		*S*	20:80	7.1	2.9	1.16
		R	20:80	7.1	5.4	1.08
		R	30:70	4.5	2.2	1.06
3 Bendroflumethiazide		*R*	20:80	4.5	19.0	1.02
		S	30:70	7.1	3.0	1.10
		S	30:70	4.5	2.0	1.10
4 Benzoin		*S*	20:80	4.5	3.4	1.04
5 Benzoin methylether		*S*	20:80	4.5	5.6	1.05
6 3-Benzylphthalide		*R*	35:65	7.1	4.4	1.07
		R	40:60	4.5	3.2	1.04
		S	30:70	7.1	4.1	1.06
		S	30:70	4.5	2.7	1.07
7 Coumachlor		*R*	35:65	7.1	6.6	1.04
		R	20:80	7.1	8.9	1.10
		S	20:80	7.1	5.4	1.09
8 5,6-Dihydro-4[(2-methylpropyl)amino]-4H-thieno{2,3-*β*}-thiopyran-2-sulfonamide-7, 7-dioxide						
9 3,5-Dinitrobenzoyl phenylglycine		*S*	50:50	4.5	2.1	1.50

TABLE I *(continued)*

Compound	Structure	Configuration of the NEC group	Mobile phase acetonitrile–buffer	pH of buffer	k'	α
10 Dyfonate		*R*	25:75	7.1	22.0	1.02
		R	30:70	4.5	12.0	1.02
11 Flurbiprofen		*S*	20:80	4.5	25.0	1.04
12 Glutethimide		*S*	30:70	7.1	4.1	1.10
13 Hydrobenzoin		*S*	20:80	4.5	1.6	1.08
14 Homophenylalanine		*S*	5:95	5.5	3.1	1.22
15 Ibuprofen		*R*	35:65	7.1	3.4	1.13
		R	40:60	4.5	5.7	1.07
		S	40:60	7.1	2.8	1.11
		S	30:70	4.5	9.5	1.07
16 Indapamide		*R*	20:80	4.5	9.4	1.04
		S	20:80	7.1	6.0	1.18
		S	20:80	4.5	3.0	1.15
17 Lorazepam		*S*	20:80	4.5	5.0	1.04
18 Oxazepam		*S*	30:70	7.1	1.7	1.15
		S	30:70	4.5	1.2	1.16
19 Pemoline		*S*	20:80	7.1	0.5	1.09
		S	20:80	4.5	0.7	1.12

(Continued on p. 88)

TABLE I *(continued)*

Compound	Structure	Configuration of the NEC group	Mobile phase acetonitrile–buffer	pH of buffer	k'	α
20 3-Phenylphthalide		*R*	35:65	7.1	4.0	1.03
		R	30:70	4.5	6.6	1.03
		S	30:70	7.1	3.6	1.06
		S	30:70	4.5	2.6	1.05
21 Ruelene		*R*	25:75	7.1	14.0	1.03
		R	30:70	4.5	7.3	1.03
22 SQ 31 579		*R*	40:60	4.5	2.1	1.05
		S	20:80	7.1	1.7	1.08
23 Temazepam		*S*	20:80	4.5	5.2	1.03
24 Terodiline		*R*	27:73	7.1	11.0	1.05
		S	27:73	7.1	6.9	1.08
25 Tetrahydrozoline		*S*	30:70	7.1	1.3	1.08
		S	20:80	4.5	0.5	1.13
26 Tetramisole		*R*	20:80	4.5	0.8	1.07
		S	30:70	7.1	2.0	1.10
		S	30:70	4.5	0.3	1.20
27 Tolperisone		*R*	10:90	4.5	6.6	1.09
		S	20:80	7.1	5.8	1.09
		S	20:80	4.5	1.3	1.11
28 Tropicamide		*S*	30:70	7.1	1.2	1.20
		S	30:70	4.5	0.9	1.20
29 West Germany (1)		*R*	20:80	4.5	12.0	1.02
30 West Germany (2)		*R*	20:80	4.5	4.4	1.09
		S	20:80	7.1	8.0	1.06

normal-phase mode. Likewise, most of the racemates previously reported resolved on the (*R*)- and (*S*)-NEC-β-CD columns in the normal-phase mode [1,5], were not resolved in this study. Thus, the NEC-β-CD column acts like two different chiral stationary phases in that it resolves two different sets of enantiomers depending on whether it is used in the normal-phase mode or the reversed-phase mode. This is logical as the separation mechanism is thought to be different in the two modes.

The question arises as to whether the selectivity of the NEC-β-CD columns, in the reversed-phase mode, is similar to that of the original native β-CD bonded phase, which also is used in the reversed-phase mode. Of the thirty compounds in Table I only one (ibuprofen) can be easily resolved on the native β-CD phase. Furthermore, compounds that are easily resolved on the native β-CD column, such dansyl-amino acids, crown ethers and a variety of other biologically active compounds [9], are generally not resolved on the NEC-β-CD column.

From these results it appears that the NEC-β-CD CSP may be the first effective multimodal chiral stationary phase for liquid chromatography. We believe that to be an effective multimodel CSP, several criteria must be met. It is not enough to simply use a normal-phase CSP briefly in the reversed-phase mode thereby achieving a poorer separation of compounds already effectively resolved in the normal-phase mode, or *vice versa*. It is necessary, but not sufficient that a multimodel CSP be stable in both normal-phase and reversed-phase solvents. On the whole, significantly different racemates should be resolved in each mode. Most likely, this would be due to differences in the retention and chiral recognition mechanisms in each mode. Also, it would be useful if there was a logical, systematic approach for the selection and optimization of the various modes. This will be addressed in a subsequent work.

Many of the current chiral stationary phases (*i.e.*, protein, adsorbed derivatized cellulose, etc.) cannot be considered multimodel because of stability problems that prevent their use with either normal- or reversed-phase solvents. Other CSPs such as the π-complex/hydrogen bonding types (Pirkle) and native CD bonded phases have

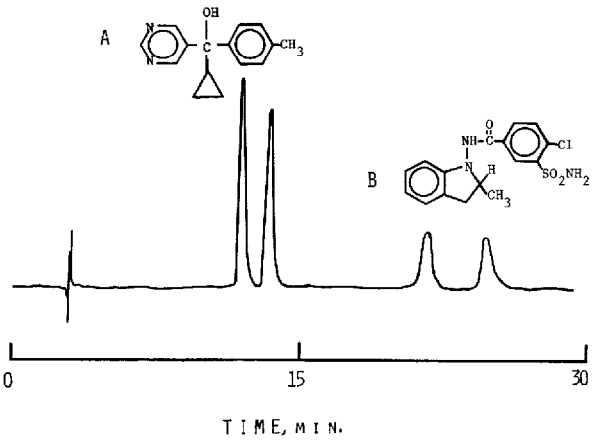


Fig. 2. Reversed-phase chromatogram showing the enantiomeric resolution of (A) the herbicide Ancymidol and (B) the diuretic Indapamide. The separation was done on a (*S*)-NEC-β-CD column. The mobile phase consisted of 1% triethylammonium acetate buffer (pH 7.1)–acetonitrile (80:20, v/v). The flow-rate was 1.0 ml/min.

been used in more than one mode [10,11]. However, thus far they are exceedingly limited in one or the other modes. For example, the CD bonded phase is stable in normal- and reversed-phase solvents. However, >95% of all enantiomers separated with this stationary phase have been in the reversed-phase mode. As far as the resolution of enantiomers is concerned it should not yet be considered an effective multimodal column. Likewise, the π-complex/hydrogen bonded phases are used almost exclusively in the normal-phase mode. Although a reversed-phase separation has been reported [10], these stationary phases often are not as stable in these solvents. More importantly, the enantioselectivity seems to be a diminished version of what is observed in the normal-phase mode.

Because of the plethora of available chiral stationary phases (>50) and their high cost, some consolidation and simplification will occur sooner or later. The development of true, effective multimodal CSPs may be the best way to reduce the total number of CSPs needed and eliminate duplicate phases. This could result in a net savings to those who are involved in enantiomeric separations.

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