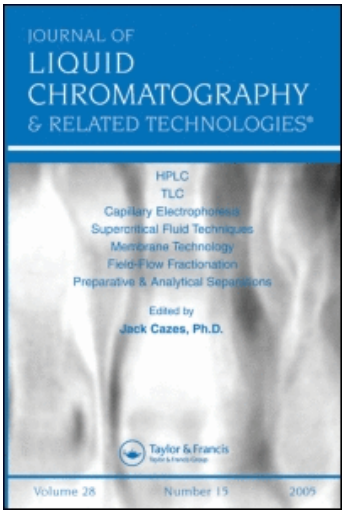

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Dynamically Generated Chiral Stationary Phase Systems with β-Cyclodextrin Derivatives

J. Zukowski[a]; R. Nowakowski[a]
[a] Institute of Physical Chemistry Polish Academy of Sciences Kasprzaka 44, Warsaw, PL, Poland

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DYNAMICALLY GENERATED CHIRAL STATIONARY PHASE SYSTEMS WITH β-CYCLODEXTRIN DERIVATIVES

J. ZUKOWSKI AND R. NOWAKOWSKI
Institute of Physical Chemistry
Polish Academy of Sciences
Kasprzaka 44, PL 01-224
Warsaw, Poland

ABSTRACT

It is demonstrated that chromatographic phase systems with a dynamically generated stationary phase with β-cyclodextrin derivatives are a powerful and versatile tools for enantioseparations. In this technique a small amount of β-cyclodextrin derivatives (di-, tri-methylated and peracetylated) is added to the eluent. These compounds are strongly adsorbed on the ODS surface where they form new adsorption sites with strong stereoselective interactions with enantiomers leading to a change in retention properties and selectivity of the phase system. This technique leads to columns with excellent time stability and good reproducibility of the enantioselectivity. The choice of a suitable β-cyclodextrin derivative offers a wide range possibilities to optimize enantioselectivity. It is shown that the kinetics of the sorption-desorption process of the solute on the chiral stationary phase largely contribute to the efficiency of the columns making an appropriate choice of the flow rate an important parameter for the optimization of the separations.

INTRODUCTION

It has been demonstrated recently that chromatography with a dynamically generated stationary phase is a powerful technique in HPLC. This method can be realized in the LLC as well as in the LSC mode.

In solvent generated LLC one liquid of a liquid - liquid phase system is used as a eluent. The second liquid phase is spontaneously generated on the surface of a suitable porous solid support [1-4]. In solvent - generated LSC a very small fraction of a strongly adsorbed component is added to the mobile phase. If this trace component has strong interactions with solute molecules its adsorption will not only block existing adsorption sites on the adsorbent surface, but also create new adsorption sites, thereby changing retention and selectivity of the phase system; it will act as column activator [5-7].

The solvent generated technique has been also successful used in chiral separation. Petersson and Stuurman have used a chiral LLC system for resolution of ephedrine enantiomers and its analogues [8]. The LSC technique has been developed for separation of various enantiomers using chiral activators eg: L-tartaric acid mono-n-octylamide [9] and L-aspartylalkylamides [10].

In previous work solvent generated chiral surface activation on an ODS support with di- and tri-methylated β-cyclodextrin derivatives has been demonstrated [11]. This method was successful in enantiomeric resolutions of barbituric acid derivatives [11] as well as glutarimide and succinimide derivatives [12].

This paper contains the results of a more detailed study of the adsorption of β-cyclodextrin derivatives (di-, tri-methylated and peracetylated) used as chiral mobile phase components on a ODS surface and the dynamics of the generation of the chiral stationary phase. We show the applicability of chromatographic systems with solvent generated chiral stationary phase for enantioseparations and discuss problems with the efficiency of the columns.

EXPERIMENTAL

Apparatus

Chromatographic experiments were carried out with a high pressure microbore liquid chromatograph Type 310 (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw) equipped with UV detector (254 nm) containing 1 μl flow cell and 0.5 μl injector. Micropreparative chromatographic purifications were carried out with an analytical high pressure liquid chromatograph Type 320 (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw) and a loop injector (Rheodyne Type 7125 & 7010, Supelco). For micropreparative chromatographic experiments and the chromatographic determination of adsorption isotherms a UV-absorption (254 nm) and a refractive index detector (Type B-5302 COBRABID, Poland) equipped with 10 μl flow cells were used.

Reagents

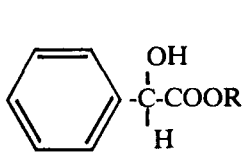
β-Cyclodextrin (β-CD) supplied by Chinoin (Budapest, Hungary) was purified by recrystallization from water. Heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) and heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) were prepared according to Szejtli et al. [13]. Peracetylated β-CD (Ac-β-CD) was synthesized according to McClenahn et al. [14]. All other reagents and solvents were of analytical-reagent grade and were used without purification. The formulae of the investigated compounds are given in Table 1.

D- and L-mandelic acid enantiomers were supplied by E. Merck and Fluka, respectively. The pure enantiomers of methyl and ethyl mandelate were prepared in simple esterification reactions from D- and L-mandelic acids in methanol or ethanol solutions with H_2SO_4 as a catalyst.

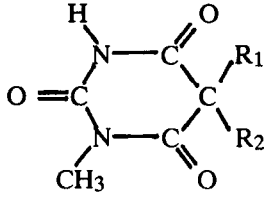
Racemic barbiturates 4 and 5 were prepared by methylation of achiral well known drugs: 5-methyl-5-phenylbarbiturate (Heptobarbital) and 5-(cyclohexen-1-yl)-5-ethylbarbiturate (Cyclobarbital) (Sandoz). The post reaction mixtures were purified by micropreparative method using 300x12

TABLE 1

Structural formulae of investigated compounds



	R
1	-H
2	-CH_3
3	-CH_2-CH_3



	R_1	R_2
4	-CH_3	
5	-CH_2-CH_3	

mm i.d. column packed with 15-25 μm LiChroprep RP 18 and MeOH:H_2O (1:1) eluent. LiChrosorb RP 18 (5 μm) and LiChroprep RP 18 (15-25 μm) (both supplied by E. Merck) were used as column packing for analytical and micropreparative chromatographic experiments, respectively.

PROCEDURE

Chromatographic experiments

Experiments were carried out with 250x1 mm i.d. column packed with 5 μm LiChrosorb RP 18. The chiral stationary phase was generated dinamically on the solid support by pumping the mobile phase phase MeOH - H_20 (0.2% H_3PO_4) solutions containing various amounts of appropriate β-CD derivatives. For comparison the same column was operated with concentrated (above 10 mM) parent β-CD in alcoholic (MeOH, EtOH) aqueous solution containing 0.2% H_3PO_4.

The elution order for mandelic acid and its derivatives was estimated by injecting pure enantiomers. The elution order of the enentiomers for the barbiturates investigated is unknown.

The plate number N and resolution R_S were calculated from chromatograms according to:

$$N = \frac{V_{Ri}^2}{\sigma V_i^2} \qquad R_S = \frac{V_{R2} - V_{R1}}{2\sigma V_1 - 2\sigma V_2}$$

where: V_{Ri} - retention volume (ml) of substance i,

σV_i - volume standard deviation (ml) of substance i.

Determination of adsorption isotherms

The adsorption isotherms of β-CD derivatives on LiChrosorb RP 18 were determinated by the non - equilibrium - peak profile method according to Huber and Gerritse [15]. The isotherms were calculated point by point from a single chromatogram of an overloaded peak to minimized the consumption of β-CD derivatives.

The experiments were carried out on 50x4 mm i.d. column packed with 5μm LiChrosorb RP 18 at 25 ± 0.1 °C. To reduce the volume of dissolved sample in eluent was 50 μl for DM- and TM-β-CD; this is about 1/10 of the column dead volume V_o. Only for hardly soluble AC-β-CD the injecting volume was 100 μl. The V_o value was determined for β-CD solute, which has a similar molecular dimension as its derivatives.

RESULTS AND DISCUSSION

Adsorption of chiral components

The adsorption isotherms of DM-β-CD, TM-β-CD and AC-β-CD on the ODS solid support are shown in Figure 1. The experimental data show two

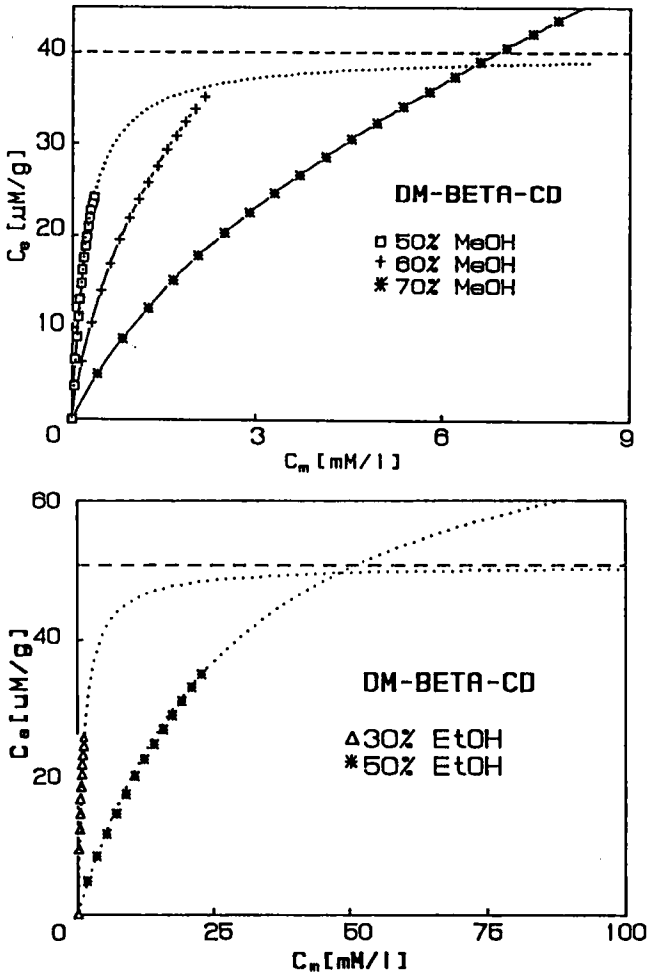


FIGURE 1. Adsorption isotherms of β-CD derivatives on a LiChrosorb RP 18 (5 μm) surface from binary solutions alcohol/water at 25 ± 0.1°C.

(continued)

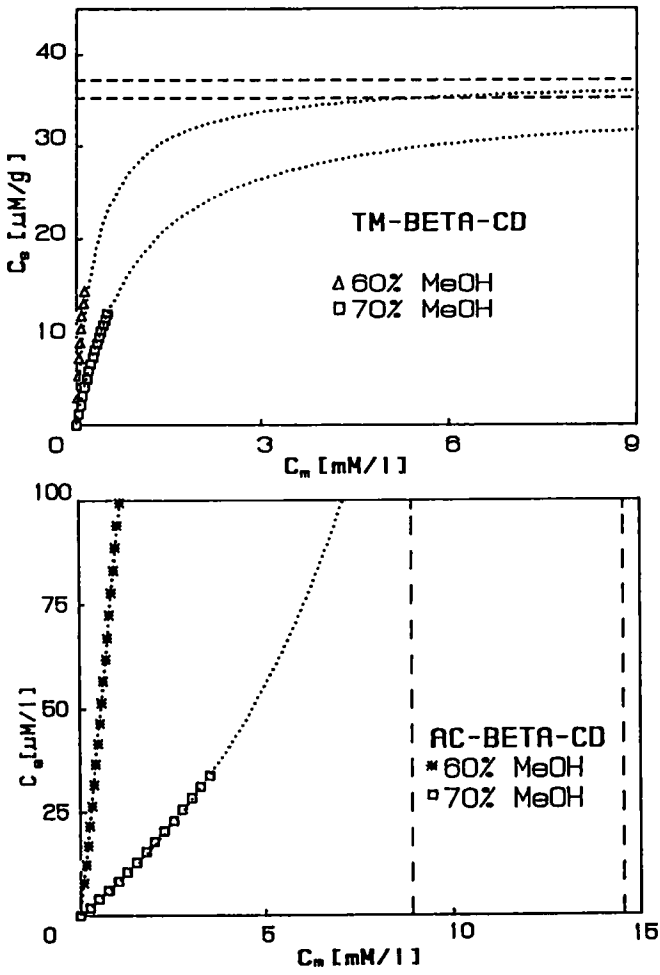


FIGURE 1 (continued)

different types of adsorption from binary solution (MeOH + H_2O and EtOH + H_2O). For methylated derivatives the experimental data indicate a typical convex Langmuir isotherms. For peracetylated β-CD rather rarely observed concave type of isotherm was found.

The convex isotherm is described by a simple Langmuir isotherm (L1 type according to [16]) assuming a monomolecular adsorbate layer:

$$C_s^* = \frac{C_m^*}{k_2/k_1 + C_m^*/Q_l} \qquad (1)$$

where: C_m,C_s - concentrations of the substance in the mobile and stationary phases,

k_1 - rate constant for the adsorption,

k_2 - rate constant for the desorption,

Q_l - capacity of the monomolecular adsorption layer,

* - signifies equilibrium values.

The concave isotherm found for peracetylated derivatives cannot be interpreted as Langmuir adsorption isotherms. This type of isotherm (type S1 according to [16]) can be found for solutions of liquids with limited miscibility [17] and for solutions of hardly soluble substances [18].

Using a simplified model [19] these isotherms can be described by:

$$C_s^* = \frac{C_m^*}{(k_2/k_1)(1 - C_m^*/Q_s)} \qquad (2)$$

where: Q_s - capacity of the mobile phase.

The equations (1) and (2) given above can be used to calculate important parameters for the characterization of the isotherms in a given chromatographic system: the ratio of the constants of the sorption process k_1/k_2 and the capacities of the stationary phase Q_L (for the convex isotherm) or the mobile phase Q_S (for the concave isotherm). In both cases k_1/k_2 is found from condition:

TABLE 2

Parameters of adsorption isotherms of β-CD derivatives from binary mixtures. Adsorbent: 5 μm LiChrosorb RP 18. Temperature 25 ± 0.1 °C.

Deriv of β-CD	eluent	Type of isotherm	Parameters of isotherm		
			K_1/K_2 [cm^3/g]	Q [μM/g]	Q [mg/g]
DM-β-CD	50%MeOH	L1	175.43	39.99	53.32
	60%MeOH	L1	40.72	57.36	76.35
	70%MeOH	L1	12.01	81.09	107.93
	30%EtOH	L1	44.57	50.82	67.64
	50%EtOH	L1	2.72	79.86	106.29
TM-β-CD	60%MeOH	L1	116.95	37.26	53.24
	70%MeOH	L1	35.71	35.23	50.34
AC-β-CD	60%MeOH	S1	80.78	8.45 μM/L	
	70%MeOH	S1	7.36	14.70 μM/L	

$$k_1/k_2 = \lim_{C_m^* \to 0} \frac{dC_s^*}{dC_m^*}$$

the inclination of the tangent at the point $C_m^* = 0$. The results of the numerical calculation are shown in Table 2. For all this isotherms the ratio of the constants of the sorption processes distinctly increases with decreasing concentration of the organic solvent in the eluent. A rough estimation of the Q_L values for the monomolecular adsorption layer assuming an adsorption surface of approximately 150 m^2/g for an ODS adsorbent and an area of 760 $Å^2$ for β-cyclodextrin derivatives leads to Q_L = 48 mg/g. This figure is in good agreement with the Q_L values found for solutions of lower methanol or ethanol concentrations indicating that a monolayer Langmuir isotherm is a realistic model for these cases.

Adsorption of β-CD derivatives on the ODS surface from eluents used in this work ie $C_{MeOH} < 50$ % is so strong that it is impossible to determinate its value by the chromatographic method discussed above. Table 3 shows k' values of β-CD derivatives on the analytical RP 18 column for different eluents. The retardations of β-CD derivatives increase steeply with decreasing MeOH concentration in the eluent.

Table 3a contains the experimental data ($65\% < C_{MeOH} < 80\%$) and Table 3b k' values for weaker eluents calculated according to

$\log k'_{CD} = b - a \cdot C_{MeOH}$. The regression parameters for this dependence are given in the Table 4.

In 65% MeOH the adsorption of DM-β-CD is two times greater than for AC-β-CD but the extrapolated adsorptions values are reversed in 30% MeOH ie the retardation of AC-β-CD exceeded the value for DM-β-CD six times.

TABLE 3

Determinated and extrapolated k' value of β-CD derivatives for different concentration of MeOH in eluent at 25 ± 0.1 °C. Stationary phase as in TABLE 2. Eluent: MeOH/H_2O (0.2% H_3PO_4).

	%MeOH	k'CD		
		DM-β-CD	TM-β-CD	AC-β-CD
a	80	3.24	4.41	0.56
	75	4.48	7.98	1.40
	70	7.86	17.67	2.96
	65	13.86	38.92	7.29
b	50	52.	338.	90.
	40	155.	1463.	487.
	30	414.	6340.	2626.

TABLE 4

Regression parameters for capacity factor and volume concentration of methanol according to : $\log k'_{CD} = b - a \cdot c_{MeOH}$ (c_{MeOH} - volume concentration of MeOH).

CD	a	b	r
DM-β-CD	0.0428	3.899	0.993
TM-β-CD	0.0637	5.712	0.998
AC-β-CD	0.0732	5.616	0.999

As can be seen from the Tables 2 and 3 the adsorption of β-CD derivatives depends strongly on the MeOH concentration in eluent. For binary eluents used in this study it decreases in the order:

TM-β-CD > AC-β-CD > DM-β-CD.

Dynamics of the generation

The chiral stationary phase was generated dynamically on the solid support by pumping diluted solution of appropriate β-CD derivatives through the column. DM-β-CD and TM-β-CD were dissolved in eluent in desired quantity (below 1 mM). Hardly soluble AC-β-CD was dissolved in stronger as optimal eluent. The formation of the chiral stationary phase was followed by the change in retention of test compounds. The results are exemplified in Figure 2. It can be seen that the retention of test solutes decreases in both instances with the volume of eluent pumped trough owing to the adsorption of chiral modifier which blocks the hydrophobic solid surface. These adsorbed chiral layer then interacts with the solutes resulting in different selectivity of the system. The generation of the stationary phase consisting of DM-β-CD and TM-β-CD is completed after pumping through approximately 15 ml corresponding to about 110 column dead volumes. A slightly different method was used for generation of chiral stationary phase consisting of AC-β-CD

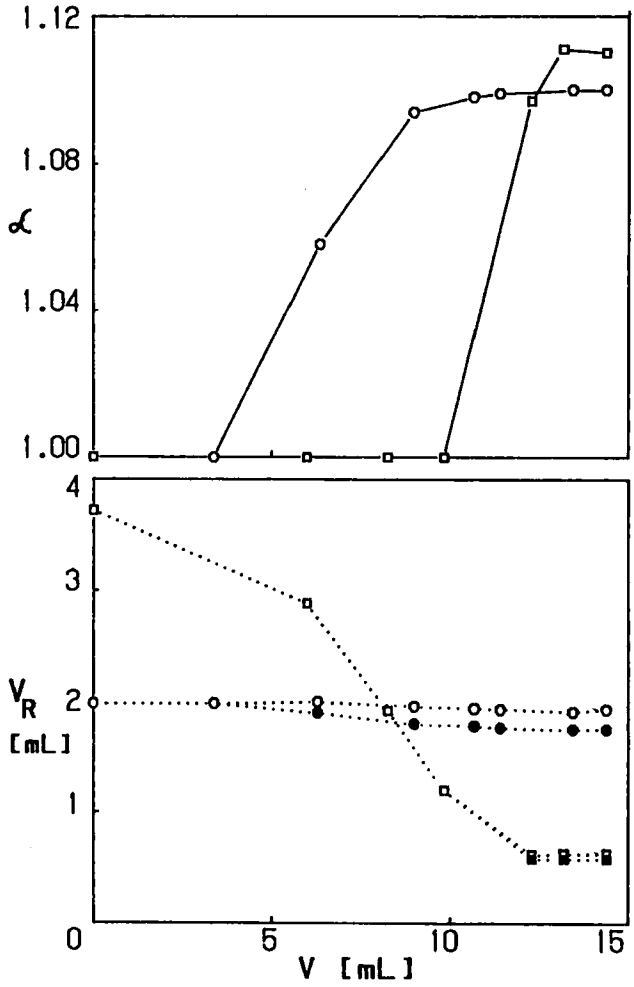


FIGURE 2. Kinetics of the generation of chiral stationary phases on Lichrosorb RP 18 (5 μm) solid support. Column dimensions: 250x1 mm. Flow rate: 40 μl /min. Eluent: □ 10 % MeOH + 1 mM TM-β-CD, test compound 2; ○ 40 % MeOH + 0.64 mM DM-β-CD, test compound 5.

because of its poor solubility in eluents. In this case a saturated solution of AC-β-CD in a stronger solvent (60 % MeOH) was used for generation of the stationary phase. The equilibrium was established after about 120 column dead volumes. Then the eluent was changed for less concentrated MeOH solution saturated with AC-β-CD. Columns obtained in this manner show excellent time stability and good reproducibility of the enantioselectivity. After removing the adsorbed layer of chiral molecules with methanol the same column can be used again to create the next chiral stationary phase dynamically without deterioration in column properties.

Selectivity

It has been demonstrated that the derivatization of CD hydroxyl groups changes its properties to a large extent. As mentioned above, methylationand acetylation enable the adsorption of β-CD derivatives on a hydrophobic ODS surface, and a change of molecular structure [20] also changes its complexing ability causing differences in enantioselectivity. This was found in gas chromatography for systems with TM-β-CD [21], in LC for systems with chemically bonded CDs [22] and in RP systems with CDs in the mobile phase [11,23].

Table 5 contains capacity factors (k') and separation factors (α) for enantiomers of barbiturates, mandelic acid and its derivatives in RP systems operated with β-CD and its derivatives in the mobile phase.

It has already been reported that enantioselectivity of systems with β-CD in eluent enables only the resolution (via complexation and recognition in a mobile phase) of barbiturates containing a chiral center in the heterocyclic ring [11,23]. Such systems exhibit lower selectivity for mandelic acid enantiomers and no selectivity for methyl mandelate [24]. The results collected in Table 5 show a new type of enantioselectivity for systems with β-CD derivatives added to the eluent. Applying different derivatives which are absorbed on the ODS surface leads to another mechanism of chiral recognition. The enantiomers of barbiturates resolved via complexation with

TABLE 5

Capacity and separation factors for investigated compounds in RP systems containing β-CD and its derivatives:

Comps	β-CD k'	β-CD α	DM-β-CD k'	DM-β-CD α	TM-β-CD k'	TM-β-CD α	AC-β-CD k'	AC-β-CD α
1 L	3.00	1.00 [a]	4.75	1.052 [c]	3.58	1.110 [c]	1.58	1.053 [e]
1 D			5.00		3.25		1.50	
2 L	10.80	1.0	5.50	1.015	6.13	1.093	5.74	1.043
2 D			5.58		5.60		5.50	
3 L	24.45	1.0	12.33	1.027	10.46	1.173	13.21	1.032
3 D			12.66		8.92		12.80	
4	2.81	1.089 [b]	5.33	1.049 [d]	2.89	1. [d]	7.80	1. [f]
	3.06		5.59					
5	6.44	1.109	16.47	1.103	7.80	1.	18.89	1.044
	7.14		18.17				19.73	

Column : 250x1 mm id packed with 5 μm LiChrosorb RP 18
Flow rate 30 μl/min.Eluents: [a] - 10% MeOH + 10 mM β-CD, [b] - 20% EtOH + 22 mM β-CD, [c] - 10% MeOH + 0.50 mM DM-β-CD or TM-β-CD, [d] - 40% MeOH + 0.64 mM DM-β-CD or TM-β-CD, [e] - 5% MeOH + saturated AC-a-CD, [f] - 40% MeOH + saturated AC-β-CD.

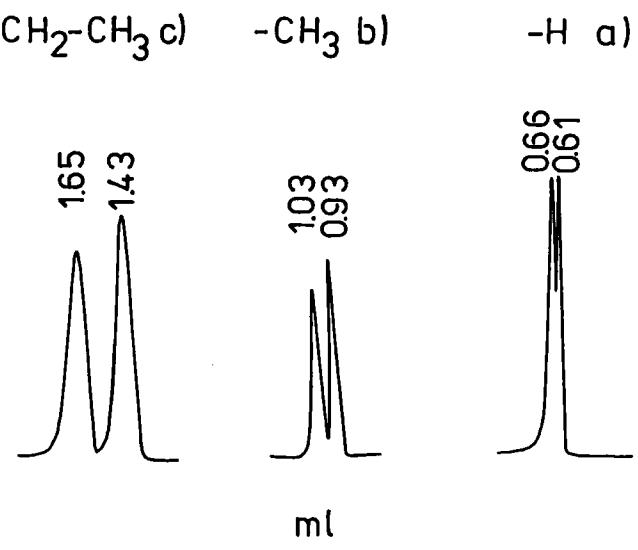


FIGURE 3. Enantiomeric resolution of mandelic acid and its derivatives obtained on a solvent generated chiral stationary phase consisting of TM-β-CD. Eluent: 10 % MeOH + 0.50 mM TM-β-CD. Flow rate: 30 μl/min. Column and solid support as in Figure 2.

β-CD in the mobile phase are also resolved in system with DM-β-CD, where chiral recognition occurs on a dynamically generated chiral stationary phase. Systems with solvent generated chiral stationary phase consisting of TM-β-CD exhibits no selectivity and the AC-β-CD systems are only poorly selective for the enantiomers of the barbiturates investigated. The enantioselectivity of these systems is quite different for mandelic acid and its derivatives. Figure 3 shows the excellent selectivity for these compounds obtained on a column with a solvent generated stationary phase consisting of TM-β-CD. The selectivity obtained in systems with DM-β-CD or AC-β-CD is much lower.

In contrast to the results obtained by Fujimura et al. [25] with columns packed with chemically bonded β-CD the elution order for all this compounds was $k'_D > k'_L$.

The results given above show that the derivatization of cyclodextrin leads to a multitude of ligands with different stereoselectivity which can be used for the optimization of chromatographic separations of different types of chiral compounds.

Column efficiency

Special attention should be paid for the efficiency of columns with phase systems of low selectivity. Unfortunately this problem is only occasionally discussed in a few papers for chiral chromatographic systems [22,26,27].

The Figures 4 and 5 show the resolution of the barbiturates 5 and 4 using the mobile phase containing β-CD (a) and in dynamically generated systems with DM-β-CD as the chiral component (b). Figure 4 shows that base line resolutions of 5 are obtained in both systems. However, the resolution obtained on a DM-β-CD generated stationary phase is better (Rs = 2.00) as in the system with β-CD in mobile phase (Rs = 1.58) for the same selectivity. The same can be observed in Figure 5, the resolution obtained in both systems is very similar, although the selectivity is higher for system recognizing via complexation in the mobile phase ($\alpha_{\beta\text{-CD}}$ = 1.089 > $\alpha_{\text{DM-}\beta\text{-CD}}$ = 1.049). These differences in resolution can be explained by differences in column efficiency.

Table 6 shows the efficiency of chromatographic column for systems without chiral component, with β-CD in the mobile phase and for dynamically generated systems with β-CD derivatives as chiral stationary phases. All the chromatographic determination were carried out on the same column. The use of chiral component always caused a decrease in column efficiency. The decrease depends on the nature of the chiral additives as well as on the solute to be separated.

Following the general non-equilibrium theory developed by Giddings [28], the overall plate high (H) for LSC can be expressed as:

$$H = A + \frac{B}{u} + C_m u + C_k u \qquad (3)$$

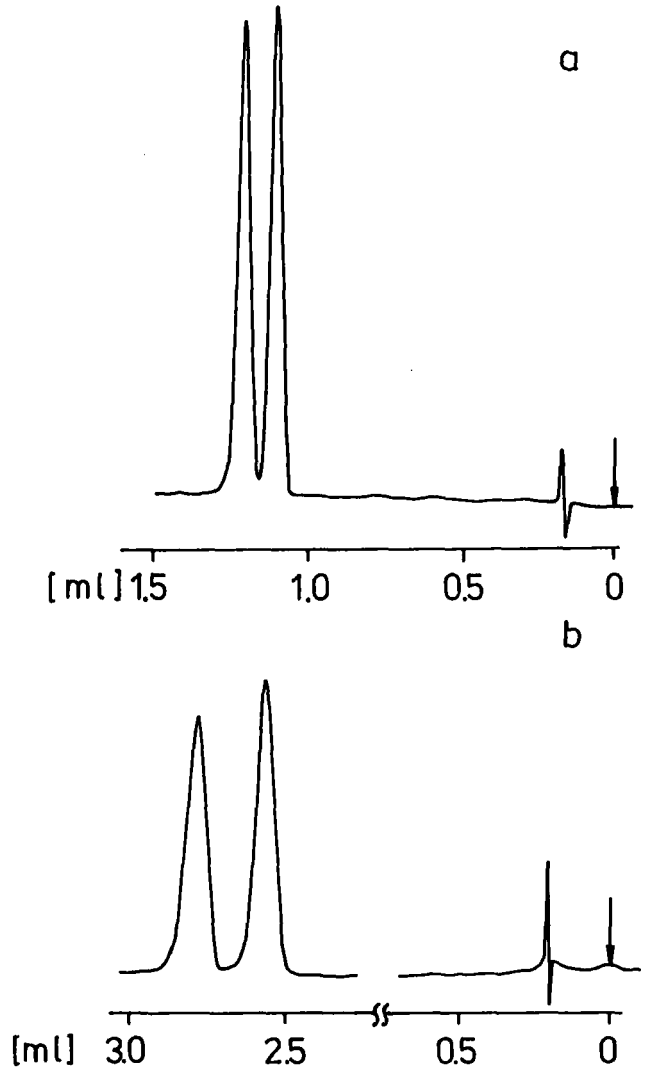


FIGURE 4. Enantiomeric resolution of barbiturate 5 on a 250x1 mm column packed with 5 μm LiChrosorb RP 18. Flow rate: 30 μl/min. Mobile phase: a) 20 % EtOH + 22 mM β-CD; b) 40 % MeOH + 0.64 mM DM-β-CD.

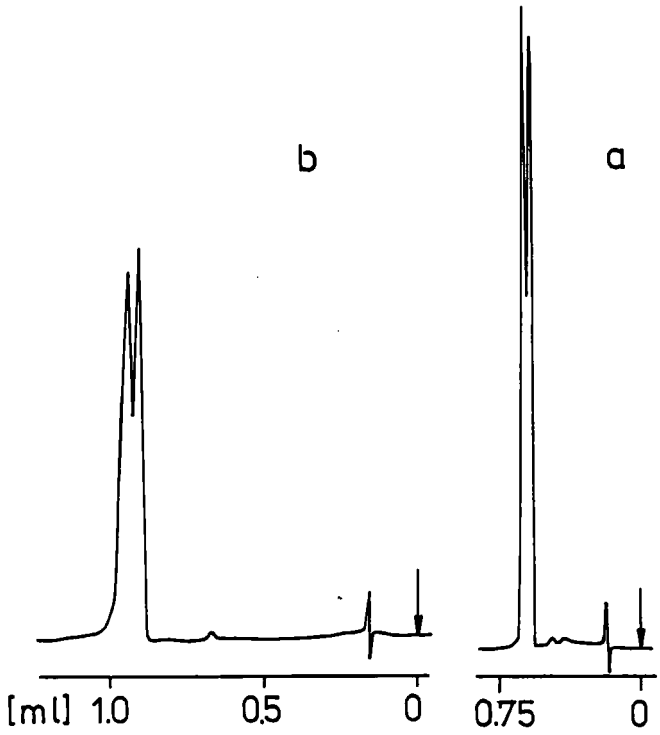


FIGURE 5. Enantiomeric resolution of barbiturate 4. Chromatographic conditions as in Figure 4.

Zone spreading is due to three independent sources: flow pattern effects (A), longitudinal diffusion (B) and mass transfer effects (C). Flow pattern effects depend on the structure of the porous support material and are independent from the eluent velocity (u). Ordinary molecular diffusion in the flow direction contributes most at low or very low velocities. Mass transfer effects contribute most in high speed runs. In adsorption chromatography they are controlled by two basically different mechanism or some combination thereof: diffusion controlled sorption and desorption rates originating in the mobile phase (C_m) and adsorption - desorption kinetics (C_k).

TABLE 6

Column efficiency for RP systems containing β-CD, its derivatives and without chiral additive. Chromatographic conditions as in Table 5.

Comps	β-CD N	DM-β-CD N	TM-β-CD N	AC-β-CD N	MeOH:H_2O k' N
1 L	10000	4530	4980	2900	-
D	10000	4670	4750	3000	
2 L	8600	4480	5070	3600	-
D	8600	5900	5940	3600	
3 L	4050	680	1440	2800	-
D	4050	580	920	2700	
5	3050	9600	-	-	4:6
	3370	7600	-	-	12.5 12200

The results presented in the Figures 6 and 7 show considerable dependence of column efficiency and therefore resolution on the eluent flow rate for all systems investigated and all compounds separated. This indicates that in the range of flow rate used in this study zone spreading is mainly caused by mass transfer effects. In systems with solvent generated chiral stationary phases the contribution of the first three terms to H is the same for both enantiomers to be separated but the mass transfer term is controlled by the kinetics of the complexation reaction between enantiomers (G) adsorbed on an ODS surface covered with β-CD derivatives:

$$(\text{deriv.-}\beta\text{-CD})_s + G_m \underset{k_d}{\overset{k_a}{\leftrightarrow}} (\text{deriv.-}\beta\text{-CD} + G)_s$$

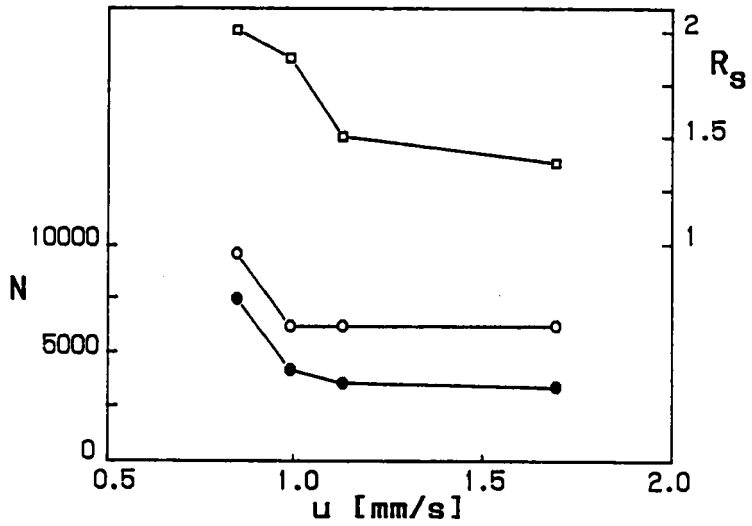


FIGURE 6. Dependence of the column efficiency and enanatio- resolution on flow rate. Test compound: barbiturate 5. Eluent: 40% MeOH + 0.64 mM DM-β-CD. Column and solid support as in Figure 2.

According to:

$$C_k = q \frac{k'}{k_d (1 + k')^2} \qquad (4)$$

where: q - geometrical parameter and k_d - desorption rate,

the significant differences in column efficiency observed indicated that the kinetics of the complexation is very different for the groups of test compounds investigated. This can very well be seen in Figure 7 and in Table 6 for the system containing DM-β-CD. The differences in column efficiency indicate that the rate of adsorption - desorption process for barbiturates is much higher as for mandelic acid and its derivatives. Moreover, the H(u) curves for barbiturates obtained point to a considerable difference in adsorption-desorption kinetics for barbiturate enantiomers: for the stronger

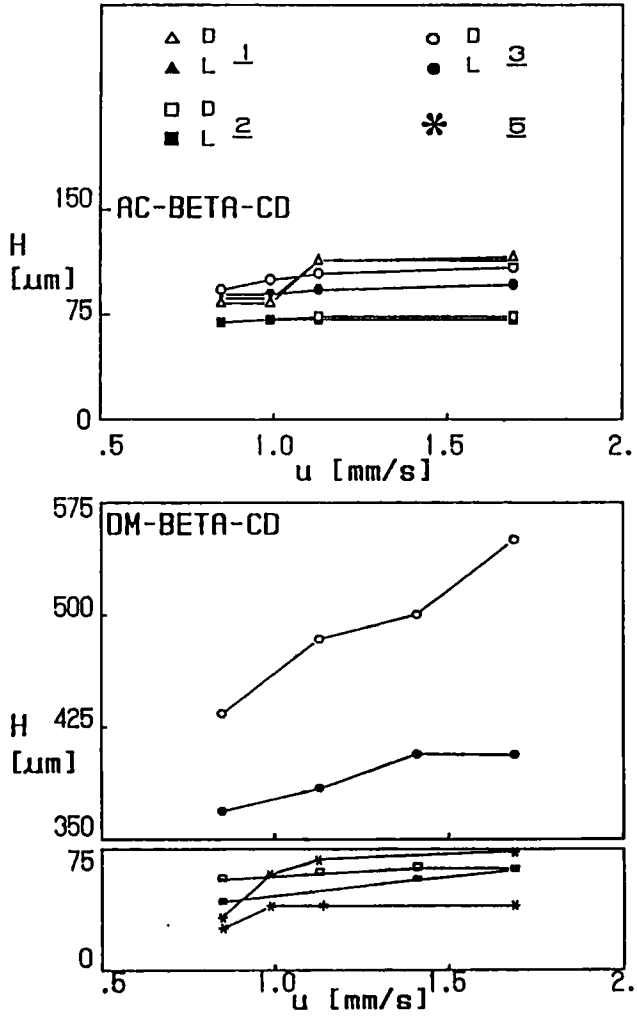


FIGURE 7. Dependence of column efficiency on flow rate in chromatographic systems with solvent generated stationary phase consisting of β-CD-derivatives. Column and solid support as in Figure 2.

(continued)

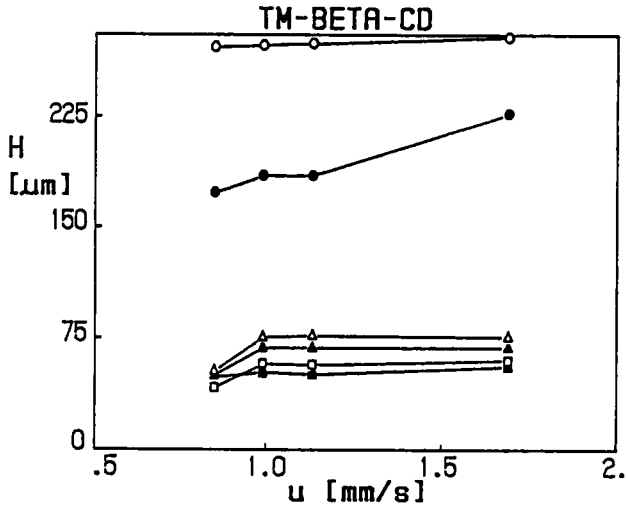


FIGURE 7 (continued)

retarded enantiomer the kinetic is much slower as for first eluted one ($N_1 >> N_2$; $k'_2 > k'_1$). In the separation of enantiomers of mandelic acid and mandelates the kinetics show big differences from substance to substance. The kinetics for ethyl derivative is especially slow resulting in a drastic decrease of column efficiency which it makes impossible to resolve these enantiomers.

A comparison with the results for the system with β-CD contained in the eluent shows the difference between a system recognizing via complexation in the mobile phase and systems with a solvent generated chiral stationary phase. For enantiomers which are not recognized in the mobile phase (like mandelic acid or mandelates) the efficiency is the same for both enantiomers and decreases with increasing capacity factor, the same dependence was discussed by Huber [29]. Substances which are recognized in the mobile phase (like barbiturates) show differences between the enantiomers and the decrease in column efficiency surpasses the decrease observed with solvent generated chiral stationary phases. This is probably due to the fact that in these cases

mass transfer effects can be controlled by the kinetics of the stereocomplexation reactions in the mobile phase and the kinetics of the adsorption - desorption process of the steroisomeric complexes on the ODS surface.

From a practical point of view the slow kinetics observed for chiral systems emphasize the importance of selecting the appropriate operating conditions which influence column efficiency: in most cases this leads to a selection of particles with a diameter as small as possible and a compromise between the analysis time and the flow rate necessary to achieve the desired resolution.

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