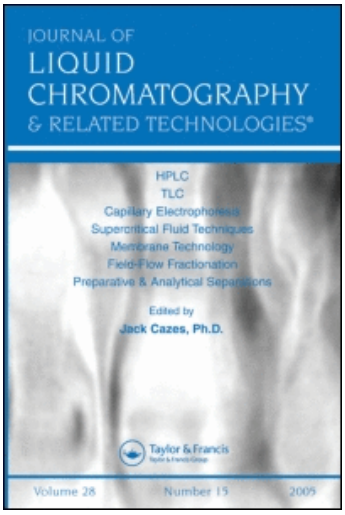

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STUDY OF MECHANISM OF ENANTIOSEPARATION. II. HPLC CHIRAL ANALYSIS OF ALKOXYSUBSTITUTED ESTERS OF PHENYLCARBAMIC ACID

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ABSTRACT

The chromatographic separation of enantiomers of a series of 2-alkoxysubstituted esters of phenylcarbamic acid (C_1-C_{10}) were studied on (β- and γ-) cyclodextrin chiral stationary phases. The pH and concentration of organic modifier in the mobile phase were optimized. It was observed that the chromatographic performance of basic compounds could be strongly enhanced by

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increasing the pH of the mobile phase. Increasing the pH produced higher values for the enantiomeric resolution and the retention factors. This work demonstrated that the nonpolar interactions between the chiral stationary phase and the neutral molecules of these enantiomers had the greatest effect on enantioresolution.

INTRODUCTION

HPLC has been a dominant method for the separation of chiral compounds. Enantiomeric selectivity usually is achieved through the appropriate choice of a chiral stationary phase and mobile phase condition. In many publications it has been demonstrated that cyclodextrins (CDs) have the ability to achieve stereoselective association with a variety of different enantiomeric molecules.[1–3] In the past twenty years, much research has centred on the use of CDs as chiral stationary phases for the LC separation of enantiomers in the reversed-phase, normal-phase, and polar-organic mode. Enantiomeric separations on native CDs in the normal-phase mode are not common. However, they can be achieved on aromatic derivatized CD stationary phases.[4] The chiral recognition mechanism in reversed-phase CD separations is thought to be the result of the formation of an inclusion complex in which the hydrophobic solute is included into the CD cavity. The separation mechanism in the polar-organic mode centers on the interaction with the hydroxyl groups on the CD stationary phase. The retention and enantioselectivity mechanisms are probably due to hydrogen bonding and dipolar interactions.[2]

Alkoxysubstituted esters of phenylcarbamic acids form a group of potential drugs that can be employed as local anaesthetics. The enantiomeric separation of derivatives of phenylcarbamic acid can be performed by means of different chromatographic techniques, including GC, TLC, or LC. In LC, different chiral stationary phases have been used for the separation of these enantiomers. Some chiral stationary phases consisted of cellulose derivatives, especially, cellulose TRIS-3,5-triphenylcarbamate.[3] Others were based on the use of β-cyclodextrin,[5] and α_1-acid glycoprotein.[6,7] Some π-complex type columns were also reported to be suitable for achieving chiral separation of derivatives of phenylcarbamic acid.[8,9]

The aim of the present study was the separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid using cyclodextrin chiral stationary phases (β- and γ-CD) in the reversed-phase mode. The effect of the structure of the compounds on the resolution of these enantiomers was studied.

EXPERIMENTAL

Chemicals and Reagents

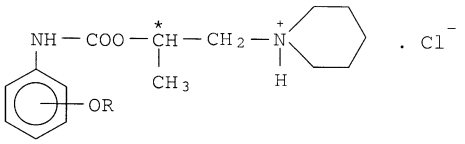
Methanol and acetonitrile of HPLC grade was obtained from Merck. All other chemicals were of analytical reagent grade. The 1-methyl-2-piperidinoethyl esters of 2-alkoxyphenylcarbamic acid were synthesised by standard procedure.[10] Structures of the compounds are shown in the Fig. 1.

Apparatus

The chromatographic system (Hewlett Packard series 1100) consists of a quaternary pump, an injection valve, Rheodyne, with 20 μL injection loop, and a photodiode array detector. The cyclodextrin chiral stationary phases, LiChroCart Chiradex BETA and LiChroCart Chiradex GAMMA (250 × 4 mm I. D. 5 μm), were obtained from Merck.

Chromatographic Conditions

The experiments were carried out at 25°C. A flow rate of 1 mL/min was used. UV detection at 240 nm was used. The analytes were dissolved in methanol (concentrations were 0.1 mg/mL).



-OR in 2- position

Nr.	R	Nr.	R
1	CH_3	6	C_6H_{13}
2	C_2H_5	7	C_7H_{15}
3	C_3H_7	8	C_8H_{17}
4	C_4H_9	9	C_9H_{19}
5	C_5H_{11}	10	$C_{10}H_{21}$

Figure 1. Chemical structures of the 1-methyl-2-piperidinoethylesters of 2-alkoxyphenylcarbamic acid that were examined.

Preparation of Mobile Phases

Mobile phases were mixtures of organic modifiers (methanol, acetonitrile) and solutions of 7.17 mmol/L triethylamine in water or 0.1 mol/L sodium acetate in water. The desired pH values (4.0–7.5) of the aqueous position of the mobile phase was obtained by addition of acetic acid, and a constant value of ion strength ($I = 0.65$ mol/L) was achieved by using lithium chloride.

The Measurement of Optical Rotation

For the measurement of optical rotation, the polarimeter Polar LμP (Na lamp, $\lambda = 589$ nm) (IBZ Messtechnik) was used. After the separation, the enantiomers were collected, sequentially, to measure their optical properties. Preconcentration of the enantiomers in the collected fractions were done by evaporation with nitrogen stream.

RESULTS AND DISCUSSION

Influence of the Organic Modifier

Optimisation of the reversed phase separations was done by controlling the nature and the amount of organic modifier added. The selectivity was affected by the type of organic modifier used, i.e., methanol or acetonitrile. Figure 2 shows the concentration effect of different organic modifiers on the retention of analyte 1 (Fig. 1). As expected, the values of the retention factors are greatest when eluting with the lowest percentage of organic modifier. The retention factors decreased with increasing concentrations of both organic modifiers. A major difference in the retention behaviour of analytes in the presence of these two organic solvents was found at higher modifier concentrations (>40%). In the acetonitrile experiments, the retention increased when the amount of modifier increased from 40% to 100% (v/v). This did not occur in the case of methanol. Similar results has been discussed in literature.[2,11] For the next series of experiments, a mobile phase containing acetonitrile as the organic modifier was used, since it produced better separations of the enantiomers studied (e.g., higher values of R_s were obtained).

The influence of the lipophilicity of the studied compounds on retention is established via the principle of reversed-phase LC. Basically, increasing the lipophilicity (i.e., the number of methylene units in the alkoxy-chain, R) of the molecule increases the values of the retention factors as shown in Table 1.

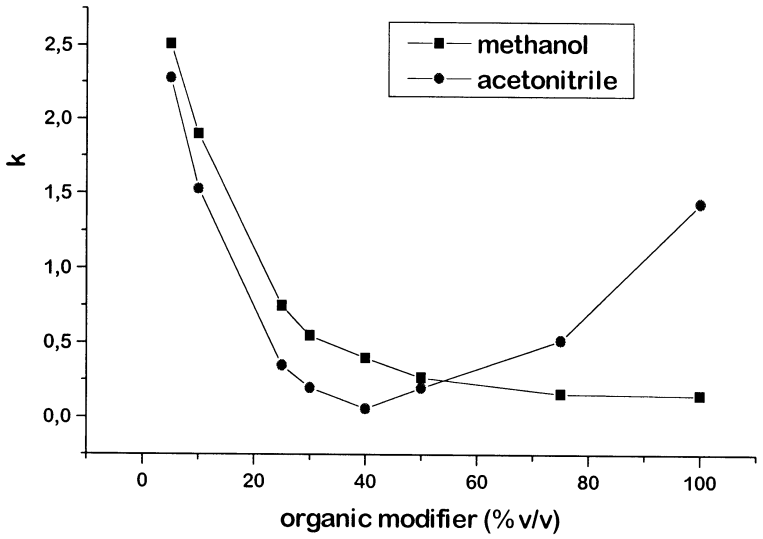


Figure 2. Reversed-phase retention of the analyte 1 as a function of mobile phase composition on β-CD column. Mobile phase: methanol/water (■), acetonitrile/water (●), other conditions: see experimental.

Table 1. The Influence of the Lipophilicity of Studied Compounds on the Retention Factor of First Eluted Enantiomers (k_1) on β-CD and γ-CD Columns. Conditions: See Experimental

Analyte	Number of C Atoms in R	β-CD		γ-CD
		k_1[a]	k_1[b]	k_1[b]
1	1	0.65	0.95	0
2	2	0.73	1.07	0
3	3	0.95	1.38	0.94
4	4	1.74	2.46	1.22
5	5	4.19	6.02	2.16
6	6	5.94	14.97	3.29
7	7	10.98	18.24	5.84
8	8	16.27	>20	10.39
9	9	>20	>20	15.85
10	10	>20	>20	>20

[a]Mobile phase: 7.17 mmol/L pH = 5 triethylamine acetate–acetic acid/acetonitrile 95/5 v/v.
[b]Mobile phase: 0.1 mol/L pH = 5 sodium acetate–acetic acid/acetonitrile 95/5 v/v.

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Influence of pH

The effect of pH of the mobile phase buffer (7.17 mmol/L triethylamine in water/acetonitrile 95/5 v/v, with pH was adjusted by addition of acetic acid) on enantioresolution (R_s) and enantioselectivity (α) is given in Figs. 3 and 4. Increasing the pH in the range from 4.0 to 7.5 had a significant influence on the values of the retention factors, but only small effects on the enantioresolution. The positive influence of increasing the pH at a constant value of ionic strength, on enantioresolution, is probably due to the fact that both retention and enantioselectivity are greater for the unionized analytes (pK_a 5.7–8.4).

Influence of Ring Size of CD and the Influence of Length of Alkoxysubstituent in Esters of Phenylcarbamic Acid

HPLC separation of enantiomers of 2-alkoxysubstituted esters of phenylcarbamic acid was studied on β- and γ-CD chiral stationary phases

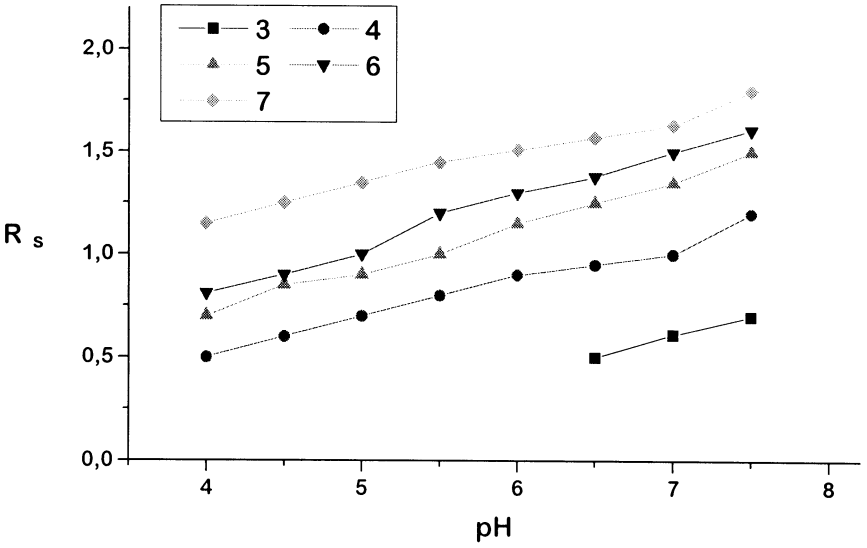

Figure 3. Effect of the pH of the mobile phase on R_s of the enantiomers of 2-alkoxysubstituted esters of phenylcarbamic acid on β-CD column. Mobile phase: 7.17 mmol/L triethylamine in water/acetonitrile 95/5 v/v, pH of triethylamine solution was adjusted with acetic acid, other conditions: see experimental.

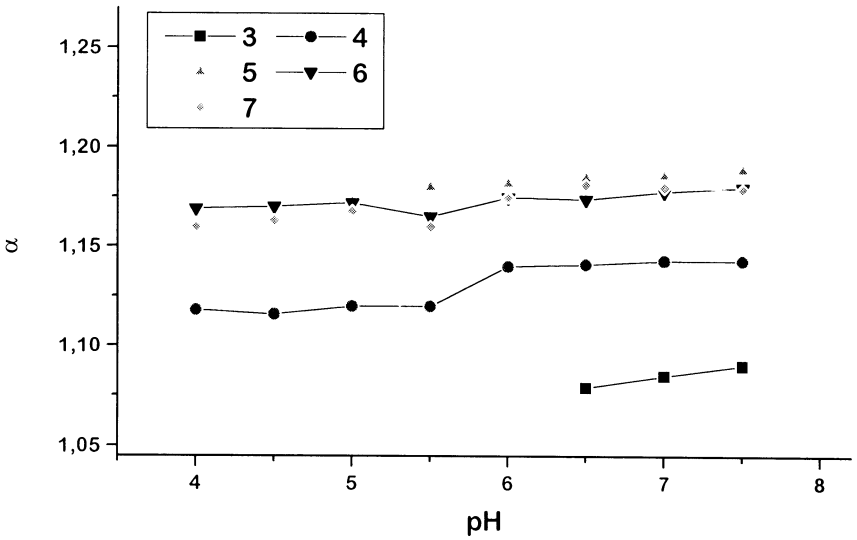

Figure 4. Effect of the pH of the mobile phase on α of the enantiomers of 2-alkoxysubstituted esters of phenylcarbamic acid on β-CD column. Mobile phase: 7.17 mmol/L triethylamine in water/acetonitrile 95/5 v/v, pH of triethylamine solution was adjusted with acetic acid, other conditions: see experimental.

using mobile phases of 0.1 mol/L pH = 5 sodium acetate–acetic acid/acetonitrile 95/5 v/v and 7.17 mmol/L pH = 5 triethylamine acetate–acetic acid/acetonitrile 95/5 v/v. The enantiomers of these compounds were separated on both chiral stationary phases. The retention factor values obtained on the chiral stationary phases are summarised in Table 1. It is evident, that by using a β-CD chiral stationary phase, higher values of retention factors were obtained compared with γ-CD. For the β-CD column, there was also a greater influence on the length of the alkoxysubstituent of the phenylcarbamic acid on the values of the retention factors. It was found, that the β-CD column is better suited for the chiral separation of analytes containing C_3–C_6 alkoxysubstituents on the aromatic ring (analytes 3–6). Enantiomers of analytes containing longer alkoxy chains (C_7–C_{10}, analytes 7–10) had very high retention factors. Lower values of the retention factors for the analytes studied were achieved by using the γ-CD column with the same composition of mobile phase.

The influence of the cyclodextrin ring size on enantioselectivity and the resolution values of the enantiomers are summarised in Table 2. It is evident that the higher resolution values were obtained on the β-CD chiral stationary phase.

Table 2. Influence of Ring Size of Cyclodextrin on Selectivity and Enantioresolution of Enantiomers of 2-Alkoxysubstituted Esters of Phenylcarbamic Acid. Conditions: See Experimental

	β-CD[a]		β-CD[b]		γ-CD[b]	
Analyte	α	R_s	α	R_s	α	R_s
3	1.009	0.51	1.001	0.50	1.000	0
4	1.120	0.73	1.126	0.79	1.000	0
5	1.173	0.96	1.158	1.00	1.000	0
6	1.172	1.15	1.151	1.10	1.000	0
7	1.168	1.35	1.155	1.25	1.000	0
8	1.170	1.52	npm	npm	1.091	1.02
9	npm	npm	npm	npm	npm	npm

[a]Mobile phase: 7.17 mmol/L pH = 5 triethylamine acetate–acetic acid/acetonitrile 95/5 v/v.
[b]Mobile phase: 0.1 mol/L pH = 5 sodium acetate–acetic acid/acetonitrile 95/5 v/v.
npm: not possible to measure because the value of the retention factor was higher than 20.

In the case of γ-CD CSPs, very poor enantioseparation was achieved for analytes containing C_1–C_7 alkoxysubstituents. Enantiomers of analyte 8 were separated with a R_s value of about 1. It was not possible to evaluate analytes 9 and 20 since their retention factors were greater than 20. Enantiomers of the analytes with the shortest alkoxysubstituents in R (C_1–C_3, analytes 1–3) were not separated on either of the tested cyclodextrins chiral stationary phases.

On the basis of the obtained results, it can be presumed that the retention of alkoxysubstituted esters of phenylcarbamic acid on cyclodextrin chiral stationary phases are primarily dependent on non-polar interactions. Clearly, increasing the number of carbon atoms in the alkoxysubstituent increases both the retention factor and the resolution of these compounds.

Cyclodextrin chiral stationary phases were not suitable for the separation of enantiomers of the related 3- and 4-alkoxysubstituted esters of phenylcarbamic acid. No enantiomeric separations were achieved for these compounds under the reversed phase conditions of this study.

Elution Order of Enantiomers

The elution order of enantiomers was determined by measuring the optical rotation of each peaks after HPLC separation for compound 1. The first eluted

enantiomer of the tested analyte rotated the plane of polarised light (wavelength 589 nm) to the right (+) and the second eluted enantiomer shows the opposite rotation. In the case of macrocyclic antibiotics chiral stationary phases (vancomycin, teicoplanin), the elution order of enantiomers with 3- and 4-alkoxysubstitution on the aromatic ring (enantiomers of the 2-derivatives were not separated) was the same.[12,13]

CONCLUSION

2-Alkoxysubstituted derivatives better fit the cavity of β-CD, and chiral separation of enantiomers was obtained in comparison with the 3- and 4-alkoxysubstituted derivatives where the enantioseparation was not achieved. The non polar interactions between the chiral stationary phase and the molecules of these enantiomers, had the greatest effect on enantioresolution. Obtained results are complimentary to those achieved on stationary phases containing macrocyclic antibiotics as chiral selector (vancomycin, teicoplanin), as each class of CSP separates enantiomers of some structural isomers, but not others.[12,13]

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