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Covalently bonded permethylated cyclodextrins, new selectors for enantiomeric separations by liquid chromatography

H. Riering, M. Sieber*

Macherey-Nagel GmbH, P.O. Box 307, D-52355, Dueren, Germany

Abstract

The properties of chiral stationary phases (CSPs), based on covalently bonded permethylated cyclodextrins, are summarized. Permethylation of cyclodextrin hydroxyl groups markedly changes the chromatographic selectivity in comparison to native cyclodextrins. Often permethylated cyclodextrins allow shorter separation times, e.g. mephobarbital can be separated in less than 10 min. The separation of optical isomers is very dependent on cyclodextrin size and can be optimized for example by temperature, volume fraction of organic modifier and the pH value of the eluent. Several 2-phenoxypropionic acids and esters can be separated very efficiently using these CSPs.

Keywords: Chiral stationary phases, LC; Cyclodextrin phases; Enantiomer separation; Chiral selectors; 2-Phenoxypropionic acids; Hexobarbital; Ciprofibrate; Chlorothalidone

1. Introduction

Cyclodextrins and their derivatives are widely used in gas chromatography (GC) [1], capillary electrophoresis (CE) [2] and high-performance liquid chromatography (HPLC) [3] for the separation of optical isomers. In liquid chromatography they are used as additives to the mobile phase [4] or as covalently bonded selectors for the preparation of chiral stationary phases (CSPs) [5–7]. The hydroxyl groups of the cyclodextrins can be modified with epoxides, carboxylic acid derivatives or isocyanates. Such derivatization steps have an great impact on the selectivities of cyclodextrin bonded CSPs towards racemates [8,9].

Stimulated by the successful use of peralkylated, especially permethylated, cyclodextrin derivatives in GC we have developed a reaction sequence for binding permethylated cyclodextrins to silica surfaces. The preparation of these sorbents was done as reported elsewhere [10]. In the first step an olefinic group is introduced into β-cyclodextrin by reaction with allyl bromide, followed by permethylation of the hydroxyl groups. This selector is covalently bonded to thiol modified silica in the presence of α,α'-azoisobutyronitrile. Here we compare permethylated and native cyclodextrins, both covalently bonded to silica, according to their ability to separate enantiomers. The chromatographic properties of these CSPs are presented.

*Corresponding author.

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2. Experimental

2.1. Materials

HPLC-grade solvents were used for the preparation of the eluents. They were obtained from Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), and Riedel-deHaën (Seelze, Germany). Triethylamine, acetic acid, sodium dihydrogen phosphate and phosphoric acid (reagent grade) were obtained from Fluka (Buchs, Switzerland). Triethylammonium acetate solutions (TEAA) were prepared by dissolving triethylamine in water (vol%) and adjusting the pH value with acetic acid. The various solutes were of reagent grade or higher purity and obtained from several sources. The chemical structures are shown in Fig. 1.

2.2. Equipment

The HPLC system used for these experiments consisted of Shimadzu LC 6A pumps, a SPD 6A UV detector and a SCL 6B system controller. Chromatograms were recorded by a Kipp and Zonen BD111 x,t-plotter.

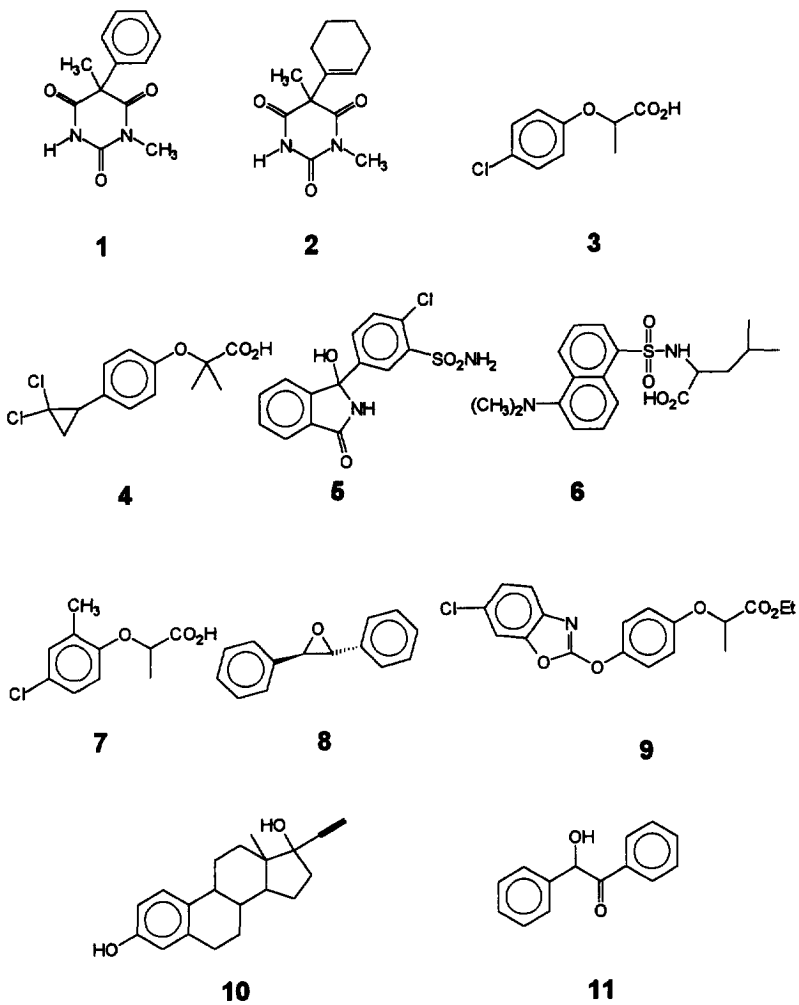


Fig. 1. Chemical structures of the analytes.

2.3. HPLC-columns

For these investigations the following HPLC columns (200×4 mm I.D.) were used: ET 200/4 NUCLEODEX β-OH, ET 200/4 NUCLEODEX α-PM, ET 200/4 NUCLEODEX β-PM and ET 200/4 NUCLEODEX γ-PM (Macherey Nagel, Dueren, Germany). The chiral selector of NUCLEODEX β-OH is native β-cyclodextrin. Permethylated α-, β- and γ-cyclodextrins are used for the sorbents NUCLEODEX α-, β- and γ-PM. The dead time (t_0) of each column was determined by injection of methanol. Capacity factors (k') were calculated as $k'=(t_1-t_0)/t_0$ and separation factors (α) as $\alpha=k'_2/k'_1$. Separations presented in this paper were done under optimized eluent conditions.

3. Results and discussion

3.1. Comparison of native and permethylated β-cyclodextrin

The separation of mephobarbital (**1**) was performed on NUCLEODEX β-OH with native β-cyclodextrin and NUCLEODEX β-PM containing permethylated β-cyclodextrin. A better selectivity at reduced capacity factors was found for the CSP with modified hydroxyl groups as shown in Fig. 2. In consequence, baseline separation of this analyte is achieved in less than 10 min. The chromatographic results of the separation of hexobarbital (**2**), 2-(*p*-chlorophenoxy)propionic acid methylester (**3**), ciprofibrate (**4**), chlorthalidone (**5**) and dansyl leucine (**6**) are summarized in Table 1. NUCLEODEX β-PM is able to resolve the first three racemates (**2**, **3** and **4**) much better than NUCLEODEX β-OH. On the other hand native β-cyclodextrin has an increased selectivity towards the optical antipodes of chlorthalidone (**4**) and dansyl leucine (**5**).

3.2. Influence of cyclodextrin size

Cyclodextrins are cyclic oligosaccharides that form cavities. They are built up from six (α-cyclodextrin), seven (β-cyclodextrin) and eight (γ-cyclodextrin) glucose units . The different number of glucose units leads to different internal diameters of

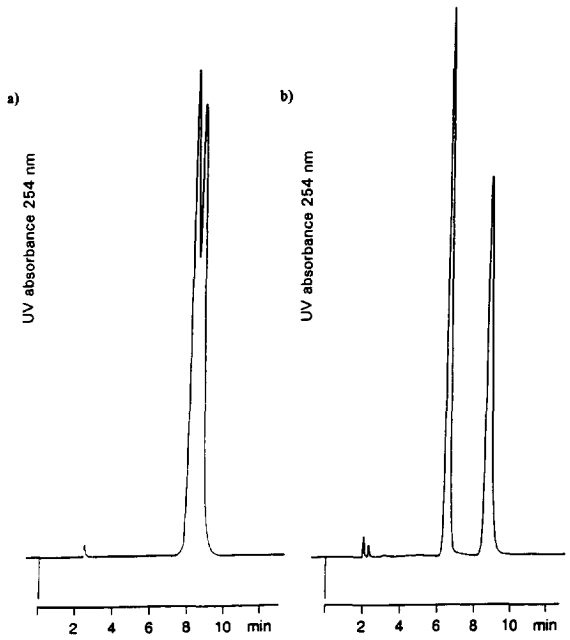


Fig. 2. Separation of mephobarbital (a) ET 200/4 NUCLEODEX β-OH, methanol–0.1% TEAA, pH 4.0 (55:45, v/v); 0.7 ml/min; UV, 254 nm. (b) ET 200/4 NUCLEODEX β-PM, methanol–0.1% TEAA, pH 4.0 (55:45, v/v); 0.7 ml/min; UV, 254 nm.

the cavities (5.7 Å for α-, 7.8 Å for β- and 9.5 Å for γ-cyclodextrin) [3]. Fig. 3 shows the change of selectivities for some racemates separated on NUCLEODEX α-PM, β-PM and γ-PM. Enantiomers of Mecoprop (**7**), a common herbicide in agriculture, can be resolved by NUCLEODEX α-PM with the smallest cyclodextrin ring. The other two permethylated selectors are not able to distinguish between the optical antipodes of this compound. Also, NUCLEODEX α-PM shows the best separation profile for the resolution of *trans*-stilbene oxide (**8**), followed by NUCLEODEX γ-PM. In this case permethylated β-cyclodextrin fails to achieve resolution of the enantiomers, but it is the column of choice for the separation of fenoxaprop ethyl (**9**). The optical isomers of ciprofibrate (**4**) can be resolved very easily with permethylated α- or β-cyclodextrin. In this case no resolution can be obtained using NUCLEODEX γ-PM. The diastereomers of the steroid ethinylestradiol (**10**) are separated by NUCLEODEX β- and γ-PM, but not by NUCLEODEX α-PM. The last compound, which has been investigated in this series, is benzoin (**11**).

Table 1
Comparison of native and permethylated β-cyclodextrin

Analyte	NUCLEODEX β-OH		NUCLEODEX β-PM	
	$k'_{(1)}$	α	$k'_{(1)}$	α
Hexobarbital (**2**)	11.5	1.10	1.64	1.45
2-(*p*-Chlorophenoxy)propionic acid methylester (**3**)	3.61	1.05	2.19	1.21
Ciprofibrate (**4**)	11.5	1.13	2.88	1.23
Chlorthalidone (**5**)	2.61	1.28	2.80	1.08
Dansyl leucine (**6**)	0.75	1.73	11.8	ca. 1

Chromatographic conditions: methanol–0.1% TEAA, different compositions; 0.7 ml/min; UV detection.

The large permethylated γ-cyclodextrin can easily distinguish between the optical antipodes of this compound.

3.3. Chromatographic conditions

Columns of the NUCLEODEX family are normally used under reversed-phase conditions. Separations using typical normal-phase eluents (*n*-heptane) did not lead to sufficient resolutions in our work up to now. Fig. 4 shows the influence of an organic modifier on the separation of ciprofibrate (**4**). As expected under reversed-phase conditions, an increased amount of methanol leads to reduced retention times. A low pH value (about 4) is necessary for reducing the ionization of the carboxylic acid group in this case. Another example of the influence of pH on the chromatographic behavior is shown in Fig. 5 for the separation of mecoprop (**7**). The protonation of the carboxylic group reduces the polarity of the analyte resulting in longer retention times acompanied by increasing selectivities. Changes in temperature have a great effect on the retention of analytes using sorbents with covalently bonded cyclodextrins. The binding constant of an analyte is significantly affected by increasing temperatures, which leads to reduced retention times [11]. A similar behavior has been observed for permethylated cyclodextrins for the separation of ethinylestradiol, which is demonstrated in Fig. 6. Higher temperatures lead to smaller k' values of the diastereomers.

3.4. Separation of 2-phenoxypropionic acids and esters

2-Phenoxypropionic acids and esters are widely used as herbicides in agriculture. Fig. 7 shows the

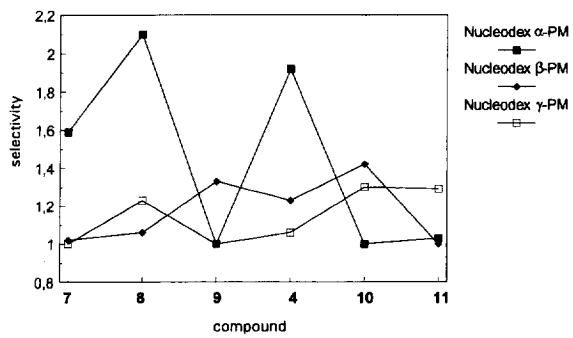


Fig. 3. Comparison of permethylated α-, β- and γ-cyclodextrin for the separation of optical isomers. Chromatographic conditions: methanol–0.1% TEAA, different compositions; 0.7 ml/min; UV detection. The numbers of the compounds refer to the structures given in Fig. 1.

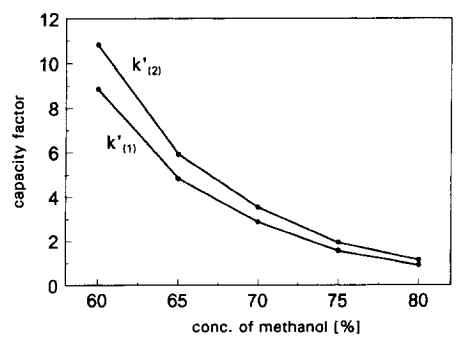


Fig. 4. Separation of ciprofibrate (**4**) using different volume fractions of methanol. Chromatographic conditions: ET 0200/4 NUCLEODEX β-PM, methanol–50 m*M* NaH_2PO_4, pH 4.0; 0.7 ml/min; UV, 230 nm.

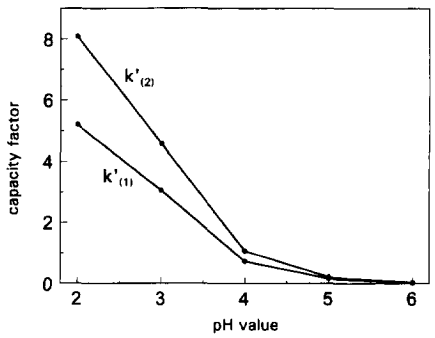


Fig. 5. Separation of mecoprop (**7**) at different pH values using ET 200/4 NUCLEODEX α-PM. Chromatographic conditions: methanol–50 m*M* NaH_2PO_4, pH 4.0; 0.7 ml/min; UV, 230 nm.

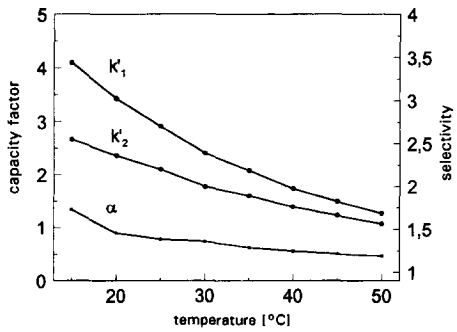


Fig. 6. Separation of ethinylestradiol (**10**) diastereomers at different temperatures using ET 200/4 NUCLEODEX β-PM. Chromatographic conditions: methanol–water (70:30, v/v); 0.7 ml/min; UV, 278 nm.

general formula for this class of substances. The α-carbon atoms of these propionic acid derivatives are chirality centres. The compounds differ by their substitution pattern at the aromatic ring (R^1, R^2, and R^3 can be, for example, H, Me, Cl or aromatic substituents). Esters and free acids are available (R^4 can be, for example, H, Me or Et).

Surprisingly, the separation of mecoprop (**7**) (R^1=Me, R^2=Cl, R^3, R^4=H) as the free acid is only possible using NUCLEODEX α-PM, whereas the methyl ester of this compound is resolved by NUCLEODEX α-PM and NUCLEODEX β-PM. The same behaviour is obtained for dichlorprop (R^1, R^2=Cl, R^3, R^4=H). Fig. 8 shows the selectivities of some mecoprop esters. They differ in the length of alcoholic component of the carboxylic ester group. NUCLEODEX α-PM shows a nearly constant selectivity towards these enantiomers. The corresponding permethylated β-cyclodextrin has a lower selectivity for the methyl ester, but the separation of the ethyl and *n*-propyl derivatives is better. The extrapolation of this series to the small hydrogen atom explains the ability of NUCLEODEX α-PM and the inability of NUCLEODEX β-PM to separate the free acid mecoprop (**7**). Stimulated by these results we have separated further 2-phenoxypropionic acids and esters on NUCLEODEX α-PM and NUCLEODEX β-PM. Table 2 summarizes the results. The class of 2-phenoxypropionic acids can be divided into three different groups. The first one has one or two small substituents such as methyl, chlorine or hydroxyl at the aromatic ring (e.g. mecoprop, dichlorprop). Columns with permethylated α-cyclodextrin are able to separate both the free acids and the esters. A further substitution (e.g. fenoprop R^1, R^2, R^3=Cl, R^4=H) leads to the second group and results in the failure of the permethylated α-cyclodextrin to achieve separation, but fenoprop can be sufficiently resolved by NUCLEODEX β-PM. The third group contains compounds like fenoxaprop or diclofop with large

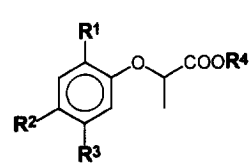


Fig. 7. General formula of the investigated 2-phenoxypropionic acids and esters. See for explanation of the substituents.

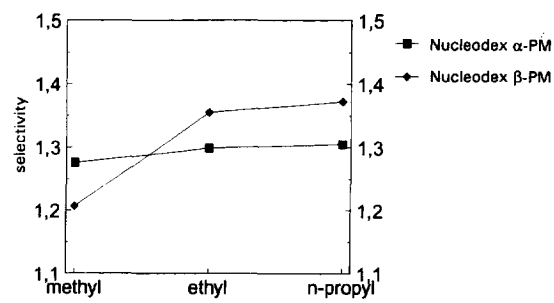
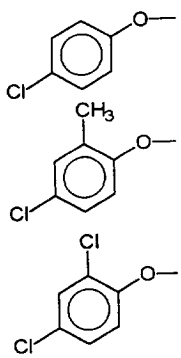
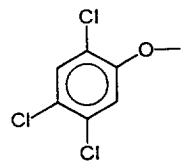
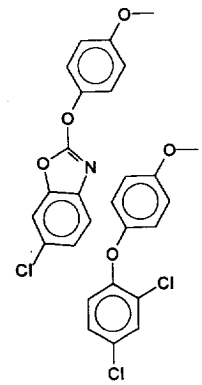


Fig. 8. Selectivities of mecoprop methyl, ethyl and *n*-propyl ester. Chromatographic conditions: ET 200/4 NUCLEODEX α-PM and ET 200/4 NUCLEODEX β-PM, methanol–0.1% TEAA, pH 4.0 (60:40, v/v); 0.7 ml/min; UV, 230 nm.

Table 2
Separation of further 2-phenoxypropionic acids and esters

	R^1, R^2=H, Cl, Me		R^1, R^2, R^3=H, Cl, Me		R^2=large substituents	
	Ester	Acid	Ester	Acid	Ester	Acid
NUCLEODEX α-PM	++	++	−−	−−	−−	−−
NUCLEODEX β-PM	++	−	+	++	−−	
Structure of the aromatic group						

++, good separation; +, separation; −, low selectivity; −−, no separation.
Chromatographic conditions: methanol/50 m*M* NaH_2PO_4, pH 3.0, different compositions; 0.7 ml/min; UV 230 nm.

substituents at the aromatic ring. In this case only the methyl or ethyl esters can be separated by permethylated β-cyclodextrin. No resolution can be obtained with NUCLEODEX α-PM.

3.5. General discussion

Native and permethylated cyclodextrins differ strongly in their selectivity towards optical isomers. The free hydroxyl groups of native β-cyclodextrin are necessary for the chiral resolution of chlorthalidone and dansyl leucine but, for example in the case of mephobarbital (**1**), these interactions lead to increased retention times for both enantiomers. Here, the permethylated cyclodextrin derivative shows an improved selectivity and a reduced retention time. The permethylation of the hydroxyl groups influences the interactions between the analytes and the cyclodextrin rim. The substitution of the hydroxy groups by methoxy groups leads to an increased hydrophobicity of the cyclodextrin rims and a change in the depth of the cavity. Another important difference between native and permethylated cyclodextrins is the ability of the selectors to form hydrogen bonds. A native cyclodextrin with free hydroxy groups is both a proton donator and acceptor while the permethylated derivative shows only proton acceptor properties.

The selectivities of sorbents based on permethylated cyclodextrins depend strongly on the size of the cyclodextrin ring. It is known that cyclodextrins can form inclusion complexes [3,12]. A part of the analyte penetrates into the lipophilic cavity. This explains why larger molecules are preferably separated with NUCLEODEX β-PM or γ-PM. Fenoxaprop methyl can be separated by NUCLEODEX β-PM but not by NUCLEODEX α-PM. Apart from the formation of inclusion complexes, the orientation of the analyte in the cavity also influences the selectivity. 2-Phenoxypropionic acids and esters differ in their substitution pattern on the aromatic ring. These substituents have a strong influence on the formation of inclusion complexes with different cyclodextrins by their different sterical demands. Multiple or large substituents on the phenyl group

result in no resolution with NUCLEODEX α-PM (small permethylated α-cyclodextrin). These compounds can be resolved by NUCLEODEX β-PM. Further important interactions with polar groups at the cyclodextrin rim take place with the carboxylic groups. This seems to be necessary for the correct fit of the analyte into the cyclodextrin cavity. Large esters (R^4=Me, Et) are better separated by NUCLEODEX β-PM, whereas NUCLEODEX α-PM is preferable for the separation of the free acids: mecoprop (**7**), dichlorprop (R^3, R^4=H, R^1, R^2=Cl) and hydroprop (R^1, R^3, R^4=H, R^2=OH).

4. Conclusions

Permethylated cyclodextrins, covalently bonded to carriers like silica, can be used in HPLC columns for the separation of optical isomers. They are able to separate many different types of enantiomers under reversed-phase conditions with good resolutions and short retention times. These sorbents expand the applicability of cyclodextrin phases. Further investigations of the properties are in progress.

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