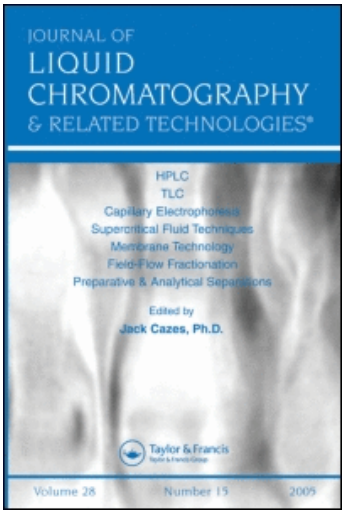

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Enantiomer Separations by Normal HPLC Systems with Permethylated β-Cyclodextrin Dynamically Coated on Silica Solid Supports

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ENANTIOMER SEPARATIONS BY NORMAL HPLC SYSTEMS WITH PERMETHYLATED β-CYCLODEXTRIN DYNAMICALLY COATED ON SILICA SOLID SUPPORTS

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ABSTRACT

The paper describes the technique of solvent-generated liquid-solid chromatography with a chiral stationary phase consisting of permethylated β-cyclodextrin coated on a bare silica surface. The chiral adsorption layer was generated dynamically by pumping diluted solutions of permethylated β-cyclodextrin in binary (hexane/different alcohols) eluents through a column packed with microparticulate silica. This technique leads to column with excellent time stability and good reproducibility of the enantioselectivity. It was found that retention characteristics e.g. enantioselectivity and capacity depend very strongly on the type and concentration of the alcohol used as mobile phase component.

INTRODUCTION

Previous work has demonstrated that solvent-generated liquid-liquid and reversed phase liquid-solid chromatographic

systems with cyclodextrins (CD) are a powerful tool for the solution of many enantioseparation problems [1-5]. Recently it has been shortly reported that the technique of solvent-generated liquid-solid chromatography can be also used to make use of the chiral separation potential of alkylated β-CD in normal phase systems [6,7]. Armstrong *and co-workers* reported the usage of normal phase systems with β-CD derivatives chemically bonded to silica for separation of enantiomers [8]. The method described here opens a second and complementary possibility to realize normal phase systems with β-CD chiral components.

This paper contains the results of a more detail study of the adsorption of the chiral agent on microparticulate silica, the optimization of the enantioselectivity by changing the eluent composition and of the stability and reproducibility of normal phase systems with permethylated β-CD (TM-β-CD) dynamically coated on a silica surface.

EXPERIMENTAL

Apparatus and Procedures

Microbore chromatographic experiments and the chromatographic determination of the retardation of TM-β-CD on hydrophilic solid support were done according to the methods and with the instruments described previously [6,7].

Reagents

TM-β-CD was supplied by Chinoin (Budapest, Hungary). All reagents and solvents were of analytical grade and were used without purification. LiChrosorb Si60 and LiChrospher Si100 both 5 μm (Merck); Partisil, 7 μm (Whatman) and Hypersil, 3 μm (Shandon) were used as hydrophilic solid support.

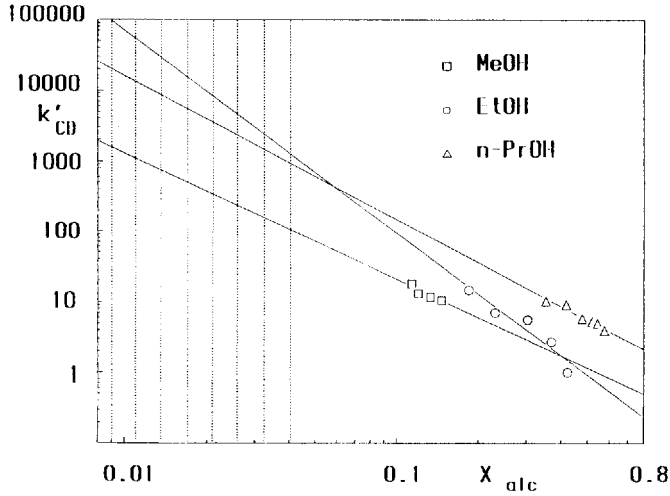

FIGURE 1. Influence of alcohol type and concentration on retardation of TM-β-CD on silica surface (LiChrosorb Si60, 5 μm) from binary hexane-alcohol solution.

DISCUSSION AND RESULTS

Adsorption of Permethylated β-CD on Silica Surface

It has previously been demonstrated that permethylated and peracetylated β-CD are strongly adsorbed from binary hexane-ethanol solution on bare silica [6]. FIGURE 1 shows the dependence of the retardation of TM-β-CD on a silica solid surface from hexane-alcohol mixtures on the type and concentration of the alcohol used. For all the alcohols investigated (e.g. MeOH, EtOH, n-PrOH), the retardation and thus adsorption of the chiral agent increases with decreasing alcohol concentration and reaches very high values for week eluents (see the dotted region in FIGURE 1).

TABLE 1

Regression parameters for capacity factor of TM-β-CD and concentration of alcohol used as modifier according to : $\log k'_{CD} = b - a \cdot \log x$, (x - mole fraction of alcohol).

Modifier	r	a	b
MeOH	0.918	1.840	0.517
EtOH	0.956	2.865	0.278
n-PrOH	0.969	1.847	0.176

TABLE 1 is a list of the regression parameters obtained for these dependencies according to:

$$\log k'_{CD} = b - a \cdot \log x$$

where: k'_{CD} - capacity factor of TM-β-CD, x - mole fraction of alcohol [9-10].

The regression data given can only serve as a rough estimate for the extend of TM-β-CD adsorption, since this compound gives an asymmetric peaks in weak eluents. The concentration dependence of the retention volumes of asymmetric peaks introduces a systematic error which causes lower regression coefficients and may result in large systematic errors for extrapolated values in the low concentration rage (the dotted range in FIGURE 1).

Dynamic Generation of the Chiral Stationary Phase

The chiral adsorption layer was generated by pumping a mobile phase consisting of TM-β-CD dissolved in binary mixtures of hexane and different alcohols through columns packed with bare silica. Before coating with chiral agent the column packed with silica must be activated by washing with ca. 100 dead volumes of absolute

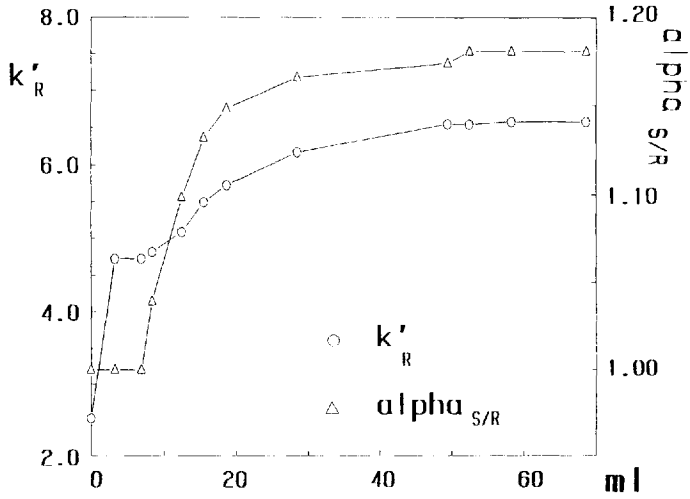

FIGURE 2. Kinetics of the generation of the CSP on LiChrosorb Si60, 5 μm. Column: 250·1 mm i.d. Eluent: hexane:n-PrOH, 150:2 (v/v) + 1 mg/ml TM-β-CD. Test compound: hexobarbital. Flow rate: 40 μl/min.

EtOH. Details of a study of the dynamic generation of the CSP on hydrophilic solid support were reported recently [7]. It was found that the dynamics of the built up of the CSP depend on the alcohol type used as modifier and on the concentration of TM-β-CD in the eluent. The date obtained formed the basis of an optimization of the generation of the CSP. A typical kinetic curve of the generation is shown in FIGURE 2. Optimal conditions were selected for this experiment e.g. we used n-PrOH as modifier, which enables to achieve the greatest selectivity in a short time and with moderate (1 mg/ml) concentration of TM-β-CD in the eluent, thus optimizing analysis time and consumption of chiral agent. The formation of the CSP was followed by the change in retention of a racemic mixture.

FIGURE 2 shows that the adsorbed chiral layer changes the retention properties and the selectivity of the phase system resulting in the enantioseparation.

TABLE 2.
Influence of alcohol used as modifier on retention and selectivity in solvent-generated chiral systems. Column: LiChrosorb Si60, 5 μm (250·1 mm). Eluent: hexane:alcohol (v:v) + 1 mg/ml TM-β-CD.

Compounds	hexane:alcohol					
	MeOH		EtOH		n-PrOH	
	k'_1	α	k'_1	α	k'_1	α
a						
Hexobarbital	15.05	1.043	7.00	1.171	6.54	1.181
Methylphenobarbital	13.57	1.064	7.60	1.158	6.73	1.181
Glutethimide	15.09	1.018	9.05	1.044	9.28	1.064
1-Methyl-5-phenyl-5-propylbarbital	10.86	1.0	6.35	1.035	6.13	1.054
b						
Benzoin	5.18	1.0	5.30	1.051	3.78	1.058
Ethyl mandelate	6.08	1.064	4.45	1.078	3.51	1.103
Methyl mandelate	7.64	1.035	7.10	1.070	6.23	1.053

a - 150 ml:2ml, b - 250 ml:0.5ml

Selectivity of the Solvent Generated Systems

The selectivity of the normal phase systems with TM-β-CD dynamically coated on the silica depends very strongly on the type and concentration of alcohol used as modifier. The influence of EtOH concentration in the mobile phase was demonstrated earlier [6]. TABLE 2 shows capacity factors for the first eluted peak and separation factors for different types of chiral compounds in normal phase systems operated with eluents containing MeOH, EtOH and n-PrOH as modifier. It can be seen from these results that the alcohol type influences the retention and selectivity of the system very strongly. For all racemates investigated, phase systems containing MeOH exhibit the greatest k' values, but lowest enantioselectivity.

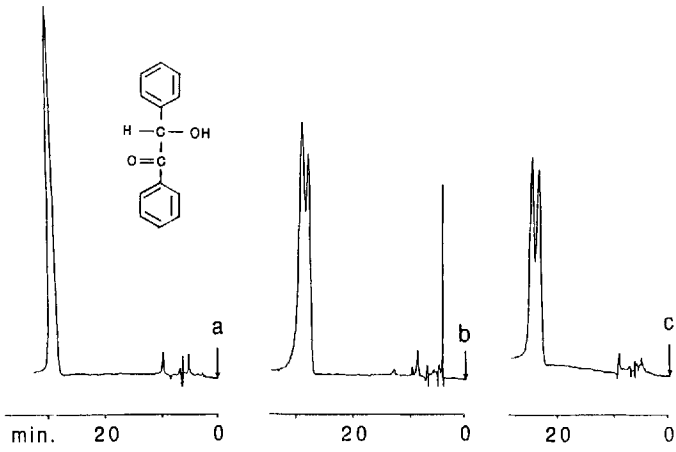

FIGURE 3. Enantiomeric resolution of benzoin enantiomers on dynamically modified LiChrosorb Si60, 5 μm. Eluent: 1 mg/ml TM-β-CD in hexane - alcohol, 150:0.5 (v/v). Flow rate: 40 μl/min. a) - MeoH, b) - EtOH, c) - n-PrOH

Applying EtOH and n-PrOH decrease the retention time and simultaneously increase the enantioselectivity. The differences in enantioseparation caused by the type of the modifier in the mobile phase are also shown in FIGURE 3 and FIGURE 4. The enantiomers of benzoin are not resolved in the phase system with MeOH as eluent component, but are separated in systems containing EtOH or n-PrOH. The same trend can be observed for the resolution of hexobarbital enantiomers. The system with n-PrOH enables base line resolution in a short time.

It seems that this phenomenon cannot be explained with a simple model. It has already been reported that the retardation of solutes strongly depends on the eluent strength [6]. This dependence on the eluent strength is also observed for the retardation of TM-β-CD on the silica surface (see FIGURE 1); the adsorption of TM-β-CD increases with decreasing eluent strength i.e. $k'_{MeOH} < k'_{EtOH} < k'_{n\text{-}prOH}$. Here we observe just opposite trend.

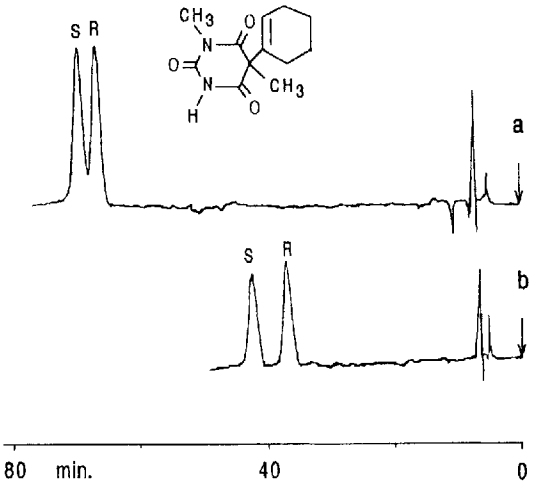

FIGURE 4. Enantiomeric resolution of hexobarbital on dynamically modified LiChrosorb Si60, 5 μm. Eluent: 1 mg/ml TM-β-CD in hexane :alcohol, 150:2 (v/v). Flow rate: 40 μl/min.
a) - MeoH, b) - n-PrOH

This can probably be explain by taking into account the mutual interaction between solute - adsorbed layer - alcohol; any explanation of this type needs additional experimental data on the stability constants of alcohol - TM-β-CD complexes for different alcohols. Such data are available for CD in aqueous eluents [11], but unfortunately not available till now for nonaqueous solution.

Stability and Reproducibility of the Normal Phase System

The reproducibility, stability and deactivation of normal phase system with dynamically generated CSP were studied by following the retention and chiral separation of enantiomers used as test analytes. TABLE 3 presents the capacity (k') and the separation

TABLE 3
Reproducibility of the retention characteristics in a chiral system generated several times on the same column. Eluent: hexane:ethanol (150:2 v/v) + 1 mg/ml TM-β-CD. Column: 250·1 mm packed with LiChrosorb Si60, 5 μm.

	I		II		III	
Compounds	k'	α	k'	α	k'	α
Hexo-barbital	7.00 8.20	1.171	7.06 8.29	1.174	6.82 7.97	1.168
Methyl-pheno-barbital	7.60 8.80	1.158	7.63 8.74	1.145	7.42 8.54	1.151

factors (α) of racemic mixtures tested for a solvent generated systems with CSP coated several times on the same column and TABLE 4 contains the retention characteristics for systems generated on Applying EtOH and n-PrOH decrease the retention time and simultaneously increase the enantioselectivity. The differences in enantioseparation caused by the type of the modifier in the mobile phase are also shown in FIGURE 3 and FIGURE 4. The enantiomers of benzoin are not resolved in the phase system with MeOH as eluent component, but are separated in systems containing EtOH or n-PrOH. The same trend can be observed for the resolution of hexobarbital enantiomers. The system with n-PrOH enables base line resolution in a short time.

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TABLE 4. Reproducibility of chiral systems generated on different silica solid supports. Eluent: hexane:n-propanol (150:0.5 v/v) + 1 mg/ml TM-β-CD. Column: 250·1 mm.

Sorbent	LiChrosorb Si60 Batch I		LiChrosorb Si60 Batch II		Partisil		Hypersil		LiChrospher Si100	
Substance	k'	α	k'	α	k'	α	k'	α	k'	α
Benzoin	3.78	1.058	4.47	1.065	3.73	1.060	3.68	1.060	3.07	1.077
	4.00		4.76		3.95		3.90		3.31	
Methyl mandelate	6.23	1.053	6.78	1.044	5.94	1.053	6.15	1.053	5.20	1.054
	6.53		7.08		6.25		6.48		5.48	
Ethyl mandelate	3.51	1.103	3.95	1.089	3.23	1.126	4.06	1.108	2.80	1.081
	3.87		4.30		3.64		4.50		3.02	
1-methyl-5-phenyl-5-propyl-barbiturate	18.20	1.078	19.10	1.089	19.05	1.074	15.31	1.052	14.51	1.070
	19.64		20.80		20.45		16.10		15.53	

i.e. $k'_{MeOH} < k'_{EtOH} < k'_{n\text{-}prOH}$. Here we observe just opposite trend. This can probably be explain by taking into account the mutual interaction between solute - adsorbed layer - alcohol; any explanation of this type needs additional experimental data on the stability constants of alcohol - TM-β-CD complexes for different alcohols. Such data are available for CD in aqueous eluents [11], but unfortunately not available till now for nonaqueous solution.

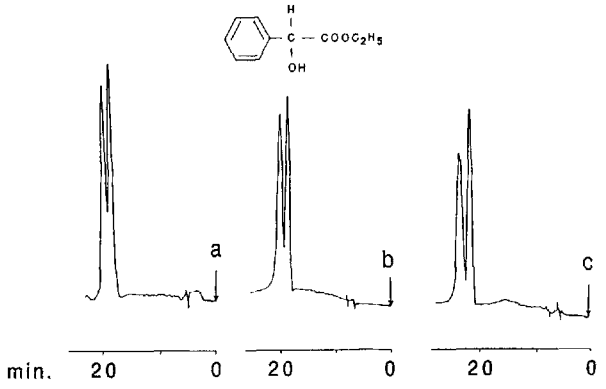

FIGURE 5. Enantiomeric resolution of ethylmandelate in normal-phase system with TM-β-CD dynamically coated on: a) LiChrosorb Si60, 5 μm; b) Hypersil, 3 μm; c) Partisil, 7 μm. Eluent: 1 mg/ml TM-β-CD in hexane - n-PrOH, 150:0.5 (v/v). Flow rate: 40 μl/min.

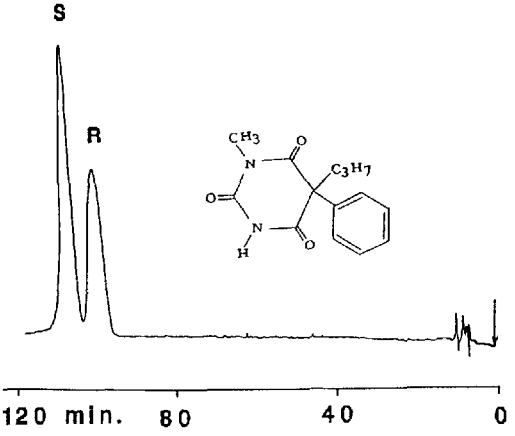

FIGURE 6. Resolution of enantiomers of 1-methyl-5-phenyl-5-propylbarbital on dynamic modified Partisil, 7 μm.
Eluent: 1 mg/ml TM-β-CD in hexane - n-PrOH, 150:2 (v/v).
Flow rate: 40 μl/min.

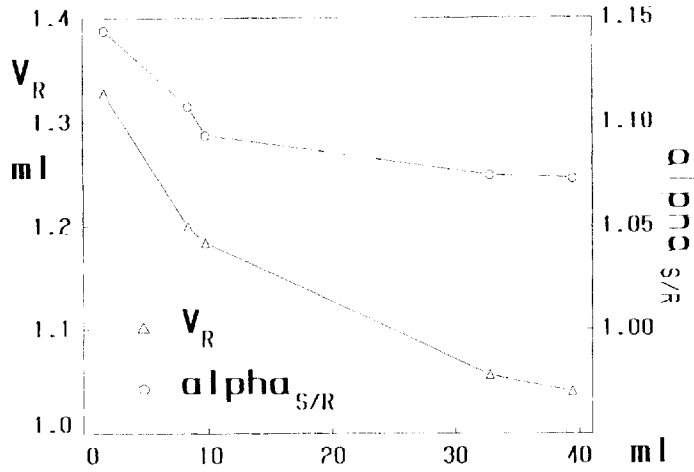

FIGURE 7. Deactivation of the chiral system - change of retention volume and selectivity factor during removal of the chiral stationary phase by achiral eluent. Column: 250·1 mm packed with LiChrosorb Si60, 5 μm dynamically coated with TM-β-CD. Eluent: hexane:EtOH, 150:2 (v/v). Flow rate: 40 μl/min. Test compound: methylphenobarbital.

Stability and Reproducibility of the Normal Phase System

The reproducibility, stability and deactivation of normal phase system with dynamically generated CSP were studied by following the retention and chiral separation of enantiomers used as test analytes. TABLE 3 presents the capacity (k') and the separation factors (α) of racemic mixtures tested for a solvent generated systems with CSP coated several times on the same column and TABLE 4 contains the retention characteristics for systems generated on different silica solid supports. In both cases the reproducibility of the retention was good and of the selectivity even very good. FIGURE 5 presents the separation of ethylmandelate enantiomers obtained on different silica supports dynamically coated with TM-β-CD. The selectivity varied from α = 1.089 for LiChrosorb Si60

batch II to α = 1.126 for Partisil, but the separation is very similar in all cases.

As reported previously [6] columns with TM-β-CD dynamically coated on silica show excellent time stability; retention volumes of the test enantiomers varied less than 0.3% during 10 days of experiment. FIGURE 7 shows the deactivations of chiral system by using an eluent without chiral agent. Retention volumes and separation factors decrease steeply with the volume of mobile phase pumped through. This reflects the slow removal of TM-β-CD coated on the silica support material by the achiral eluent. However, the enantioseparation can be observed even after pumping more than 250 column dead volumes, which shows a good stability of the system and on the other hand indicates that chiral recognition occurs really on the adsorbed chiral layer and not in the mobile phase.

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